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Influence of Rhizobia Inoculation and supplementation with phosphorus and potassium in soybean-maize intercropping system

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INFLUENCE OF RHIZOBIA INOCULATION AND SUPPLEMENTATION WITH PHOSPHORUS AND POTASSIUM IN SOYBEAN-MAIZE INTERCROPPING SYSTEM

Daniel Nyoki

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Life Science of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

A field experiment was carried out at Tanzania Coffee Research Institute (TaCRI) farm, for two consecutive years (2015 and 2016). The objective of this study was to assess the effects of cropping systems, *Rhizobium* inoculation and fertilization with P and K on farm productivity of both soybean and maize. The experiment was laid out in split-split plot design with 2 x 4 x 7 factorial arrangements and replicated thrice. The main plots comprised two rhizobia inoculation treatments, while the sub plots were comprised of: Maize pure stand (75 x 60 cm); Soybean pure stand (75 x 40 cm); maize-soybean intercropping (75 x 60 cm and 75 x 20 cm), maize and soybean respectively; and the last cropping system was maize-soybean intercropped (75 x 60 cm and 75 x 40 cm), maize and soybean respectively. The sub-subplots were assigned the following fertilizer levels (kg ha^{-1}): control (0 kg ha^{-1}); 20 K; 40 K; 26 P; 52 P; 26 P + 20 K; 52 P + 40 K. The 3-way analysis of variance (ANOVA) in factorial arrangement was performed. The STATISTICA software program was used. The fisher's least significance difference (L.S.D.) was used to compare treatment means at $p = 0.05$ level of significance. The results indicated that rhizobia inoculation and fertilization of crops with P and K significantly improved mineral composition in the rhizosphere soil of soybean; nutrient uptake in soybean shoots; nitrogen fixation and chlorophyll concentration in soybean. Furthermore, rhizobia inoculation and P and K fertilization significantly improved plant growth and final yield of both soybean and maize. Cropping systems were also assessed and found that intercropping was advantageous since the values of land equivalent ratios (LER) were greater than one. In general, several parameters tested in this study have shown to perform better in combined lower rates (20 kg K ha^{-1}+26 kg P ha^{-1}) of P and K. It is therefore recommended that the combined lower rates of these fertilizers should be adopted and be used by farmers in areas with similar characteristics as that of study area.

DECLARATION

I, **DANIEL NYOKI** do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Daniel Nyoki

Name of candidate	**Signature of candidate**	**Date**

The above declaration is confirmed

Prof. Patrick A. Ndakidemi

Name of supervisor	**Signature of supervisor**	**Date**

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CERTIFICATION

The undersigned certify that they have read the dissertation titled **Influence of rhizobia inoculation and supplementation with phosphorus and potassium in soybean-maize intercropping system** and recommend for examination in fulfilment of the requirements for the degree of Doctor of Philosophy of Life Science of the Nelson Mandela African Institution of Science and Technology.

Professor Patrick A. Ndakidemi	Date

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DEDICATION

This dissertation is dedicated to my beloved wife Anna Baltazari, daughter Diella Daniel and son Adriel Daniel for their patience during my studies.

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M+B (A)	Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively (Narrower Spacing)
M+B (B)	Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively (Wider spacing)
M+B(A)+R	Maize intercropped with Rhizobium inoculated soybean at narrower spacing
M+B(B)+R	Maize intercropped with Rhizobium inoculated soybean at wider spacing
M+B(A)-R	Maize intercropped with un-inoculated soybean at narrower spacing
M+B(B)-R	Maize intercropped with un-inoculated soybean at wider spacing
+R	With rhizobium
-R	With out Rhizobium
*	Significant at $p \leq 0.05$
**	Significant at $p \leq 0.01$
***	Significant at $p \leq 0.001$

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BNF	Biological Nitrogen Fixation
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Chl T	Chlorophyll total
CR	Competitive Ratio
CroSyt	Cropping Systems
DMSO	Dimethyl Sulphoxide
DTPA	Diethylenetriaminepentaacetic acid
EC	Electrical Conductivity
Fert	Fertilizers
HI	Harvest Index
IPNI	International Plant Nutrition Institute
ISFM	Integrated Soil Fertility Management
L.S.D	Least Significance Difference
LER	Land Equivalent Ratio
MAI	Monetary Advantage Index
MOP	Muriate of Potash
NM-AIST	Nelson Mandela African Institution of Science and Technology
ns	Not significant
OC	Organic Carbon
PGPR	Plant Growth Promoting Rhizobacteria
RCC	Relative Crowding Coefficient
Rhiz	Rhizobium
SB	Sole soybean
SE	Standard error
SM	Sole maize
SSA	Sub Saharan Africa
TaCRI	Tanzania Coffee Research Institute
TNF	Total Nitrogen Fixation
TSP	Triple Super Phosphate
WAP	Weeks after Planting

CHAPTER ONE

1.1. Background information

Intercropping is an old and common agricultural practice of growing more than one crop in the same field at the same time. This is a common practice in sub Saharan Africa (SSA), and it is mostly practiced by smallholder famers. Most common crop combinations in intercropping systems includes: maize-cowpea, maize-pigeon pea, maize-soybean, maize-groundnuts, maize-beans, maize-lablab sorghum-cowpea, millet-groundnuts, and rice-pulses (Matusso *et al.,* 2012). This cropping practice aims to match efficiently crop demands to the available growth resources and labour **(**Dahmardeh *et al.*, 2010; Lemlem, 2013). The efficient use of available growth resources in a given piece of land and eventually maximizing productivity is the primary advantage of intercropping crops of different height, canopy structure, rooting ability, and nutrient requirements **(**Lemlem, 2013; Ghanbari, *et al*., 2010**)**. Many studies on intercropping in SSA have shown that legumes-cereal intercropping is an important productive and sustainable system due to its resource facilitation and significantly enhancing crop productivity as compared with that of monoculture crops (Jensen, 1996; Ghanbari *et al*., 2010; Dahmardeh *et al*., 2010).

In an effort to improve food security, intercropping cereals with legumes plays an important role by providing a farmer with both carbohydrates and proteins for their dietary needs. Apart from nutritional composition of component crops in an intercropping, it has been also reported that intercropping improves soil fertility through biological nitrogen fixation, increases soil conservation through greater ground cover than sole cropping (Lemlem, 2013), and provides better protection against crop pests and diseases than when grown in monoculture (Matusso *et al*., 2012).

Leguminous crops are well known for their ability to fix atmospheric nitrogen, which can be used by the legume plant themselves or might be excreted out of legume's root structures called nodules into the rhizosphere soil and be utilized by other plants growing nearby in an intercropping systems (Andrew, 1979). The fixed nitrogen can be transferred from legumes to cereals or other non fixing crops in intercropping systems during the co-growing period, and this nitrogen is an important resource for cereals growth and development (Shen and Chu, 2004). For example, Shen and Chu (2004) reported that at the low rate of applied N; rice (cereals) could utilize some N from peanut (legumes) during the period of their co-growth. Furthermore, it was reported that interspecific root interactions between faba beans intercropped with maize played a significant role in the yield benefit of maize in an intercropping system (Li *et al*., 1999; Zhang and Li, 2003). Following the yield advantage in an intercropping system, it was thought that the nitrogen that was fixed by faba beans may have been transferred to maize and increase the maize yield (Zhang and Li, 2003) suggesting the importance of intercropping legumes with cereals.

1.2. Problem Statement and Justification

Food security in SSA has been constrained by many factors including poor soil fertility (Buerkert *et al*., 2001). Nutrient (e.g. Nitrogen [N], Phosphorus [P] and Potassium [K]) deficiency is the most limiting factor for crop growth, development and production (Singh *et al*., 2011). The low yields pronounced in grain legumes and cereals are often associated with low N, P and K levels in the soil among other factors. While it is expected that intercropping legume with cereals would increase yields due to inter-specific interactions and facilitations of the component crops, still most farmers in Tanzania gets low yield. This might be due to low levels of phosphorus and potassium, and unavailability of specific rhizobia strain that would increase nitrogen fixation, and consequently improve yields in intercropping systems. Application of moderate levels of chemical fertilizers can improve grain yields of both legumes and cereals (Ndakidemi *et al*., 2006). However, farmers are not always using these inputs either because of their high prices (Ndakidemi *et al*., 2006; Chianu *et al*., 2011), lack of awareness on their economic returns, or both (Ndakidemi *et al*., 2006).

On the other hand, most intercropping research conducted have focused on the assessment of yield performance and revealed that there are yield benefits of intercropping than monoculture cropping (Li *et al*., 1999; Li *et al*., 2001; Ghosh, 2004; Lemlem, 2013). However, there is little information on the response of soybean and maize in an intercropping system, supplied with phosphorus, potassium and rhizobia inoculation. Intercropping systems, rhizobia inoculation and supplementation with moderate phosphorus and potassium has great potentials for changing the response of these crops not only on their yield performance but also in terms of chemical composition of their grains, nutrient uptake, nitrogen fixation and transfer to the nearby crops and chlorophyll formation which eventually leads to production of sugars. The current study was carried out in order to investigate and have better understanding of the agronomical and biochemical effects of phosphorus, potassium fertilization and inoculation of rhizobia on growth performance, yield, chemical changes in the rhizosphere soil, nutrient uptake, N-fixation, and chlorophyll content of soybean intercropped with maize in the depleted soils in northern Tanzania.

1.3. Hypothesis

The study was guided by the hypothesis that rhizobia inoculation, intercropping system, and fertilization with P and K will improve farm productivity of both soybean and maize.

1.4. Objectives

1.4.1. General objective

The general objective of this study was to enhance integrated soil management and different cropping systems for improve farm productivity.

1.4.2. Specific objectives

i. To assess the effects of rhizobia inoculation supplemented with phosphorus, potassium and intercropping system on the mineral composition in the rhizosphere of soybean
ii. To determine the effects of intercropping and rhizobia inoculation supplemented with phosphorus and potassium on nutrient uptake in soybean.
iii. To determine the effects of rhizobia inoculation supplemented with phosphorus and potassium on nitrogen fixation in soybean intercropped with maize.
iv. To assess the effects of rhizobia inoculation supplemented with phosphorus, potassium and intercropping systems on chlorophyll formation in soybean.
v. To assess growth performance of both soybean and Maize as affected by intercropping and rhizobia inoculants supplemented with phosphorus and potassium
vi. To determine the of land equivalent ratio of maize intercropped with rhizobia inoculated soybean and supplementation with P and K
vii. To determine the effects of intercropping, rhizobia inoculation supplemented with phosphorus and potassium on yield performance of both Soybean and Maize.

CHAPTER TWO

INTERCROPPING SYSTEM, RHIZOBIA INOCULATION, PHOSPHORUS AND POTASSIUM FERTILIZATION: A STRATEGY OF SOIL REPLENISHMENT FOR IMPROVED CROP YIELD[1]

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Abstract

Loss of soil fertility is the most significant constraint to legumes and cereal crop production in most sub-Saharan Africa countries. The most limiting soil nutrients are nitrogen (N), phosphorus (P) and potassium (K) which to the great extent cause low grain yields. The main reason for declining of these nutrients in the soil is the mining through continued cultivation without external input application. These nutrients are not usually applied by farmers because of their high prices leading to poor crop growth, development and finally poor yield. Leguminous crops have ability to form symbiotic relationship with rhizobia and fix atmospheric nitrogen. The fixed nitrogen can be used by legume plant themselves or might be transferred and be utilized by other plants growing nearby in intercropping systems or can be used by plants grown in the subsequent season. This review focused on understanding how rhizobia inoculation, intercropping system, and fertilization with P and K influences nitrogen fixation; mineral composition in the crop rhizosphere; nutrient uptake in plants; plant growth; photosynthesis and leaf chlorophyll formation; land equivalent ratio and ultimately yield performance of legumes and cereals. The results from different literatures cited showed that

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rhizobia inoculation and supplementation with phosphorus and potassium had positive significant effects on all parameters measured. Therefore, based on the findings reported, it can be recommended, to use rhizobia inoculants supplemented with optimum levels of phosphorus and potassium in intercropping systems as a strategy for improving crop production.

***Keywords**:* Biological nitrogen fixation, soil fertility, nutrient uptake, land equivalent ratio, food security.

2.1. Introduction

Loss of soil fertility is one of the important constraints to legumes and cereal crop production in sub-Saharan Africa countries (Buerkert *et al*., 2001). The most limiting nutrients are nitrogen (N), phosphorus (P) and potassium (K) (Bekunda *et al*., 2004), which to the great extent cause low grain yields. However, N is abundantly (80%) available in the air, existing in a form that cannot be used by plants (Santi *et al*., 2013) until it is fixed in either natural ways or through biological agents of nitrogen fixation. This nitrogen is very important for plant/crop growth and development, short of its supply to plants results in stunted growth. The deposits of K are relatively plenty, but the phosphate reserves are increasingly becoming scarce (Roy, 2006). The dependence of crop growth on nitrogen and the limited bioavailability of this element have resulted in a massive N-based fertilizer industry worldwide which led to increased use of nitrogenous fertilizers to meet the global food demand (Westhoff, 2009; Santi *et al*., 2013). However, these nitrogenous fertilizers go in opposite direction with the current global theme of climate smart agriculture as they cause greenhouse gas emission (N_2O).

Leguminous crops are well known for their ability to fix atmospheric nitrogen (Ledgard and Steele, 1992; Peoples *et al*., 1995). This nitrogen is used by the legume crops themselves or might be excreted out of legume's root structures called nodules into the rhizosphere soil and be utilized by other plants growing nearby in intercropping systems (Andrew, 1979; Shen and Chu, 2004). Furthermore, the fixed nitrogen can be used by plants grown in the succeeding season following the death and subsequent mineralization of diazotrophs (James, 2000). For example, Shen and Chu (2004) reported that at the low rate of applied N; rice could utilize some N from peanut during the period of their co-growth. Furthermore, it was reported that inter specific root interactions between faba beans intercropped with maize played a significant role in the yield benefit of maize in an intercropping system (Li *et al*., 1999; Zhang and Li, 2003). Following the yield advantage in an intercropping system, it was thought that the nitrogen that was fixed by faba beans may have been transferred to maize and increase the maize yield (Zhang and Li, 2003) suggesting the importance of intercropping legumes with cereals.

Intercropping is an old and common agricultural practice of growing more than one crop in the same field at the same time (Sanchez, 1976). It is mainly practiced in sub Saharan Africa (SSA), by smallholder famers (Matusso *et al*., 2014). Most common crop combinations in intercropping systems include: maize-cowpea, maize-pigeon pea, maize-soybean, maize-groundnuts, maize-beans, maize-lablab, sorghum-cowpea, millet-groundnuts, and rice-pulses (Matusso *et al*., 2012). This cropping practice aims to match efficiently crop demands to the available growth resources and labour (Dahmardeh *et al*., 2010; Lemlem, 2013). The efficient use of available growth resources in a given piece of land and eventually maximizing productivity is the primary advantage of intercropping crops of different height, canopy structure, rooting ability, and nutrient requirements (Lemlem, 2013; Ghanbari *et al*., 2010). Many studies on intercropping have shown that legumes-cereal intercropping is an important productive and sustainable system due to its resource facilitation and significantly enhancing crop productivity as compared with that of monoculture crops (Jensen, 1996; Ghanbari *et al*., 2010; Dahmardeh *et al*., 2010). In an effort to improve food security, intercropping cereals with legumes plays an important role by providing a farmer with both carbohydrates and proteins for their dietary needs. Apart from nutritional composition of component crops in an intercropping, it has been also reported that intercropping improves soil fertility through biological nitrogen fixation, increases soil conservation through greater ground cover than sole cropping (Lemlem, 2013), and provides better protection against crop pests and diseases than when grown in monoculture (Matusso *et al*., 2012).

Despite of increased global mineral fertilizer use accelerated by global food demand, smallholder farmers in SSA usually experience low crop productivity (Mwangi, 1996). This might be due to continued cropping without addition of external inputs leading to low levels of soil nutrients. On the other hand, unavailability of specific rhizobia strain would reduce the biological nitrogen fixation, and consequently result in low grain yields in intercropping systems. Grain yields of both legumes and cereals can potentially improve from the application of moderate levels of chemical fertilizers (Ndakidemi *et al*., 2006). However, these inputs are rarely used by farmers either because of their skyrocketing prices (Ndakidemi *et al*., 2006; Chianu *et al*., 2011), lack of farmer's awareness on their economic returns, or both (Ndakidemi *et al*., 2006). The use of these inorganic fertilizers has also made prices of many agricultural commodities to skyrocket (Masarirambi, 2010). Therefore, there is a need to find out simple, cheap and environmentally friendly methods of improving agricultural productivity through Integrated Soil Fertilty Management (ISFM). Rhizobia inoculation, intercropping systems, and fertilization with moderate levels of phosphorus and potassium may have great potentials as an ISFM strategy for changing the response of crops in different parameters.

The aim of this article is to critically review and explore how rhizobia inoculation, intercropping system, and fertilization with P and K influences nitrogen fixation; mineral composition in the crop rhizosphere; nutrient uptake in plants; plant growth; photosynthesis and leaf chlorophyll content; land equivalent ratio and finally yield performance of legumes and cereals.

2.2. Biological nitrogen fixation in legumes under rhizobia inoculation, phosphorus and potassium fertilization, and its associated benefits to the cereal component

2.2.1. Biological nitrogen fixation and their associated benefits to the cereal crop

Rhizobia are microorganisms that are employed to improve the availability of nutrients such as nitrogen through atmospheric N_2 fixation. These microorganisms are also called biofertilizers. In recent years, biofertilizers have emerged as a vital component for biological nitrogen fixation providing an economically attractive and ecologically sound way for increasing nutrient supply (Shridhar, 2012). Legumes such as soybean, lablab, common bean, cowpea and ground nuts are important hosts for these microorganisms to perform biological nitrogen fixation. Biological N_2-fixation and mineral soil or nitrogenous fertilizers are the major sources of meeting the N requirement of high yielding legumes. Recently, it was reported that about 50–60% of soybean N demand was met by biological N_2 fixation (Salvagiotti *et al*., 2008). Soybean (*Glycine max*) is a crop grown in different parts of the world. It is a popular nutritious crop providing human with a very high proteins and it is of high economic importance (Raji, 2007). The popularity of this crop is not based only on its high protein content but also its ability to fix atmospheric nitrogen thereby contributing to soil N and improve soil quality. When legume crops are inoculated with the right strain of rhizobia, they are able to fix atmospheric nitrogen and contribute to the soil nitrogen to meet plant N requirements (Salvagiotti *et al*., 2008). In a natural ecosystem, legumes can fix nitrogen in the range of 25 – 75 lb which is equivalent to 11.34 – 34.02 kg of nitrogen per acre per year (Flynn and Idowu, undated). In cropping systems for example perennial legumes such as Alfalfa, sweet clovers, true clovers, and vetch may fix up to 250 – 500 lb of nitrogen per acre per year (Walley *et al*., 1996). Likewise, grain legumes such as peanuts, cowpeas, soybeans, and fava beans, can fix up to 250 lb which is equivalent to 113.4 Kg N ha^{-1} (Flynn and Idowu, undated). The fixed nitrogen is of beneficial to the cropping systems as it is not only used by the fixing crop but also non fixing crops growing nearby may consume this nitrogen when are released out of the fixing plants (Shen and Chu, 2004). For example, a total of 17.08 kg N ha^{-1} was transferred from legumes to the non-legumes in the mixture (Frankow-Lindberg and Dahlin, 2013). However, studies on dinitrogen fixation in complex cereal/legume mixtures are few (Stern, 1993; Peoples *et al*., 2002) as reviewed by Ndakidemi (2006). Therefore, there is a need to conduct study that will explore the response of legumes inoculated with rhizobia on nitrogen fixation so as to add knowledge on existing information. Furthermore, studies are also required to quantify the amount of nitrogen that can be fixed by specific legumes in different environments and cropping systems and how much of these nitrogen can be used by cereal crops in an intercropping systems.

2.2.2. Phosphorus and potassium fertilization on nitrogen fixation in legumes

N_2-fixation by *Rhizobium* bacteria in leguminous plants is favoured by similar conditions necessary for good growth, vigour and dry matter production of the host plant. These conditions include availability of mineral elements such as starter N, phosphorus (P) and potassium (K). The primary source of nutrients (P and K) is weathering of bedrock, and the availability trend of these nutrients tends to decline with time as soils age (Hedin *et al*., 2003). Apart from their biochemical and physiological functions in the plants, these elements have other function of enhancing biological nitrogen fixation. The influence of phosphorus on symbiotic N_2-fixation in leguminous plants has been studied intensively and many researchers have reported that phosphorus improved nitrogen fixation in legumes (Tang *et al*., 2001; Ndakidemi *et al*., 2006; Zafar *et al*., 2011). Israel (1987), reported that severe phosphorus deficiency significantly impaired both host plant growth and symbiotic N_2

fixation, indicating that N_2-fixation has a higher phosphorus requirement for optimal functioning than that required for host plant growth and nitrate assimilation. Potassium plays an important role in the process of nitrogen fixation (Mengel *et al*., 1974). Potassium is essential in photosynthesis, as it maintains and balances the electrical charges at ATP production site, and also helps to promote translocation of photosynthetic substances (carbohydrate) to storage organs (fruits or roots) (Uchida, 2000). Carbohydrate produced by the host plant is also translocated to other parts of the plants including nodules where it is used by nitrogen fixing bacteria as a source of energy to fix atmospheric nitrogen (Mengel *et al*., 1974). Regardless of the effects of these mineral elements (P and K) on dinitrogen fixation, there is a need to conduct a study to assess their combined effects on nitrogen fixation in legumes growing in association with maize.

2.3. Mineral composition in the rhizosphere of legumes and cereals under intercropping system, rhizobia inoculation, phosphorus and potassium fertilization

2.3.1. Effects of rhizobia inoculation on rhizopheric mineral composition

Inoculation of legumes with specific strain of *Rhizobium* is well known for its ability to increase N_2 fixation, plant yield and also improve the seed quality (Saini *et al*., 2004; Bambara and Ndakidemi, 2010). A group of soil dwelling and beneficial non pathogenic bacteria are referred to as plant growth promoting rhizobacteria (PGPR). PGPR colonizes the rhizosphere of diverse plant species and confer beneficial effects, such as increased plant growth by providing plants with fixed nitrogen and reduced susceptibility to diseases resulting from plant pathogenic bacteria, viruses, fungi, and nematodes (Kloepper *et al*., 2004; Yang *et al*., 2009). Some PGPR also shows physical or chemical changes in the rhizosphere which is related to plant growth and plant defense (Yang *et al*., 2009). A study conducted by Bambara and Ndakidemi (2010) on common bean (*P. vulgaris*) showed that *Rhizobium* inoculation significantly increased soil pH, Ca and Na availability. In their study, they also reported a significant increase in available micronutrients such as Fe, Cu, Zn and Mn following *Rhizobium* inoculation when compared with the control. However, little information is available about the effect of rhizobia inoculation on the chemical composition of rhizosphere of intercropped plants. Studies are needed to explore more information about the effects of rhizobia inoculation on mineral composition in the rhizosphere of intercropped plants.

2.3.2. Rhizospheric mineral composition under legume-cereals mixtures

In past few decades, intensification of agricultural systems have increased and reduced crop diversity to one or few species that are sometimes genetically homogenous with the uniform planting arrangements (Mobasser *et al*., 2014). Traditionally, small-holder subsistence farmers in the tropics have the tendency of intercropping their land to keep the associated risks of monocultures and assure stable income and nutrition (Francis, 1986). Intercropping cereal with grain legume crops facilitate the improvement and maintenance of soil fertility, because legume crops such as cowpea, mungbean, soybean and groundnuts are reported to accumulate from 80 to 350 kg nitrogen (N) ha^{-1} (Peoples and Craswell, 1992). Intercropping have been reported to have indirect effect in the rhizospheres of intercropped species by enhanced nutrient mineralization because of the changes in soil organic matter decomposition rates, resulting from the addition of fresh organic matter (Blagodatskaya and Kuzyakov, 2008; Mobasser *et al*., 2014). A study done by Bolan *et al*. (1991) has shown that plants fixing nitrogen may cause changes in soil pH, which may limit the availability of some mineral elements. Other studies have reported that there were changes in physical and chemical characteristics of rhizosphere following intercropping (Zhang *et al*., 2004). Specifically, Song *et al*. (2007) reported that intercropping augmented microbial biomass and increased the availability of C, N and P in the rhizosphere. However, there is little information on mineral composition of rhizosphere influenced by association of cereals and legumes inoculated with rhizobia. Hence, calling for more studies to explore on how these interactions and association affects chemical and mineral composition of rhizosphere soil in cereals and legumes.

2.4. Nutrient uptake in legumes and cereals under intercropping, rhizobia inoculation, phosphorus and potassium fertilization

2.4.1. Below ground interaction of legumes and cereals affects nutrient uptake

Many studies on intercropping have generally paid attention on the legume-cereal intercropping and assess yield performance of the crops taking advantage better resource utilization (Li *et al*., 1999; Andersen *et al*., 2007; Agegnehu *et al*., 2008; Hauggaard-Nielsen *et al*., 2009). When plants are grown in mixture they have potentials of modifying nutrient availability in the soil by releasing exudates from their roots (Raynaud *et al*., 2008). These exudates may contain various chemical compounds like organic anion, amino acids, protons, sugars and enzymes which are believed to modify nutrient availability for the plants and hence improve yield (Raynaud *et al*., 2008). Morris and Garrity (1993) have reported the close association between yield advantage and plant nutrient uptake by intercropped plant species. Further studies by Hauggaard *et al*. (2009) showed that accumulation of nutrients such as phosphorus (P), potassium (K), and sulphur (S) may be enhanced by the nutrient complementarity of intercropped pea and barley and further postulated that these might have influenced the overall crop yield and thereby increasing competitive ability of capturing and utilization of other resources. P uptake has been reported to be influenced by intercropping in many studies (Mobasser *et al*., 2014). Specifically, it was reported that there were increased uptake of P in white lupin intercropped with wheat (Gardner and Boundy, 1983; Cu *et al*., 2005). Other study by Ae *et al*. (1990) showed that pigeon pea influenced the uptake of P in the sorghum in an intercropping. The literature has pointed out that intercropping legume with cereals may improve uptake of some mineral element, however, we would like explore how uptake of both macro and micro nutrients is affected by plant grown in an intercropping systems.

2.4.2. Influence of rhizobia inoculation on nutrient uptake in plant tissues

Uptake of plant nutrients is an essential process as these nutrients needed by plants for normal growth and development. Nutrient uptake by plants depends on the amount, concentration, rhizosphere processes and the capacity of soil to replenish nutrient in the soil (Makoi *et al*., 2013). Microorganisms such as rhizobia as well as other plant growth promoting rhizobacteria, are said to change the chemistry of nutrients in the soil and make them available for uptake by plants (Saharan and Nehra, 2011). Rhizobial inoculants are reported to increase uptake of nutrients such as N and P though the biological nitrogen fixation thereby improving N availability to plants (Ndakidemi *et al*., 2011). They can also mobilize both organic and inorganic phosphorus from organic as well as inorganic sources making them available in the rhizosphere for uptake by plant (Matiru and Dakora, 2004). Recent studies (Fatima *et al*., 2007; Ndakidemi *et al*., 2011; Makoi *et al*., 2013; Nyoki and Ndakidemi 2014a, b; Tairo and Ndakidemi 2014) have reported that the rhizobia inoculation have influenced and increased the uptake of different nutrients in plants. For example, Makoi *et al*. (2013) reported a significant increase in the uptake of P, K, Ca, and Mg in plant tissues. Similarly, Ndakidemi *et al*. (2011) working on *P. vulgaris* reported a significant increase in uptake of micronutrients Fe, Cu, Zn, Mn, B, Mo in different plant tissues. Regardless of many studies conducted on intercropping there are few research reports specifically in Tanzania about the role of intercropping and rhizobia inoculation on nutrient uptake in legumes intercropped with cereals. Therefore, there is a need to conduct research investing the influence of cereal-legumes intercropping systems and rhizobia inoculation supplemented with phosphorus and potassium on plant nutrient uptake.

2.4.3. P and K fertilization on other nutrient uptake by plants

Nutrients such as phosphorus and potassium play different important roles in plant growth and development thereby increasing biomass and grain yield. Bioavailability and uptake of these nutrients is constrained by different factors including their concentration in the soil (Makoi *et al*., 2013), pH of the soil (Bambara and Ndakidemi, 2010) and the nature of exudates produced by the plants (Raynaud *et al*., 2008). P is reported to facilitate plant roots development and enhances nodules of the legume plants so that increases seed yields (Hayat *et al*., 2010). Plants supplied with mineral elements P and K will easily capture and take up the supplied elements and may influence the uptake of other nutrients. For example, Islam *et al*. (2008) reported an increased phosphorus uptake in rice with increasing application of P rates. Akram *et al*. (2007) showed that nitrogen uptake in sorghum was improved with application of P and K, pointing out that their combined use exceeded their alone application. In recent study conducted by Nyoki and Ndakidemi (2014a, b), it was reported that phosphorus supplementation improved micro and macro nutrient uptake in different tissues of cowpea grown under the field and screen house condition. Another study reported that application of K helped the release of fixed NH^{4+} ion from the soil and this enabled the crop to better uptake of nitrogen (Sharma and Ramna, 1993). To obtain the maximum yield, plants need to be supplied with the optimum mineral nutrients they require. However, the crops are not supplied with these nutrients by many smallholder farmers in sub-Saharan Africa, leading to poor crop growth, development and finally poor yield. More studies are proposed to assess the factors influencing nutrient uptake in P and K treated crops and what are the associated benefits of improved nutrient uptake to the human diet.

2.5. Growth performance of legumes and cereals as affected by rhizobia inoculation supplemented with phosphorus and potassium in intercropping system

2.5.1. Growth performance of crops under intercropping systems

Growth performance is one of the indicators of crop yield performance. Plant growth is affected either positively or negatively by different factors including cropping patterns (Carr *et al*., 2004; Dusa and Stan, 2013). The effects of intercropping on growth performance of intercropped crops have been studied for a long time and many researchers have reported different findings. Hirpa (2014) reported that there was significant increase in maize height just by delaying planting date of haricot bean for three weeks after planting maize as compared with the simultaneous planting maize and haricot bean. In another study, Hirpa (2013) reported that there was a significant interaction of intercropped legume species and intercropping time resulting in an increase in maize height simultaneously planted with legumes and gave the reason that maize height could have been contributed by inter-specific competition to avoid over shading. Lemlem (2013) recorded a significant difference in plant height where it was found that the height of sole maize was significantly higher than maize-lablab and maize-cowpea intercropping. However, there is little information reported on the effects of legume-cereals intercropped at different spacing on growth performance particularly in depleted soils. Studies on intercropping cereals with legumes at different spacing would provide more information on growth performance of crops grown in mixture and different spacing.

2.5.2. Growth performance of crops as affected by rhizobia inoculation

Rhizobia inoculation is well known for its effects on biological nitrogen fixation when comes in symbiotic relationship with leguminous plants. The improved nitrogen fixation is very important for the crop growth and development. Several studies have shown that there is evidence of improved plant growth following rhizobia inoculation. For example, Yamanaka *et al*. (2005) reported that there was a significant increase in biomass in the *Alnus sieboldiana* seedlings inoculated with Frankia and *Gigaspora margarita* when compared with un-inoculated seedlings. Unavailability of specific strain of rhizobia reduces the growth of leguminous crops to the great extent (Vincent *et al*., 1979). Poor symbiosis between *Rhizobium* and legumes are reported to reduce the amount of fixed nitrogen in legumes resulting in reduced plant growth (Bambara and Ndakidemi, 2009). Furthermore, a study done by Bambara and Ndakidemi (2010) showed the presence of significant increase in fixed nitrogen in different plant tissues of *Phaseola vulgaris* relative to un-inoculated treatments. The improved N nutrition improves plant growth as well as yield performance. Many research on influence of rhizobia on plant focus on growth performance of the fixing crop without considering the effect of rhizobia on growth of neighbouring non fixing plants. It is therefore important to conduct studies to assess how rhizobial inoculation may influence growth performance of both fixing and non-fixing plant.

2.5.3. Growth performance of crops under phosphorus and potassium fertilization

Mineral elements such as N, P and K plays important roles in plant growth and development and ultimately determination of crop yield (Uchida, 2000). Both elements are essential macronutrients required in relatively large amount by plants. Being one of the important element for plant growth, phosphorus is found in every living plant cell playing role in various plant functions including energy transfer, photosynthesis, translocation of sugars and starches as well as movement of nutrients within the plant (Brady, 2002; Shahid *et al*., 2009). Potassium is required by plants for a number of vital physiological processes including the following: activation of several enzymes, synthesis and degradation of carbohydrates, production of proteins as well as regulation of stomata pores for gas exchange and photosynthesis (Lissbrant *et al*., 2009). However, P and K are usually very low in the soils, a condition which limit proper plant growth resulting in stunted crops and hence poor yields. Therefore, for proper plant growth and development, more studies are of utmost important to investigate the effects of different levels of P and K on plant growth in different soil condition and different cropping systems.

2.6. Photosynthesis and chlorophyll formation as affected by rhizobia inoculation, phosphorus and potassium fertilization in legume-cereals mixtures

2.6.1. Photosynthesis and chlorophyll formation in crops as influenced by rhizobia inoculation

Chlorophyll can be referred to as a green molecule found in plant cells which plays the central function in photosynthesis. Photosynthesis is a process by which plants captures sun light and converts it to useful chemical energy in presence of water, carbon dioxide and chlorophyll (Amesz, 1987). Life on earth would be not possible without photosynthesis because it creates living matter out of inert organic material, replenishes the reservoirs of oxygen in the atmosphere and store light energy from sun to support the life activities of nearly all organisms (Rabinowitch and Govindjee, 1969; Gaidos, 1999). Inoculation of rhizobia may affect the whole plant photosynthesis because they tend to improve plant nutrition and growth by increasing total leaf area (Kaschuk *et al*., 2009). Another study showed that *P. vulgaris* L. inoculated with rhizobia had an increased leaf chlorophyll content compared with un-inoculated plants (Bambara and Ndakidemi, 2009). Research evidence shows that *Rhizobium* inoculation increases the chlorophyll content of leaves (Arumugam *et al*., 2010), and hence improves plant biomass production. However, rhizobia inoculation under cereal-legume intercropping systems still needs more studies to assess its effects on leaf chlorophyll content of both components of intercropping.

2.6.2. Phosphorus and potassium fertilization on the photosynthesis and chlorophyll formation in crops

Declining soil fertility, especially mineral nutrients such as N, P and K has continued to cause low yield for many farmers in SSA. The limited supply of these elements is reported to impair plant growth in terms of cell division and expansion, and photosynthesis (Hossain *et al*., 2010; Longstreth and Nobel, 1980). Potassium (K^+) is one of the abundant ion in the plant cells being required for various functions including maintenance of electrical potential gradients across plasma membrane and also it activates the function of various enzymes (Britto and Kronzucker, 2008). Apart from these functions in plants P and K play an important role in the photosynthetic activities and chlorophyll formation in plants. For example, in the past few years one group of researchers reported an increase in chlorophyll content following application of phosphorus on the seedlings of *Larix olgensis* (Wu *et al*., 2006). Recent studies have also shown that the plants treated with relatively high levels of P and K improved chlorophyll a, b and ab production in cotton leaves (Onanuga *et al*., 2011). This report is in line with the previous study by Lamrani *et al*. (1996) who reported that K nutrition promoted formation of both chlorophyll a and b in cucumber leaves, and that K deficient is associated with low chlorophyll content on cotton (Zhao *et al*., 2001).

2.6.3. Photosynthesis and chlorophyll formation as affected by intercropping systems:

Intercropping has been reported to bring about yield advantages over sole crop by many researchers (Giller and Wilson, 1991; Khogali *et al.*, 2011; Lemlem, 2013). However, this may lead to the suppression of one of the companion crop in the mixture by preventing the sunlight from reaching the crop. Sunlight is normally captured by plant leaves and converted into chemical energy to be used for various plant activities. It was previously reported by Islam *et al*. (1993) that Mungbean intercropped with sorghum suffered a shading stress at different growth stages. It was further reported that grain filling stage is very much light sensitive. For instance, Yoshida and Hara, (1977) reported low light intensity causes a slight delay in the grain filling of the whole panicle and reduced the percentage of filled grains on the lower branches of Indica and Japonica rice. Therefore, there is a need to conduct further studies to assess the effects of intercropping on chlorophyll formation in legumes intercropped with cereals. This will help us better understand how intercropping may affect chlorophyll formation and photosynthesis there by affecting grain and biomass production.

2.7. Yield performance of legumes and cereals as influenced by rhizobia inoculation and P and K fertilization in intercropping systems

2.7.1. Yield performance of legumes and cereals in mixed culture

Intercropping is an agricultural practice of growing more than one crop in the same piece of land at the same time aiming at efficiently matching the available growth resources to the crop demands (Banik and Bagchi, 1993; Zhu *et al*., 2000; Xu *et al*., 2008). Many studies have reported that most advantage of intercropping is production of greater yield on a given piece of land (Giller and Wilson, 1991; Ndakidemi and Dakora, 2006; Khogali *et al*., 2011; Lemlem, 2013). Intercropping maize with grain legumes is the traditional farming practice believed to reduce the risk of crop failure, and add some N to the system through biological N fixation (Whitbread, 2004). The most probable reason for production of greater yield in an intercropping system is the addition of N in the soil from biological nitrogen fixation (BNF) (Whitbread, 2004; Khogali *et al*., 2011), better utilization of available growth resources (water, nutrients, light and air) (Morris and Garrity, 1993; Zhu *et al*., 2000; Li *et al*., 2003), better use of available piece of land (Singh and Usha, 2003), and interspecific interactions and facilitation of the component crops (Zhang, 2003; Fan *et al*., 2006). Li *et al*. (2001) stated that the crops grown in the mixture, such as cereals and legumes may have a series of complex inter- and intra-specific interactions which leads to an increased crop yield. However, Ndakidemi *et al*. (2006) reported that African soils are heavily mined for nutrients, especially N and P, with a consequent decline in crop yields. Although many researches have been done on yield advantage of legumes intercropped with cereals, there is a need to conduct further studies on the factors influencing greater yield in an intercropping systems.

2.7.2. Rhizobia inoculation on yield performance of legumes and cereals in mixture

Crop production in most smallholder farmers of sub-Saharan Africa is characterized by continuous cropping with low or no external inputs application resulting in reduced soil fertility and low agricultural productivity. As poor and hungry people cannot afford to purchase mineral fertilizers (Ndakidemi *et al*., 2006), they need low cost and readily available technologies and practices to increase food production (Pretty *et al*., 2003). Inoculation of rhizobia could be simple and affordable technology from which a farmer can increase crop yield. Rhizobia are soil bacteria which colonizes the roots of leguminous plants and form nodules in which biological nitrogen fixation takes place (Mia and Shamsuddin, 2010). Nitrogen is a macro element being required by plants in a relatively large amount than other elements (Cechin and de Fátima, 2004). It is required in large quantity by crops for maximum growth and development. Many studies have shown that rhizobia inoculation improved both crop growth and grain yields (Menaria *et al*., 2004; Popescu, 1998; Zahran, 1999; Vargas *et al*., 2000; Hernandez and Cuevas, 2003). In an intercropping of cereals and legumes, rhizobia inoculation enables nitrogen fixation and the fixed nitrogen is used by both legumes and cereals growing together in an intercropping systems thereby enhancing yield performance of cereals.

2.7.3. Phosphorus and potassium fertilization on yield performance of cereals and legumes in the mixed systems

For proper plant growth and development, the soil must be fertile and contain appropriate levels of essential mineral elements (Bationo *et al*., 2002; White *et al*., 2012). A fertile soil provides essential mineral nutrients for crop plant growth, supports a varied and active biotic community (Mäder *et al*., 2002). The essentiality of elements is based on Arnon and Stout (1939), who stated that "an element is not considered essential unless: i) a deficiency of it makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle; ii) such deficiency is specific to the element in question, and can be prevented or corrected only by supplying this element; and iii) the element is directly involved in the nutrition of the plant quite apart from its possible effects in correcting some unfavorable microbiological or chemical condition of the soil or other culture medium". The most important plant nutrients for production of high yields are nitrogen (N), phosphorus (P) and potassium (K). Among these elements, N is abundant in the air, and deposits of K are relatively plenty, but the phosphate reserves are increasingly becoming scarce (Roy, 2006). Potassium is involved in the translocation of photosynthetic products (sugars) for plant growth or storage in fruits or roots (Uchida, 2000). Phosphorus performs many functions in plants including the following: it is a part of the RNA and DNA structures which are the main components of genetic information; it is required in large quantities in young cells, such as shoots and root tips where metabolism is high and cell division is rapid; it aids in root and nodules development, flower initiation, and seed and fruit development (Uchida, 2000; Mokwunye and Bationo, 2002). Studies have shown that plants supplied with appropriate amount of P has resulted in increased yields over the control (Ndakidemi *et al*., 2006; Zafar *et al*., 2011). However, most soils in some Eastern Africa countries have negative balances of N, P and K which limits crop production (Bekunda *et al*., 2004) (Table 1). The limited availability of soil nutrients, calls upon crop scientist to conduct studies to investigate the response of crops supplied with P and K at different levels in an intercropping systems on crop yields.

Table 1: Calculated nutrient balances of N, P and K (kg $ha^{-1}year^{-1}$) of the arable land for some Eastern Africa countries

Country	N		P		K	
	1982-84	2000	1982-84	2000	1982-84	2000
Kenya	-41	-47	-6	-7	-29	-36
Tanzania	-27	-32	-4	-5	-18	-21
Rwanda	-54	-60	-9	-11	-47	-61

(Bekunda, et al., 2004)

2.7.4. Land equivalent ratio (LER)

Intercropping of cereals with legumes has been an ordinary cropping system in different arid and semi-arid areas of SSA. In an effort to assess the efficiency of intercropping over monocropping, scientists use different competition indices (Hiebesch and McCollum, 1987).

However, Land Equivalent Ratio (LER) is the most used convention for intercrop versus sole crop comparisons (Agegnehu, 2006). LER provides an accurate assessment of the competitive relationship between the component plants in an intercropping, as well as the overall productivity of intercrop systems (Zada *et al*., 1988). LER measures how efficient are intercropping or mixture. The LER makes comparison of land areas required under single or sole cropping to give the yields obtained from the component crops of the mixture (Federer and Schwager, 1982). If the intercropped crops have the same agro-ecological characteristics, their total LER should be 1.0 and their partial LER should be 0.5 for each crop. Dariush *et al*. (2006) and Mohammed (2011) pointed out that if a total of LER is greater than 1.0 signifies that the positive inter-specific interference that exist in the monoculture is intensive than that in the mixture. The LER value of 1.0 indicates that the yield of intercrop are the same as those of the collections of monocultures and any value greater than 1.0 indicates the advantage for intercropping (Mead and Willey, 1980; Mazaheri and Moveysi, 2004; Solanki *et al*., 2011). The comparative advantages of intercropped crops over sole crops may be influenced by many factors such as crop density and soil nutritional status. Rhizobia inoculation and supplementation of phosphorus and potassium may influence yield performance of intercropped crops, and therefore, it is important to assess their effects on land equivalent ratio.

2.8. Conclusion

This review focused on the potential effects of rhizobial inoculation, phosphorus and potassium fertilization in legume-cereal intercropping systems on nitrogen fixation; mineral composition in the crop rhizosphere; nutrient uptake in plants; plant growth; photosynthesis and leaf chlorophyll content; yield performance of legumes and cereals and finally land equivalent ratio. The results from different literatures cited showed that *Rhizobium* inoculation and supplementation with phosphorus and potassium had positive significant effects on all parameters measured. Therefore, when these bio-fertilizers are used and supplemented with optimum levels of phosphorus and potassium they can significantly increase both legumes and cereals production. Based on these results, it is recommended to use rhizobia inoculants supplemented with optimum levels of phosphorus and potassium in the intercropping systems for production of high yield in highly depleted soils. However, more studies are required to explore whether the increased plant performances are mainly due to plant-microbes interactions or due to other underlying factors.

CHAPTER THREE

SELECTED CHEMICAL PROPERTIES OF SOYBEAN RHIZOSPHERE SOIL AS INFLUENCED BY CROPPING SYSTEMS, *RHIZOBIUM* INOCULATION AND THE SUPPLY OF PHOSPHORUS AND POTASSIUM AFTER TWO CONSECUTIVE CROPPING SEASONS[2]

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Abstract

The field experiment was carried out in northern Tanzania to assess the effects of intercropping systems, *Rhizobium* inoculation and fertilization with P and K on chemical properties of soybean rhizosphere soil. The experiment was laid out in split-split plot design with 2x4x7 factorial arrangement replicated thrice. The main plots had two inoculation treatments and the sub plots were comprised of four cropping systems which were: sole-maize, sole-soybean and two intercropping at different soybean spacing (75x20 and 75x40cm). The fertilizer levels (kg/ha) control (0 kg/ha); 20K; 40K; 26P; 52P; 26P + 20K; and 52P + 40K were assigned to sub-subplots. The rhizosphere soil of soybean was sampled at 50% pod formation and its chemical properties were determined. Statistical analysis was performed using ANOVA. Least Significant Difference was used to compare treatment means at p=0.05 significance level. The results indicated that rhizosphere soil chemical properties such as pH, Organic carbon (OC), macro and micro-nutrients (N, P, Ca, Mg, Na and) and (Fe, Cu, Mn, and Zn) respectively were significantly increased in the *Rhizobium*

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inoculated soybean over the control. The supply of P and K fertilizers significantly increased the rhizosphere content of macro nutrients (P, K, Ca, and Mg) and also they altered the pH and EC of the rhizosphere soil relative to control.

***Keywords*:** Rhizosphere, pH, electrical conductivity, mineral elements, agro-ecosystems, cereal-legume intercropping

3.1. Introduction

Plants require 17 nutrients to perform different plant functions related with growth, development and reproduction. However, most soils in sub-Saharan Africa are depleted and are deficient in mineral elements to sustain crop production (Sanchez, 2002; 2005; Sanginga and Woomer, 2009). Each of these plant nutrients is needed in deferent amount by plants and they differ in their mobility and availability in plants and soil. In agro ecosystems where most farmers prefer to grow more than one crop in the same piece of land at the same time; there are maximum interactions between plant roots and soil (Ndakidemi, 2006). The concentration of plant nutrients in the zone of soil-roots interactions (rhizosphere) is reported to be different from bulk soil (Hinsinger *et al*., 2005). Compared with the bulk soil, rhizosphere soil is said to have high concentration of mineral elements and soil microorganisms (both beneficial and harmful) (Cabala *et al*., 2004; Cheng and Gershenson, 2007). Interactions between roots and soil during plant growth induce changes in the soil that make rhizosphere soil to differ from bulk soil (Wang and Zabowski, 1998; Makoi *et al*., 2014). These changes in the rhizosphere may be caused by root uptake of nutrients, microbial activity, and/or components of root exudates (Hinsinger *et al*., 2005; Huang *et al*., 2014). Plants release several low and high molecular weight organic compounds such as sugars, organic acids, amino acids and phenolics into the rhizosphere (Marschner and Römheld, 1996; Hinsinger *et al*., 2005). The compounds that are released can lead to dissolution of primary minerals and precipitation or crystallization of secondary compounds and/or minerals, and eventually transformation of mineral components in the rhizosphere (Cabala *et al.* 2004).

Plant Growth Promoting Rhizobacteria (PGPR) also do concentrate in the rhizosphere soil and confer the plants with beneficial effects such as solubilization of mineral nutrients, fixation of nitrogen and disease suppression resulting from plant pathogens (Gupta *et al*., 2000; Weller *et al*., 2002; Kloepper *et al*., 2004; Yang *et al*., 2009; Mendes *et al*., 2011;

Tahir *et al*., 2016). Bambara and Ndakidemi (2010) reported a significant increase in soil pH, Ca and Na following *Rhizobium* inoculation on *Phaseolus vulgaris*. Crop diversity in the field increases concentration of microbes in the rhizosphere soil compared with the single species because of specificity of microbes to plant species. Intercropping cereal with legume crops such as cowpea, mungbean, soybean and groundnuts can fix and accumulate nitrogen ranging from 80 to 350 kg ha^{-1} per year (Peoples and Craswell, 1992) thereby improving soil fertility. Intercropping also can enhance nutrient mineralization because it improves the decomposition rates of soil organic matter (Blagodatskaya and Kuzyakov, 2008; Mobasser *et al*., 2014). Biological nitrogen fixation induces changes in the soil pH resulting in limited availability of some plant nutrients in the soil (Bolan *et al*., 1991).

Currently, there is limited information about the effect of *Rhizobium* inoculation on the chemical composition in the rhizosphere of soybean intercropped with maize, supplemented with lower and higher rates of phosphorus (P) and potassium (K) fertilizers. Therefore, the current study was carried out to determine the effects of intercropping systems, *Rhizobium* inoculation and fertilization with different levels of P and K singly or combined application on chemical properties of soybean rhizosphere soil after two consecutive cropping seasons.

3.2. Material and methods

3.2.1. Experimental design and treatments

The field experiment was conducted in the same place for two consecutive years (2015 and 2016 cropping seasons). The treatments for year one were repeated in year two in the same spots. The experimental trials were set at Tanzania Coffee Research Institute (TaCRI) farm in northern Tanzania. The experiment followed a split-split plot design with factorial arrangement and replicated thrice. The plot measured 3 x 3 m. The main plots had two Rhizobia inoculation treatments, while the sub plots were comprised of the following treatments; Maize (sole crop) at a spacing of 75 x 60 cm; Soybean (sole crop) at a spacing of 75 x 40 cm; Maize/soybean (intercropping system) at a spacing of 75 x 60 cm and 75 x 20 cm, Maize and soybean respectively; and the last cropping system was Maize/soybean (intercropping system) at a spacing of 75 x 60 cm and 75 x 40 cm, Maize and soybean respectively. The sub-subplots were treated with the following fertilizer levels (kg ha^{-1}):

control (0 kg ha^{-1}); 20 K; 40 K; 26 P; 52 P; 26 P + 20 K; 52 P + 40 K. The sources of these elements were Triple Super Phosphate (TSP for P) and Muriate of Potash (MOP) for K

3.2.2. Sample collection

The Rhizosphere soil used in this study was collected during the second year of the experimentation when the soybean was at 50% pod formation. The rhizosphere soils were sampled from five plants of middle rows for each plot excluding the border plants. This was achieve by carefully excavation of soil from around each plant down to about 10 – 20 cm depending on root depth of the respective plant, and removed with the plant and its roots intact inside the bulge of soil. The rhizosphere soil adhering to plant roots was shaken in the labeled bags, air dried in the laboratory and sieved (2 mm) ready for determination of pH, organic carbon and analysis of nutrients. The samples collected from each plot for the two cropping season was pooled together and well mixed to form one sample per treatment.

3.2.3. Determination of plant-available nutrients in rhizosphere soil

Total N was determined by the method of micro-Kjeldahlas described by Bremner (1965). Phosphorus was determined by the molybdenum blue method (Murphy and Riley, 1962). Concentrations of elements such as Ca, Mg, K and Na were determined by method described in Hesse (1971). The trace elements such as Cu, Zn, Fe and Mn were extracted by diethylenetriaminepentaacetic acid (DTPA) (Lindsay and Norvell, 1978) and determined by an atomic absorption spectrophotometer. The rhizosphere soil pH was analysed in 1:2.5 (soil:water) suspension, by the electrometric method (Chapman, 1965), and electrical conductivity (EC), measured in a 1:5 (soil:water) suspension, using the electrometric method (Chapman, 1965). The organic carbon was determined by the Walkley and Black method (Walkely and Black, 1934).

3.2.4. Statistical analysis

The collected data was analysed using statistical software called STATISTICA. The statistical analysis was performed using analysis of variance (ANOVA) in factorial arrangement. The fisher's least significance difference (L.S.D.) was used to compare treatment means at p = 0.05 level of significance (Steel and Torrie, 1980).

3.3. Results

3.3.1. Effects of cropping systems on rhizosphere soil chemical properties

The results indicated that except for sodium (Na), cropping systems had no significant effects on the chemical properties of the soybean rhizosphere soil. Only sodium was observed to be greater in the rhizosphere soil of soybean grown under maize intercropping systems compared with the rhizosphere soil of soybean pure stand (Table 2).

3.3.2. Effects of *Rhizobium* inoculation on rhizosphere soil chemical properties

Rhizobium inoculation was observed to alter the most of the chemical properties of the rhizosphere soil of soybean compared with the rhizosphere soil collected from un-inoculated soybean. Rhizosphere soil chemical properties that were significantly increased in the inoculated soybean includes macro and micro nutrients (N, P, Ca, Mg, Na and OC) and (pH, Fe, Cu, Mn, Zn) respectively. In this study, *Rhizobium* inoculation treatment did not significantly alter the concentration of EC and K in the rhizosphere soil (Table 2 and 3). Specifically, *Rhizobium* inoculation increased the concentration of chemical (N, P, Ca, Mg, Na and OC) in the soil by 10.5, 120.6, 14.2, 16.7, 33.3 and 17.8% respectively. Similarly, the rhizosphere pH and micro nutrients (Fe, Cu, Mn and Zn) were respectively increased by 1.3, 10.6, 31.4, 41.7, and 25% in *Rhizobium* inoculated soybean relative to un-inoculated soybean.

3.3.3. Effects of P and K fertilization on rhizosphere soil chemical properties

Fertilization of crop (soybean) with P and K did not significantly change the concentration nitrogen, sodium and the organic matter content of rhizosphere soil. However, application of these fertilizers significantly increased the rhizosphere content of macro nutrients such as P, K, Ca, and Mg (Table 2). P and K fertilization also altered the pH and EC of the rhizosphere soil relative to control (Table 3).

Table 2: Effects of Rhizobium inoculation, Cropping Systems and the supply of P and K on macro nutrients in the rhizosphere soil measure in 2016 season

Treatments	N	OC	Av. P	K	Ca	Mg	Na
	← % →		Bray 1. mg/kg	← Meq/100g →			
Crop. System							
SB	0.039±0.001a	2.65±0.05a	3.56±0.56a	2.20±0.15a	8.60±0.41a	1.67±0.06a	0.16±0.01b
M+B(A)	0.041±0.001a	2.65±0.05a	3.50±0.59a	2.25±0.17a	7.95±0.39a	1.58±0.06a	0.18±0.01a
M+B(B)	0.040±0.001a	2.67±0.04a	3.42±0.66a	2.27±0.18a	8.55±0.44a	1.62±0.06a	0.19±0.01a
Rhizobia							
With out	0.038±0.001b	2.59±0.04b	2.18±0.40b	2.32±0.15a	7.81±0.28b	1.50±0.04b	0.15±0.01b
With	0.042±0.001a	2.72±0.03a	4.81±0.52a	2.16±0.12a	8.92±0.37a	1.75±0.05a	0.20±0.01a
Fertilizers							
Control	0.038±0.002a	2.61±0.06a	0.60±0.17b	1.51±0.23c	6.92±0.39c	1.59±0.06b	0.14±0.01a
20K	0.039±0.001a	2.71±0.06a	2.47±0.95b	2.52±0.15b	6.44±0.42c	1.52±0.10b	0.17±0.01a
40K	0.039±0.001a	2.74±0.07a	0.71±0.16b	2.81±0.19ab	6.46±0.40c	1.42±0.06b	0.18±0.02a
26P	0.040±0.001a	2.62±0.07a	5.60±0.95a	1.32±0.08c	10.14±0.57ab	1.85±0.08a	0.17±0.02a
52P	0.041±0.002a	2.66±0.05a	5.76±1.04a	1.45±0.12c	10.46±0.72a	1.85±0.11a	0.20±0.02a
20K+26P	0.041±0.002a	2.56±0.08a	4.69±0.81a	2.83±0.20ab	8.77±0.43b	1.50±0.05b	0.18±0.02a
40K+52P	0.041±0.001a	2.69±0.06a	4.63±0.96a	3.24±0.29a	9.38±0.57ab	1.63±0.10ab	0.18±0.01a
3-Way ANOVA F-statistics							
CroSyt	0.475ns	0.06ns	0.02ns	0.103ns	1.15ns	0.62ns	3.41*
Rhiz	16.746***	5.76**	21.71***	1.478 ns	8.40**	15.97***	18.79***
Fert	0.810ns	0.90ns	8.86***	19.195***	11.65***	4.04***	1.75ns
CroSyt*Rhiz	1.091ns	0.54ns	0.90ns	2.027ns	0.48ns	0.19ns	0.97ns
CroSyt*Fert	0.898ns	1.02ns	0.66ns	2.578**	0.61ns	0.52ns	0.71ns
Rhiz*Fert	1.467ns	0.58ns	1.77ns	1.225ns	0.25ns	0.19ns	0.48ns
CroSyt*Rhiz*Fert	0.830ns	0.70ns	1.01ns	0.567ns	1.41ns	0.86ns	0.85ns

CroSyt: Cropping Systems; Fert: Fertilizers; Rhiz: Rhizobium; SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; Values presented are means ± SE; *,**, ***: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

Table 3: Effects of rhizobium inoculation, Cropping Systems and the supply of P and K on soil pH, EC and micro nutrients in the rhizosphere soil measure in 2016 season

Treatments	pH	EC	Fe	Cu	Mn	Zn
Crop. System	Water 1:2.5	mS/cm	←	ppm		→
SB	5.39±0.03a	0.21±0.01a	40.88±1.00a	15.61±0.72a	9.51±0.44a	0.74±0.03a
M+B(A)	5.35±0.03a	0.24±0.02a	42.04±1.11a	15.87±0.57a	9.80±0.48a	0.69±0.03a
M+B(B)	5.35±0.03a	0.24±0.02a	41.34±1.05a	17.86±1.10a	10.71±0.70a	0.73±0.03a
Rhizobia						
With out	5.33±0.03b	0.22±0.01a	39.34±0.67b	14.22±0.54b	8.28±0.29b	0.64±0.02b
With	5.40±0.03a	0.23±0.01a	43.50±0.89a	18.68±0.69a	11.73±0.48a	0.80±0.02a
Fertilizers						
Control	5.21±0.05c	0.18±0.02d	41.22±1.25a	15.97±1.28a	9.16±0.67a	0.70±0.05a
20K	5.34±0.06b	0.21±0.01bcd	42.00±1.89a	15.92±1.36a	9.90±0.80a	0.70±0.03a
40K	5.43±0.05ab	0.20±0.02cd	42.11±1.66a	15.90±1.13a	10.08±1.03a	0.73±0.05a
26P	5.31±0.03bc	0.24±0.03abc	43.13±1.57a	17.88±1.13a	9.09±0.70a	0.63±0.03a
52P	5.36±0.03b	0.26±0.02ab	40.95±1.26a	14.37±1.01a	10.36±0.70a	0.71±0.05a
20K+26P	5.42±0.05ab	0.26±0.02ab	39.54±1.85a	17.42±1.10a	11.93±1.26a	0.81±0.05a
40K+52P	5.49±0.05a	0.27±0.02a	41.01±1.36a	17.67±1.75a	9.52±0.49a	0.73±0.04a
3-Way ANOVA F-statistics						
CroSyt	0.7ns	0.98ns	0.33ns	2.51ns	1.57ns	0.93ns
Rhiz	4.9*	0.33ns	12.50*	24.74***	35.64***	27.69***
Fert	4.2***	2.99*	0.53ns	1.14ns	1.61ns	1.77ns
CroSyt*Rhiz	5.0**	2.30ns	0.21ns	1.05ns	0.39ns	0.31ns
CroSyt*Fert	1.6ns	1.29ns	1.36ns	1.37ns	0.41ns	0.71ns
Rhiz*Fert	1.1ns	0.90ns	0.60ns	0.23ns	1.26ns	0.84ns
CroSyt*Rhiz*Fert	0.5ns	1.55ns	0.30ns	0.28ns	0.66ns	0.79ns

CroSyt: Cropping Systems; Fert: Fertilizers; Rhiz: Rhizobium; SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; Values presented are means ± SE; *,**, ***: significant at p ≤ 0.05, p ≤ 0.01, p ≤ 0.001 respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

3.3.4. Interactions of factors on rhizosphere K and pH

The current study showed that there were significant interactions between cropping systems and fertilizers on rhizosphere soil K content and between cropping systems and *Rhizobium* inoculation on rhizosphere soil pH. Fertilization with two levels of K significantly increased the rhizosphere K over the plots fertilized with P and the control. The plots that were fertilized with P had statistically the same rhizosphere K with the control (Fig. 1). The highest rhizosphere K level was recorded in soybean intercropped with maize at wider spacing and fertilized with doubled combined fertilizers, while the lowest level of rhizopsphere K was recorded in the control plots (Fig. 1). The rhizosphere soil pH was significantly higher in *Rhizobium* inoculated plots over un-inoculated one throughout the cropping systems. The highest pH value was recorded in *Rhizobium* inoculated pure stand while the lowest was recorded in un-inoculated pure stand soybean (Fig. 2).

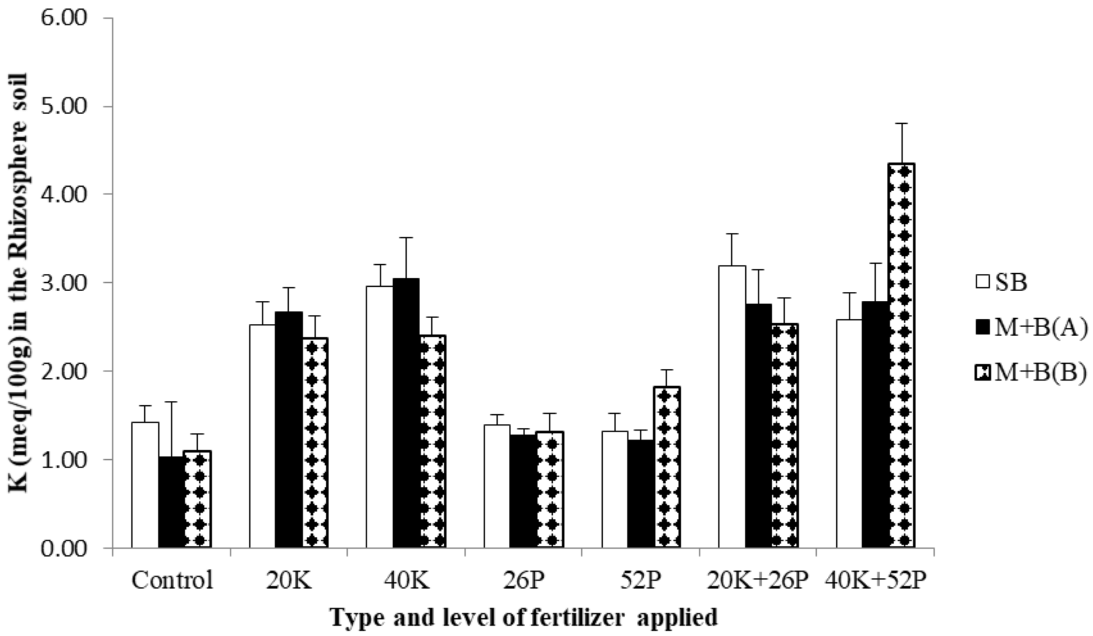

Figure 1: Interactive effect of cropping systems and fertilizers on rhizosphere soil K content

SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively

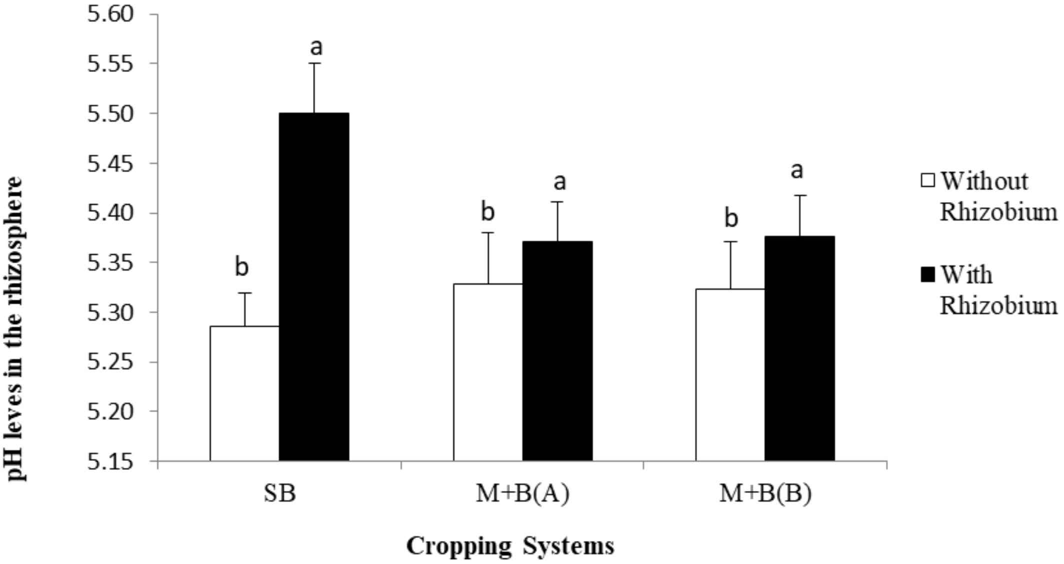

Figure 2: Interactive effect of cropping systems and rhizobium inoculation on rhizosphere soil pH

SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively.

3.4. Discussion

The findings of this study showed that *Rhizobium* inoculation altered the chemical composition of rhizospheres soil relative to un-inoculated treatments. *Rhizobium* inoculation reduced the soil acidity by increasing the rhizosphere soil pH relative to un-inoculated treatments. Similar findings were reported by Bambara and Ndakidemi (2010) who found that *Rhizobium* inoculation significantly increasing the soil pH in the rhizosphere of *P. vulgaris*. Furthermore, *Rhizobium* inoculation altered the chemical properties of rhizosphere whereby most of the mineral elements were increased in rhizosphere soils of inoculated soybean over the control. There are several possible explanations for increased concentration of macro and micro nutrients in the rhizosphere soils of inoculated soybean. Firstly, it is due to increased soil pH which favoured the availability of most plant nutrients (Bagayoko *et al*., 2000; Condron *et al*., 1993). Increased availability of nutrients in the rhizosphere soil provides normal growth of plants and eventually increased yield. Normally, if there is low soil pH, the soil is acidic which results in poor plant growth and development as most of plant nutrients becomes unavailable for plants. Secondly, mineralisation activities of rhizopheric microorganisms tend to solubilise mineral elements such as P and make it available in the soil

(Dakora and Phillips, 2002). Thirdly, *Rhizobium* produces Fe career compound called siderophores which tends to increase the Fe content in the rhizosphere soil (Wang *et al*., 1993; White and Broadley, 2009). Fourthly, the decaying cells of microorganisms releases nutrients and make them available in the rhizosphere soil (McCulley 2001). Fifthly, mineral elements can be excreted in the rhizosphere soil as exudates from plant roots (Ae *et al*., 1990).

All these processes are taking place in the soil and in one way or another may have attributed to the increased chemical properties of rhizosphere soil of *Rhizobium* inoculated soybean. The current study also showed that organic carbon significantly increased in the rhizosphere soil of *Rhizobium* inoculated soybean over the control. The increase in organic carbon content might be attributed to better root growth and deposition of organic materials in first cropping season since these data were taken in the second cropping season. Similar findings were reported by Sharma *et al*. (2009) and Sharma and Verma (2011). However, our findings on organic carbon content in the rhizosphere differed with that of Yusif *et al*. (2016) who found that *Rhizobium* inoculation decreased the soil pH and the organic carbon. They urged that decrease of organic carbon may have been attributed by increased microorganisms which hasted decomposition of organic carbon in the rhizosphere.

The results also showed that P and K fertilization increased the concentration of P, K, Ca and Mg in the rhizosphere over the control (Table 2). Increased concentration of Ca and Mg in the rhizosphere soil may have been attributed by synergistic effect of P and K which made these nutrients to concentrate more in the rhizosphere soil. The significant increase of P and K in the rhizosphere soil has been attributed by P and K fertilization which increased the availability of these nutrients. Furthermore, root exudates may have contributed to the increased macro nutrients in the plots treated with P and K. It was also noted that the rhizosphere soil pH and electrical conductivity (EC) were significantly higher in P and K fertilised plots relative to control. Soil electrical conductivity (EC) provides the measurement of the amount of salts in soil (salinity of soil) (Shainberg *et al*., 1980; Grisso *et al*., 2009). EC affects crop yields, crop suitability, plant nutrient availability, and activity of soil microorganisms which influence key soil processes. However, EC does not provide a direct measurement of specific ions or salt compounds. Researchers have correlated it to concentrations of ions such as N, P, K, Ca, Mg, Na, Mn, Zn, and Cu (Heiniger *et al*., 2003; Grisso *et al*., 2009; Hamzehpour and Abasiyan, 2016). Their finding are in line with our results which showed the increased EC in plots treated with P and K fertilizers compared with the control.

There were significant interactions between cropping systems and fertilizers on rhizosphere soil K content and between cropping systems and *Rhizobium* inoculation on rhizosphere soil pH. Potassium fertilization significantly interacted with cropping system and contributed to the available K in the rhizosphere soil. The highest K level was recorded in rhizosphere soil of soybean intercropped with maize at wider spacing and fertilized with doubled combined fertilizers, while the lowest level of rhizopsphere K was recorded in the control plots. The rhizosphere soil pH was significantly higher in *Rhizobium* inoculated plots over un-inoculated one throughout the cropping systems. The highest pH value was recorded in *Rhizobium* inoculated pure stand while the lowest was recorded in un-inoculated pure stand soybean suggesting that microorganisms such as *Rhizobium* can help to reduce soil acidity.

3.5. Conclusion

Rhizobium inoculation altered most of the chemical properties of the rhizosphere soil of soybean in this study. The rhizosphere soil chemical properties such as pH, OC, EC, macro and micro nutrients (N, P, Ca, Mg, and Na) and (Fe, Cu, Mn, and Zn) respectively were significantly increased in the *Rhizobium* inoculated soybean over the control. These results strongly support the use of microorganism to improve soil chemical properties for improved plant growth, development and production. The supply of P and K fertilizers significantly increased the rhizosphere content of macro nutrients such as (P, K, Ca, and Mg) and also they altered the pH and EC of the rhizosphere soil relative to control.

CHAPTER FOUR

RHIZOBIUM INOCULATION REDUCES P AND K FERTILIZATION REQUIREMENT IN CORN-SOYBEAN INTERCROPPING[3]

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Abstract

The field experiment was carried out at Tanzania Coffee Research Institute (TaCRI) for two consecutive years (2015 and 2016) to assess the effects of *Rhizobium* inoculation, supplemented with phosphorus and potassium on nutrient uptake in soybean intercropped with maize. The experiment was laid out in split-split plot design with 2x4x7 factorial arrangement replicated thrice. The main plots had two rhizobial inoculation treatments, while the sub plots comprised of four cropping systems: Maize (sole crop), Soybean (sole crop) and two intercropping at different spacing. The sub-subplots were assigned to fertilizer levels (kg ha^{-1}): control; 20K; 40K; 26P; 52P; 26P + 20K; 52P + 40K. The statistical analysis was performed using ANOVA. The fisher's L.S.D. was used to compare treatment means at p=0.05 level of significance. The results indicated that soybean pure stand significantly improved the uptake of Mg over the soybean under intercropping systems for the two cropping seasons. This was contrary to the uptake of Fe which was increased in intercropped soybean for the first cropping season relative to soybean pure stand. *Rhizobium* inoculation significantly improved the uptake of all other macro nutrients (N, K, P, and Mg) and micronutrients (Fe, Cu, Zn, Mn) in soybean shoots over un-inoculated soybean. Fertilization

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of soybean with P and K significantly increased the uptake of N, P and K for both cropping seasons. However, the uptake of macro nutrients such as calcium (Ca) and Magnesium (Mg) and micro nutrients such as Fe and Cu decreased with the increase of P and K fertilizers. P and K fertilization did not significantly affect the uptake of Mg and Mn for the two cropping seasons. Based on the findings of this study, *Rhizobium* inoculation and P and K fertilization at lower rates are recommended for improved uptake of macro and micro nutrients in legumes such as soybean.

***Keywords**:* Soybean, *glycine max*, rhizobium, fertilizer, maize intercropping

4.1. Introduction

Intercropping is an important cropping system of growing two or more crops in the same field at the same time (Sanchez, 1976). It is particularly important cropping system not only in tropical but also in temperate areas (Vandermeer, 1989). Most common crop combinations in intercropping systems include: maize-soybean, maize-cowpea, maize-pigeon pea, maize-groundnuts, maize-beans, maize-lablab, sorghum-cowpea, millet-groundnuts, and rice-pulses (Matusso *et al*., 2012; Nyoki and Ndakidemi, 2016). Maize-soybean intercropping have been reported to increase the efficiency of land use through improved soil productivity (Kebebew, 2014; Zhang *et al*., 2015), increasing the total crop yield per unit area relative to monocropping through better use of resources by the component crops (Ghanbari, *et al*., 2010; Lemlem, 2013; Kebebew, 2014). Intercropping is also an effective control of pests and diseases, good ecological services and economic profitability (Thierfelder, 2012; Jensen, 1996; *Zhang et al*., 2015). Legume-cereal intercropping, is a productive and sustainable cropping system due to its resource utilization (water, light, nutrients), and its effect on N input from symbiotic nitrogen fixation into the cropping system (Jensen, 1996; Willey, 1979; Whitbread and Ayisi, 2004; Khogali *et al*., 2011). The use of nitrogen fixing bacteria (Rhizobia) in soybean-corn intercropping can reduce to the large extent the need for external fertilizer N as soybean can fix reasonable amount of nitrogen for itself and for the cereal component crop (Van Groenigen *et al.,* 2015). Since both soybean and corn needs nitrogen for proper growth and development, they tend to compete for nitrogen forcing soybean to fix atmospheric N_2 in symbiosis with rhizobia (Corre-Hellou *et al*., 2006; Zhang *et al*., 2015). In Tanzania, rhizobia is not commonly used by farmers in legume production but researchers have reported several advantages of these microorganisms including improved nutrient supply in the soil (Saharan and Nehra, 2011). Inoculation of legumes with effective strains of rhizobia can potentially influence nutrient uptake by component crops of intercrop due to the spread of roots, which determines the uptake and utilization of water and nutrients (Gao *et al*., 2010).

However, the supply and bioavailability of these mineral elements in most soils of sub-Saharan Africa and other parts of the world is limiting and continuously declining leading to low crop production (Buerkert *et al*., 2001; Sandeep *et al*., 2008; White and Broadley, 2009; Nyoki and Ndakidemi, 2014b). Humans require sufficient intakes of many mineral elements for their wellbeing (White and Broadley, 2005; Stein, 2010). The limited supply of essential mineral elements in the soil create deficiency of these minerals in human diet as plant tissues will not have enough to supply for human needs (Govindaraj, 2011; White *et al*., 2012; Arunachalam *et al*., 2013). Some efforts need to be done to improve soil fertility which will lead to improved uptake of plant nutrients and eventually improve crop yields. The current study was carried out with the aim of improving nutrient uptake in *Rhizobium* inoculated soybean (*Glycine max* (L) Merr.) intercropped with maize, and supplemented with phosphorus and potassium. Soybean was used in this study because of its richness in nutrients for human diet and livestock feeds (Myaka *et al*., 2005; Keino *et al*., 2015) and its ability to fix large amount of nitrogen for its requirements and for the non-fixing crops growing together in intercropping (Vanlauwe *et al*., 2003; Keino *et al*., 2015). Under this system maize was included in order to study how intercropping may facilitate the uptake of nutrients soybean.

4.2. Material and methods

4.2.1. Description of the study area

The field experiment was carried out at Tanzania Coffee Research Institute (TaCRI) for two consecutive years (2015 and 2016) to assess the effects of *Bradyrhizobium japonicum* inoculation, supplemented with phosphorus and potassium on nutrient uptake in soybean intercropped with maize. The study area (TaCRI) is located at the foot of mount Kilimanjaro at the elevation of 1330 m above the sea level in Kilimanjaro region, Tanzania having latitude (3˚13'58.99"S) and longitude (37˚14'53.03"E). The field experiment was conducted in an area with bimodal rainfall pattern and mean annual rainfall of 1200 mm.

4.2.2. Plant materials, fertilizers and inoculation procedure

The crop plant used for this experiment were corn variety SEEDCO 513 was bought from seed company and Soybean variety Uyole Soya 2 (SH 2) was obtained from Uyole Agricultural Research Institute, Tanzania. The fertilizers used in this study were Triple Super Phosphate (TSP) for phosphorus and Murate of Potash (MOP) for potassium. The BIOFIX legume inoculants (*Bradyrhizobium japonicum)* were obtained from *MEA* Company Nairobi-Kenya, sold under the license from the University of Nairobi. The *B. japonicum* inoculants were applied following manufacturers' instructions as follows: three (30) gram of gum Arabic was added to 300 ml of water and mixed to form a solution. 15 kg of Soybean seeds was weighed and 300 ml of gum Arabic solution was added and mixed well. 50 gm of legume inoculants was added and mixed well so that all seeds are coated. The inoculated seeds were put under shade and the seeds were then sown immediately in a moist soil.

4.2.3. Experimental design and treatments

The experiment was laid out in split-split plot design with 2 x 4 x 7 factorial arrangement. The plot size was 3 x 3 m. The main plots had two rhizobia inoculation treatments, while the sub plots comprised: Maize (sole crop) at a spacing of 75 x 60 cm; Soybean (sole crop) at a spacing of 75 x 40 cm; Maize/soybean (intercropping system) at a spacing of 75 x 60 cm and 75 x 20 cm, Maize and soybean respectively; and the last cropping system was Maize/soybean (intercropping system) at a spacing of 75 x 60 cm and 75 x 40 cm, Maize and soybean respectively. The sub-subplots were assigned the following fertilizer levels (kg ha^{-1}): control; 20 K; 40 K; 26 P; 52 P; 26 P + 20 K; 52 P + 40 K. Each treatment was replicated three times and the treatments were randomised to minimise errors.

4.2.4. Sample preparation

At early stage of pod formation, five soybean plants were sampled in the middle rows of each plot. The sampled plants were careful excavated with their entire roots and the above ground (shoots) oven-dried at 70 °C for 48 h, weighed and ground into a fine powder for a complete plant analysis.

4.2.5. Determination of nutrients in plants

The micro-Kjeldahl method was used to determine the total N (Bremner, 1965). Phosphorus was determined by the molybdenum blue method as described by Murphy and Riley (1962). Ca, Mg, and K concentrations in plant extracts were determined by method described in Hesse (1971). Micronutrients (Cu, Zn, Fe and Mn) were extracted by diethylenetriaminepentaacetic acid (DTPA) (Lindsay and Norvell, 1978) and determined by an atomic absorption spectrophotometer. Then nutrient uptake (mg.plant^{-1}) was calculated as the product of nutrient concentration (mg.g^{-1}) and the weight of the plant part dry matter (g.plant^{-1}).

4.2.6. Statistical analysis

The collected data was analysed using statistical software called STATISTICA. The statistical analysis was performed using analysis of variance (ANOVA) in factorial arrangement. The fisher's least significance difference (L.S.D.) was used to compare treatment means at p = 0.05 level of significance (Steel and Torrie, 1980)

4.3. Results

4.3.1. Effects of cropping systems, *Rhizobium* inoculation and P and K fertilizers on macro and micronutrient uptake

The results presented in Table 4 showed that cropping systems had no significant ($p \leq 0.05$) effects on macronutrient uptake except for Mg which was significantly affected by cropping systems in the two cropping seasons. Soybean pure stand significantly ($p \leq 0.05$) improved the uptake of Mg over the soybean under intercropping systems for the two cropping seasons. Except for Ca in the second cropping season, *Rhizobium* inoculation significantly improved the uptake of all other macro nutrients (N, K, P, and Mg) in soybean shoots over un-inoculated soybean (Table 4). The results also indicated that there were significant effects of P and K fertilization on the macronutrient uptake in soybean for the two cropping seasons. Fertilization of soybean with P and K significantly increased the uptake of N, P and K for both cropping seasons. For the two cropping seasons, the uptake of calcium (Ca) and

Magnesium (Mg) decreased with P and K fertilization. The results also showed that there were interactions between factors on some nutrient uptake.

In this study, micronutrients uptake were differently influenced by some or all factors such as cropping systems, *Rhizobium* inoculation and Fertilization with P and K. With exception of Fe in the first cropping season, cropping systems had no significant ($p \leq 0.05$) effects on other micronutrients (Fe (2^{nd} season), Cu, Zn, Mn) uptake in this study (Table 5). Except for the Mn in the second season, *Rhizobium* inoculation significantly increased the uptake of all micronutrients (Fe, Cu, Zn, and Mn) relative to the un-inoculated plots (Table 5). The results of this study also showed that P and K fertilization significantly influenced the uptake of Fe and Cu compared with the control. It was observed that the uptake of Fe and Cu was reducing with the increase of P and K fertilizers for the two cropping seasons. However, P and K fertilization did not significantly affect the uptake of Mg and Mn for the two cropping seasons (Table 5).

4.3.2. Interactive effects of cropping systems, *Rhizobium* inoculation, and P and K fertilization on nutrients uptake

The current study showed that there were significant interactions between the *Rhizobium* inoculation and cropping systems on the uptake of P, Ca, Mg and Mn in soybean shoots in different cropping seasons (Fig. 3a-d). Likewise, there was significant interaction of Cropping systems, *Rhizobium* inoculation and P and K fertilization of manganese (Mn) uptake in the soybean shoots for the second cropping season (2016). Generally, in this interaction there was a declining trend of Mn uptake with addition of P and K fertilizers. It was also observed that Mn uptake was lower in plots that were not inoculated with *Rhizobium* compared with those under *Rhizobium* inoculation. Under the cropping systems, intercropping at narrower spacing improve Mn uptake over the other two cropping systems (Fig. 4)

Table 4: Effects of cropping systems, rhizobium inoculation, and the supply of P and K on macro nutrient uptake in soybean shoots

Treatments	Macro nutrient uptake (%) Season one					Macro nutrient uptake (%) Season two				
	N	K	P	Ca	Mg	N	K	P	Ca	Mg
Crop. System										
SB	2.35±0.12a	3.80±0.24a	0.34±0.02a	2.31±0.09a	0.56±0.02a	3.58±0.14a	5.78±0.21a	0.35±0.02a	2.08±0.10a	0.50±0.03a
M+B(A)	2.46±0.11a	3.10±0.22a	0.36±0.02a	2.27±0.10a	0.48±0.03b	3.27±0.15a	5.42±0.21a	0.33±0.02a	1.91±0.12a	0.45±0.03ab
M+B(B)	2.35±0.11a	4.28±0.20a	0.36±0.02a	2.45±0.10a	0.47±0.02b	3.37±0.11a	5.50±0.19a	0.32±0.02a	1.91±0.09a	0.42±0.03b
Rhizobia										
With	2.68±0.08a	4.55±0.19a	0.38±0.02a	2.50±0.08a	0.58±0.02a	3.55±0.12a	5.90±0.19a	0.38±0.02a	1.98±0.09a	0.50±0.02a
With out	2.10±0.08b	3.51±0.14b	0.33±0.02b	2.19±0.08b	0.43±0.01b	3.26±0.11b	5.23±0.12b	0.29±0.01b	1.95±0.07a	0.41±0.02b
Fertilizers										
Control	1.50±0.17d	2.80±0.03c	0.21±0.02e	2.60±0.17a	0.61±0.05a	2.12±0.21f	4.12±0.17c	0.20±0.02d	2.04±0.15ab	0.58±0.05a
20K	1.98±0.10c	3.72±0.19b	0.26±0.01de	2.55±0.16a	0.61±0.03a	2.93±0.10e	5.19±0.15b	0.25±0.02cd	2.26±0.17a	0.53±0.03a
40K	2.40±0.12b	4.54±0.28ab	0.30±0.01cd	2.44±0.14a	0.53±0.03b	3.46±0.13cd	6.68±0.27a	0.30±0.03bc	2.16±0.11ab	0.49±0.03ab
26P	2.37±0.12b	3.69±0.30b	0.35±0.02c	2.46±0.10a	0.50±0.03bc	3.19±0.11de	4.76±0.17b	0.36±0.03ab	2.07±0.16ab	0.49±0.04ab
52P	2.81±0.12a	4.23±0.36ab	0.42±0.02b	2.23±0.07ab	0.46±0.02cd	3.79±0.12bc	5.23±0.15b	0.40±0.03a	1.89±0.14bc	0.41±0.03bc
20K+26P	2.75±0.11a	4.65±0.36a	0.42±0.02b	2.31±0.19a	0.44±0.03cd	4.12±0.06ab	6.25±0.23a	0.42±0.03a	1.79±0.19bc	0.35±0.03c
40K+52P	2.91±0.18a	4.51±0.33ab	0.51±0.03a	1.83±0.13b	0.39±0.03d	4.22±0.18a	6.72±0.31a	0.41±0.03a	1.56±0.11c	0.35±0.03c
3-Way ANOVA F-statistics										
CroSyt	0.64ns	1.38	0.90ns	0.96ns	8.25***	2.85ns	1.94ns	0.81ns	1.03ns	4.13**
Rhiz	41.91***	19.72***	10.43**	7.74**	57.45***	7.44**	18.40***	24.14***	0.05ns	12.20***
Fert	18.14***	4.59***	28.64***	3.30**	10.78***	26.90***	23.44***	12.58***	2.44*	7.62***
CroSyt*Rhiz	0.90ns	0.05	3.76*	2.25ns	0.56ns	0.27ns	0.54ns	0.49ns	3.22*	8.22***
CroSyt*Fert	0.75ns	0.31	0.31ns	0.53ns	0.67ns	0.29ns	0.41ns	0.63ns	0.88ns	0.87ns
Rhiz*Fert	0.36ns	0.55	0.31ns	1.04ns	0.87ns	0.61ns	1.40ns	0.85ns	0.77ns	0.59ns
CroSyt*Rhiz*Fert	1.03ns	0.41	0.69ns	0.52ns	0.95ns	0.72ns	0.73ns	0.22ns	0.72ns	1.18ns

CroSyt: Cropping Systems; Fert: Fertilizers; Rhiz: Rhizobium; SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; Values presented are means ± SE; *,**, ***: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

Table 5: Effects of cropping systems, rhizobium inoculation, and the supply of P and K on micro nutrient uptake in soybean shoots

Treatments	Micro nutrient uptake (mg/kg) Season one				Micro nutrient uptake (mg/kg) Season two			
	Fe	Cu	Zn	Mn	Fe	Cu	Zn	Mn
Crop. System								
SB	1030.77±61.07b	30.21±2.71a	147.32±8.03a	282.80±13.32a	534.41±35.94a	13.36±1.05a	124.88±7.48a	223.69±10.93a
M+B(A)	1197.38±91.88ab	29.96±2.76a	139.35±7.06a	275.18±15.65a	525.24±28.18a	12.96±1.00a	120.63±6.77a	254.58±15.60a
M+B(B)	1305.54±82.47a	29.39±2.69a	139.73±8.05a	272.74±16.39a	535.57±33.17a	13.94±1.12a	115.42±5.71a	242.98±20.07a
Rhizobia								
With	1280.71±61.11a	34.52±2.22a	166.15±6.25a	293.85±11.97a	556.85±25.08a	14.65±0.92a	134.09±6.21a	241.51±14.50a
With out	1075.08±68.57b	25.20±2.03b	118.11±4.66b	259.96±12.36b	506.63±27.50b	12.19±0.76b	106.53±3.88b	239.33±11.51a
Fertilizers								
Control	1592.36±155.45a	43.50±3.99a	124.44±13.26a	256.94±26.45a	827.29±29.87a	19.61±2.23a	120.97±11.39a	214.17±29.07a
20K	1370.00±65.43ab	36.06±3.85ab	158.19±11.13a	303.75±19.31a	690.42±30.84b	17.58±0.92ab	142.78±8.47a	279.17±20.97a
40K	1106.11±45.83bc	34.86±3.85ab	137.56±10.53a	269.31±22.85a	587.64±22.47c	14.56±0.74bc	121.94±7.50a	214.44±10.86a
26P	1167.22±83.26bc	29.31±3.87bc	127.78±11.49a	265.69±22.83a	522.92±22.36cd	14.28±1.00bc	112.78±7.58a	224.58±17.45a
52P	1108.89±71.15bc	25.83±3.40bcd	142.08±11.89a	268.89±17.42a	458.06±21.86de	10.97±1.09cd	108.89±11.10a	218.89±15.17a
20K+26P	933.47±82.32c	22.83±3.41cd	158.06±10.05a	281.67±26.38a	386.67±26.43e	7.94±1.06d	125.64±13.94a	291.67±44.07a
40K+52P	967.22±209.11c	16.61±3.47d	146.81±12.89a	292.08±26.23a	249.17±29.20f	9.00±1.61d	109.17±8.95a	240.00±11.44a
3-Way ANOVA F-statistics								
CroSyt	3.49*	0.03ns	0.50ns	0.13ns	0.10ns	0.32ns	0.54ns	0.96ns
Rhiz	5.78*	9.46**	42.35***	4.13*	5.84*	6.08*	13.55*	0.01ns
Fert	4.20***	5.10***	1.88ns	0.56ns	48.95***	10.83***	1.44ns	1.74ns
CroSyt*Rhiz	2.12ns	0.33ns	1.87ns	2.94ns	0.06ns	0.32ns	0.29ns	3.56***
CroSyt*Fert	0.87ns	0.18ns	1.07ns	0.74ns	1.11ns	1.12ns	0.98ns	0.45ns
Rhiz*Fert	1.03ns	0.50ns	1.44ns	0.31ns	0.31ns	1.31ns	0.68ns	0.67ns
CroSyt*Rhiz*Fert	0.49ns	0.18ns	1.42ns	2.33**	0.36ns	0.60ns	0.72ns	1.01ns

CroSyt: Cropping Systems; Fert: Fertilizers; Rhiz: Rhizobium; SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; Values presented are means ± SE; *,**, ***: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

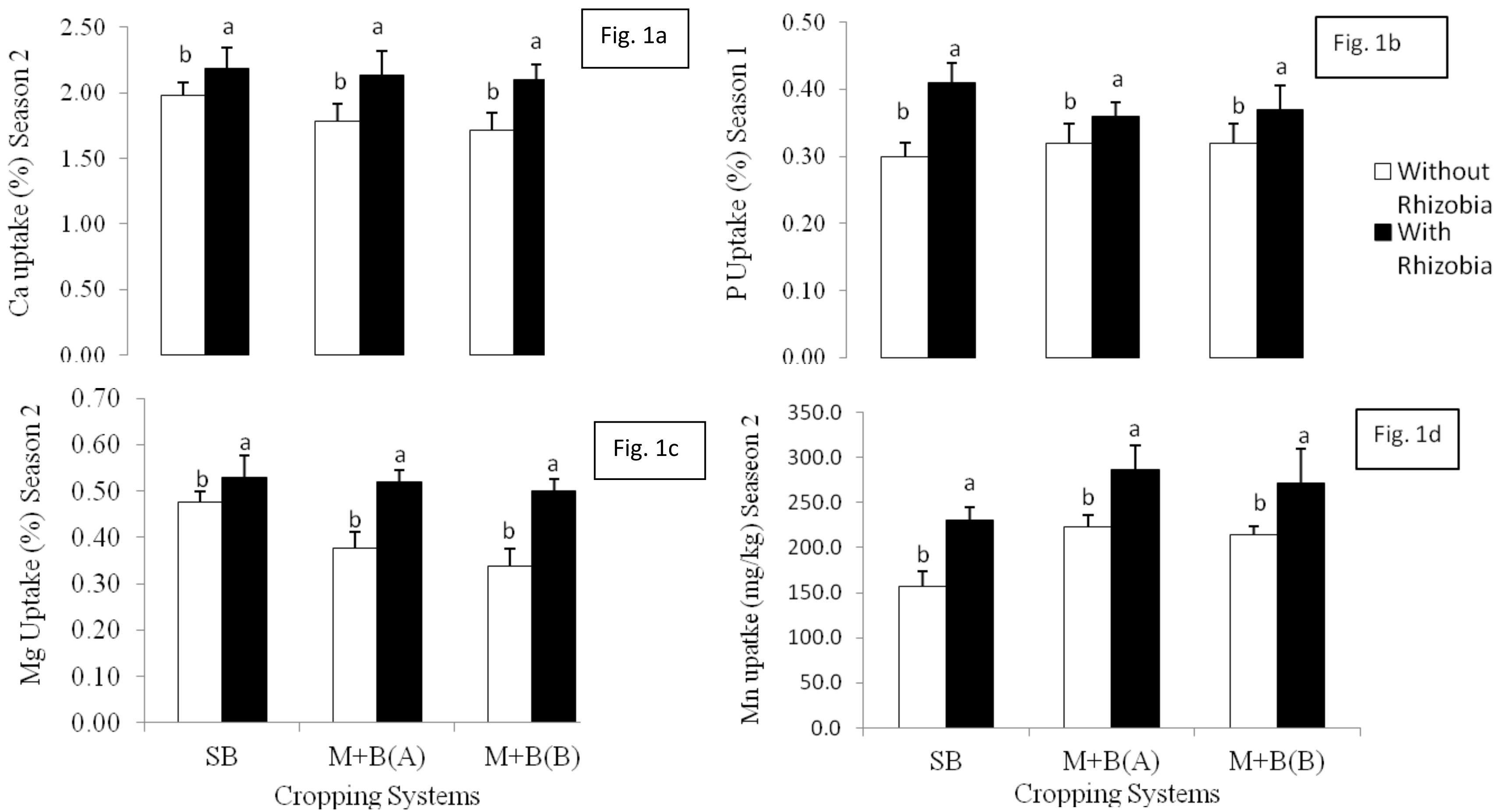

Figure 3 a, b, c, d.: Interactive effects of cropping systems and rhizobium inoculation on Ca uptake (a), P uptake (b), Mg uptake (c) and Mn uptake (d).

Error was estimated for each mean and bars represent mean ± standard error. Bars followed by similar letter are not significantly different from each other. Key: SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively

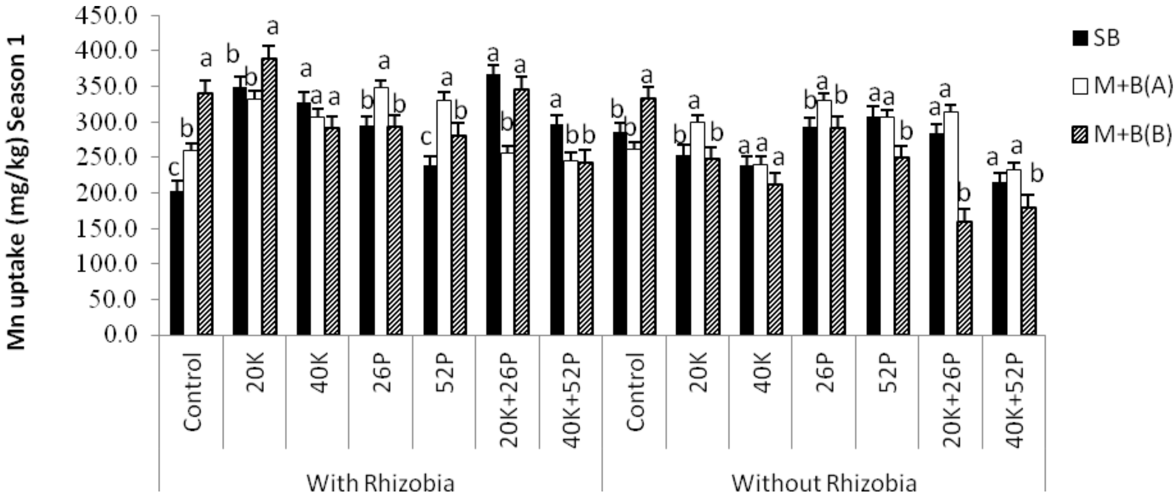

Figure 4: Interactive effects of cropping systems, Rhizobium inoculation, and P and K fertilization on Mn uptake.

Each treatment was replicated trice and the error was estimated for each mean. Key: SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively

4.4. Discussion

4.4.1. Effects of cropping systems on nutrients uptake

The results indicated that cropping systems had no significant ($p \leq 0.05$) effects on macronutrient uptake except for Mg. The uptake of Mg was significantly increased in Soybean pure stand over the soybean under intercropping systems for the two cropping seasons. The possible reason could be due to the fact that there was interspecific competition for nutrients between maize and soybean in intercropping which resulted to lower Mg uptake in soybean compared with soybean pure stand. The studies by (Zhang *et al*., 2001; Zhang and Li, 2003) revealed that interspecific competition and facilitation between the intercropped crops could account for the increased or decreased uptake of nutrients in plants. The current study indicated that Fe uptake was significantly increased in soybean intercropped with maize compared with the soybean under pure stand indicating the positive interactions (Interspecific facilitations) of maize and soybean on Fe uptake in soybean (Li *et al*., 2003). However, the mechanisms for decreased and increased uptake of Mg and Fe respectively are not clear, and hence further research may reveal the mechanisms behind their uptake in intercropping systems.

4.4.2. Effects of *Rhizobium* inoculation on nutrients uptake

The results of the current study showed that *Rhizobium* inoculation significantly improved the uptake of macro nutrients (N, K, P, and Mg) and micro nutrients (Fe, Cu, Zn, Mn) in soybean shoots relative to the un-inoculated soybean which had significantly lower macro and micro nutrients uptake. It is clear that high shoot nitrogen content in *Rhizobium* inoculated soybean is due to biological nitrogen fixation which made nitrogen readily available for plants uptake. The increased uptake of major and trace elements in *Rhizobium* inoculated soybean indicate the necessity of using these microorganisms in agricultural systems. Similar to our findings, several other studies (Baqual and Das, 2006; Ndakidemi *et al*., 2011; Makoi *et al*., 2013; Nyoki and Ndakidemi, 2014a, b) have reported the improved uptake of plant nutrients following *Rhizobium* inoculation. The mechanisms of this improved uptake of nutrients in *Rhizobium* inoculated soybean are still not clear, however, it is thought that these microorganisms can change the soil pH to the level which favours the uptake of plant nutrients (Bambara and Ndakidemi, 2010; Ndakidemi *et al*., 2011). Other studies have reported that *Rhizobium* inoculation can releasing to the soil dead cells which may contain plant nutrients or chemical molecules that can mobilize unavailable nutrients to a form that can be utilized by plants (Halder and Chakrabartty, 1993; Abd-alla, 1994; Saharan and Nehra, 2011; Makoi *et al*., 2013). Furthermore, the increased uptake of elements such as Fe in Rhizobium inoculated soybean may have been attributed by production of iron carrier compound called siderophores (White and Broadley, (2009). This compound facilitates formation of soluble Fe^{3+} complexes which is then reduced by enzyme ferric reductases to F^{2+}, a form that is usable by plants and hence more Fe in inoculated soybean than uninoculated one (White and Broadley, (2009). In other studies rhizobia inoculation have been reported to increase phosphorus by mobilizing it from organic and inorganic sources in the soil rhizopshere and make it available for uptake by plants (Matiru and Dakora, 2004; Saharan and Nehra, 2011).

4.4.3. Effects of P and K fertilization on nutrients uptake

The current study showed that P and K significantly increased the uptake of macro nutrients N, P and K for the two cropping seasons but decreased the uptake of macro nutrients Ca and Mg. The uptake of Fe and Cu was also reduced with the addition and increase of P and K fertilizers for the two cropping seasons. However, P and K fertilization did not significantly affect the uptake of Mg and Mn for the two cropping seasons. Similar to this study, other researchers have reported the similar results as follows: intercropped faba bean increased N and P uptake by 58 and 56% respectively with 33 kg P ha^{-1} (Li *et al*., 2003), Phosphorus application significantly increased the uptake of N, P, K, Ca, Mg, S, Fe and Zn in shoot of chickpea in the first year (Togay *et al*., 2008), and another study by (Das and Sen, 1980) showed that nitrogen, phosphorus and potassium deficiency reduced the uptake of ^{32}P-phosphate, ^{35}S-sulphate, ^{24}Na-, ^{42}K-, ^{45}Ca-, ^{54}Mn-, ^{59}Fe- and ^{65}Zn- by *Cicer arietinum* (Bengal gram) cv B-75 indicating that the supply of N,P and K is necessary for the uptake of other nutrients. However, in the current study, P and K fertilization showed antagonistic effects on the uptake of mineral elements such as Ca, Mg, Fe and Cu in soybean contradicting with the previous study by Li *et al*. (2003) which showed increased Ca, Mg, Fe and Cu with P and K fertilization. The mechanisms for reduced uptake of Ca, Mg, Fe and Cu with P and K fertilization are complex. However, some studies have reported that potassium, calcium, sodium and magnesium ions are quite similar in size and charge and hence, exchange sites cannot distinguish the difference between the ions (Fageria, 2001; Malvi, 2011). Therefore, increasing one of the nutrients in the growth media will definitely limit the uptake of other nutrients with similar characteristics since they compete for site of adsorption, absorption, transport, and function on plant root surfaces or within plant tissues (Robson and Pitman, 1983; Fageria, 2001). This kind of interaction is therefore called negative (antagonism) interaction. In the current study we have also seen the decreased uptake of Ca, Mg, Fe and Cu with addition of P indicating the antagonistic interactions between these elements and P. Similar to our findings, Malvi (2011) reported that excessive amounts of phosphorus reduces uptake of cationic micronutrients like iron, manganese, zinc and copper. In our study, there were synergistic effects of macro nutrients whereby the application of P and K significantly increased the uptake of N, P and K in soybean shoots for the two cropping seasons.

4.4.4. Interactions of the three factors on nutrients uptake

Significant interactions between the *Rhizobium* inoculation and cropping systems on the uptake of P, Ca, Mg and Mn in soybean shoots in different cropping seasons was observed in the current study. There was also significant interaction of Cropping systems, *Rhizobium* inoculation and P and K fertilization of manganese (Mn) uptake in soybean shoots for the second cropping season (2016). The interactions of these factors on the uptake of nutrients indicated that both factors are important and have both contributed in the uptake of nutrients in the soybean shoots.

4.5. Conclusion

This study has revealed that interspecific facilitations occurred between maize and soybean which enhanced the uptake of Fe in intercropped soybean relative to un-inoculated soybean. However, it was also noted that intercropping reduced the uptake of Mg is soybean under intercropping relative to pure stand soybean. *Rhizobium* inoculation also significantly increased the uptake of both macro and micro nutrients in soybean relative to un-inoculated soybean suggesting the use of *Rhizobium* for soybean production. This study also showed that P and K fertilization significantly increases the uptake of N, P and K for both cropping seasons. However, the uptake of calcium (Ca) and Magnesium (Mg) decreased with P and K fertilization. The results of this study also showed that the uptake micronutrients Fe and Cu were decreased with the application of P and K. The decreased uptake of nutrients is an indication of antagonistic movement of these nutrients. The increased concentration of one element in the soil decreases the uptake of others in plant tissues. Generally, both factors have differently influenced the uptake of both macro and micro nutrients in soybean. The increased uptake of nutrients in soybean is an indication that these nutrients will be made available for human and animal bodies when feed on these crops.

CHAPTER FIVE

ROOT LENGTH, NODULATION AND BIOLOGICAL NITROGEN FIXATION OF *RHIZOBIUM* INOCULATED SOYBEAN (*GLYCINE MAX* [L.] MERR.) GROWN UNDER MAIZE (*ZEA MAYS* L.) INTERCROPPING SYSTEMS AND P AND K FERTILIZATION[4]

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Abstract

A two years field trial was carried out to investigate the effects of *Rhizobium* inoculation supplemented with P and K on root length, nodulation and N_2-fixation in soybean intercropped with maize. The split-split plot design with 2 x 4 x 7 factorial arrangement replicated thrice was used. The main plots had two rhizobial inoculation treatments, while the sub plots comprised of four cropping systems namely Maize (sole crop), Soybean (sole crop) and two intercropping at different spacing. The sub-subplots were assigned to fertilizer levels (kg ha^{-1}): control; 20K; 40K; 26P; 52P; 26P + 20K; 52P + 40K. Dried plant sample were ground for determination of N_2-fixation. N_2-fixation was estimated using total nitrogen difference method where the total nitrogen obtained from none-fixing plants (Maize) was subtracted from total nitrogen obtained from fixing plants (Soybean). The results revealed that cropping systems, Rhizobium inoculation and P and K fertilizers have differently affected the root length, number of nodules and/or nitrogen fixation in soybean. Intercropping increased the number of nodules relative to sole soybean. Inoculated soybean significantly increased root length, number of nodules and nitrogen fixation over un-

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inoculated. Root lengths were increased by 7.5% and 7.3% in 2015 and 2016 respectively. P and K fertilization also increased the number of nodules and nitrogen fixation over the control. There was also a significant interaction of *Rhizobium* inoculation and fertilizers on number of nodules and nitrogen fixation in 2015 cropping season. The use of combined fertilizers at lower rates (20K+26P) was generally seen to be better.

Keywords: Symbiotic relationship, biological nitrogen fixation, plant hormones, mixed cropping, phosphorus, Potassium.

5.1. Introduction

All plants need relatively large amounts of nitrogen (N) for proper growth and development (Uchida, 2000). Nitrogen element is critical because it is the major component of essential biomolecules such as chlorophyll, an important pigment for photosynthesis; amino acids, which are the key building blocks of proteins and other biomolecules such as ATP and nucleic acids (Wagner, 2012). Nitrogen is added in the soil through addition of industrial nitrogenous fertilizers (Van Groenigen *et al.,* 2015) the decomposition of soil organic matter and redistribution of organic materials, natural processes of converting atmospheric N_2 through lighting and biological nitrogen fixation (BNF).

Several researchers have stated the principal sources of N for crop production are biological N_2 fixation, organic resources recycled within the cropping field or concentrated from a larger area, and mineral N fertilizers (Giller *et al*., 1997; Van Groenigen *et al.,* 2015). Of these sources of N, mineral fertilizers have raised a global environmental concern resulting from the large amounts of N entering the global food production system (Galloway *et al*., 2013; Van Groenigen *et al.,* 2015). Studies have also shown that excess N has negative effects on water, air, and ecosystem and human health (Compton *et al*., 2011; Davidson *et al*., 2012; Van Groenigen *et al.,* 2015). Apart from environmental effects of mineral N fertilizers, the cost input is high to afford by small holder farmers and it increases the costs of production (Ndakidemi *et al*., 2006; Chianu *et al*., 2011). To minimize the harmful effects of excessive N form mineral fertilizers and to reduce the costs of production, researchers and farming communities have struggled to maintain soil fertility levels relying mostly on BNF (Van Groenigen *et al.,* 2015).

BNF is the term used for a process whereby atmospheric nitrogen (N=N) is reduced to ammonia in the presence of nitrogenase (Rees *et al*., 2005; Dashora, 2011). Nitrogenase is a biological catalyst found naturally only in certain microorganisms such as the symbiotic *Rhizobium* and *Frankia*, or the free-living *Azospirillum* and *Azotobacter* (Bohlool *et al*., 1992; Van Groenigen *et al.*, 2015). The process of BNF is only possible in a select group of plants, with the help of soil microorganisms.

Symbiotic relationship between plants and microbes has been studied and a group of soil dwelling bacteria have long been used to improve the availability of nitrogen through atmospheric nitrogen fixation. These microorganisms stimulate plant growth by a plethora of mechanisms, hence are called plant growth promoting rhizobacteria (PGPR) (Vessey, 2003). Recently, biofertilizers have emerged as a fundamental component for biological nitrogen fixation which provides an ecologically sound and economically attractive way of improving nutrient supply in the soil (Saharan and Nehra, 2011). Important hosts for these microorganisms to perform biological nitrogen fixation are legumes such as Soybean, Common bean, Lablab, Groundnut, Cowpea, Pigeon pea, Mung bean, Faba bean, Chickpea, Alfalfa, etc. Although BNF has long been a component of many farming systems throughout the world, its importance as a primary source of N for agriculture has diminished as there is increasing use of fertilizer-N for the production of food and cash crops (Peoples *et al*., 1995).

In this study, we are focused on Soybean (*Glycine max*) as a host plant of nitrogen fixing bacteria. Soybean is a nutritious grain legume grown in diverse parts of the world. It is of economic importance and a nutritious crop which provides human with high proteins (Raji, 2007). The crop was introduced in Tanzania for the first time at Amani, Tanga by the German traders and has been grown since 1907 (Myaka *et al*., 2005). It contains 20% non-cholesterol oil and 45% protein compared to 20 and 13% protein content in meat and egg, respectively (Malema, 2005). Areas with the greatest potential for soyabean production in Tanzania include Ruvuma, Mbeya, Rukwa, Morogoro and Iringa, all in south-western Tanzania (Ronner and Giller, 2012) and in northern Tanzania (Ndakidemi *et al*., 2006). Apart from its high protein content soybean has a high nitrogen fixing ability (Vanlauwe *et al*., 2003) for its requirements and contribute to soil N thereby improve soil quality and fertility. None fixing crops growing nearby these legumes or grown in the subsequent season can benefit from nitrogen released out of the fixing plants to the rhizosphere (Shen and Chu, 2004).

For effective nitrogen fixation by *Rhizobium* bacteria, there must be favourable conditions similar to those necessary for growth of the host plant. Among of the conditions necessary for plant growth include availability of macro nutrient such as N, P and K. Phosphorus has been reported to influence symbiotic N_2-fixation in leguminous plants by many researchers (Tang *et al*., 2001; Ndakidemi *et al*., 2006; Zafar *et al*., 2011). Severe deficiency of this element in the soil can significantly impair growth of host plant and symbiotic N_2 fixation (Israel, 1987). Israel (1987) further pointed out that N_2-fixation has higher phosphorus requirements for optimal functioning than the host plant requires for its growth and nitrate assimilation. Another important element in the process of dinitrogen fixation is Potassium (Mengel *et al*., 1974). The process of photosynthesis requires potassium which is essential in maintenance and balance of the electrical charges at ATP production site (IPNI, 1998; Nyoki and Ndakidemi, 2016). Translocation of photosynthetic substances (carbohydrate) to storage organs (fruits or roots) is also mediated under the help of potassium (IPNI, 1998; Uchida, 2000). Under the storage organs such as root nodules, carbohydrate produced by host plant is used by nitrogen fixing bacteria as source energy to fix atmospheric nitrogen (Mengel *et al*., 1974).

However, currently there is limited information regarding the combined effects of P and K and rhizobia inoculation on root length, nodulation and nitrogen fixation in soybean intercropped with maize. Therefore, the current study aimed to determine the effects of rhizobia inoculation supplemented with P and K on root length, nodulation and nitrogen fixation in soybean intercropped with maize.

5.2. Material and methods

5.2.1. Experimental design and treatments

The field experiment was carried out at Tanzania Coffee Research Institute (TaCRI) for two consecutive years (2015 and 2016 cropping seasons). The experiment was laid out in split-split plot design with 2 x 4 x 7 factorial arrangement replicated thrice. The plot size was 3 x 3 m. The main plots had two Rhizobia inoculation treatments, while the sub plots comprised: Maize (sole crop) at a spacing of 75 x 60 cm; Soybean (sole crop) at a spacing of 75 x 40 cm; Maize/soybean (intercropping system) at a spacing of 75 x 60 cm and 75 x 20 cm, Maize and soybean respectively; and the last cropping system was Maize/soybean (intercropping system) at a spacing of 75 x 60 cm and 75 x 40 cm, Maize and soybean respectively. The sub-subplots were assigned the following fertilizer levels (kg ha^{-1}): control; 20 K; 40 K; 26 P; 52 P; 26 P + 20 K; 52 P + 40 K.

5.2.2. Data collection

i. Plant harvest and sample preparation

At 50% flowering, soybean crop was sampled for nitrogen fixation analysis. Plants were excavated carefully from the soil with their entire root system, washed, nodules were counted and recorded, and root length were also measured and recorded. The above ground part (shoots) of the plants were oven-dried at 70 °C for 48 hrs, weighed and ground into a fine powder for determination of nitrogen fixation.

ii. Estimation of N fixation

Nitrogen fixation was estimated using Total Nitrogen Difference (TND) method where the total nitrogen obtained from none-fixing plants (Maize) was subtracted from total nitrogen obtained from fixing plants (Soybean) (Unkovich *et al*., 2008).

Thus

$$\mathrm{N_2\ fixed = Total\ N\ in\ legume\ - Total\ N\ in\ reference\ crop}$$

$$\mathrm{Total\ N_2\ fixed\ in\ plants\ (kg\ ha^{-1}) = \frac{(Dry\ matter\ weight\ (kg\ ha^{-1})\ X\ \%\ N\ in\ plants)}{100}}$$

5.2.3. Statistical analysis

The collected data was analysed using statistical software called STATISTICA. The statistical analysis was performed using analysis of variance (ANOVA) in factorial arrangement. The fisher's least significance difference (L.S.D.) was used to compare treatment means at p = 0.05 level of significance (Steel and Torrie, 1980).

5.3. Results

5.3.1. Root length

The current study showed that cropping systems had no significant effects on the root length of soybean in both cropping seasons. However, this study showed that the roots of soybean were influenced by *Rhizobium* inoculation in all (2015 and 2016) cropping seasons. *Rhizobium* inoculated soybean resulted in significantly longer roots compared with the un-inoculated soybean. In 2015 cropping season, *Rhizobium* inoculation increased root length by 7.5% and in 2016 root length was increased by 7.3%. In this study, P and K fertilization did not show any significant (*p=0.5*) effects on the soybean root length for all two years (Table 6).

5.3.2. Number of nodules

The results of this study showed that cropping systems significantly affected the number of nodules in the two cropping seasons (Table 6). Intercropped soybean produced high number of nodules than the pure stand soybean in both cropping seasons. The percentage increase on the number of nodules in intercropped soybeans over sole soybean was 20.41% and 27.36% for 2015 and 2016 cropping seasons respectively. On the other hand, *Rhizobium* inoculation also significantly increased number of nodules over un-inoculated soybean. The percentage increase on the number of nodules in inoculated plots was 95.97% and 78.17% relative to un-inoculated plots in 2015 and 2016 cropping seasons respectively. Likewise, P and K fertilization significantly increased the number of nodules over the control in both cropping seasons. The highest mean number of nodules (13.25 and 20.46) was recorded in 20 K+26 P (kg ha^{-1}) for 2015 and 2016 cropping seasons respectively, while the lowest mean number of nodules (6.65 and 12.11) of was recorded control plots for both 2015 and 2016 respectively (Table 6).

Table 6: Effects of cropping systems, Rhizobium inoculation supplemented with P and K on soybean root length and number of nodules in 2015 and 2016

Treatments	Root Length		Number of nodules	
	Season one (2015)	Season two (2016)	Season one (2015)	Season two (2016)
Cropping System				
SB	19.64±0.37a	20.30±0.43a	8.58±1.60b	14.60±2.10b
M+B(A)	18.60±0.37a	20.50±0.74a	10.44±1.79a	16.45±2.65ab
M+B(B)	18.81±0.44a	20.52±0.53a	10.78±1.77a	20.10±2.46a
Rhizobia				
With	19.71±0.32a	21.16±0.53a	19.09±1.03a	27.99±1.66a
With out	18.33±0.31b	19.72±0.38b	0.77±0.43b	6.11±1.13b
Fertilizers				
Control	19.36±0.46a	20.02±0.79a	6.65±2.11d	12.11±2.50b
20K	18.30±0.45a	21.41±0.63a	7.79±1.97cd	14.78±3.33ab
40K	18.40±0.67a	19.59±0.35a	9.41±2.82bcd	16.11±4.01ab
26P	19.95±0.58a	22.02±1.01a	9.68±2.43abcd	18.37±4.55ab
52P	19.74±0.58a	19.44±1.46a	11.20±2.49abc	18.57±3.64ab
20K+26P	18.54±0.66a	20.56±0.82a	13.25±3.41a	20.46±3.94a
40K+52P	18.84±0.79a	20.06±0.65a	11.52±2.93ab	18.94±3.85ab
3-Way ANOVA F-statistics				
CroSyt	2.162ns	0.046ns	1.9149*	2.45*
Rhiz	10.154**	4.884*	345.4335***	111.90***
Fert	1.355ns	1.221ns	3.0209**	1.11*
CroSyt*Rhiz	0.989ns	1.037ns	0.9663ns	0.84ns
CroSyt*Fert	1.022ns	1.161ns	1.3582ns	0.26ns
Rhiz*Fert	1.688ns	0.601ns	2.6283*	1.46ns
CroSyt*Rhiz*Fert	0.980ns	1.188ns	1.5647ns	0.65ns

CroSyt: Cropping Systems; Fert: Fertilizers; Rhiz: Rhizobium; SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; Values presented are means ± SE; *,**, ***: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

5.3.3. Interactive effects of *Rhizobium* inoculation and P and K fertilization on number of nodule in 2015 cropping seasons

There were significant interactions between *Rhizobium* inoculation and fertilizers in the cropping year 2015. In this study, *Rhizobium* inoculation interacted well with fertilizers leading to increased number of nodule. Un-inoculated plots produced nodules that were below five, and the fertilizer level of 20 K and 40K+52P (kg ha^{-1}) produced higher number of nodules compared with other fertilizer levels. *Rhizobium* inoculation significantly increased the number of nodules regardless of whether it was fertilized or not. However, the plots treated with 26 P resulted in high number of nodules over all other treatments followed by 40K+52P (kg ha^{-1}) (Fig. 5)

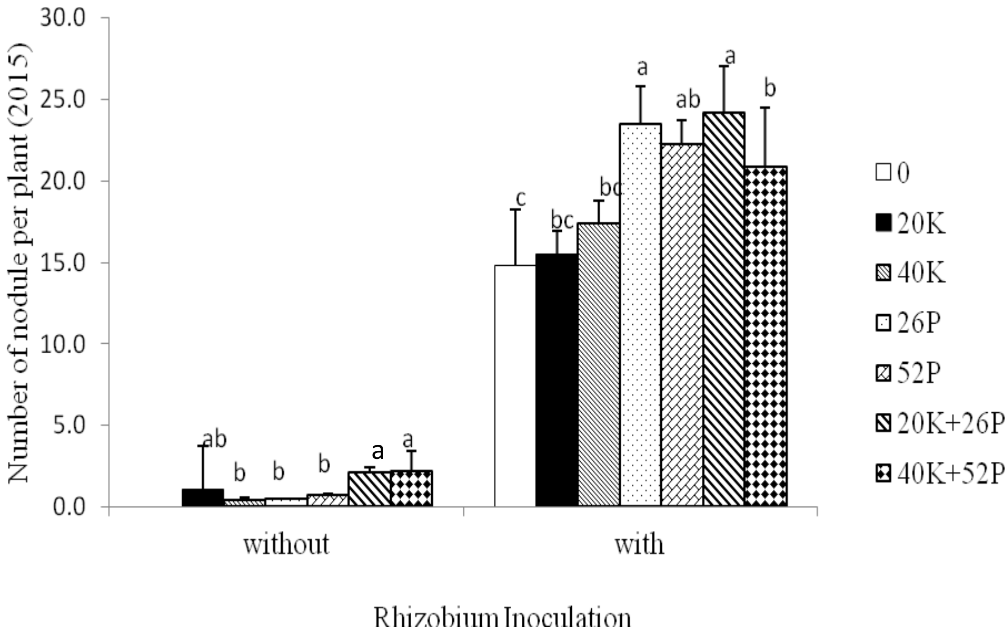

Figure 5: Interactive effects of rhizobia inoculation and P and K fertilization on number of nodule in 2015

5.3.4. Nitrogen fixation

The results of the current study indicated that cropping systems had no significant effects on nitrogen fixation for the two cropping seasons. However, numerically there was slight increase in nitrogen fixation under intercropping relative to the sole cropped soybean (Table 7). Rhizobium inoculation showed a highly significant effect on nitrogen fixation over un-inoculated treatments with an increase of 63 and 55.16 (kg ha^{-1}) in 2015 and 2016 respectively. In both cropping seasons, P and K fertilization significantly improved nitrogen fixation over the control. The highest value of fixed nitrogen was found in the plots fertilized with 52 P (kg h^{-1}) while the lowest values were recorded in the control plots for the two cropping seasons (Table 7).

Table 7: Effects of cropping systems, Rhizobium inoculation supplemented with P and K on nitrogen fixation in 2015 and 2016

	N Fixed (kg/ha)	
Level of Factor	Season one (2015)	Season two (2016)
Cropping Systems		
SB	81.92±8.27a	127.25±9.53a
M+B(A)	84.96±6.90a	129.32±9.33a
M+B(B)	88.40±10.48a	130.63±8.18a
Rhizobium inoculation		
With	116.97±7.51a	156.66±7.29a
With out	53.21±3.25b	101.47±5.50b
Fertilization		
Control	57.85±8.51c	79.51±9.29c
20K	78.32±9.38bc	118.51±7.95b
40K	77.83±8.10bc	117.05±9.05b
26P	89.53±14.24ab	146.70±16.36ab
52P	108.10±22.05a	158.23±15.04a
20K+26P	88.67±7.45ab	142.68±14.70ab
40K+52P	95.35±14.64ab	140.76±13.77ab
3-way ANOVA (F-statistics)		
CropSystem	0.25ns	0.06ns
Rhizobia	71.78***	46.11***
Fertilizer	2.54***	6.05***
CropSystem*Rhizobia	0.63ns	1.32ns
CropSystem*Fertilizer	1.08ns	0.90ns
Rhizobia*Fertilizer	2.98**	1.46ns
CropSystem*Rhizobia*Fertilizer	1.23ns	1.16ns

CropSystem: Cropping Systems; SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; Values presented are means ± SE; **, ***: significant at $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

5.3.5. Interactive effects of *Rhizobium* inoculation and P and K fertilization on nitrogen fixation in 2015 cropping season

The results of this study also showed a significant interactions between *Rhizobium* inoculation and P and K fertilizers for the first (2015) cropping season on nitrogen fixation. The combination of fertilizers and *Rhizobium* showed a good performance in nitrogen fixation where the best combination was observed in plots which received 52 kg of phosphorus and 20 K + 26 P (kg ha^{-1}). Inoculation alone without fertilizers resulted in lower nitrogen fixation compared with the *Rhizobium* plus fertilizers (Fig. 6). Compared with *Rhizobium* inoculated, un-inoculated plots recorded significantly lower amount of fixed nitrogen with fertilizer application. Under un-inoculated plots, the combined lower rates of fertilizers (20 K + 26 P (kg/ha)) improved the nitrogen fixation over all other treatments.

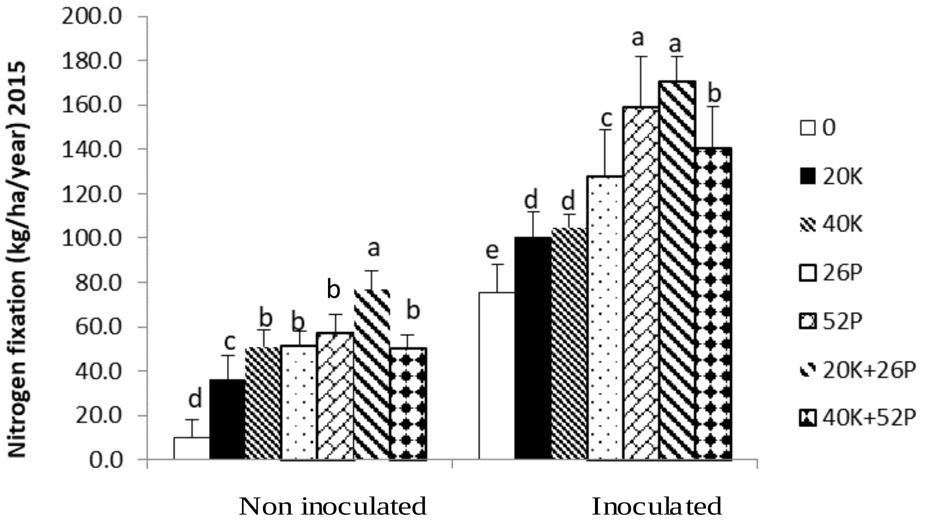

Figure 6: Interactive effects of rhizobia inoculation and P and K fertilization on nitrogen fixation in 2015

5.4. Discussion

The current study showed that cropping systems, *Rhizobium* inoculation and fertilizers improved the root length, number of nodules and nitrogen fixation. In this study, cropping systems and fertilizers did not show significant effects on root length for the two cropping season. However, roots length was significantly improved with *Rhizobium* inoculation. The improved root length in *Rhizobium* inoculated plots could have been caused by nitrogen fixation, which eventually resulted into available nitrogen for plant growth. Furthermore, the increased root length in the *Rhizobium* inoculated plots could have been attributed by PGPR which functions through production of plant hormones such as auxins, and cytokinins (Keating *et al*., 1998; Hardarson, 1993; Hayat *et al*., 2010).

The number of nodules was significantly increased with Cropping systems, *Rhizobium* inoculation and fertilization with P and K. The intercropped plots produced more nodules compared with sole grown soybean. The increased number of nodules in intercropped soybean could have been attributed by and enhancement with flavonoids found in root exudates of both soybean and maize. The similar findings were obtained by Liu *et al*. (2017) who conducted the study on “Intercropping influences component and content change of flavonoids in root exudates and nodulation of Faba bean”. In their study, they found the increased number of nodules and nodules dry weight in Faba bean intercropped with wheat compared with those found in monocropping and attributed it to the enhancement with flavonol, isoflavone, chalcone and hesperetin from their root exudates. The increased number of nodules in intercropped legumes over the intercropped one is in agreement with the previous related studies (Banik and Sharma, 2009; Cun *et al*., 2014). The concept behind these finding is that root exudates contains flavonoids which are signal molecules acting as nod gene inducers for the nodules forming symbiotic *Rhizobium*, hence increased number of nodules over the pure stand legumes (Liu *et al*., 2017). Apart from intercropping, P and K fertilization also increased number of nodules over the control. This study revealed unfertilized plots (control plots) significantly lowered the number of nodules as compared with any level of P and K whether singly or applied in combination. The combined application was superior in enhancing nodule formation relative to single application. These results showed that P and K are important elements for nodules formation, which finally enhance nitrogen fixation in soybean. Other studies have similarly reported the increase in nodule number under P sufficient treatments relative to P deficient treatments (Gentili and Huss-Danell, 2002; Seresinhe and Pathirana, 2002; Kouas *et al*., 2005; Gentili *et al*., 2006; Chmelkov and Hejcman, 2014). Potassium was reported to contribute to good root growth and has been shown to improve the number and size of nodules on roots (Becker *et al*., 1991; Sangakkara *et al*., 1996; IPNI, 1998; Hayat *et al*., 2010; Chmelkov and Hejcman, 2014). In another study on the influence of potassium supply on nodulation, nitrogenase activity and nitrogen accumulation of soybean (*Glycine max* L. Merrill) grown in nutrient solution it was found that nodule parameters (nodule number and fresh weight of nodule per plant, average weight of nodule) increased with increasing K-supply (Premaratne and Oertli, 1994).

Following the effects of nodulation in soybean, the study also determined the effects of cropping systems, *Rhizobium* inoculation and P and K fertilization on nitrogen fixation through N difference method. Except for the cropping systems, *Rhizobium* inoculation and P and K fertilization increased nitrogen fixation for the two cropping seasons. Although the nodulation was increased in intercropped soybean this increase was not reflected in nitrogen fixation. However, there was a numerical increase of nitrogen in intercropped soybean compared with sole soybean which correlates with the number of nodules in intercropping system. The increased nitrogen fixation in *Rhizobium* inoculated plots is an indication of effective legume-microbes symbiosis in which legumes confer sources of carbon to the bacteria and in turn the bacteria fix atmospheric nitrogen for the host plant. Similar to our findings, several researchers have reported an increase in nitrogen fixation following *Rhizobium* inoculation in legumes (Ledgard and Steele, 1992; Peoples *et al*., 1995; Ndakidemi, 2006; Salvagiotti *et al*., 2008). As previously reported in this study, the increase in number of nodules in P and K fertilized plots, was positively reflected in nitrogen fixation. Phosphorus and potassium significantly enhanced biological nitrogen fixation in this study relative to un-fertilized treatments. A number of studies (Israel, 1987; Tang *et al*., 2001; Ndakidemi *et al*., 2006) have reported the increased nitrogen fixation in different legumes following P fertilization and that P deficient reduced nitrogen fixation. Potassium also has been similarly reported to increase nitrogen fixation in different legumes (Becker *et al*., 1991; Sangakkara *et al*., 1996). These results suggest the importance of these mineral elements in enhancing nitrogen fixation, eventually growth and development of crops.

5.5. Conclusion

This study revealed that cropping systems, *Rhizobium* inoculation and P and K fertilizers have differently affected the root length, number of nodules and/or nitrogen fixation in soybean. In this study, intercropping significantly increased the number of nodules relative to sole soybean. The inoculation of soybean with *Bradyrhizobium japonicum* significantly increased the soybean root length, number of nodules per plant and nitrogen fixation over un-inoculated soybean. P and K fertilization significantly increased the number of nodules per plant and nitrogen fixation over the control. The best combination of fertilizers which increased the number of nodules in this study was 20 K + 26 P (kg/ha). For the nitrogen fixation, supplying 52 kg of P resulted in higher values compared with other treatments. The amount of nitrogen fixed in plots supplied with 52 kg of P was statistically similar to 26P, 20K+26P and 40K+52P (kg ha^{-1}). There was also a significant interaction of *Rhizobium* inoculation and fertilizers on number of nodules and nitrogen fixation in 2015 cropping seasons. Since all elements are important in nodulation and nitrogen fixation, we can conclude by recommending the use of combined fertilizers at lower rates (20K+26P) in areas with similar characteristics. Doubling of the combined fertilizers may not significantly increase nodulation and nitrogen fixation but rather a cost burden to a farmer.

CHAPTER SIX

EFFECTS OF RHIZOBIA INOCULATION, PHOSPHORUS AND POTASSIUM ON CHLOROPHYLL CONCENTRATION OF SOYBEAN GROWN UNDER MAIZE INTERCROPPING SYSTEM[5]

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Abstract

The study was conducted to assess the effects of Rhizobia inoculation, supplemented with phosphorus (P) and potassium (K) under intercropping system on soybean chlorophyll content. The design of the experiment was split-split plot with three factors factorial and replicated thrice. The experiment was carried for consecutive years 2015 and 2016 at the Tanzania Coffee Research Institute farm in Northern Tanzania. There were two inoculation treatments, four intercropping systems and seven fertilizer levels (kg ha^{-1}): Control, 20, 40 K, 26, 52 P, 26 P + 20 K and 52 P + 40 K. Chlorophyll concentrations were extracted using dimethyl sulphoxide (DMSO). Spectrophotometer was used reed the absorbance values at 645 nm (Chlorophyll b) and 663 nm (Chlorophyll a). The results showed that *Rhizobium* inoculation significantly (p=.05) increased total soybean leaf chlorophyll content from 4.25±0.30 to 5.32±0.34 and 7.20±0.27 to 7.88±0.29 in 2015 and 2016 cropping seasons respectively. P and K fertilization also significantly (p=.05) increased soybean total leaf chlorophyll content from 1.69±0.23 to 7.17±0.51 and 4.62±0.33 to 9.87±0.48 in 2015 and 2016 cropping seasons respectively. The combined fertilizers had higher mean values of chlorophyll concentration over all other treatments in both 2015 and 2016 cropping seasons. Therefore, for improved chlorophyll concentration, P and K should be applied in combination at low rate of 20 K + 26 P (kg ha^{-1}). Doubling of these fertilizers may be costly and will not significantly change the leaf chlorophyll content

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Keywords: Intercropping systems, BNF, plant pigments, photosynthesis, soybean, plant nutrition.

6.1. Introduction

Photosynthesis is a process by which green plants and other photosynthetic organisms use the energy from sunlight and convert it to produce useful chemical energy in presence of water, carbon dioxide and chlorophyll (Roy *et al.*, 2006). Chlorophyll may be referred to as a green pigments found in photosynthetic organisms such as plants, algae, and photosynthetic bacteria. For the purpose of this article, we will be referring to Soybean chlorophyll. The molecule plays the central function in photosynthesis (Arnon, 1971). Therefore, decreased chlorophyll concentration may inhibit photosynthesis (Abd El-Mageed *et al.*, 2016), and hence reduce production of food in crops.

The general equation for the photosynthesis process is shown bellow

$$6CO_2 + 6H_2O \rightarrow \frac{\text{Chlorophyll}}{\text{Sun light}} \rightarrow C_6H_{12}O_6 + 6O_2$$

Where:

CO_2 = carbon dioxide } Input
H_2O = water

$C_6H_{12}O_6$ = glucose } Output
O_2 = oxygen

Since the chlorophyll is necessary for the photosynthesis process (Marchesini *et al.*, 2016), which is vital for the life of nearly all organisms, it is important to enhance it in the cropping systems to allow production of enough food to feed the sky-rocketing human population. One way of enhancing chlorophyll concentration in the cropping systems is to improve nutrition and adequate exposure of plants to sunlight.

Several researches have been done to assess photosynthetic activities of plant and their responses under different factors. For example, studies have shown that plant beneficial microorganisms (Rhizobia) have enhanced photosynthesis because they improve plant nutrition hence increased leaf area that reflects photosynthesis (Kaschuk *et al*., 2009), In another study done by Nyoki and Ndakidemi (2014), it was reported that total leaf chlorophyll content of cowpea was significantly increased following inoculation of *Bradyrhizobium japonicum*. The same results were found in another study by Bambara and Ndakidemi (2009), which showed that *P. vulgaris* L. inoculated with Rhizobia had increased leaf chlorophyll content compared with that of control plants. However, much of these studies have focused on inoculation of legumes grown as monocrop. There is little information on chlorophyll content of inoculated legumes grown under intercropping systems. Therefore, there was a need to conduct a study assessing the chlorophyll content of inoculated soybean and un-inoculated soybean grown under maize intercropping systems

A supply of different mineral elements is another factor which is reported to enhance chlorophyll concentration and photosynthesis in general. Potassium and phosphorus are particularly important in plant chlorophyll concentration and photosynthesis. Hossain *et al*. (2010) and Longstreth and Nobel (1980) pointed out that the limited supply of these elements impaired plant growth in terms of cell division and expansion, and photosynthesis. Wu *et al*. (2006) reported an increase in chlorophyll content following application of phosphorus on the seedlings of *Larix olgensis*. Furthermore, Onanuga *et al*. (2011) observed that the plants treated with relatively high levels of P and K improved chlorophyll a, b and a/b production in cotton leaves. Study by Zhao *et al*. (2001) showed that K deficient was associated with low chlorophyll content in cotton leaves. In another study, Lamrani *et al*. (1996), who investigated the influence of nitrogen, phosphorus, and potassium on pigment concentration in cucumber leaves, observed that K nutrition promoted formation of both chlorophyll a and b.

Another factor that may affect chlorophyll content and photosynthesis is by growing crops of different height in the mixture (intercropping). This practice has been reported to improve yield over sole crop by many researchers (Giller and Wilson, 1992; Li *et al*., 1999; Zhang and Li, 2003; Khogali *et al*., 2011; Lemlem, 2013). Though, this may result in the suppression of one crop in the mixture by preventing the sunlight from reaching the crop. It was previously reported that Mungbean suffered a shading stress when it was intercropped with sorghum at different growth stages (Islam *et al*., 1993). The grain filling stage is very much light sensitive. Therefore, if one has to improve and maximize yield, grain filling stage needs to be given special attention in intercropping systems (Islam *et al*., 1993).

It is evident from different literature cited that *Rhizobium* inoculation and mineral elements supplemtation increases the chlorophyll content of leaves, and hence improves plant biomass production. However, these treatments need to be studied under cereal-legume intercropping systems to assess their effects on leaf chlorophyll content of legumes. Therefore, the objective of this study was to assess the effects of Rhizobia inoculation, supplemented with phosphorus (P) and potassium (K) under intercropping system on chlorophyll synthesis in soybean.

6.2. Materials and methods

6.2.1. Experimental design and treatments

The experiment was carried out at Tanzania Coffee Research Institute (TaCRI) for two consecutive cropping seasons (April – September 2015 and April – September 2016). The experiment was laid out in Split-split plot with three factors factorial and replicated thrice. The plot size was 3 x 3 m, with main plot comprised two inoculation treatments: i). With rhizobia inoculation and ii). Without rhizobia inoculation. The subplots was assigned with cropping systems as follows: maize (sole crop) at a spacing of 75 x 60 cm; soybean (sole crop) at a spacing of 75 x 20 cm; maize/soybean (intercropping system) at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; and the last cropping system was Maize/ soybean (intercropping system) at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively. The following fertilizer levels (kg ha^{-1}) were assigned to the sub-subplots. (i) Control (Without fertilizer). (ii) 20 K. (iii) 40 K. (iv) 26 P. (v) 52 P. (vi) 26 P + 20 K. (vii) 52 P + 40 K.

6.2.2. Chlorophyll extraction and determination

Chlorophyll concentrations were extracted using dimethyl sulphoxide (DMSO) as it was previously described in Hiscox and Israelstam (1979). In this method, a third leaf from the top of the plant for each treatment was collected for chlorophyll extraction. From the sampled leaves, a hundred (100) mg of the middle portion of fresh leaf slices was placed in a 15 mL vial containing 7 mL DMSO and incubated at 4°C for 72 h. After the incubation, the extract was diluted to 10 mL with DMSO. This technique helps to extract chlorophyll from shoot tissue without grinding or maceration Hiscox and Israelstam (1979). From the chlorophyll extract, 3 mL sample was transferred into curvets for absorbance determination. A spectrophotometer (UV/Visible Spectrophotometer, Pharmacia LKB Ultrospec II E) was used to determine absorbance values at 645 (Chlorophyll b) and 663 nm (Chlorophyll a), which were then be used in the equation proposed by Arnon (1949) to determine total leaf chlorophyll contents against DMSO blank, expressed as mg L^{-1} as follows:

$$\text{Chlorophyll total (Chlt} = 20.2D_{645} + 8.02D_{663})$$

Where "D" is the density at the respective wavelengths which was obtained from spectrophotometer

Visual assessment of plant color was done in a scale of 1 – 5. This assessment was based on previous studies by Xu *et al*. (2000), Maher *et al*. (2003) and Ndakidemi and Makoi (2009). In this study, the scale of 1 was assigned to plots which were observed to be more dark green and 5 was assigned to plots with yellowish color. This scale enabled the researcher to quantify the color intensity of plants in different treatments.

6.2.3. Statistical analysis

The statistical analysis was performed using the 3-way analysis of variance (ANOVA) in factorial arrangement. The computation was performed with the software program STATISTICA. The Fisher's least significance difference (L.S.D.) was used to compare treatment means at $p = 0.05$ level of significance (Steel and Torrie, 1980)

6.3. Results

6.3.1. Soil results

The results of selected chemical properties of the soil from the study area before the start of experiment are presented in Table 8.

Table 8: The selected chemical properties of soil

pH 1:2.5		TOTAL N	AVAIL. P, Bra-I	K
H_2O	KCl	%	mg/kg	meq/100g
6.43	6.14	0.183	5.21	0.93

6.3.2. Effect of cropping systems on chlorophyll content of soybean

The results presented in Table 9, indicated that for the year 2015, the cropping systems had no significant effect on the chlorophyll a, b and total of the soybean leaves. The chlorophyll concentrations were almost the same in sole soybean and maize-soybean intercropped at different spacing. In the second season (2016 cropping season), cropping systems did not show significant differences in chlorophyll a, b and total concentration. However, soybean intercropped with maize at a spacing of 75 x 20 cm, and 75 x 60 cm soybean and maize respectively numerically had lower chlorophyll a, b and total concentration when compared with the soybean in monocrop (Table 10).

6.3.3. Effects of *Rhizobium* inoculation (*Bradyrhizobium japonicum*) on chlorophyll content in soybean

In both cropping seasons, i.e. 2015 and 2016, Rhizobia (*Bradyrhizobium japonicum*) inoculation had a positive effect and significantly (P = .05) increased the chlorophyll a, b and total concentration over the control (Table 9 and 10). In 2015 cropping season (Table 9), the concentration of chlorophyll a, b and total were increased by 27, 23 and 25% respectively in the inoculated plots over the control (un-inoculated plots). In 2016 cropping season (Table 10), Rhizobia inoculation significantly improved chlorophyll a, b and total relative to un-inoculated plots. Inoculation significantly increased chlorophyll a, b and total by 8.40, 10.70 and 9.35% respectively.

6.3.4. Effects P and K fertilization on chlorophyll content in soybean

Different levels of K and P significantly affected soybean leaf chlorophyll concentrations. In both cropping seasons (2015 and 2016), the higher rate of potassium fertilizer (40 kg ha^{-1}) significantly increased chlorophyll a, b, and total compared with the lower rate (20 kg ha^{-1})

and the control. Furthermore, when compared with the control, the lower rate of potassium (20 kg ha^{-1}) significantly increased the concentration of chlorophyll a, b and total (Table 9 and 10). Following potassium fertilization, the concentration of chlorophyll in both 2015 and 2016 cropping seasons followed a trend of control <20 <40 (kg ha^{-1}). The data presented in Table 9 (2015 cropping season), showed that the higher rate of potassium (40 kg ha^{-1}) increased chlorophyll a, b, and total by 137, 133 and 135% respectively over the control. Likewise, in Table 10 (2016 cropping season), the higher rate of potassium (40 kg ha^{-1}) significantly increased chlorophyll a, b, and total by 58, 41 and 50% respectively relative to the control.

Referring to the Table 9 and 10, phosphorus fertilization significantly increased chlorophyll a, b and total. The concentration levels of chlorophyll in phosphorus fertilized plots followed the same trend (control <26 <52 (kg ha^{-1}) as those in potassium treated plots. For both cropping seasons, doubled treatment of phosphorus (52 kg ha^{-1}) significantly increased chlorophyll a, b and total chlorophyll over the lower rate (26 kg ha^{-1}) and the control. In 2015 cropping season, application of phosphorus at the level of 52 kg ha^{-1} significantly increased chlorophyll a, b and total by 18, 19 and 18% respectively over 26 kg P ha^{-1} treated plots and by 251, 243 and 245% respectively over the control. For the 2016 season, application of phosphorus at the level of 52 kg ha^{-1} significantly increased chlorophyll a, b and total by 10, 1 and 6 % respectively over the 26 kg ha^{-1} treated plots and by 83, 67 and 76% respectively over the control.

The application of the combined P and K fertilizers significantly increased chlorophyll a b and total over all the treatments in the two (2015 and 2016) cropping seasons. However, the doubling of combined P and K did not show any significant difference between the lower rate (20 K + 26 P (kg ha^{-1}) and the doubled rate (40 K + 52 P (kg ha^{-1}) (Table 9 and 10).

6.3.5. Interactive effects of rhizobia, cropping systems and fertilizer levels

The results from this study did not show any significant interactions of the main plots and subplots

Table 9: Effect of cropping systems, Rhizobia inoculation, P and K fertilization on concentration of soy bean leaf chlorophyll a, b and total in 2015 cropping season

Treatments	Chl a 2015	Chl b 2015	Chl T 2015
Cropping System			
SB	2.08±0.16a	2.11±0.19a	4.19±0.35a
M+B (A)	2.24±0.22a	2.71±0.25a	4.95±0.47a
M+B (B)	2.31±0.18a	2.54±0.20a	4.85±0.37a
Rhizobia			
With out	1.95±0.14b	2.31±0.16b	4.25±0.30b
With	2.47±0.17a	2.85±0.18a	5.32±0.34a
Fertilizer levels (kg ha^{-1})			
Control	0.76±0.12e	0.93±0.13e	1.69±0.23f
20 K	1.60±0.26d	1.66±0.16d	3.26±0.38e
40 K	1.80±0.15cd	2.17±0.17cd	3.97±0.32de
26 P	2.26±0.17bc	2.69±0.21bc	4.95±0.39cd
52 P	2.67±0.22ab	3.19±0.28ab	5.86±0.49bc
20 K + 26 P	3.31±0.26a	3.86±0.26a	7.17±0.51a
40 K + 52 P	3.06±0.29a	3.53±0.35a	6.59±0.63ab
3-Way ANOVA F-statistics			
CroSyt	0.59ns	0.52ns	0.43ns
Rhiz	9.17**	8.47**	9.29**
Fert	15.22***	18.40***	17.71***
CroSyt*Rhiz	0.71 ns	1.40 ns	1.02 ns
CroSyt*Fert	0.55 ns	0.39 ns	0.45 ns
Rhiz*Fert	0.53 ns	0.57 ns	0.56 ns
CroSyt*Rhiz*Fert	0.30 ns	0.15 ns	0.18 ns

CroSyt: Cropping Systems; Fert: Fertilizers; Rhiz: Rhizobium; Chl: Chlorophyll; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; Values presented are means ± SE; **, ***: significant at $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p = 0.05 according to Fischer least significance difference (LSD).

Table 10: Effect of cropping systems, Rhizobia inoculation, P and K fertilization on concentration of soy bean leaf chlorophyll a, b and total 2016 cropping season

Treatments	**Chl a 2016**	**Chl b 2016**	**Chl T 2016**
Cropping System			
SB	4.27±0.18a	3.64±0.16a	7.91±0.33a
M+B (A)	4.05±0.17a	3.36±0.17a	7.41±0.33a
M+B (B)	3.97±0.20a	3.32±0.19a	7.30±0.38a
Rhizobia			
With out	3.93±0.14b	3.27±0.13b	7.20±0.27b
With	4.26±0.16a	3.62±0.15a	7.88±0.29a
Fertilizer levels (kg ha^{-1})			
Control	2.46±0.18e	2.17±0.17e	4.62±0.33e
20 K	3.28±0.14d	2.71±0.12d	5.99±0.24d
40 K	3.88±0.14c	3.06±0.16cd	6.94±0.28cd
26 P	4.11±0.14bc	3.59±0.14bc	7.69±0.25bc
52 P	4.51±0.17b	3.63±0.15b	8.14±0.29b
20 K + 26 P	5.13±0.22a	4.40±0.23a	9.53±0.44a
40 K + 52 P	5.33±0.22a	4.54±0.28a	9.87±0.48a
3-Way ANOVA F-statistics			
CroSyt	1.75ns	2.00ns	2.09ns
Rhiz	5.79*	6.14*	6.67*
Fert	31.39***	21.08***	28.78***
CroSyt*Rhiz	0.87 ns	0.99 ns	0.88 ns
CroSyt*Fert	0.54 ns	0.82 ns	0.65 ns
Rhiz*Fert	1.04 ns	0.24 ns	0.60 ns
CroSyt*Rhiz*Fert	0.49 ns	0.77 ns	0.60 ns

CroSyt: Cropping Systems; Fert: Fertilizers; Rhiz: Rhizobium; Chl: Chlorophyll; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; 3Values presented are means ± SE; *, ***: significant at p≤ 0.5, p ≤ 0.001 respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p = 0.05 according to Fischer least significance difference (LSD).

6.3.6. Visual assessment of crop pigmentation

Dark green colour is an indication of healthy plants. Dark green colour is also an indication that active growth and active photosynthesis is taking place. Visual assessment of plant greenness showed that for the two cropping seasons (2015 and 2016) Rhizobia inoculation and fertilizer (P and K) application significantly increased plant greenness over the control. The cropping systems did not show any significant difference in plant greenness for the 2015 season. However, cropping systems significantly affected the crop greenness in the second season (2016) whereby soybean planted as monocrop were greener compared with those in intercropped plots (Table 11). It is clearly seen in the Image (1D) that soybean intercropped with maize without Rhizobia inoculation suffered both effects of shading and nitrogen deficiency compared with monocropped soybean which suffered only nitrogen deficiency (Image 1A). Rhizobia inoculated soybean under intercropping system did not suffer shading effect from its companion crop (Image 1C). The greenness of rhizobial inoculated soybean under monocropping was not different from that of Rhizobia inoculated soybean under intercropping systems (Image 1 C and B).

Table 11: Visual assessment of plant greenness scored in a scale of 1 – 5

Treatments	Greenness	
Cropping System	2015 Cropping season	2016 Cropping season
SB	2.56±0.22a	2.00±0.19b
M+B (A)	2.48±0.25a	2.54±0.19a
M+B (B)	2.79±0.25a	2.39±0.18a
Rhizobia		
With out	3.92±0.11a	3.13±0.14a
With	1.29±0.09b	1.35±0.06b
Fertilizer levels (kg ha^{-1})		
Control	3.33±0.35a	3.06±0.26a
20 K	2.53±0.39ab	2.50±0.35b
40 K	2.56±0.36ab	2.44±0.30b
26 P	2.56±0.37ab	1.83±0.23c
52 P	2.61±0.37ab	1.72±0.23c
20 K + 26 P	2.03±0.31b	2.11±0.27bc
40 K + 52 P	2.04±0.42b	2.03±0.31bc
3-Way ANOVA F-statistics		
CroSyt	1.86 ns	4.12*
Rhiz	375.21***	176.39***
Fert	1.37 *	6.69***
CroSyt*Rhiz	0.25 ns	0.59ns
CroSyt*Fert	1.12 ns	0.77ns
Rhiz*Fert	2.38ns	0.85ns
CroSyt*Rhiz*Fert	1.08 ns	1.05ns

CroSyt: Cropping Systems; Fert: Fertilizers; Rhiz: Rhizobium; Chl: Chlorophyll; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; Values presented are means ± SE; *, ***: significant at $p \leq 0.5$, $p \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p = 0.05 according to Fischer least significance difference (LSD).

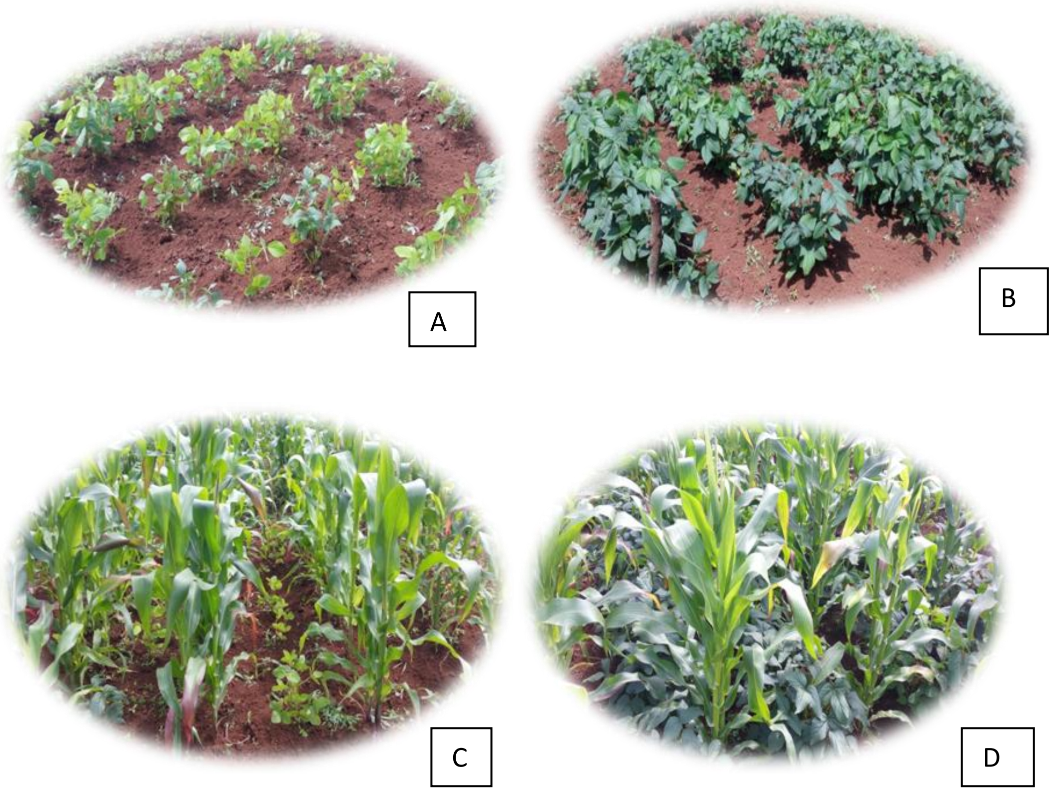

Image 1: A. Soybean monocropped without rhizobia inoculation; B. Soybean monocropped with rhizobia inoculation; C. Soybean maize intercropping without rhizobia inoculation; D. Soybean maize intercropping with rhizobia inoculation

6.4. Discussion

Chlorophyll concentration of the plants is generally affected by the treatments received by the respective plants. The current study examined the effects of cropping systems, Rhizobia inoculation and P and K fertilizers on chlorophyll concentration in soybean leaves. Form this study; it was generally observed that cropping systems had no significant effect on chlorophyll concentration in leaves of soybean.

Rhizobia inoculation in the two cropping seasons significantly affected the concentration of chlorophyll a, b and total when compared with un-inoculated treatments. These findings are in line with the previous report (Sekhon *et al*., 2002; Bambara and Ndakidemi, 2009; Nyoki and Ndakidemi, 2014; Tajini *et al*., 2008; Vollmann *et al*., 2011) which showed that Rhizobia strain significantly increased chlorophyll concentration in the crops. Since Rhizobial inoculation increases chlorophyll contents and bearing in mind that chlorophyll in necessary in the photosynthesis, it is also ideal to say Rhizobia is necessary for the increased leaf photosynthesis (Zhou *et al*., 2006). The relationship between Rhizobia inoculation and chlorophyll content is found in the biological nitrogen fixation, a process by which plants convert atmospheric nitrogen into a usable form by plant. The fixed nitrogen is responsible for the increases greenness of the plant leaves (Cabrera, 2004) and the greenness of plant leaves is an indicator of the improved chlorophyll content of the plant leaves (Bojović and Marković, 2009). Since nitrogen is a structural element of chlorophyll (Tucker, 2004), hence its availability to plants results in increased chlorophyll content (Melton and Dufault, 1991).

P and K fertilization also improved chlorophyll concentration of soybean leaves. From the current study, increasing the level of fertilizers had positive effects on chlorophyll content of soybean leaves. Interestingly, in the two cropping seasons, the lower (26 kg ha^{-1}) and higher (52 kg ha^{-1}) rate of phosphorus fertilizer had higher mean values of chlorophyll relative to the potassium fertilized plots and the unfertilized plots. The related findings were previously reported that phosphorus increased leaf chlorophyll content (Melton and Dufault, 1991; Nyoki and Ndakidemi, 2014). However, contrary to our results in which higher rate of phosphorus increased chlorophyll content, Melton and Dufault (1991) reported that the higher P rate significantly decreased chlorophyll content in their 1^{st} year of experiment. Furthermore, when compared with the control, potassium fertilization increased chlorophyll content of soybean leaves. The findings of the current study agree with the Zhao *et al*. (2001) who reported that potassium deficient was associated with the low chlorophyll content in cotton leaves. Doubling potassium rate from 20 to 40 (kg ha^{-1}) significantly increased the leaf chlorophyll content in the two cropping seasons. The combined fertilizer treatments at their lower rates (20 K + 26 P (kg ha^{-1}) resulted in higher mean values of chlorophyll content compared with the different fertilizer levels when applied singly. However, doubling of combined fertilizers (40 K + 52 P (kg ha^{-1}) did not significantly change the chlorophyll content of the soybean leaves. From this study, we learn that P and K deficiency reduced leaf chlorophyll content of soybean. This observation agrees with Watanabe and Yoshida (1970) who stated that deficiency phosphorus and potassium causes changes in the structure of chloroplasts and may affect the biochemical activity of chloroplast resulting to low leaf chlorophyll content.

6.5. Conclusion

The results from current study indicated the importance of mineral elements in chlorophyll formation in soybean leaves. It can be generalised that N, P and K are equally necessary for the formation of chlorophyll in crops thereby improving final yields. We have tested these elements, P and K from mineral fertilizers and N from BNF and found that both of them significantly increased soybean leaf chlorophyll content. The combined P and K at the lower rate resulted in higher mean values of chlorophyll content. From this observation it is recommended that for improved chlorophyll concentration, P and K should be applied in combination at low rate of 20 kg K ha^{-1}+26 kg P ha^{-1}. Doubling of these fertilizers may be costly and will not significantly change the leaf chlorophyll content.

CHAPTER SEVEN

GROWTH RESPONSE OF *BRADYRHIZOBIUM* INOCULATED SOYBEAN GROWN UNDER MAIZE INTERCROPPING SYSTEMS, AND P AND K FERTILIZATION[6]

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Abstract

The field experiment was carried out for two consecutive years to assess the effects of cropping systems, *Rhizobium* inoculation supplemented with P and K on growth performance of soybean. The experiment was laid out in a split-split plot design with the main plots comprised of Rhizobia inoculation (with and without). The sub plots comprised of three cropping systems and the sub-sub plots having seven fertilizer levels (kg ha^{-1}): Control, 20, 40 K, 26, 52 P, 26 P + 20 K and 52 P + 40 K. The experiment was replicated thrice. The results indicated that both treatments have influenced most of the growth parameters of soybean assessed. Over un-inoculated treatments, Rhizobia inoculation significantly improved the growth of all parameters of soybean in this study. Similarly, P and K fertilization improved the growth of soybean over the control. Most of the parameters performed supper in plots treated with Rhizobia inoculation supplied with 26, 52 P and 20 K + 26 P kg ha^{-1} levels of P and K. The positive interactive effects of cropping systems, *Rhizobium* inoculation and P and K supplementation have been observed on different growth parameters assessed. The positive interaction of these treatments indicated their importance for improving growth of crops in the study area.

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***Keywords*: *Rhizobium*, plant nutrition, plant growth, legumes, biological nitrogen fixation**

7.1. Introduction

In recent years, there has been increased interest in production of Soybean [*Glycine max* (L.) Merr.] and its yield have substantially increased since 1961 (Lobell and Field, 2007). The crop has been gaining global attention due to its health, nutritional and economical importance to human (Mateos-Aparicio *et al*., 2008), animals feeds and soil fertility (Dwivedi *et al*., 2015). To achieve the desired yield performance, the crops must grow vigorously and free from biotic and abiotic stress. Among other many factors yield performance of crops is influenced by plant growth. There are diverse factors that may affect plant growth including cropping patterns and soil nutrition among others. Intercropping is one of the cropping patterns which may affect crop growth.

Mineral elements such as N, P and K play important roles in plant growth (Nyoki and Ndakidemi, 2014a). These macronutrients are required in relatively large amount by plant. Phosphorus is used for various plant functions including energy transfer, photosynthesis, translocation of sugars and starches as well as movement of nutrients within the plant (Brady, 2002; Shahid *et al*., 2009). Potassium plays a number of vital physiological processes such as activation of several enzymes, synthesis and degradation of carbohydrates, production of proteins as well as regulation of stomata pores for gas exchange and photosynthesis (Lissbrant *et al*., 2009). Nitrogen is the key element for plant growth as well as yield performance (Nyoki and Ndakidemi, 2014b). Legumes are good at fixing own nitrogen and contribute to the soil through decomposition of crop litter and release of fixed nitrogen from the root nodules. Vincent *et al*. (1979) previously studied and found that unavailability of specific strain of rhizobia reduced growth of leguminous crops. Several studies on rhizobia inoculation have reported that inoculated legumes improved growth over the control (Yamanaka *et al*., 2005; Bambara and Ndakidemi, 2009; Bambara and Ndakidemi, 2010). It has been reported that major elements (N, P and K) are continuously declining in East Africa. The current study aimed at investigating and exploration of soybean growth potentials under cereal-legume intercropping, rhizobia inoculation and P and K fertilization.

7.2. Material and methods

7.2.1. Experimental design and treatments

The field experiment was carried out at Tanzania Coffee Research Institute (TaCRI) for two consecutive years (2015 and 2016). The experiment was laid out in split-split plot design with 2 x 4 x 7 factorial arrangements and replicated thrice. The plot size was 3 x 3 m, with main plots having two rhizobia inoculation treatments, while the sub plots comprised: Maize (sole crop) at a spacing of 75 x 60 cm; Soybean (sole crop) at a spacing of 75 x 40 cm; Maize/soybean (intercropping system) at a spacing of 75 x 60 cm and 75 x 20 cm, Maize and soybean respectively; and the last cropping system was Maize/soybean (intercropping system) at a spacing of 75 x 60 cm and 75 x 40 cm, Maize and soybean respectively. The sub-subplots were assigned the following fertilizer levels (kg ha^{-1}): control; 20 K; 40 K; 26 P; 52 P; 26 P + 20 K; 52 P + 40 K.

7.2.2. Data collection

The growth and development parameters of soybean which were measured includes: plant heights measured at different stages of the plants growth, number of leaves per plant; Leaf area, stem girth. Plant height was measured using a meter ruler, Stem girth (cm) was measured with digital veneer calliper at physiological maturity, while the leaf area (cm^2) was calculated as the product of the total length and width at the broadest point of the longest leaf on the plant as described in Bhatt and Chanda (2003).

$$LA= 11.98 + 0.06LW. \text{ Where; } L= \text{leaf length and } W= \text{leaf width}$$

7.2.3. Statistical analysis

The collected data was analysed using statistical software called STATISTICA. The statistical analysis was performed using the 3-way analysis of variance (ANOVA) in factorial arrangement. The Fisher's least significance difference (L.S.D.) was used to compare treatment means at $p = 0.05$ level of significance (Steel and Torrie, 1980)

7.3. Results

The current study assessed how different variables are affected by cropping systems, rhizobia inoculation and fertilization with P and K. some of the factors had no significant effect on parameters measured. Therefore, we only report the significant data

7.3.1. Plant height

The results presented in study showed that all the factors tested had the significant effects on plant height (Table 12). Cropping systems significantly affected the height of soybean plants where by in all cases sole soybean (SB) recorded the lowest plant height from 2, 4 and 6 weeks after planting (WAP) for the two cropping seasons. The highest plant height was recorded in the soybean intercropped with maize at narrower spacing of M+B(A) regardless of time taken from planting to measurement. However, the intercropped soybean statistically had plants of the same height measured at 6WAP which were significantly higher compared with sole bean (SB) (Table 12). Furthermore, the current study showed that rhizobia inoculated soybean significantly increased plant height over the un-inoculated plots measured at 2, 4 and 6 WAP for the two cropping seasons (Table 12). Fertilization of crops with P and K significantly increased the height of soybean measured at 2, 4 and 6 WAP over the control for the two cropping seasons (Table 12). In the first cropping season (2015), the two levels of P (26 and 52 kg ha^{-1}) significantly produced taller plants over all other treatments and the control. In the second cropping season, 52 P; 20 K + 26 P and 40 K + 52 P kg ha^{-1} significantly produced taller plants compared with the other treatments. In all measurements, the control plots gave the sorter plants relative to other fertilizer applied plots for the two cropping seasons (Table 12).

Table 12: Effects of cropping systems, rhizobia inoculation, and P and K fertilization on plant height.

	Plant Height (cm) 2WAP		Plant Height (cm) 4WAP		Plant Height (cm) 6WAP	
Treatments	2015	2016	2015	2016	2015	2016
Cropping ystem						
SB	13.44±0.31a	15.09±0.22b	20.94±0.51b	24.14±0.39c	26.08±0.57b	31.42±0.67b
M+B(A)	13.99±0.34a	15.77±0.31a	22.34±0.55a	27.71±0.59a	28.12±0.66a	36.37±0.90a
M+B(B)	13.88±0.32a	14.90±0.31b	21.72±0.55ab	25.34±0.48b	27.49±0.79a	34.72±0.92a
Rhizobia						
With	15.02±0.21a	16.25±0.21a	23.92±0.38a	27.11±0.46a	30.58±0.43a	36.38±0.59a
With out	12.52±0.21b	14.25±0.19b	19.41±0.28b	24.35±0.35b	23.88±0.30b	31.96±0.75b
Fertilizers (kg ha^{-1})						
0	11.59±0.52c	13.41±0.30c	17.59±0.52d	22.52±0.42c	25.87±0.79b	33.89±1.51
20K	13.57±0.40b	14.50±0.26b	20.93±0.56c	23.98±0.43b	26.07±0.77b	31.93±1.41
40K	13.76±0.44ab	14.85±0.32b	21.13±0.60bc	25.31±0.73b	28.61±1.11a	33.15±1.50
26P	14.41±0.55a	15.19±0.37b	23.28±0.88a	25.11±0.58b	28.89±1.26a	33.69±1.35
52P	14.44±0.50a	16.26±0.41a	23.22±0.78a	27.48±0.91a	28.93±1.18a	35.94±1.22
20K+26P	12.54±0.42ab	16.44±0.48a	23.21±0.75a	27.65±0.84a	25.88±0.71b	34.37±1.17
40K+52P	13.07±0.48ab	16.13±0.43a	22.30±0.79ab	28.06±0.86a	26.35±1.18b	36.24±1.26
3-Way ANOVA F-statistics						
CroSyt	1.389	6.43**	6.31**	29.19***	10.61***	12.589***
Rhiz	75.482***	92.84***	193.88***	50.40***	327.64***	28.983***
Fert	4.110***	16.22***	22.96***	16.54***	9.25***	1.962
CroSyt*Rhiz	0.843ns	6.97**	3.02ns	5.36**	7.23***	0.887
CroSyt*Fert	0.482ns	0.41ns	0.37ns	0.61ns	0.86ns	1.557
Rhiz*Fert	0.692ns	0.71ns	2.14ns	0.47ns	4.93***	1.597
CroSyt*Rhiz*Fert	0.909ns	0.65ns	1.05ns	1.08ns	2.67**	1.334

CroSyt: Cropping Systems; Fert: Fertilizers; Rhiz: Rhizobium; SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; Values presented are means ± SE; *,**, ***: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

7.3.2. Interactive effects of rhizobia and cropping systems on plant height measured 2 and 4 weeks after planting in 2016 cropping season

The current study showed a significant interaction between Rhizobia and cropping systems on plant height measured measured 2 and 4 weeks after planting in 2016 cropping season. Rhizobia inoculation influenced the plant height across the cropping systems over un-inoculated plots (Fig. 7[A and B]). Intercropped soybean with rhizobia inoculation significantly gave taller plants over sole soybean and un-inoculated soybean (Fig. 7[A and B]).

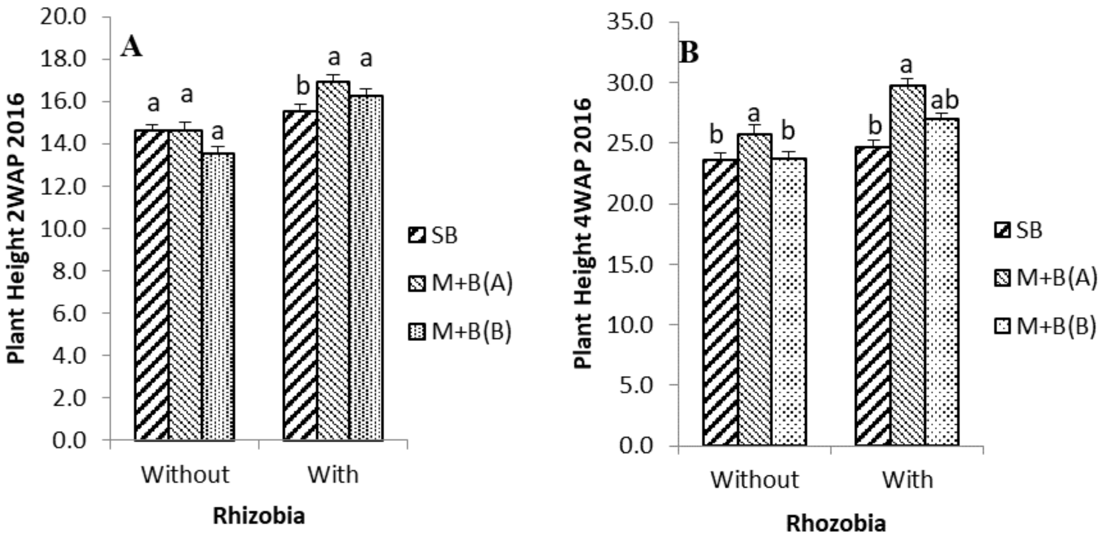

Figure 7: Interaction between rhizobia and cropping systems on plant height measured 2(A) and 4(B) weeks after planting in 2016 cropping season

SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively. WAP = Weeks after planting. Bars followed by similar letter(s) are not significantly different from each other

7.3.3. Interactive effects of rhizobia and cropping systems (A) and interactive effects of rhizobia and fertilizers (B) on plant height measured 6 weeks after planting in 2015 cropping season

Rhizobia inoculation influenced the plant height across the cropping systems over un-inoculated plots (Fig. 8[A and B]). Intercropped soybean with rhizobia inoculation significantly gave taller plants over sole soybean and un-inoculated soybean (Fig. 8A). In Fig. 8B, inoculation of soybean with rhizobia produced taller plants over un-inoculated plants. Regardless of the applied fertilizers, un-inoculated treatments didn't have significant effect on plant height. However, fertilizer application had significant effects on plant height in rhizobia inoculated soybean (Fig. 8B).

7.3.4. Interactions between Cropping systems, Rhizobia and fertilizers on plant height measured at 6 weeks after planting in 2015 cropping season

There were significant interactions between cropping systems, Rhizobia inoculation and fertilizers on plant height measured at 6 weeks after planting in 2015 cropping season.

Rhizobia inoculation significantly increased plant height relative to un-inoculated plots across all the cropping systems. Generally, the plots treated with 26, 52 P; 26 P +20 K and 52 P + 40 K (kg ha^{-1}) performed better in terms of soybean plant height. Furthermore, whether inoculated or not inoculated intercropped soybean at narrower spacing (M+B (A)) produced significantly taller plants compared with sole soybean (SB) and intercrop at wider spacing M+B (B) (Fig. 9).

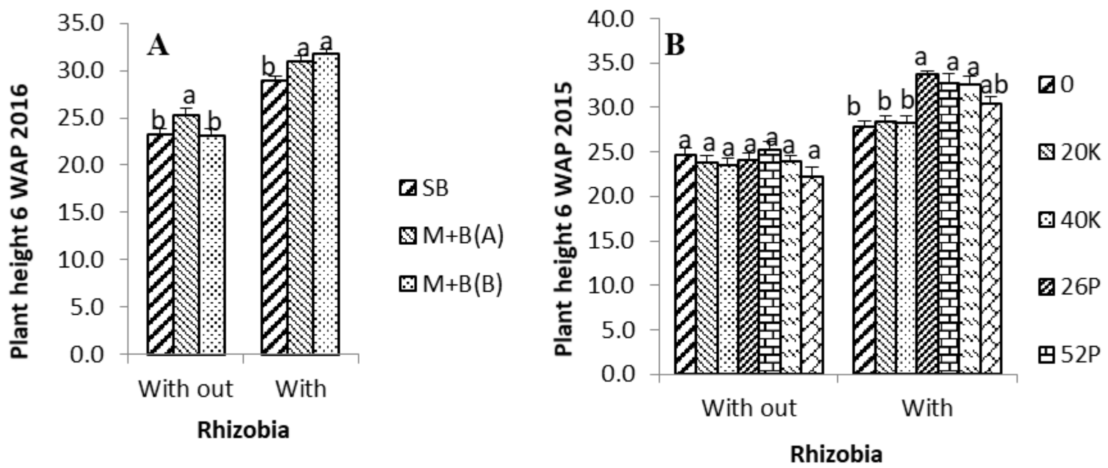

Figure 8 A and B**: Interaction between Rhizobia and cropping systems (A) and interaction between Rhizobia and fertilizers (B) on plant height measured 6 weeks after planting in 2015 cropping season**

SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively. WAP = Weeks after planting. Bars followed by similar letter(s) are not significantly different from each other

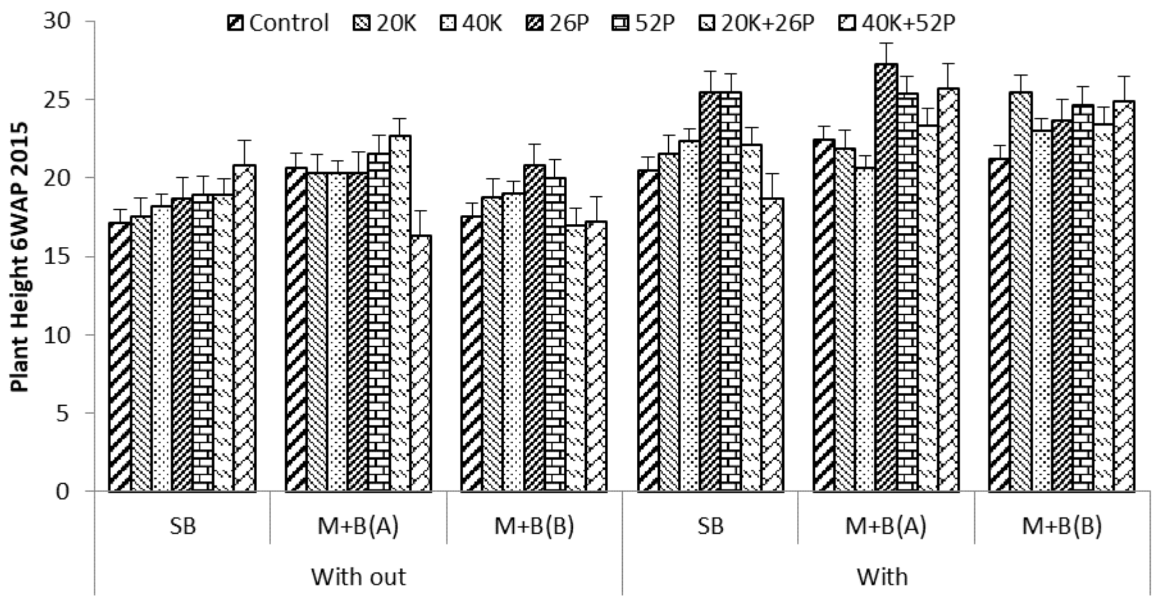

Figure 9: Interactions between cropping systems, rhizobia and fertilizers on plant height measured at 6 weeks after planting in 2015 cropping season

SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively. WAP = Weeks after planting. Bars followed by similar letter(s) are not significantly different from each other.

7.3.5. Stem girth

Soybean grown as mono-crop (SB) had significantly greater stem girth compared with those in intercropped plots for the two cropping season. Regardless of the spacing, intercropped soybean gave statistically the same stem girths which are lower than those in mono crop for the two cropping seasons. Similarly, Rhizobia inoculated soybean had significantly greater stem girth compared with un-inoculated soybean for the two cropping seasons. P and K fertilization also increased stem girth over the control. For the two cropping seasons, the plots that received 52 kg P ha^{-1} resulted in greater stem girth over all treatments (Table 13). The plots treated with 52 kg P ha^{-1} increased the stem girths by 14 and 30 % from the control in 2015 and 2016 cropping season respectively (Table 13). The combined fertilizers whether applied at lower dose or doubled dose resulted in statistically the same stem girth which are the same as those recorded in 52 kg P ha^{-1}.

7.3.6. Pant vigour

From the current study, cropping season had no significant effects on the pant vigour. Rhizobia inoculated plots recorded highly vigorous crops than un-inoculated crops. Fertilization of soybean with P and K significantly increased plant vigour over the control. The pots that were treated with P either at lower or higher dose resulted in significantly vigorous soybean compared with other treatments for the cropping seasons (Table 13).

7.3.7. Number of branches per plant

The results of this study showed that Rhizobia inoculation significantly increased number of soybean leaves per plant over un-inoculated treatments. The rhizobia inoculated soybean produced mean leaf number of 5.02±0.16 and 4.81±0.34 compared with un-inoculated treatments which produced 3.43±0.13 and 4.13±0.09 for the 2015 and 2016 cropping season respectively (Table 13). P and K fertilization also significantly increased the number of soybean leaves over the control for both two cropping seasons. The highest number of leaves was recorded in plots treated with 26 kg of P per hectare for the two cropping seasons.

7.3.8. Interactive effects of cropping systems, rhizobia inoculation and fertilizers on number of branches per plant for 2015 cropping season

For the 2015 cropping season, there were significant interactions between i) cropping systems and fertilizers and ii) Rhizobia inoculation and fertilizers on the number of branches per plant (Fig. 10 and 11). Number of branches was higher in mono cropped soybean compared with intercropped one (Fig. 10). Fertilizer application also significantly increased the number of branches per plant over the control (Fig. 10). Rhizobia inoculation and fertilization with P and K also increased the number of branches per plant over the control (Fig. 11).

Table 13: Effects of cropping systems, rhizobia inoculation and P and K fertilization on stem girth, plant vigour and number of leaves per plant

Treatments	Stem Girth (mm)		Plant Vigour		Number of branches 4WAP	
	2015	2016	2015	2016	2015	2016
Cropping System						
SB	5.34±0.22a	8.33±0.32a	2.74±0.19a	2.33±0.18a	4.25±0.21a	4.48±0.12a
M+B(A)	4.97±0.18b	5.95±0.23b	2.65±0.23a	2.15±0.19a	4.25±0.20a	4.24±0.12a
M+B(B)	4.87±0.21b	5.95±0.23b	2.95±0.23a	2.43±0.19a	4.16±0.24a	4.68±0.50a
Rhizobia						
With	6.14±0.10a	7.29±0.25a	1.65±0.10b	1.37±0.07b	5.02±0.16a	4.81±0.34a
With out	3.99±0.09b	6.19±0.24b	3.91±0.11a	3.25±0.12a	3.43±0.13b	4.13±0.09b
Fertilizers						
0	4.66±0.24b	5.88±0.35c	3.39±0.33a	2.44±0.25ab	4.22±0.29ab	4.07±0.17b
20K	4.77±0.33b	5.93±0.38bc	2.84±0.35abc	2.67±0.34a	4.11±0.32ab	4.24±0.15b
40K	5.32±0.34a	6.78±0.43abc	3.03±0.33ab	2.83±0.28a	3.93±0.31b	4.26±0.15b
26P	5.02±0.32ab	6.82±0.46abc	2.53±0.35bc	1.94±0.26c	4.78±0.28a	5.69±1.14a
52P	5.29±0.32a	7.64±0.47a	2.60±0.32bc	1.94±0.29c	4.67±0.31a	4.22±0.15b
20K+26P	5.26±0.30a	7.17±0.55a	3.19±0.27a	2.17±0.26bc	3.96±0.38b	4.41±0.25b
40K+52P	5.12±0.36ab	6.96±0.59ab	2.89±0.37abc	2.14±0.29bc	3.89±0.38b	4.39±0.16b
3-Way ANOVA (F-statistics)						
CroSyt	5.045**	32.32***	1.678 ns	1.53ns	0.12ns	0.57ns
Rhiz	285.998***	15.63***	271.659*	210.37***	72.58***	4.01*
Fert	2.403*	2.99**	2.533***	4.17***	2.15*	1.48*
CroSyt*Rhiz	2.180 ns	0.28ns	0.892 ns	0.71ns	0.47ns	1.40ns
CroSyt*Fert	0.776 ns	0.54ns	1.159 ns	0.75ns	1.95*	0.66ns
Rhiz*Fert	1.101 ns	0.73ns	2.110 ns	0.32ns	2.39*	1.42ns
CroSyt*Rhiz*Fert	1.860ns	1.19ns	1.080 ns	0.97ns	1.26ns	1.39ns

CroSyt: Cropping Systems; Fert: Fertilizers; Rhiz: Rhizobium; SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; Values presented are means ± SE; *,**, ***: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD). Note: plant vigour was assessed in a scale of 1-5. 1=Good; 5= Bad.

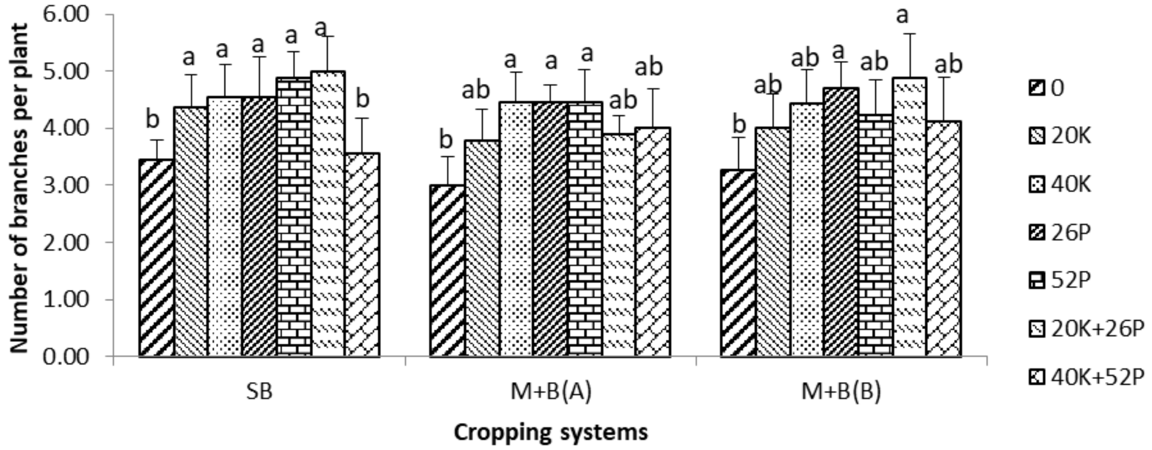

Figure 10: Interactive effects of cropping systems and fertilizers on number of branches per plant for 2015 cropping season

SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively. WAP = Weeks after planting. Bars followed by similar letter(s) are not significantly different from each other

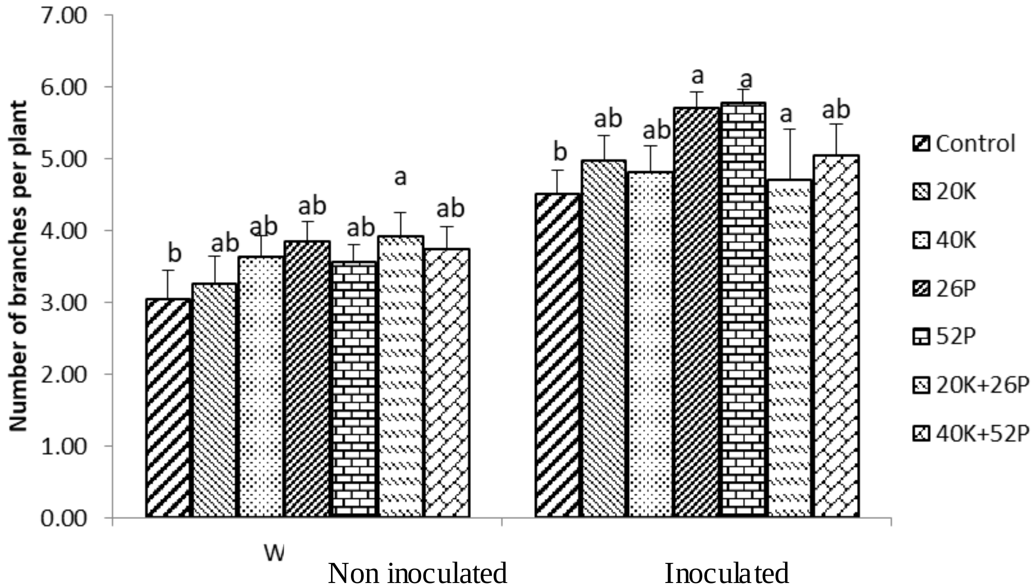

Figure 11: Interactive effects of Rhizobia and fertilizers on number of braches per plant for 2015 cropping season

SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively. WAP = Weeks after planting. Bars followed by similar letter(s) are not significantly different from each other.

7.3.9. Number of leaves per plant

The results presented in Table 14 indicated that for the 2015 cropping season, intercropped soybean produced many leaves measured at 2 weeks after planting (2 WAP) over the sole cropped soybean. The number of leaves counted at 4 weeks after planting (4 WAP) showed that cropping systems had no significant effect for the two cropping seasons (Table 14). Rhizobia inoculation significantly increased the number of leaves compared with un-inoculated soybean for the two cropping seasons (Table 14). Furthermore, fertilization of soybean with P and K significantly increased the number of leaves counted at 2 WAP and 4 WAP over the control for the two cropping seasons (Table 14).

7.3.10. Leaf area

All the treatments applied in this experiment had statistical effect on the leaf area of soybean measured at 50% pod formation. The results presented in Table 14 showed that sole soybean had smaller leaves measured in terms of leaf area compared with the intercropped soybean which gave significantly higher leaf area for the 2015 cropping season. In the second cropping season, the cropping systems did not significantly increase leaf area. However, numerically leaf areas of soybean were higher in intercropped plots compared with sole crops (Table 14). Rhizobia inoculated soybean significantly increased leaf areas relative to un-inoculated soybean for the two cropping seasons. It was also observed that fertilization of soybean with P and K significantly increased the leaf areas over the control. The combined P and K applied at their lower rate significantly gave higher values of leaf areas compared with all other treatments for the two cropping seasons.

7.3.11. Interactive effects of cropping systems and rhizobia on number of leaves counted 2 weeks after planting for 2015 cropping season

Significant interactions between cropping systems and Rhizobia inoculation on number of leaves per plant were observed in this study. Soybean grown alone had more number of leaves compared with intercropped soybean. Similarly, Rhizobia inoculated soybean had more number of leaves compared with un-inoculated soybean (Fig. 12).

Table 14: Effects of cropping systems, rhizobia inoculation and fertilizer levels on number of leaves per plant and leaf area of soybean

	Number of Leaves 2WAP		Number of leaves 4WAP		LA	
Cropping ystem	2015	2016	2015	2016	2015	2016
SB	5.09±0.06b	6.02±0.09a	8.54±0.29a	14.33±0.41a	221.39±11.18b	263.73±11.92a
M+B(A)	5.25±0.05a	6.06±0.15a	8.62±0.27a	14.37±0.48a	253.10±13.47a	283.95±13.86a
M+B(B)	5.28±0.07a	6.18±0.16a	8.87±0.31a	14.26±0.48a	236.90±14.66ab	292.55±12.03a
Rhizobia						
With	5.40±0.05a	6.41±0.12a	9.94±0.21a	16.12±0.33a	304.61±7.41a	307.23±9.95a
With out	5.02±0.046b	5.77±0.09b	7.41±0.12b	12.52±0.25b	169.65±5.78b	252.92±9.65b
Fertilizers						
Control	5.00±0.11c	5.70±0.14b	7.28±0.25c	11.80±0.44d	177.45±16.41d	241.01±23.36c
20K	5.15±0.10bc	5.81±0.11b	8.31±0.35b	12.72±0.46cd	214.35±19.53c	282.69±17.63ab
40K	5.20±0.07abc	5.70±0.17b	8.22±0.38b	13.28±0.48c	227.85±18.78bc	249.14±18.50bc
26P	5.39±0.09a	6.41±0.26a	9.37±0.46a	14.70±0.55b	244.28±19.60ab	293.21±20.81ab
52P	5.26±0.08ab	6.48±0.25a	9.26±0.49a	15.41±0.66ab	257.65±21.04a	294.86±19.19ab
20K+26P	5.31±0.08ab	6.37±0.19a	9.34±0.44a	16.39±0.75a	268.21±20.57a	303.13±15.63a
40K+52P	5.13±0.11bc	6.15±0.23ab	8.96±0.46ab	15.93±0.72a	270.13±18.86a	296.50±17.18ab
3-Way ANOVA F-statistics						
CroSyt	3.98*	0.47ns	0.97ns	0.04ns	6.15**	1.51ns
Rhiz	40.19***	20.60***	153.23***	132.38***	334.21***	15.28***
Fert	2.63*	3.46**	8.28***	17.72***	11.70 ***	1.82*
CroSyt*Rhiz	4.07*	2.37ns	2.55ns	2.43ns	3.09ns	1.50ns
CroSyt*Fert	0.58ns	0.52ns	0.55ns	0.69ns	1.10ns	0.66ns
Rhiz*Fert	0.36ns	1.15ns	1.24ns	0.74ns	1.05ns	0.76ns
CroSyt*Rhiz*Fert	1.36ns	1.13ns	1.26ns	0.89ns	0.74ns	0.84ns

CroSyt: Cropping Systems; Fert: Fertilizers; Rhiz: Rhizobium; SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; Values presented are means ± SE; *,**, ***: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

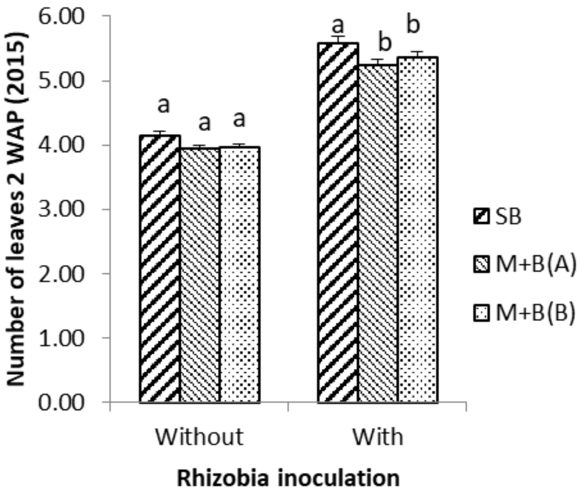

Figure 12: Interactive effects of cropping systems and rhizobia on number of leaves counted 2 weeks after planting for 2015 cropping season.

SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively. WAP = Weeks after planting. Bars followed by similar letter are not significantly different from each other

7.4. Discussion

From this study, analysis of variance showed that all treatments had significant effects on almost all parameters measured. Supplying soybean with *Rhizobium* inoculants significantly increased growth parameters of soybean. Specifically, Rhizobia inoculation significantly increased plant height, stem girth, number of branches per plant, number of leaves per plant and soybean plant vigour over un-inoculated treatments. Similar to this study, other related studies have reported the increase in growth parameters of leguminous plant following Rhizobia inoculation (Raj *et al*., 2003; Pirlak and Kose, 2009; Wu *et al*., 2013; Nyoki and Ndakidemi, 2014a; Fageria *et al*., 2014; Bai *et al*., 2016). The increased growth parameters of soybean might have been attributed by rhizobia inoculation which facilitates plant growth through: addition of nitrogen through biological nitrogen fixation and decomposition of plant residuals (Wagner, 2011); production of plant growth hormone (Yasmeen and Bano, 2014); increases surface area for nutrients uptake; or control diseases by inhibiting colonization of plant roots from phytopathogens (Wall *et al*., 2000; Doornbos *et al*., 2012; Verma *et al*., 2012).

Similarly, cropping systems and fertilization of soybean with P and K significantly increased some growth parameters of soybean. For example, for the two cropping seasons, cropping systems had significant effects on plant height and stem girth. The plant height measured 2 WAP had no significant different from each treatment for first cropping season. This could be due to the fact that both crops were of the same height hence there was no completion for light. Plant heights measured at 4 and 6 WAP significantly differed from each cropping system, where the soybeans were significantly taller in intercropped plots compared with mono cropped soybean. Similar to our findings, Hamd-Alla *et al*. (2014) reported the increased cowpea height in intercropping relative to mono cropped cowpea. Furthermore, Hirpa (2014) reported that there was an increase in height of Haricot bean intercropped with maize compared with sole grown Haricot bean. The increased plant height in intercropping over sole crop may be due to the effect of shading from maize which normally grows taller and faster than soybean (Hamd-Alla *et al*., 2014) and below ground interactions of intercropped plant (Ndakidemi, 2006). Therefore, in the struggle for accessing sunlight, the intercropped soybean becomes taller than those in mono cropped plots. Similarly, other researchers have reported the increased legume plant height in intercropping compared with sole crop (Megawer *et al*., 2010; Ali and Mohammad, 2012;) and reduced number of leaves per plant in intercropping relative to sole crop (Maluleke *et al*., 2005). Furthermore, the findings of this study showed that the stem girths were increased in plots where soybeans were grown as mono crop relative to those under intercropping. The possible explanation for this could be due to reduced completion for growth resources in mono cropped soybean relative to those under intercropping.

P and K fertilization significantly improved growth parameters of such as plant height, stem girth, number of branches per plant, number of leaves per plant and soybean plant vigour over the control treatment. It is well known that plants require nutrients for proper growth. From the current study we found that P and K fertilizers whether applied singly or combined, at lower rates or doubled significantly increased growth parameters of soybean relative to the control. Generally, almost all growth parameters of soybean were greatly improved in plots received 26 P, 52 P and 20 K + 26 P kg ha^{-1} relative to other treatments.

The current study showed significant interactions between main plot treatments and sub plot treatments on some growth parameters of soybean. Interaction of treatments were observed in plant height measured at different growth stages and at different cropping seasons, number of branches per plant for first cropping season, and number of leaves per plant. Regardless of the growth stage and cropping season from which the parameter was measured, existence of significant interactions means both treatments have better contributed to the performance of specific parameter. This argument is supported by Bambara and Ndakidemi (2010) who observed the presence significant interaction between *Rhuzobium*, molybdenum and lime on yield attributes of *P.vulgaris*. In this study, there were significant interactions between 1. Rhizobia and cropping systems on plant height measured 2 and 4 WAP in 2016 cropping season; 2. Rhizobia and cropping systems on plant height measured at 6 WAP in 2015 cropping season; 3. Rhizobia and fertilizers on plant height measured at 6 WAP in 2015 cropping season; 4. Rhizobia, cropping systems and fertilizers on plant height measured at 6 WAP in 2015 cropping season. 5. Cropping systems and fertilizers on number of branches in 2015 cropping season 6. Rhizobia inoculation and fertilizers on number of branches per plant counted in 2015 cropping season and 7. Rhizobia and cropping systems on number of leaves per plant counted 2 WAP in 2015 cropping season. Similar to the current study, Onduru *et al*.

(2008) reported the steady vigorous growth of cowpeas in the treatment, *Rhizobium* and TSP postulating that it could be due to interactive effects of Rhizobium and TSP.

7.5. Conclusion

According to the data of the current study, significant differences have been observed among the treatments of the main plots and the sub plots. The data showed that cropping systems influenced plant height whereas intercropped soybeans were taller compared with mono cropped one. The stem girths of soybean were high in pure stand soybean than in intercrop. *Rhizobium* inoculation significantly increased the plant height, stem girth, number of leaves and number of branches per plant, leaf area and finally plant vigour over un-inoculated treatments. P and K fertilization also significantly improved growth parameters of soybean that were assessed. However, each parameter was affected differently from each other with the level and type of fertilizer applied (Table 12, 13 and 14). Our general observation is that fertilizer levels of 26 P, 52 P and 20 K + 26 P kg ha^{-1} performed better in terms of improving growth parameters of soybean. Significant interactions were reported by inoculating the soybean with *Rhizobium*, and supplying P and K, in intercropping systems indicating the need for these inputs combination in the study area.

CHAPTER EIGHT

GROWTH RESPONSE OF MAIZE (*ZEA MAYS*) INTERCROPPED WITH *RHIZOBIUM* INOCULATED SOYBEAN (*GLYCINE MAX* (L.) MERR.) AND P AND K FERTILIZATION[7]

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Abstract

The research was carried out for the two consecutive cropping seasons in northern Tanzania to evaluate the effects of cropping systems and P and K fertilization on maize growth. A split-split plot design experiment with 2 x 4 x 7 factorial arrangements and replicated thrice was conducted. The main plots comprised of two rhizobia inoculation treatments, the sub plots comprising sole maize (SM) at a spacing of 75 x 60 cm; sole soybean at a spacing of 75 x 40 cm; maize-soybean intercropped at 75 x 60 and 75 x 20 cm, maize and soybean respectively; and the last cropping system was maize-soybean intercropped at 75 x 60 and 75 x 40 cm, maize and soybean respectively. The fertilizer levels: control; 20 K; 40 K; 26 P; 52 P; 26 P + 20 K; 52 P + 40 K (kg ha^{-1}) were assigned to sub-subplots. The results indicated that both cropping systems and P and K fertilization improved maize growth for the two cropping seasons. The plant height was significantly higher in M+B(A)+R and M+B(B)+R. The stem girth, plant vigor and greenness were statistically similar in sole maize (SM), M+B(A)+R and M+B(B)+R for both seasons. Any level of P and K significantly increased all growth attributes measured. The 40K+52P (kg ha^{-1}) performed better than all other

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fertilizers treatments. Therefore, intercropping maize with *Rhizobium* inoculated soybean at wider spacing and supplemented with P and K applied at higher rate of 40K+52P (kg ha^{-1}) will result in improved plant growth and hence final yield.

Keywords: Legume-cereals intercropping, NPK, plant height, stem girth, plant vigor.

8.1. Introduction

Maize (*Zea mays* L.) is the most important grain crop being grown in different parts of world and is produced under diverse environments. In sub-Saharan Africa (SSA), maize is the most important cereal crop and staple food for about 1.2 billion people (IITA, 2009) and occupy a third of the cultivated area (Blackie, 1990). Tanzania is one of the major maize producers in the world, ranked 1, 5, and 18 top maize producing countries in East Africa (EA), Africa and in the world respectively (FAOSTAT, 2015; United States Department of Agriculture, 2016). Successful maize production depends on the correct application of production inputs and correct agronomic practices that will sustain the environment as well as agricultural production (du Plessis, 2003). These includes: adapted and improved cultivars; plant population; soil tillage; fertilisation; weed, insect and disease control and harvesting (du Plessis, 2003). Management of these inputs results in a better growth performance of crops which eventually results in higher crop yield performance. Among other factors, soil fertility is the most critical input for crop growth, development and production. In many regions of East Africa, soils have negative balances of nutrients such as NPK affecting crop production (Bekunda *et al*., 2004). Growing crops by intercropping cereals and legumes may also influence the growth of respective crop and improve yields (Carr *et al*., 2004; Dusa and Stan, 2013). The main parameter of plant growth is its height which can be measured at different growth stages of plant or at the end (at physiological growth). Several studies have shown different growth responses as a result of different treatments applied to crop plants. The varied crop growth following intercropping or cropping patterns have been reported by several researchers (Lemlem, 2013; Hirpa, 2013; Hirpa, 2014; Nyoki and Ndakidemi, 2017). Availability or deficiency of soil nutrients such as N, P and K is another factor affecting plant growth. Availability of these nutrients for plants will improve plant growth and finally increase yield, while their deficiency will result in poorly developed crops there by reducing the final yields.

Each of these elements has important function(s) in plant growth and development. For example, in plant cell, phosphorus plays role in a variety of plant functions such as energy transfer, photosynthesis, translocation of sugars and starches as well as movement of nutrients within the plant (Brady, 2002; Shahid *et al*., 2009). Plants physiological processes such as activation of several enzymes, synthesis and degradation of carbohydrates, production of proteins and regulation of stomata pores for gas exchange and photosynthesis are regulated in presence of potassium (Lissbrant *et al*., 2009). Currently, there is little information on the growth response of maize intercropped with *Rhizobium* inoculated soybean, supplemented with P and K fertilizers. Therefore, the current study was carried out to assess the growth response of maize intercropped with *Rhizobium* inoculated soybean, and fertilized with different levels of P and K applied singly and combined.

8.2. Materials and methods

8.2.1. Experimental design and treatments

The field experiment was carried out at Tanzania Coffee Research Institute (TaCRI) farm for two consecutive years (2015 and 2016). The experiment was laid out in split-split plot design with 2 x 4 x 7 factorial arrangements and replicated thrice. The plot size was 3 x 3 m, with main plots having two rhizobia inoculation treatments, while the sub plots comprised: Maize pure stand at a spacing of 75 x 60 cm; Soybean pure stand at a spacing of 75 x 40 cm; Maize-soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; and the last cropping system was Maize-soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, Maize and soybean respectively. The sub-subplots were assigned the following fertilizer levels (kg ha^{-1}): control; 20 K; 40 K; 26 P; 52 P; 26 P + 20 K; 52 P + 40 K.

8.2.2. Data collection

Growth and development parameters of maize that were measured includes: plant eight (H), number of leaves per plant, stem girth. The plant height was measured at different growth stages using a meter ruler, while stem girth (cm) was measured with veneer caliper. This was done three times at 2 weeks interval during the growing period of the crops.

8.2.3. Statistical analysis

The collected data was analyzed using statistical software called STATISTICA. The statistical analysis was performed using the 3-way analysis of variance (ANOVA) in factorial arrangement. The Fisher's least significance difference (L.S.D.) was used to compare treatment means at $p = 0.05$ level of significance (Steel and Torrie, 1980).

8.3. Results

8.3.1. Plant height

The results of the current study indicated that the cropping systems had significant effect on the plant height where the intercropped maize appeared taller than pure stand maize. For the two cropping season and across the growth stages, pure stand maize were significantly shorter than the intercropped ones. The data recorded from maize that were intercropped with *Rhizobium* inoculated soybean showed that maize were significantly taller than those intercropped with un-inoculated soybean and the pure stand maize for the two cropping seasons. Although there were slight differences of plant height with regard to the spacing, the differences were not significant (Table 15). The results of this study also showed that fertilizers had significant effects on plant height. When compared with the control (unfertilized plots), all other fertilizer treated plots significantly improved the maize height for both cropping season. The data recorded at different growth stages showed that whether applied singly or combined, at lower rates or doubled rates, P and K fertilizers improved plant height over the control for the two cropping seasons (Table 15).

Table 15: Plant height measured at different growth stages of maize as affected by cropping systems and P and K fertilizers for the two cropping seasons

	Mean plant height (cm) 2015 cropping season			**Mean plant height (cm) 2016 cropping season**		
	2 WAP	**4 WAP**	**6 WAP**	**2 WAP**	**4 WAP**	**6 WAP**
SM	16.24±0.51d	28.78±1.09a	67.32±2.33b	20.70±0.64b	38.06±0.94c	145.89±5.73c
M+B(A)-R	17.92±0.37c	28.94±0.64a	66.13±2.14b	21.75±0.91b	40.62±1.21b	155.95±4.54ab
M+B(B)-R	18.40±0.50bc	29.49±0.79a	68.17±1.93b	21.83±0.76b	39.22±1.42bc	149.86±3.06bc
M+B(A)+R	19.19±0.54ab	29.05±0.83a	69.30±2.60ab	23.48±0.62a	43.11±1.27a	154.05±4.17ab
M+B(B)+R	19.32±0.50a	31.11±1.12a	72.27±2.20a	24.21±0.80a	42.98±1.15a	157.24±5.16a
Fertilizer (kg ha^{-1})						
0	15.38±0.40f	25.09±0.82e	54.80±1.38e	18.24±0.70e	33.49±1.02f	121.02±4.69f
20K	16.51±0.40e	27.22±0.80de	60.73±1.20d	20.07±0.56d	36.22±0.91e	137.76±3.13e
40K	17.73±0.32d	28.29±0.78cd	65.18±1.49c	21.47±0.59cd	38.82±0.75d	147.24±2.68d
26P	18.11±0.43cd	29.31±0.92cd	68.53±1.43bc	21.89±0.58bc	40.53±0.62d	155.93±2.38c
52P	19.07±0.39bc	30.29±0.85bc	72.00±1.33b	23.33±0.74b	42.64±0.54c	160.87±2.04bc
20K+26P	19.87±0.60ab	32.18±0.79ab	78.40±1.31a	25.18±0.70a	45.13±0.89b	166.47±2.29b
40K+52P	20.82±0.59a	33.93±0.87a	80.82±2.12a	26.56±0.65a	48.76±1.02a	178.89±3.28a
F-Statistics						
CropSyst	15.43***	1.51 ns	3.45**	7.40***	16.25***	3.52*
Fert	25.44***	10.63***	39.07***	21.69***	63.03***	42.33***
CropSyst*Fert	0.59ns	0.21 ns	0.60 ns	0.329ns	1.01 ns	0.84 ns

CroSyt: Cropping Systems; Fert: Fertilizers; SM: Sole maize; WAP: Weeks after Planting; M+B(A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; –R and +R un-inoculated and inoculated soybean respectively; Values presented are means ± SE; *,**, ***: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

8.3.2. Number of leaves

The results presented in Table 16 showed that for the first cropping season, both cropping systems and fertilizers had no significant effect on the number of leaves. In the second cropping season, data recorded from 4 Weeks after Plant (4 WAP) and 6 Weeks after Plant (6 WAP) indicated that P and K significantly increased the number of leaves over the control. All levels of fertilizers significantly increased the number of leave compared with unfertilized plots. However, the highest number of leaves was recorded in plots treated with (kg ha^{-1}) 52 P, 20 K +26 P and 40 K + 52 P (Table 16)

Table 16: Mean number of leaves counted at different growth stages of maize as affected by cropping systems and P and K fertilizers for the two cropping seasons

	Mean number of Leaves 2015			Mean number of Leaves 2016		
	2 WAP	4 WAP	6 WAP	2 WAP	4 WAP	6 WAP
SM	5.56±0.08a	7.30±0.14a	11.05±0.19a	6.48±0.15a	9.97±0.23a	13.11±0.13a
M+B(A)-R	5.75±0.09a	7.43±0.07a	11.46±0.21a	6.76±0.09a	10.27±0.20a	12.83±0.17a
M+B(B)-R	5.56±0.07a	7.47±0.11a	11.19±0.16a	6.43±0.09a	9.75±0.13a	12.98±0.17a
M+B(A)+R	5.63±0.08a	7.56±0.11a	11.22±0.17a	6.51±0.10a	10.09±0.17a	12.97±0.22a
M+B(B)+R	5.65±0.07a	7.70±0.13a	11.40±0.19a	6.56±0.08a	10.27±0.18a	13.06±0.19a
Fertilizer (kg ha^{-1})						
0	5.51±0.10a	7.38±0.14a	11.02±0.32a	6.27±0.12a	9.27±0.19d	12.18±0.22c
20K	5.69±0.09a	7.51±0.10a	10.93±0.19a	6.47±0.09a	9.84±0.17c	12.73±0.23b
40K	5.56±0.06a	7.44±0.10a	11.24±0.17a	6.44±0.09a	9.96±0.19bc	13.22±0.16ab
26P	5.71±0.11a	7.44±0.14a	11.49±0.24a	6.56±0.11a	10.43±0.25ab	12.73±0.24b
52P	5.76±0.07a	7.67±0.10a	11.67±0.18a	6.73±0.15a	10.16±0.23abc	13.38±0.13a
20K+26P	5.64±0.12a	7.43±0.18a	11.24±0.20a	6.64±0.13a	10.27±0.16abc	13.29±0.14a
40K+52P	5.53±0.10a	7.56±0.18a	11.24±0.18a	6.71±0.16a	10.56±0.19a	13.40±0.13a
F-Statistics						
CropSyst	0.93 ns	1.76 ns	0.69 ns	1.51 ns	1.78 ns	0.50 ns
Fert	0.98 ns	0.53 ns	1.14 ns	1.79 ns	4.87***	6.29***
CropSyst*Fert	0.78 ns	1.34 ns	0.39 ns	0.80	0.99 ns	1.18ns

CroSyt: Cropping Systems; Fert: Fertilizers; SM: Sole maize; WAP: Weeks after Planting; M+B(A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; –R and +R un-inoculated and inoculated soybean respectively; Values presented are means ± SE; ***: significant at $p \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

8.3.3. Stem girth

The results of the current study showed that the cropping systems had significant effects on the maize stem girth. The maize pure stand and maize intercropped with inoculated soybean planted at wider spacing had significantly higher stem girth compared with other cropping systems for the two cropping seasons (Table 17). Phosphorus and potassium also significantly increased the maize stem girth over the control (Table 17). The stem girths were increasing with the increasing fertilizer levels and this trend was observed in the two cropping seasons. It was generally observed that the combined doubled P and K resulted in greater stem girth relative to other treatments.

8.3.4. Plant vigor and greenness

The cropping systems and P and K fertilization had significant effects on plant vigor and greenness for the two cropping seasons. For the two cropping seasons, maize pure stand (SM) and maize intercropped with inoculated soybean planted at wider spacing (M+B(B)+R) significantly improved the plant vigor over the rest of the cropping systems used in this study (Table 17). In the 2015 cropping season, maize intercropped with inoculated soybean planted at wider spacing (M+B(B)+R) significantly improved the greenness of the plants. However, this was statistically the same with maize intercropped with inoculated soybean planted at narrower spacing (M+B(A)+R) and the maize grown as pure stand (SM). In the second cropping season (2016), maize grown as pure stand (SM) and maize intercropped with inoculated soybean planted at wider spacing (M+B(B)+R) had significantly greener maize plants over all other cropping systems (Table 17). On the other hand, plant vigor and greenness were strongly and significantly improved in the plots treated with both P and K over the control. Of all the treatment, 52 P, 20 K+26 P, 40 K+52 P (kg ha^{-1}) had excellent plant vigor and the greenness for the two cropping seasons (Table 17).

Table 17: Stem girth, vigor and greenness of plant as affected by cropping systems and P and K fertilizers for the two cropping seasons

Cropping Systems	2015 cropping season			2016 cropping season		
	Stem Girth (mm)	Plant Vigor	Greenness	Stem Girth (mm)	Plant Vigor	Greenness
SM	13.51±0.25a	2.00±0.21b	2.02±0.21b	14.76±0.32a	1.79±0.19c	1.76±0.19c
M+B(A)-R	12.89±0.31bc	2.55±0.23a	2.48±0.25a	13.98±0.41b	2.25±0.28a	2.37±0.29a
M+B(B)-R	12.74±0.35c	2.12±0.24b	2.12±0.22ab	13.57±0.39b	2.14±0.25ab	2.14±0.22ab
M+B(A)+R	13.35±0.27ab	2.07±0.20b	2.00±0.21b	13.81±0.27b	2.11±0.26ab	2.11±0.26ab
M+B(B)+R	13.42±0.30ab	2.00±0.21b	1.91±0.21b	14.62±0.37a	1.98±0.21bc	2.03±0.23bc
Fertilizer (kg ha^{-1})						
0	11.55±0.21d	3.53±0.13a	3.63±0.12a	11.84±0.34e	3.71±0.14a	3.81±0.16a
20K	12.19±0.23cd	3.00±0.13b	2.87±0.12b	13.19±0.28d	3.22±0.10b	3.12±0.13b
40K	12.86±0.21bc	2.40±0.16c	2.23±0.19c	14.01±0.27c	2.30±0.15c	2.27±0.17c
26P	13.13±0.25b	2.07±0.17c	1.97±0.19cd	14.03±0.22c	1.77±0.17d	1.77±0.22d
52P	13.45±0.27b	1.57±0.16d	1.53±0.17de	14.54±0.29bc	1.30±0.11e	1.37±0.11e
20K+26P	14.23±0.21a	1.27±0.15d	1.20±0.15e	15.24±0.23b	1.07±0.05ef	1.13±0.08e
40K+52P	14.87±0.26a	1.20±0.14d	1.30±0.15e	16.19±0.30a	1.00±0.00f	1.11±0.07e
F-Statistics						
CropSyst	2.86*	3.11*	2.48*	5.19***	4.22**	3.60**
Fert	22.40**	33.32***	28.64**	27.17***	109.74***	59.12***
CropSyst*Fert	0.45	0.50 ns	0.36ns	0.63 ns	1.64 ns	0.97 ns

CroSyt: Cropping Systems; Fert: Fertilizers; SM: Sole maize; WAP: Weeks after Planting; M+B(A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; –R and +R un-inoculated and inoculated soybean respectively; Values presented are means ± SE; *,**, ***: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD). Note: plant vigor and Greenness was assessed in a scale of 1-5. 1=Good; 5= Bad.

8.4. Discussion

In the current study we examined the effects of cropping systems and P and K fertilization on growth parameters of maize intercropped with inoculated and un-inoculated soybean. The results showed that both cropping systems and fertilizers had significant effects on almost all growth parameters such plant height; number of leaves; stem girth; plant vigor and greenness of plant leaves. Intercropping maize with inoculated soybean resulted in taller maize that those intercropped with un-inoculated soybean and those under the pure stand. The reason for that might be due to the effects of competition for light because inoculated soybean grew quicker and pose enough competition to maize in early weeks of plant growth leading to increased plant height. When soybean stopped growing the other factor that may have contributed to increased plant height in maize is the effects nitrogen fixed from legume component of intercrop. This argument is supported by many researchers who reported the presence of direct nitrogen transfer from legumes to cereals in intercropping leading to improved growth of both crop components (Giller and Wilson, 1991; Giller *et al*., 1991; Shen and Chu, 2004). Maize intercropped with un-inoculated soybean were relatively shorter than those under *Rhizobium* inoculated soybean because there was no strong competition as un-inoculated soybean could not strongly compete for light with maize, and there was no additional nitrogen from legumes. Maize grown as pure stand were relatively of the same height with those intercropped with un-inoculated soybean except those recorded 2 WAP in the first cropping season and 4 and 6 WAP in the second cropping season which were shorter than those under intercropping. Our findings do not agree with those found by Ndiso *et al*. (2017) who reported that the height of both cowpea and maize were shorter in intercropping than those under respective pure stand crops. They argued that their results could have been attributed by competition for resources among the component crops. In our study cropping systems did not have significant effect on the number of leaves of maize plant for the two cropping seasons. However, there were significant effects of the cropping systems on the maize stem girth, plant vigor and greenness. For the two cropping seasons, the maize pure stand and maize intercropped with inoculated soybean at wider spacing resulted in improved and greater stem girth over the maize intercropped with inoculated soybean at narrower spacing and un-inoculated soybean. These results could have been attributed by lack of completion in maize sole crop. The improved maize stem girth in maize intercropped with inoculated soybean at wider spacing could have been attributed by less competition due to enough spacing and also the fixed nitrogen (data not presented in this paper) from soybean (Karim *et al*., 1993; Oliveira *et al*., 2016). Plant vigor and greenness was significantly influenced by cropping systems where maize plants were greener and grew vigorously in plots that had maize intercropped with soybean at wider spacing compared with other treatment. This could have been contributed by the fixed nitrogen which improved the plant vigor and greenness over other treatments. Although intercropping at narrower spacing (M+B(A)+R) also had fixed nitrogen, but the was strong completion which led to thinner maize stem girth.

On the other hand, phosphorus and potassium fertilization also showed a significant effect on plant growth parameters measured in this study. The plant height of P and K fertilized maize was significantly higher compared with the unfertilized maize, indicating the needs of these elements in plant growth. The number of leaves was also increased in fertilized plots relative to the control plots. Maize stem girth, plant vigor and greenness were also improved following P and K fertilization. Generally, any level of P and K has significantly contributed to the growth performance of the crop. Similar to our findings Yilmaz (2008) reported that there was a significant increase in plant height following fertilization with higher (75 kg ha^{-1}) rate of phosphorus on Narbon Vetch. Phosphorus is an essential element required in large quantities in young cells, such as shoots and root tips, where metabolism is high and cell division is rapid making its availability to improve growth of plants (Uchida, 2000). Other function of phosphorus is root development. Well-developed roots can explore enough growth resources leading to the improved plant growth than in P deficient Plants (Brady, 2002; Shahid *et al*., 2009). Apart from phosphorus, potassium also has contributed much in the improved growth of maize in this study because K is known to be an enzyme activator that promotes metabolism (Lissbrant *et al*., 2009; Barragán *et al*., 2012). Other functions of K is to promote the translocation of photosythates (sugars) for plant growth, K also assists in regulating the plant's use of water by controlling the opening and closing of leaf stomata (Talbott and Zeiger, 1996; Uchida, 2000; Lissbrant *et al*., 2009; White and Karley, 2010; Andrés *et al*., 2014). It was observed in this study that fertilization with P and K at higher rates whether singly or combined significantly increased plant growth parameters than when they were applied at lower rates. The combined doubled fertilizers (40K+52P kg ha^{-1}) showed super performance in improving plant growth over all other fertilizers treatments for the two cropping seasons. Similar to our study, Mallarino *et al*. (1999) have reported the increased in early growth of corn following fertilization with P and K. In another study conducted by Zafar *et al*. (2011) it was reported that different sources of P applied along with plant Growth Promoting Rhizobacteria (PGPR) significantly increased morphological parameters of the plants in *P. vulgaris*. In this study, there were no significant interactions of cropping systems and fertilizer application

8.5. Conclusion

The results of this study have showed that both cropping systems and P and K fertilization has contributed much to the improved maize growth over the period of two cropping seasons. Plant height was significantly higher in plots that were intercropped maize with inoculated soybean compared with other treatments. It was generally observed that stem girth, plant vigor and greenness were found to be statistically similar in sole maize (SM) and maize intercropped with *Rhizobium* inoculated soybean at both narrower and wider spacing for the two cropping seasons. P and K fertilization significantly increased plant growth parameters such as plant height, number of leaves, stem girth, plant vigor and greenness over the control. The higher rates of these fertilizers significantly increased the plant growth parameters over the lower rates. Interestingly, their combined application significantly increased growth traits than when they were singly applied. When the combination of these fertilizers was doubled (40K+52P (kg ha^{-1})) they showed a super performance in improving plant growth over all other fertilizers treatments for the two cropping seasons. Therefore, based on these findings we recommend that maize should be intercropped with *Rhizobium* inoculated soybean at the recommended spacing and supplemented with combined P and K applied at higher rate of 40K+52P (kg ha^{-1}).

CHAPTER NINE

ASSESSING THE LAND EQUIVALENT RATIO (LER) OF MAIZE (*ZEA MAYS* L.) INTERCROPPED WITH *RHIZOBIUM* INOCULATED SOYBEAN (*GLYCINE MAX* [L.] MERR.) AT VARIOUS P AND K LEVELS[8]

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Abstract

A 2 years field experiment was carried out in northern Tanzania with the aim of assessing the effects of maize-soybean intercropping systems, *Rhizobium* inoculation and P and K supplementation on Land Equivalent Ratio. A three replicate experiment was laid out in a split-split plot design with the main plots comprised of Rhizobia inoculation (with and without). The sub plots comprised of three cropping systems and the sub-sub plots having seven fertilizer levels (kg ha^{-1}): Control, 20, 40 K, 26, 52 P, 26 P + 20 K and 52 P + 40 K. The results indicated that compared with pure stand, intercropping maize with soybean was advantageous because all the values of LER were above 1.0. Supplementation of inputs such as *Rhizobium* inoculants and P and K fertilizers significantly ($p<0.05$) increased the LERs over the control. The rhizobial inoculated plots gave the highest LER of 1.73 and 1.61 grain biological yield compared with un-inoculated plots which gave the lowest LER of 1.31 and 1.39 grain biological yield respectively. P and K also significantly increased LER over the control. When compared with the narrower spacing, wider spacing of soybean resulted to a greater LER values suggesting the use of wider spacing for legume-cereals intercropping. Hence, this study suggests that farmers should be advised to intercrop maize with soybean at a recommended spacing, and supplying with the recommended inputs above. However,

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application of P and K fertilizers will depend on the fertility status of the soil in respective area under consideration.

***Keywords*:** Legumes, cereals, intercropping, monoculture, biological nitrogen fixation, yield advantage.

9.1. Introduction

In agro ecosystems, intercropping allows better resource use efficiency hence reducing the needs for external inputs and moving towards agricultural sustainability (Beets, 1994; Dariush *et al*., 2006). Intercropping is the practice of growing two or more crops in the same piece of land at the same time (Sanchez, 1976). It plays an important role in subsistence food production in developing countries (Tsubo *et al*., 2005). It is has been well established that intercropping offers so many potential advantages such as: improved utilization of growth resources by the intercropped species (Banik *et al*., 2006); direct nitrogen transfer from legumes to cereals in intercropping (Giller and Wilson, 1991); Enhanced productivity due to nitrogen fixation (Maingi *et al*., 2001; Banik *et al*., 2006); used as a method of controlling weeds, insect pests, diseases (Smith and Mcsorley, 2000) and control of soil erosion (Jabbar *et al*., 2009; Matusso *et al*., 2012). However, intercropping may results in positive interactions (facilitations) or negative interactions (competitions) of the intercropped crop components. Positive interaction is good because the component crops under intercropping facilitate each other to achieve maximum yielding or productivity (Ghaffarzadeh *et al*., 1994; Ghosh, 2004; Trydemanknudsen *et al*., 2004). On the other hand, a negative interaction reduces the yield of the less competitive crops in intercropping. There are many indices/methods that have been developed to assessing these interactions in intercropping. These includes: relative crowding coefficient (RCC) (Gosh, 2004), competitive ratio (CR) (Willey and Rao (1980), land equivalent ratio (LER) (Mead and Willey, 1980), aggressivity (A) (McGilchrist and Trenbath, 1971), and monetary advantage index (MAI) (Gosh, 2004).

Of these indices, the LER is mostly preferred and used index for comparisons of intercrop versus sole crop (Agegnehu, 2006; Esmaeili *et al*., 2011). LER is an accurate method of assessing the competitive relationship between the intercropped crops, and the overall productivity of intercropping system (Zada *et al*., 1988). It also measures how efficient are intercropping, it compares land areas required under monoculture or sole cropping to give the same yields as that obtained from the component crops of the intercrop (Federer and Schwager, 1982; Brintha and Seran, 2009; Nyoki and Ndakidemi, 2016). Based on the advantages of using LER in comparing intercropped crops, this study focused on LER as an index of assessing overall productivity and comparing intercropped crops. According to Gliessman (2007), the total LER of the intercropped crops should be 1.0 and their partial LER should be 0.5 for each crop if the intercropped crops have the same agro-ecological characteristic. The resulting number from LER is a ratio that indicates the amount of land required to grow both crops together relative to the amount of land needed to grow sole crop of each and give the same yield (Amanullah *et al*., 2016). The LER with value greater than 1.0 indicates that intercropping is advantageous while the LER less than 1.0 shows that intercropping is disadvantageous (Dariush *et al*., 2006; Mohammed, 2011). For instance, a LER 1.25 indicates that an area planted sole crop or monoculture, would require 25% more land to produce the same yield as the same area planted in an intercrop (Laster and Furr, 1972; Dariush *et al*., 2006). On the other hand the LER of 0.75 shows that the yield of intercropped crops was only 75% of the yield of pure stand. Regardless of the yield advantages in intercropping, there is little information on how variation and combination of inputs such as *Rhizobium* inoculants and P and K fertilizers may influence the yield advantages in intercropping over sole cropping. The objective of the current study was to assess the land equivalent ratio of the maize intercropped with soybean at different soybean spacing under Rhizobia inoculation and different levels of singly applied and combined P and K.

9.2. Material and methods

9.2.1. Experimental design and treatments

The field experiment was carried out at Tanzania Coffee Research Institute (TaCRI) for two consecutive years (2015 and 2016 cropping seasons). The experiment was laid out in split-split plot design with 2 x 4 x 7 factorial arrangement replicated thrice. The plot size was 3 x 3 m. The main plots had two Rhizobia inoculation treatments, while the sub plots comprised: Maize (sole crop) at a spacing of 75 x 60 cm; Soybean (sole crop) at a spacing of 75 x 40 cm; Maize/soybean (intercropping system) at a spacing of 75 x 60 cm and 75 x 20 cm, Maize and soybean respectively; and the last cropping system was Maize/soybean (intercropping system) at a spacing of 75 x 60 cm and 75 x 40 cm, Maize and soybean respectively. The sub-subplots were assigned the following fertilizer levels (kg ha^{-1}): control; 20 K; 40 K; 26 P; 52 P; 26 P + 20 K; 52 P + 40 K.

9.2.2. Data collection

At physiological maturity, the plants in the middle rows of each plot were counted and harvested for assessing grain yield and yield components of both soybean and maize. The border row and border plants were excluded in the determination of yield. For yield components, 10 plants of both crops were sub-sampled from each plot to determine the biological yield in both soybean and maize. All pods and cobs from each plot were manually threshed separately and allowed to dry to 13% moisture content for determination of gain yield.

9.2.3. Determination of land equivalent ratio (LER)

Intercropping was assessed, relative to sole crops, by use of Land Equivalent Ratios (LERs), which is referred to as the proportion/amount of land area that is needed for sole cropping to produce the same yields as the intercropping (Mead and Willey, 1980).

$$LER = L1 + L2 = \frac{YI1}{YS1} + \frac{YI2}{YS2}$$

L1 and L2 are the LERs for the individual crops (soybean and Maize), (YI1 and YI2 are the individual crop yields in intercropping, where YS1 and YS2 are their yields as sole crops. The partial LERs (L1 and L2) were then summed up to give the total LER for the intercrop.

9.2.4. Statistical analysis

The collected data was analysed using statistical software called STATISTICA. The statistical analysis was performed using analysis of variance (ANOVA) in factorial arrangement. The fisher's least significance difference (L.S.D.) was used to compare treatment means at $p = 0.05$ level of significance (Steel and Torrie, 1980)

9.3. Results

9.3.1. Land equivalent ratio (LER) for grain yield

Statistical analysis of the data showed that combination of Rhizobia inoculation, intercropping systems and P and K fertilization had significant effects on LER for the two cropping seasons (Table 18). An LER was significantly higher in plots that were inoculated with Rhizobia relative to un-inoculated plots for the two consecutive years and. It is well shown in Table 18 that intercropping at different spacing had significant effects on LER. The narrower spacing of M+B(A) produced lower total LER compared with the wider spacing of M+B(B) which produced significantly higher LER (Table 18). The highest Total LER of 1.73 was obtained in Rhizobium inoculated plot and intercropping at wider spacing of M+B(B) in 2015 cropping season while the lowest total LER of 1.31 was obtained at intercropping with narrower spacing of M+B(A) without Rhizobia inoculation (Table 18). The results of this study further indicated that P and K fertilization also significantly increased the values of LER over the control. The highest LERs (1.48) were recorded in plots treated with 40 K and 20 K + 26 P for 2015 cropping season at a narrower spacing. In the same season, the wider spacing gave the highest LER of 1.57 recorded from 26 P and 40 K + 52 P (kg ha^{-1}) (Table 18). In the second cropping season, the highest LER of 1.59 was recorded from plots treated with 40 K and 40 K + 52 P (kg ha^{-1}) at narrower spacing of M+B(A). The wider spacing of intercropping produced significantly higher (1.68) LER which was found in plots treated with 40 kg of K per hectare. Regardless of the cropping season and the spacing applied under intercropping, lowest LERs were recorded in the control plots (Table 18).

9.3.2. Land equivalent ratio (LER) for biological yield:

As for grain yield, the biological yield also resulted in greater LER values in plots inoculated with Rhizobia compared with un-inoculated treatments. When comparing intercrop spacing, and rhizobia inoculation, the LER was higher (1.61) in Rhizobia inoculated plots and the lowest LER was 1.39 recorded in un-inoculated plots with wider spacing of intercrop in 2015 cropping season (Table 19). Furthermore, the current study has indicated that P and K significantly improved the total LER over the control (Table 19). The highest total LER of **1.64** was recorded in plot treated with 26 kg of P and wider spacing of intercrop in the second cropping season. The lowest LER of **1.31** was recorded in control plots and both narrower and wider spacing of intercrop for the first cropping season.

Table 18: Partial and total LER for grain yield of soybean and maize for 2015 and 2016 cropping season as affected by varied spacing, rhizobia inoculation and P and K fertilization

Treatment	2015 cropping season						2016 cropping season					
	Partial LER at M+B(A)		Total LER	Partial LER at M+B(B)		Total LER	Partial LER at M+B(A)		Total LER	Partial LER at M+B(B)		Total LER
	Soybean	Maize		Soybean	Maize		Soybean	Maize		Soybean	Maize	
Rhizobia												
With out	0.66	0.69	**1.35b**	0.78	0.71	**1.49b**	0.66	0.65	**1.31b**	0.69	0.66	**1.35b**
With	0.80	0.74	**1.54a**	0.94	0.79	**1.73a**	0.75	0.68	**1.43a**	0.86	0.72	**1.58a**
Fertilizers (kg ha^{-1})												
0	0.65	0.68	**1.33e**	0.76	0.62	**1.38d**	0.73	0.69	**1.42e**	0.59	0.65	**1.24f**
20K	0.67	0.68	**1.35d**	0.70	0.75	**1.45c**	0.87	0.65	**1.52c**	0.85	0.69	**1.54d**
40K	0.70	0.78	**1.48a**	0.70	0.79	**1.49b**	0.90	0.69	**1.59a**	0.97	0.71	**1.68a**
26P	0.70	0.73	**1.43b**	0.82	0.75	**1.57a**	0.74	0.75	**1.49d**	0.82	0.7	**1.52d**
52P	0.69	0.69	**1.38c**	0.85	0.71	**1.56a**	0.80	0.76	**1.56b**	0.87	0.74	**1.61b**
20K+26P	0.89	0.59	**1.48a**	0.78	0.69	**1.47bc**	0.78	0.64	**1.42e**	0.89	0.67	**1.56c**
40K+52P	0.74	0.73	**1.47a**	0.84	0.73	**1.57a**	0.72	0.87	**1.59a**	0.76	0.66	**1.42e**
Level of significant												
Rhizobia			***			***			***			***
Fertilizers			***			***			***			***

LER: Land Equivalent Ratio, M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; Values presented are means; ***: significant at $p \leq 0.001$; Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

Table 19: Partial and Total LER for biological yield of Soybean and Maize for 2015 and 2016 cropping seasons as affected by varied spacing, Rhizobia inoculation and P and K fertilization

Treatment	2015 cropping season						2016 cropping season					
	Partial LER at M+B(A)		Total LER	Partial LER at M+B(B)		Total LER	Partial LER at M+B(A)		Total LER	Partial LER at M+B(B)		Total LER
	Soybean	Maize		Soybean	Maize		Soybean	Maize		Soybean	Maize	
Rhizobia												
With out	0.68	0.86	**1.54b**	0.66	0.73	**1.39b**	0.76	0.72	**1.48b**	0.75	0.76	**1.51b**
With	0.71	0.89	**1.60a**	0.82	0.79	**1.61a**	0.76	0.78	**1.54a**	0.74	0.84	**1.58a**
Fertilizers (kg ha^{-1})												
0	0.61	0.70	**1.31d**	0.60	0.71	**1.31e**	0.72	0.67	**1.39c**	0.54	0.81	**1.35e**
20K	0.74	0.78	**1.52c**	0.65	0.76	**1.41d**	0.70	0.73	**1.43cb**	0.79	0.78	**1.57b**
40K	0.69	0.86	**1.55b**	0.81	0.73	**1.54b**	0.71	0.75	**1.46b**	0.62	0.86	**1.48d**
26P	0.73	0.88	**1.61a**	0.75	0.79	**1.54b**	0.69	0.83	**1.52a**	0.78	0.86	**1.64a**
52P	0.76	0.85	**1.61a**	0.8	0.69	**1.49c**	0.81	0.73	**1.54a**	0.81	0.77	**1.58b**
20K+26P	0.68	0.91	**1.59a**	0.76	0.73	**1.49c**	0.62	0.81	**1.43cb**	0.78	0.75	**1.53c**
40K+52P	0.69	0.87	**1.56b**	0.8	0.81	**1.61a**	0.72	0.74	**1.45b**	0.83	0.79	**1.62a**
Level of significant												
Rhizobia			***			***			***			***
Fertilizers			***			***			**			***

LER: Land Equivalent Ratio, M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; Values presented are means; **, ***: significant at $p \leq 0.01$, $p \leq 0.001$ respectively; Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

9.4. Discussion

The results of the current study has proved that growing two or more crops in a piece of land at the same time, is advantageous and farmer who practice intercropping gets more crops compared with the one growing sole crops. The yield advantage in intercropping is indicated by the LER greater than 1.0 (Esmaeili *et al*., 2011). From the results above, all the total LERs were greater than which notifying that there was yield advantages in intercropping relative to mono culture (Dariush *et al*., 2006; Esmaeili *et al*., 2011). Interestingly, Rhizobia inoculation and P and K fertilization significantly increased the total LERs of both grain and biological yield over the control. This shows the necessity of these inputs in the study area when the crops are intercroped. For the two cropping seasons, wider spacing intercrop under Rhizobia inoculation significantly increased the total LERs of grain yield by 24 and 23 % over narrower spacing which increased total LERs by 19 and 12 % for the 2015 and 2016 cropping seasons respectively. Moreover, there was a significant biological yield advantage of 6% in inoculated plots with narrower spacing over un-inoculated plots for the two cropping seasons. The wider spacing and Rhizobia inoculation resulted in yield advantage of 22 and 7 % for first and second cropping seasons respectively. In general, the Rhizobia inoculated plots with wider spacing of intercrop gave 73% grain yield advantage of intercrop over sole cropping in 2015 cropping season. From this point the farmer would require 73% of more land to grow sole crops in order get the same grain yield as that obtained in the intercrop. Likewise, a farmer would require 61% of more land for sole crop to achieve the same biological yield as that obtained in intercropping.

The highest value of LER for grain yield was 1.68, indicating that a farmer would need 68 % of more land to grow sole crops in order to achieve the same grain yield as obtained from intercropping. For biological yield, the highest LER was 1.64 which indicates the yield advantage of 64 % in intercropping over sole crop. Therefore, a farmer would require 64 % of more land for sole crops to achieve the yield obtained in the intercropping. Similar to our findings, several studies (Hugar and Palled, 2008; Yilmaz *et al*., 2008; Dahmardeh *et al*., 2010; Solanki *et al*., 2011; Amanullah *et al*., 2016) have reported the LER greater than 1.0 indicating the intercropping advantages over sole cropping. From this study, we have noticed reduced values of LER in narrower spacing compared with wider spacing. The reduced LER in narrower spacing of soybean intercropped with maize can be explained by the findings of Ofori and Stern (1986) who reported that light is the determinant of LER of maize and soybean and that LER declines when legume becomes severely shaded. Ijoyah and Jimba, (2012) have reported reduction in number of pods of okra intercropped with maize stating the reason being the effects of nutrient and light completion. Furthermore, Santalla *et al*. (2001) reported a reduction of common bean yield in intercropping compared with pure stand due to the effect of shading.

9.5. Conclusion

From this study, intercropping maize with soybean was advantageous because all the values of LER were above 1.0. Supplementation of inputs such as *Rhizobium* inoculants and P and K fertilizers significantly ($p<0.05$) increased the LERs over the control. The system was more beneficial in rhizobial inoculated plots which gave the highest LER of 1.73 and 1.61 grain biological yield compared with un-inoculated plots which gave the lowest LER of 1.31 and 1.39 grain biological yield respectively. P and K also greatly contributed to the increased LER over the control. Wider spacing of soybean resulted to a greater LER compared with narrower spacing suggesting the use of wider spacing for legume-cereals intercropping. Therefore, this study suggests that farmers may be advised to intercrop maize with soybean at a recommended spacing, and supplying with the tested inputs above. However, application of P and K fertilizers will depend on the level of these nutrients in respective soil under consideration.

CHAPTER TEN

YIELD RESPONSE OF INTERCROPPED SOYBEAN-MAIZE UNDER RHIZOBIA (*BRADYRHIZOBIUM JAPONICUM*) INOCULATION AND P AND K FERTILIZATION[9]

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Abstract

The field study was carried out in two consecutive years (2015 and 2016) at the Tanzania Coffee Research Institute farm in northern Tanzania. The aim of this study was to assess the response of soybean yield attributes to cropping systems, Rhizobia (*Bradyrhizobium japonicum*) inoculation, and P and K fertilization. The study was laid out in a split-split plot design replicated three times. The results showed that both cropping systems, Rhizobia (*Bradyrhizobium japonicum*) inoculation, and P and K fertilization significantly (*p*=0.05) influenced most of the yield parameters measured. For example, cropping systems significantly (*p*=0.05) improved number of pods per plant, biological yield, grain yield and harvest index (2015 and 2016) of soybean. Rhizobia inoculation also significantly (*p*=0.05) improved soybean yield attributes such as number of pods, 100 seed weight, biological yield and grain yield of soybean (2015 and 2016) and harvest index in soybean (2015). P and K fertilization also significantly (*p*=0.05 improved different yield attributes of soybean over the control. It was noted that doubling of K from 20 kg to 40 kg improved most of the yield parameters of soybean relative to control. Doubling of P from 26 kg to 52 kg per hectare may not significantly change the soybean yield parameter. Doubling the combined fertilizers did not significantly increase the yield parameters of soybean suggesting the use of lower dose of combined fertilizers. Intercropping maize with rhizbial inoculated soybean significantly improved maize yield compared with intercrop without inoculation. Maize grown as

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monocrop gave relatively the same yield with intercropped maize and inoculated soybean. Fertilization with P and K also improved yield attributes of maize over the control

***Keywords**:* Biological nitrogen fixation, harvest index, legumes, intercropping.

10.1. Introduction

Soybean (*Glycine max* [L.] Merr.) is an important grain legume native to Eastern Asia. Currently, the crop is grown in diverse parts of the world. Soybean is preferred because of its high nutritional contents (Raji, 2007), high economic importance and its ability to form symbiotic relationship with nitrogen fixing bacteria (Ndakidemi *et al*., 2006). From this relationship, soybean produces much of its nitrogen requirement amounting to 50-60% (Salvagiotti *et al*., 2008). Despite of the nutritional and economical importance of soybean to human, its production is still low in most parts (Middle, Western and Eastern Africa) of Africa (Fig.13) (Ndakidemi *et al*., 2006). With reference to the increasing food and nutritional demand geared by increasing human population, legumes production by small holder farmer in Sub Saharan African needs to be advocated. The best agronomic practice is of paramount important for increasing legumes production such as soybean. Therefore, for improved plant growth, there must be favouring environment such as soil nutrients among other factors (Reckling, 2014).

Production of legume may be improved by using good agricultural inputs such as improved seeds, and fertilizers. Resource poor farmers who always harvest little from their field cannot afford to purchase good agricultural inputs (Ndakidemi *et al*., 2006). The most important plant nutrients for crop production are nitrogen (N), phosphorus (P) and potassium (K). However, studies have shown that these macronutrients are increasingly declining in the soil resulting to poor crop yields (Ndakidemi *et al*., 2006; Nyoki and Ndakidemi, 2014; Siddique *et al*., 2012). Soybean and other legumes are self-sufficient in their N needs and may contribute to N economy of the entire cropping system by adding fixed N to the soil pool (Siddique *et al*., 2012). Selection of specific nitrogen fixing bacteria for specific legume species is very important in order to attain good expected yield performance.

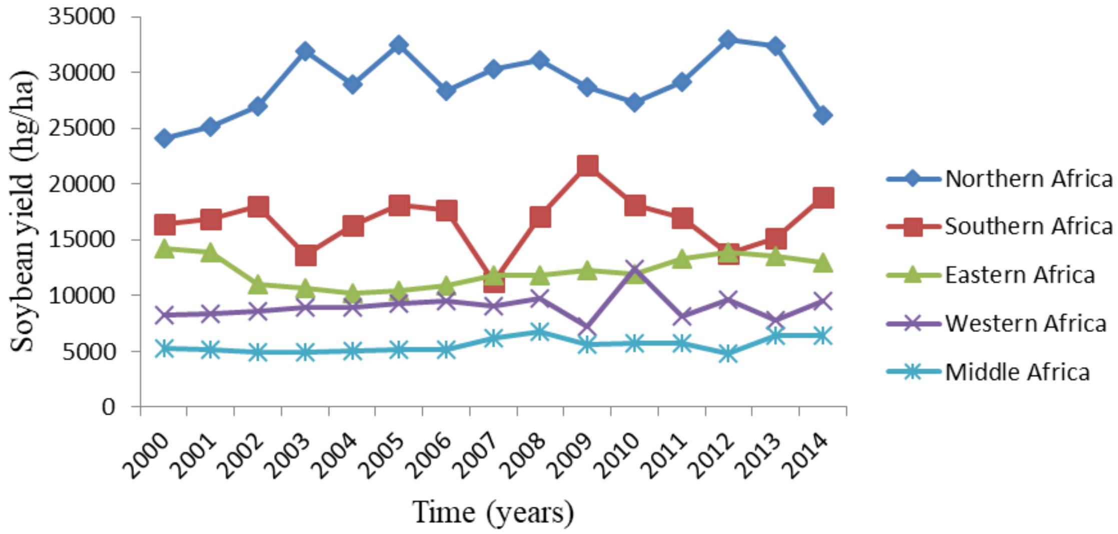

Figure 13: Time series showing Soybean production in different zones of Africa

Source: Data extracted from FAOST

In the farmer's field, legumes are always intercropped with cereals. Legume-Cereal intercropping has many advantages. Firstly, the farmer will get both carbohydrates and protein for their daily diet; secondly, addition of nitrogen to the soil through biological nitrogen fixation (BNF) (Whitbread and Ayisi, 2004; Khogali *et al*., 2011); thirdly, intercropping reduces the risk of crop failure; fourthly, efficiently utilization of growth resources (Morris and Garrity, 1993; Banik and Bagchi, 1993; Zhou *et al*., 2000; Li *et al*., 2003; Xu *et al*., 2008.) and fifthly, better use of land where the intercropped crops can interact and influence each other in terms of yield production (Zhang, 2003; Singh and Usha, 2003; Fan *et al*., 2006; Khogali *et al*., 2011; Lemlem, 2013).

In order to fill the gap between the potential yield and actual yield, it is important to conduct studies that will explore how farmers can just use little and cheap resources to attain high yield. Therefore, it was the interest of this study to determine yield performance of soybean and maize grown in intercropping systems under rhizobia inoculation and supplementation with phosphorus and potassium.

10.2. Material and methods

10.2.1. Experimental design and treatments

The field experiment was carried out at Tanzania Coffee Research Institute (TaCRI) for two consecutive cropping seasons (2015 and 2016). The experiment was laid out in split-split plot design with 2 x 4 x 7 arrangements and replicated thrice. The plot size was 3 x 3 m, with main plots comprised two inoculation treatments (with and without). The cropping systems were assigned to the subplots as follows: Maize (sole crop [SM]) at a spacing of (75 x 60 cm); Soybean (sole crop [SB]) at a spacing of (75 x 40 cm); Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm (M+B[A]) maize and soybean respectively; and the last cropping system was Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, (M+B[B]) maize and soybean respectively. The sub-subplots were treated with the following ertilizer levels (kg ha^{-1}): i). Zero control, ii). 20 K, iii). 40 K, iv). 26 P, v). 52 P, vi). 26 P + 20 K, vii). 52 P + 40 K.

10.2.2. Yield data collection

At physiological maturity, the plants in the middle rows of each plot were counted and harvested for assessing grain yield and yield components of both soybean and maize. The border row and border plants were excluded in the determination of yield. For yield components, 10 plants of both crops were sub-sampled from each plot to determine the number of pod per plant and number of seeds per pod, 100 seed weight and biological yield in soybean; cob length and cob weight in maize. All pods and cobs from each plot were manually threshed separately and allowed to dry to 13% moisture content for determination of gain yield. Harvest index was calculated according to Bell *et al*. (1995) using the formula,

$$HI = \frac{\text{Economic yield}}{\text{Biological yield}}$$

10.2.3. Statistical analysis

The statistical analysis was performed using the 3-way analysis of variance (ANOVA) in factorial arrangement. The computation was performed with the software program

STATISTICA. The fisher's least significance difference (L.S.D.) was used to compare treatment means at $p = 0.05$ level of significance (Steel and Torrie, 1980)

10.3. Results

10.3.1. Soybean yield attributes

i. Number of pods

The results from current study showed that the yield and yield attributes were significantly influenced by cropping systems, rhizobia inoculation and P and K fertilization for both cropping systems (Table 20). Number of pods per plant was significantly different in different cropping systems. Soybean grown as monocrop produced more pods compared with those in intercropping systems. Furthermore, rhizobia inoculation significantly increases number of pods in the two cropping seasons. For example, the mean number of pods under inoculation was 22.32±0.47 and 27.50±1.11 in first and second cropping seasons respectively compared with mean number of pods obtained in un-inoculated plots (17.13±0.63 and 23.94±1.09) for the two cropping seasons (Table 20). P and K fertilization also had positive effects on number of pods for both cropping seasons. In the first season, 20kg of K+26kg of P and 40 kg K+52 kg of P per hectare statistically produced same number of pods which were higher compared with other fertilizer treatments and the zero control. In 2015, the highest mean pods was 22.94±0.68 recorded at plots which received 40 kg K+52 kg of P and the lowest mean number of pods was 15.03±0.93 recorded from control plots (Table 20). In the second season (2016), the highest mean number of pods (28.04±2.13) was recorded at plots treated with 26 kg P per ha, while the lowest mean number of pods was 22.37±2.07 recorded from the control plots (Table 20).

ii. Number of seeds per pod

The results showed that the number of seed per pods was not significantly affected by cropping systems and fertilizer application in both 2015 and 2016 cropping seasons (Table 20). For the 2016 cropping season, Rhizobia inoculation had significant effect on soybean seeds per pod. The mean number of seeds per pod recorded in rhizobia inoculated plots was higher (2.06±0.04) compared with mean number of seeds per pod 1.88±0.04 recorded from the control (non inoculated plots) (Table 20).

iii. Hundred (100) seed weight (g)

Only rhizobia inoculation significantly increased seed weight for both cropping seasons. For example, the mean 100 seed weight in rhizobia inoculated plots was 22.17±0.39 and 23.15±0.17 compared with the control 20.02±0.47 and 21.77±0.23 for 2015 and 2016 cropping seasons respectively (Table 20).

Table 20: Effects of cropping systems, rhizobia inoculation and P and K fertilization on number of pods per plant, number of seeds per pod, and 100 seed weight for 2016 and 2015 cropping season

	2015 Cropping season			2016 Cropping season		
	Number of pods/plant	Number of Seeds/pod	100 Seed Weight (g)	Number of pods/plant	Number of Seeds/pod	100 Seed Weight (g)
Cropping Systems						
SB	22.02±0.74a	2.06±0.03a	21.02±0.50a	28.46±1.73a	1.89±0.05a	22.52±0.29a
M+B(A)	19.25±0.69b	2.05±0.03a	20.98±0.62a	24.22±1.01b	2.01±0.05a	22.19±0.27a
M+B(B)	17.90±0.82c	2.11±0.05a	21.29±0.52a	24.47±1.21b	2.01±0.06a	22.68±0.25a
F Statistics	**19.819*** **	**0.750ns**	**0.117ns**	**3.709***	**2.02ns**	**1.05ns**
Rhizobia						
With	22.32±0.47a	2.06±0.03a	22.17±0.39a	27.50±1.11a	2.06±0.04a	23.15±0.17a
With out	17.13±0.63b	2.08±0.03a	20.02±0.47b	23.94±1.09b	1.88±0.04b	21.77±0.23b
F Statistics	**90.480*** **	**0.143ns**	**14.668*** **	**6.234** **	**9.81** **	**24.50*** **
Fertilizer						
Control	15.03±0.93c	2.11±0.08a	20.44±0.64a	22.37±2.07b	1.88±0.07a	22.16±0.54a
20 K	17.44±1.09b	2.00±0.00a	20.17±0.82a	25.01±1.41ab	2.02±0.09a	22.30±0.31a
40 K	19.15±1.31b	2.08±0.06a	21.56±1.08a	25.12±1.74ab	1.82±0.09a	22.57±0.25a
26 P	19.25±1.16b	2.14±0.07a	21.33±0.86a	28.04±2.13a	2.03±0.08a	22.89±0.35a
52 P	21.59±0.89a	2.11±0.06a	21.83±0.70a	27.69±2.03a	2.08±0.08a	22.87±0.41a
20 K +26 P	22.63±1.18a	2.00±0.00a	20.50±1.04a	25.03±2.63ab	1.93±0.06a	22.49±0.41a
40 K + 52 P	22.94±0.68a	2.06±0.06a	21.83±0.64a	26.74±2.46ab	2.02±0.07a	21.95±0.55a
F Statistics	**16.028*** **	**0.988ns**	**0.899ns**	**1.07***	**1.67ns**	**0.91ns**
Interactions						
CrSyst*Rhiz	0.894ns	1.536ns	7.033**	0.02ns	1.99ns	0.02ns
CrSyst*Fertili	1.028ns	0.792ns	1.556ns	0.90ns	0.71ns	1.53ns
Rhiz*Fertili	1.388ns	0.893ns	1.352ns	1.13ns	0.77ns	1.56ns
CrSyst*Rhiz*Fertili	1.388ns	1.161ns	1.001ns	2.73**	1.11ns	1.07ns

CroSyt: Cropping Systems; Fert: Fertilizers; Rhiz: Rhizobium; SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; Values presented are means ± SE; *,**, ***: significant at p ≤ 0.05, p ≤ 0.01, p ≤ 0.001 respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

iv. Interactive effects of cropping systems and rhizobia inoculation on 100 seed weight

There was positive interaction between cropping systems and rhizobia inoculation on 100 seed weight in the 2015 cropping season. Fig. 14 shows that rhizobia inoculation significantly increased 100 seed weight in intercropping than in sole soybean over the control which had lowered 100 seed weight in intercropping than in sole soybean.

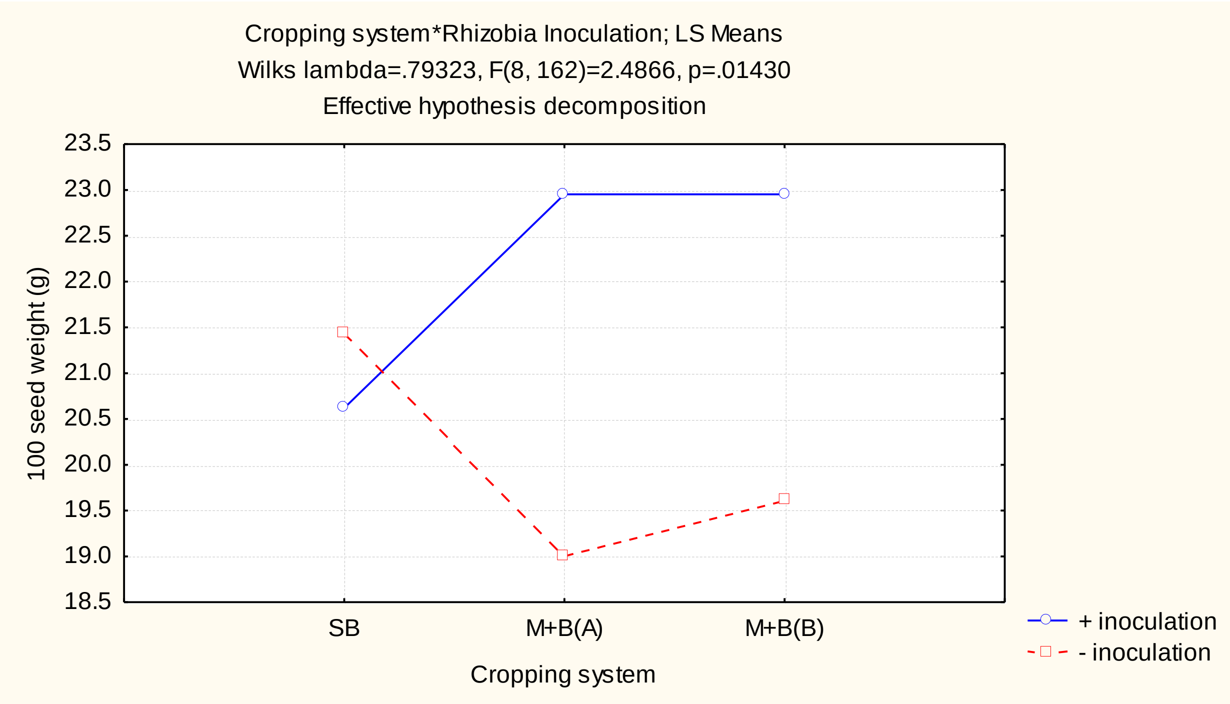

Figure 14: Interactive effects of cropping systems and rhizobia inoculation on 100 seed weight 2015 cropping season

v. Interactive effects of cropping systems, rhizobia inoculation and fertilizer levels on number of pods per plant for the 2016 cropping season

In 2016 cropping season, there was significant interaction between the cropping systems, Rhizobia inoculation and fertilizer levels on number of pods per plant. Generally, rhizobia inoculated plots in all cropping systems, produced many number of pods per plant relative to un-inoculated plots. Comparing number of pods per plant in the three cropping systems, the sole soybean (SB) produced many number of pods compared with the rest cropping systems. Interestingly, intercropping at narrower spacing [M+B(A)] produced many number of pods in un-inoculated plots compared with the wider spacing of [M+B(B)] (Fig. 15).

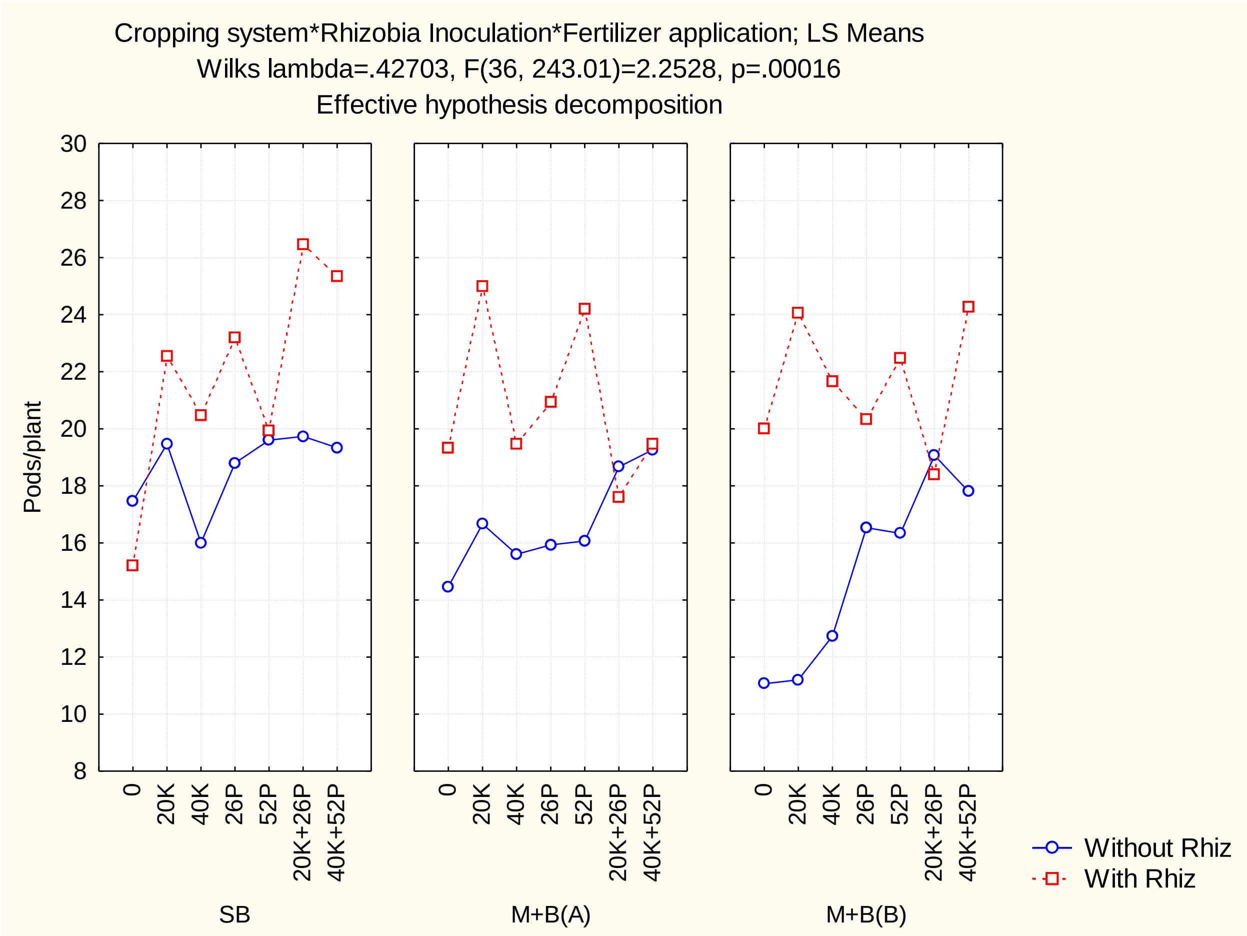

Figure 15: Interactive effects of cropping systems, rhizobia inoculation and fertilizer levels on number of pods per plant for the 2016 cropping season

vi. Biological yield (kg/plot)

For both cropping seasons, all treatments had significant effect on soybean biological yield. Sole soybean (SB) produced high biological yield of 4.41±0.24 and 4.27±0.29 for both 2015 and 2016 cropping seasons respectively, compared with intercropping at different spacing. The mean biological yield obtained from M+B(A) and M+B(B) were statistically the same for both cropping seasons (Table 21). The Rhizobia inoculated plots significantly produced high biological yield 4.69±0.15 and 3.92±0.15 for 2015 and 2016 cropping seasons respectively, relative to un-inoculated plots which gave 2.96±0.16 and 2.76±0.22 for the two cropping seasons respectively (Table 21). Furthermore, biological yield were significantly increased following P and K fertilization when compared with the control for both 2015 and 2016 cropping seasons. For example, the highest biological yield (4.97±0.33) in 2015 cropping season were recorded from plot treated with 20 kg of K+26 kg of P per ha, and the lowest (2.32±0.22) was recorded from the control. In 2016 cropping season, the highest biological yield (3.85±0.53) was recorded from plots treated with 40 kg of K + 52 kg of P per ha. However, this was statistically the same with plots treated with 20 kg of K+26 kg of P and 26 kg of P. The lowest biological yield of 2.65±0.28 was recorded from control plots (Table 21).

vii. Gran yield/ha (Mt)

The results for grain yield presented in Table 21 clearly show that cropping systems, Rhizobia inoculation, and fertilizers had significant effect on grain yield of soybean for the two cropping seasons. The highest mean grain yield of 1.74±0.06 and 1.55±0.10 were recorded in sole soybean (SB) plots while the lowest mean grain yield of 1.36±0.08 and 1.21±0.06 were harvested in M+B(A) plots for the 2015 and 2016 cropping seasons respectively. Rhizobia inoculation had very high significant effect on soybean grain yield where it produced 1.84±0.05 and 1.57±0.07 over the un-inoculated plots which produced 1.24±0.06 and 1.16±0.05 for 2015 and 2016 cropping seasons respectively. Phosphorus and potassium fertilization also significantly improved grain yield per hectare. The highest grain yield of 1.97±0.10 and 1.81±0.16 metric tons were harvested in plots which received doubled combined fertilizers 40kg K+52 kg P per hectare, while the lowest yield of 1.09±0.10 and 0.82±0.06 metric tons were harvested in control plots for 2015 and 2016 cropping season respectively.

viii. Harvest index

The results of the current study indicated that Harvest Index (HI) was significantly affected by cropping systems, Rhizobia inoculation, and P and K fertilization (Table 21). Rhizobia inoculation had significant effect on HI for 2015 cropping season only. Inoculated plots produced significantly lower (0.36±0.01) harvest index relative to un-inoculated plots (0.40±0.01). In 2016 cropping season, inoculation had no significant effect on harvest index. The sole soybean produced higher HI of 0.40±0.02 in 2015 cropping season while the results were different in 2016 cropping season where SB produced lower HI 0.35±0.02 compared with the intercropped soybean which produced statistically the same harvest index. Phosphorus and potassium fertilization also significantly affected the HI where the plots treated with 20 kg K+26 kg P recorded the highest HI (0.44±0.02) in 2015, and the doubled combined fertilizers (40 kg K+52 kg P) resulted in higher (0.48±0.03) harvest index over all other treatments in 2016 cropping season. The lowest harvest index of 0.36±0.02 and 0.33±0.04 was recorded from the control plots in 2015 and 2016 cropping seasons respectively. Rhizobia inoculation had no significant effect on HI for 2015 cropping season.

Table 21: Effects of cropping systems, rhizobia inoculation and P and K fertilization on soybean yield attributes for 2016 and 2015 cropping season

	2015 Cropping season			2016 Cropping season		
	Biological Yield Kg/plot	Gran Yield/Ha (mt)	HI	Biological Yield kg/plot	Gran Yield/Ha (mt)	HI
Cropping Systems						
SB	4.41±0.24a	1.74±0.06a	0.40±0.02a	4.27±0.29a	1.55±0.10a	0.35±0.02b
M+B(A)	3.41±0.20b	1.36±0.08c	0.36±0.01b	2.92±0.18b	1.21±0.06b	0.40±0.02ab
M+B(B)	3.65±0.23b	1.51±0.09b	0.39±0.01ab	2.84±0.19b	1.32±0.07b	0.45±0.02a
F Statistics	23.95***	19.19***	2.42*	17.24***	12.83***	6.70***
Rhizobia						
With out	2.96±0.16b	1.24±0.06b	0.40±0.01a	2.76±0.22b	1.16±0.05b	0.42±0.02a
With	4.69±0.15a	1.84±0.05a	0.36±0.01b	3.92±0.15a	1.57±0.07a	0.39±0.01a
F Statistics	196.71***	139.43***	6.03**	26.92***	53.80***	2.61ns
Fertilizer (kg ha^{-1})						
Control	2.32±0.22e	1.09±0.10e	0.36±0.02b	2.65±0.28b	0.82±0.06d	0.33±0.04d
20 K	2.96±0.26d	1.28±0.11d	0.36±0.02b	2.75±0.28b	1.04±0.07c	0.36±0.02cd
40 K	3.33±0.28d	1.34±0.11cd	0.37±0.02b	3.09±0.28ab	1.20±0.07bc	0.39±0.03cd
26 P	3.88±0.31c	1.52±0.11c	0.36±0.01b	3.77±0.40a	1.37±0.07b	0.37±0.03bcd
52 P	4.51±0.31b	1.71±0.11b	0.38±0.02b	3.47±0.34ab	1.62±0.08a	0.47±0.04a
20 K +26 P	4.97±0.33a	1.87±0.10ab	0.44±0.02a	3.81±0.38a	1.68±0.13a	0.42±0.03ab
40 K + 52 P	4.79±0.31ab	1.97±0.10a	0.40±0.02ab	3.85±0.53a	1.81±0.16a	0.48±0.03a
F Statistics	37.24***	23.49***	2.25*	3.02**	24.10***	3.39**
Interactions						
CrSyst*Rhiz	8.49***	2.99*	4.53**	0.40	3.02*	0.95
CrSyst*Fertili	1.44	1.48	1.52	0.59	1.25	1.03
Rhiz*Fertili	1.11	0.78	0.53	2.044	0.40	2.29
CrSyst*Rhiz*Fert	0.58	0.47	0.59	1.48	1.48	1.77

CroSyt: Cropping Systems; Fert: Fertilizers; Rhiz: Rhizobium; SB: Sole soybean; M+B (A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; Values presented are means ± SE; *,**, ***: significant at p ≤ 0.05, p ≤ 0.01, p ≤ 0.001 respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

ix. Interactive effects of cropping systems and rhizobia inoculation on soybean biological yield, grain yield and harvest index (2015 cropping season)

There was a significant interaction between the cropping systems and Rhizobia inoculation on soybean biological yield, grain yield and harvest index in the 2015 cropping season. The results indicated that inoculated plots were superior in biological yield and grain yield over un-inoculated plots. On the side of cropping systems, SB produced higher biological and grain yield per plot over the other two cropping systems [M+B(A) and M+B(B)]. Furthermore, the grain yield were significantly higher in M+B(B) compared with M+B(A) both inoculated and un-inoculated plots (Fig. 16).

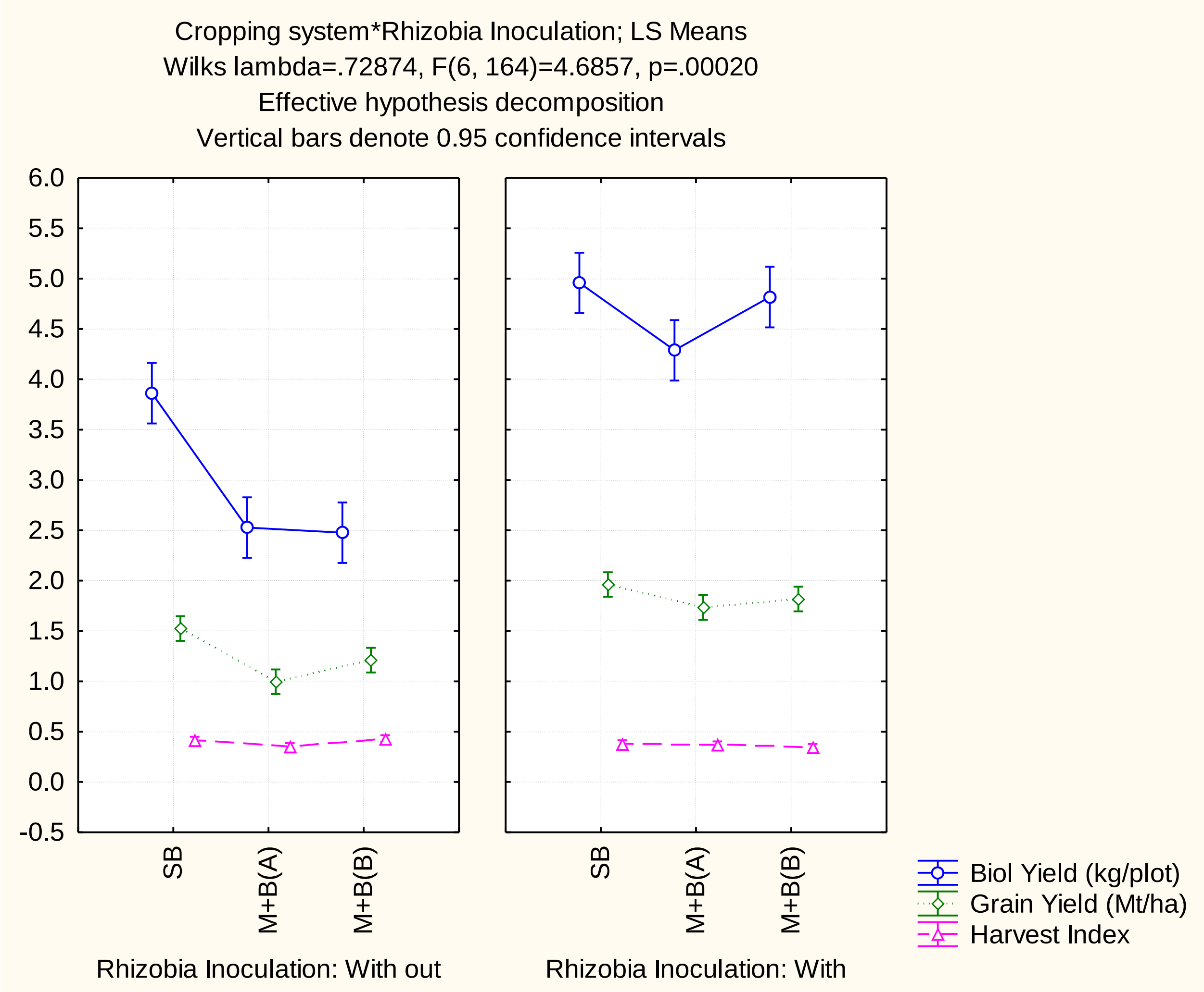

Figure 16: Interactive effects of cropping systems and rhizobia inoculation on soybean biological yield, grain yield and harvest index (2015 cropping season)

x. Interactive effects of cropping systems and Rhizobia inoculation on soybean grain yield per plot and grain yield per hectare (2016 cropping season)

The results presented in Fig. 17 showed the significant interaction between cropping systems and Rhizobia inoculation on soybean grain yield per plot and grain yield per hectare. It is clear from Fig. 17 that the grain yields were higher in inoculated plots compared with un-inoculated plots. Furthermore, the SB produced higher grain yield over other cropping systems in both inoculated and un-inoculated plots. In un-inoculated plots, intercropping at different spacing did not give significant effects on grain yield. However, in inoculated plots the grain yield this trend SB> [M+B(B)]> [M+B(A)] (Fig.17). Again the effects of maize shading have been seen here where the intercropping at narrower spacing [M+B(A)] produced significantly lower grain yield over other cropping systems.

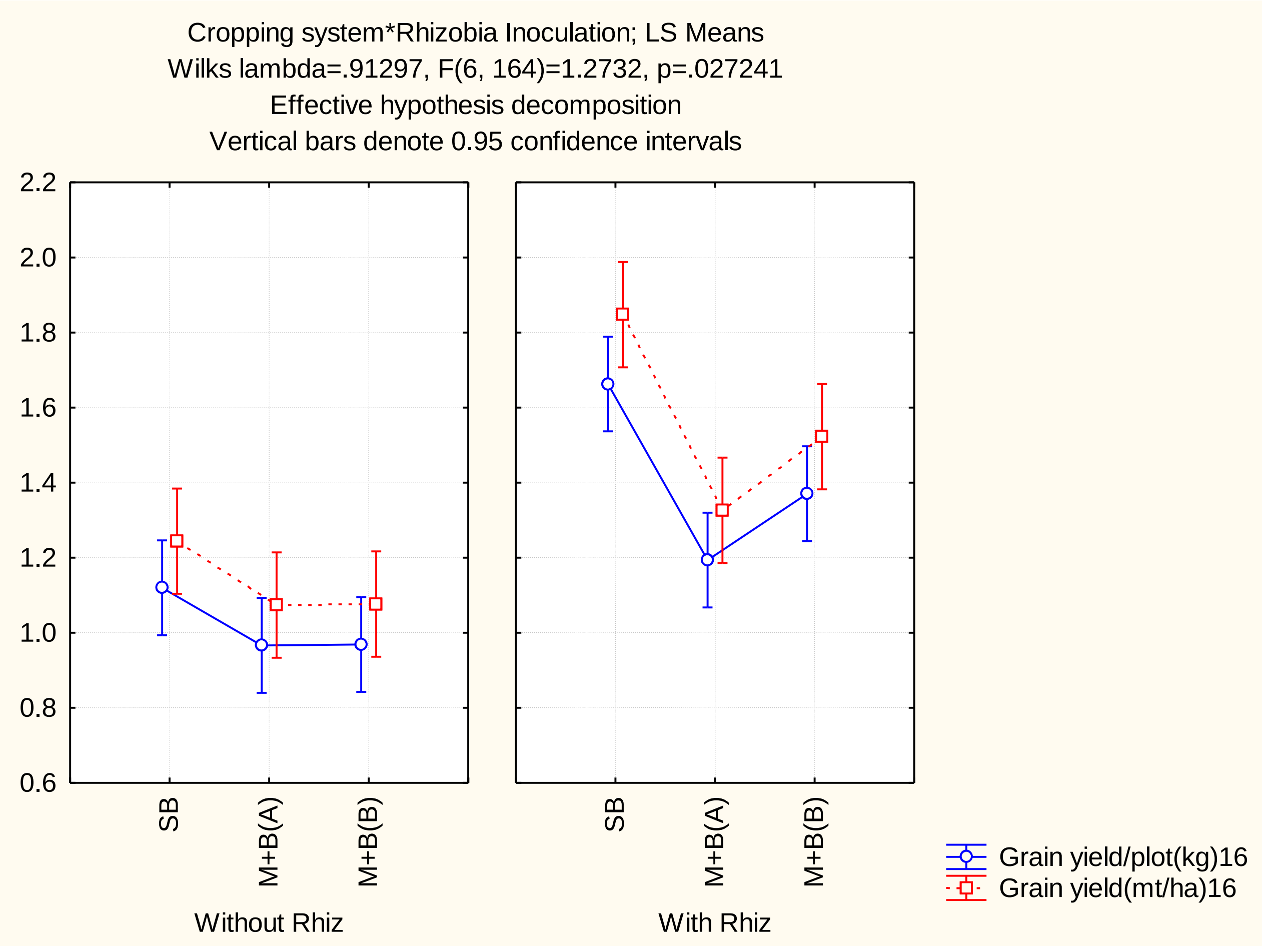

Figure 17: Interactive effects of cropping systems and rhizobia inoculation on soybean grain yield per plot and grain yield per hectare (2016 cropping season)

10.3.2. Maize yield attributes

i. Maize cob length and weight

Cropping systems significantly improved cob length and cob weight in this study. Intercropping at a wider spacing with *Rhizobia* inoculated soybean [M+B(B)+R] significantly produced long cobs compared with narrower spacing [M+B(A)+R], intercrop without inoculation [M+B(A)-R and M+B(B)-R] and sole maize (SM) for the two cropping seasons. Cropping systems had significant effect on cob weight for the 2016 cropping season only. Likewise, the maize cob were significantly heavy in wider spacing and inoculated soybean [M+B(B)+R] over all other treatments (Table 22).

P and K fertilization also significantly improved the cob length and weight in the current study. Application of 52 kg of P and combined P and K at lower doses and their doubled dose statistically produced cobs of the same length and weight which were significantly higher relative to other treatments. The control plots produced shorter and light cobs when compared with other fertilizer treatments for all cropping seasons (Table 22).

Table 22: Effect of cropping systems, P and K fertilization on cob length and cob weight of maize grown under soybean intercropping system

	2015 Cropping Season		2016 Cropping Season	
	Cob length (cm) 2015	Cob weight (kg) 2015	Cob length (cm) 2016	Cob weight (kg) 2016
Cropping Systems				
SM	12.47±0.25b	0.15±0.007a	13.70±0.29b	0.18±0.006bc
M+B(A)-R	12.81±0.19b	0.16±0.008a	13.59±0.27b	0.17±0.005c
M+B(B)-R	12.93±0.32b	0.15±0.009a	13.73±0.23b	0.18±0.008bc
M+B(A)+R	13.48±0.27a	0.14±0.007a	14.06±0.31b	0.19±0.008b
M+B(B)+R	13.78±0.32a	0.16±0.007a	14.84±0.20a	0.21±0.006a
Fertilizer (kg ha^{-1})				
Control	11.51±0.25d	0.14±0.007a	12.69±0.29d	0.14±0.005d
20 K	12.25±0.18c	0.14±0.009a	13.92±0.28bc	0.17±0.009c
40 K	12.87±0.24bc	0.15±0.007a	13.33±0.27cd	0.18±0.006bc
26 P	12.97±0.22b	0.17±0.008a	14.13±0.31ab	0.19±0.006b
52 P	13.77±0.25a	0.17±0.011a	14.67±0.19a	0.20±0.007a
20 K +26 P	14.01±0.31a	0.14±0.007a	14.53±0.36ab	0.21±0.005a
40 K + 52 P	14.27±0.28a	0.15±0.011a	14.61±0.26a	0.21±0.006a
F-Statistics				
CropSyst	7.97***	1.66ns	6.51***	11.09***
Fert	20.27***	1.72ns	9.87***	23.00***
CropSyst*Fert	0.91ns	0.95ns	1.98**	1.47ns

CroSyt: Cropping Systems; Fert: Fertilizers; SM: Sole maize; M+B(A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; –R and +R un-inoculated and inoculated soybean respectively; Values presented are means ± SE; **, ***: significant at $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

ii. Maize grain and biological yield

The results presented in Table 23, showed that cropping systems significantly influenced the gain and biomass yield of maize for the two cropping seasons. In 2015 cropping season, maize grown as mono crop (SM) and intercropping at a wider spacing with Rhizobia inoculated soybean [M+B(B)+R] statistically produced the same gain and biological yield over other cropping systems. Intercropping at a narrower spacing with Rhizobia inoculated soybean [M+B(A)+R] and intercropping at wider and narrower spacing un-inoculated soybean produced lower grain and biological yield (Table 23). In 2016 cropping season, maize grown as mono crop (SM) produced higher grain yield over all other cropping systems. Intercropping at a wider spacing with Rhizobia inoculated soybean [M+B(B)+R] significantly produced higher biological yield over other cropping systems in 2016 copping season, followed by maize grown as mono crop (SM). For the two cropping seasons, intercropping at a narrower spacing without soybean inoculation produced lower grain and biological yield relative to all other cropping systems (Table 23).

For the two (2015 and 2016) cropping seasons, maize grain and biological yield were influenced by P and K fertilization. Application of P and K at any level significantly increased grain and biological yield of maize in the two cropping seasons over the control (Table 23). The combined fertilizers at lower dose and doubled dose yielded higher grain and biological yield relative to singly applied fertilizers and the control. However, P applied at 52 kg ha^{-1}, yielded relatively the same grain and biological yield as the combined P and K at lower and doubled dose (Table 23).

iii. Maize harvest index (HI)

For the two (2015 and 2016) cropping seasons, there was no significant effect of cropping systems on harvest index of maize. In the year 2016, fertilizer application also had no significant effect on harvest index of maize. P and K fertilization significantly influenced harvest index of maize. The HI was higher in the plot fertilized with 20 kg K ha^{-1} and the control relative to other treatments (Table 23).

Table 23: Effect of cropping systems, P and K fertilization on yield of maize grown under soybean intercropping system

	2015 cropping season			2016 cropping season		
	Grain Yield (kg/ha) 2015	Biomass (kg/ha) 2015	HI	Grain Yield (kg/ha) 2016	Biomass (kg/ha)2016	HI
Cropping Systems						
SM	1164±0.62a	2109±0.59ab	0.55±0.02a	1307±0.48a	2676±1.62ab	0.50±0.02a
M+B(A)-R	1017±0.44b	1862±0.67c	0.55±0.02a	1096±0.34c	2254±1.09c	0.50±0.02a
M+B(B)-R	1050±0.51b	1940±0.68c	0.54±0.02a	1121±0.49c	2397±1.16bc	0.47±0.02a
M+B(A)+R	1096±0.59ab	1995±0.90bc	0.56±0.03a	1141±0.45bc	2471±1.42abc	0.48±0.02a
M+B(B)+R	1178±0.49a	2166±0.99a	0.56±0.03a	1202±0.37b	2717±1.60a	0.47±0.02a
Fertilizer (kg ha^{-1})						
Control	791±0.31c	1595±0.93e	0.52±0.04a	897±0.34e	1786±0.78d	0.52±0.03a
20 K	956±0.31b	1787±0.54d	0.54±0.02a	1037±0.32d	2025±0.70cd	0.52±0.02a
40 K	1028±0.34b	1873±0.58d	0.56±0.03a	1096±0.29cd	2240±0.79bc	0.50±0.02ab
26 P	1060±0.35b	2072±0.65c	0.52±0.02a	1169±0.28c	2479±0.89b	0.48±0.02ab
52 P	1255±0.62a	2100±0.61bc	0.61±0.04a	1297±0.30b	2830±1.32a	0.47±0.02ab
20 K +26 P	1292±0.59a	2271±0.68ab	0.58±0.04a	1378±0.37a	3046±1.91a	0.47±0.02ab
40 K + 52 P	1326±0.47a	2401±0.76a	0.56±0.03a	1339±0.42ab	3116±1.30a	0.44±0.02c
F-Statistics						
CropSyst	3.74**	5.06**	0.09ns	13.79***	3.75**	0.68ns
Fertil	21.18***	18.69***	0.92ns	43.00***	18.95***	1.50ns
CropSyst*Fert	0.87ns	0.84ns	0.94ns	1.17ns	0.47ns	0.52ns

CroSyt: Cropping Systems; Fert: Fertilizers; SM: Sole maize, M+B(A): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 20 cm, maize and soybean respectively; M+B (B): Maize/soybean intercropped at a spacing of 75 x 60 cm and 75 x 40 cm, maize and soybean respectively; –R and +R un-inoculated and inoculated soybean respectively; Values presented are means ± SE; **, ***: significant at p ≤ 0.01, p ≤ 0.001 respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

10.4. Discussion

The results of the current study showed that cropping systems significantly affected the number of pods per plant. The results showed that soybean grown as monocrop produced more pods compared with those in intercropping systems in two cropping seasons. These results concur with that of Zerihun *et al*. (2014) who found that sole soybean produced more pods than those intercropped with maize. The same results were found by Nyasasi and Kisetu (2014) and Zerihun *et al*. (2014) who reported that sole cowpea and sole soybean produced more pods than those intercropped with maize. The possible explanation for this is that the intercropped soybean experienced the effects of shading from maize which lowers the number of pods. The results also showed interactive effect between cropping systems, rhizobia inoculation and fertilizer levels. The intercropping at narrower spacing [M+B(A)] produced many number of pods in un-inoculated plots compared with the wider spacing of M+B(B). The reason could be due to intra specific competition of soybean for fertilizers in

M+B(B) which had four seeds per hole compared with M+B(A) which had two seeds per hole. Furthermore, the results found in this study showed that cropping systems influenced biological yield of soybean whereby sole soybean produced higher biological yield than the intercropped soybean. The grain yield of soybean was significantly influenced by cropping systems in this study. The highest grain yield was obtained from sole soybean compared with intercropped soybean in both cropping seasons. The results from the current agree with the study by Mbah and Ogidi (2012) who reported that higher yield of soybean was obtained in sole soybean. In this study, there was significant interaction between the cropping systems and Rhizobia inoculation on soybean biological and grain yield. Rhizobia inoculation increased the biological and grain yield over un-inoculated treatments. Furthermore, sole soybean produced high biological and grain yield over the intercropped soybean. Moreover, the grain yield were significantly higher in M+B(B) compared with M+B(A) for both inoculated and un-inoculated plots. The possible explanation for reduced biological and grain yield in intercropped soybean could be due to effect of shading from maize which suppressed the yield development of intercropped soybean. The effect of shading was also significant in narrower spaced intercropping relative to the wider spacing. This could have been attributed by increased inter-specific competition for growth resources among the crops under intercrop. Number of seed per pod and 100 seed weight were not significantly increased by cropping systems.

Rhizobia inoculation also significantly influenced the number pods per plant, seed per pod, 100 seed weight, biological yield, grain yield and harvest index of soybean over the control (un-inoculated treatments). These findings are in line with previous researchers (Popescu, 1998; Zahran, 1999; Vargas *et al*., 2000; Hernandez and Cuevas, 2003; Menaria *et al*., 2004; Ndakidemi *et al.,* 2006; Bambara and Ndakidemi, 2010; Sajid *et al*., 2010; Nyoki and Ndakidemi, 2013; Mfilinge *et al*., 2015) who reported the significance of rhizobia inoculation on yield attributes. The improved yield attributes under rhizobial inoculation, might have been attributed by biological nitrogen fixation which improved nutrition and ultimately yield of the crops.

The current study used different level of P and K applied as single fertilizer and as combined fertilizers to assess their effects on yield attributes of soybean. The findings of this study indicated that P and K fertilization had positive influence on different yield parameters measured for both 2015 and 2016 cropping seasons. Specifically, P and K fertilization increased the number of pods per plant, biological yield per plot and grain yield per hectare over the control plots. In the 2015 cropping season, the applied fertilizer rate of 52 kg P, 20 kg K+26 kg P, and 40 kg K+52 kg P statistically produced the higher number of pods over all other treatments. This observation suggests that increasing combined fertilizers may not have significant effect on the number of pods per plant. Control treatments produced lowest number of pods (15.03±0.93) compared with all other fertilizer treatment. The highest number of pods (28.04±2.13) in 2016 was obtained from the plots treated with 26 kg of P and the lowest number was obtained from the control plot. This suggests the importance of these elements for pod formation in legumes and eventually improved grain yield. Biological yield of soybean was increasing with the increase in fertilizer level and type. For example, the trend of biological yield was increasing with the increase in K following this sequence, 0 kg K<20 kg K< 40 kg K, for the two cropping seasons. However, 20 kg K and 40 kg K had no statistical differences between them. The current study findings agrees with the recent findings by Mfilinge *et al*. (2015) who reported on the increase of yield parameters of legumes supplied with 20 kg of potassium. The results above may have been caused by the functions of K in crop which is responsible for water absorption, root growth, maintenance of turgidity, transport and stomatal regulation (Khurana and Sharma, 2000; Singh and Kataria, 2012). Furthermore, these results might be attributed by potassium which is involved in the translocation of photosynthetic products from the site of production to the plant storage parts such as fruits or roots (Uchida, 2000; Nyoki and Ndakidemi, 2016).

Supplementation with phosphorus significantly improved the yield attributes of soybean measured in this study. Phosphorus improved number of pods per plant, biological yield per plot; grain yield and harvest index of soybean in the two cropping seasons. Our finding are in agreement with previous researcher who reported that plants supplied with appropriate amount of P has resulted in increased yields over the control (Ankomah *et al*., 1995; Bolland *et al*., 2001; Ndakidemi *et al*., 2006; Magani and Kuchinda, 2009; Zafar *et al*., 2011; Ndor *et al*., 2012; Nyoki and Ndakidemi, 2013 Mfilinge *et al*., 2015). The increases soybean yield parameters have been attributed by phosphorus which is very important for photosynthesis (Nyoki and Ndakidemi, 2016), root nodulation, pod formation and grain filing (Nyoki and Ndakidemi, 2013; Mokwunye and Bationo, 2002; Nkaa *et al*., 2014). Interestingly, the combined fertilizers whether applied at lower dose (20 kg K+26 kg P) or doubled (40 kg K+52 kg P) statistically, gave the same results in most of soybean yield attributes measured for the two cropping seasons. This suggest that when applying P and K together, the lower dose could results in the desired outcome and doubling of the fertilizers may not add significant yield over the lower dose. Our results are in agreement with that of Ayub et al. (2012) who reported that increasing the rate of PK from 70-70 and 100-100 kg ha^{-1} resulted in statistically similar green forage yield. The reason for reduced or constant yield parameters at higher dose of fertilizers such as P and K is still unclear (Barben *et al*., 2007). However, the anticipated reasons could be due to the effect of toxicity of these nutrients. Interaction of P with other elements such as Zn in the soil solution could lower the yield parameters of crops (Barben *et al*., 2007).

Harvest index was significantly affected by all factors applied in this experiment. In both cropping season, the HI was higher in sole soybean compared with that of intercropped one for the 2015 cropping season. However, it was differently observed in 2016 cropping season where sole soybean (SB) produced lower harvest index over intercropped one. With Rhizobia inoculation, HI was lowered in inoculated plots compared with the un-inoculated plots which gave higher mean HI values. Again, the significant increase was observed in the first year of experiment only. Harvest index describes plant capacity to allocate biomass (assimilates) into the formed reproductive parts (Wnuk *et al*., 2013). The possible explanation for lower HI in inoculated plots may be due to the fact that inoculation increased nitrogen fixation which eventually leads to the increased shoot growth compared with un-inoculated plots. It is thought that the increased shoot growth (biomass) in inoculated plots happened before the formation of reproductive parts of soybean. Therefore, assimilates were not allocated to the reproductive parts (Reddy *et al*., 2003). P and K fertilization also influenced the HI of the soybean, where in 2015, the HI was significantly higher in plots treated with 20 kg of K+26 kg of P and in 2016, the highest HI was obtained in plots treated with 40 kg of K+52 kg of P per hectare.

Maize yield in this study was significantly influenced by both cropping systems and P and K fertilization. The yield attributes were high where maize was intercropped with Rhizobia inoculated soybean relative to intercropped plots without inoculation. The reason for improved maize yield could be attributed by biological nitrogen fixation in legumes (Li *et al*., 2005; Zhang *et al*., 2010; Latati *et al*., 2014). However, the yield of maize grown as monocrop was relatively similar to that obtained from intercrop with Rhizobia inoculated soybean. It is thought that intercropping may lead to crop competition for growth resources which eventually could reduce the yield of intercropped maize compared with sole maize.

The current study suggested that we could improve the yield of maize in intercropping systems by inoculating legumes with good strain of *Rhizobium.* Fertilization of crops with P and K at any level improved maize yield attributes for the two cropping seasons over the control. This indicates that P and K are of utmost important for crop production (Dawson and Hilton, 2011).

10.5. Conclusion

In conclusion, cropping systems, Rhizobia inoculation and fertilization with P and K significantly increased the yield and yield attributes of soybean measured in this trial. From our observation, application of K alone gave lower yield attributes of soybean compared with that of P which was seen to increase more yield parameters tested in this study. However, doubling of P from 26 kg to 52 kg per hectare may not significantly change the yield parameter of soybean. The combined P and K at their lower dose of 20 kg K + 26 kg P were observed to be the ideal combination for this study. Doubling the combined fertilizers may not significantly increase the soybean yield but rather a burden of production costs to farmers. The current study showed that doubling of K from 20 kg to 40 kg improved most of the yield parameters of soybean relative to control. From this we suggest further studies to test different combination of K and P to see how they may influence the yield parameters. Intercropping maize with rhizbial inoculated soybean significantly improved maize yield compared with intercrop without inoculation. P and K fertilization also significantly improved yield attributes over the control

CHAPTER ELEVEN

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

11.1. General discussion

Globally, N, P and K are the most limiting mineral nutrients to plant growth and crop production (Buerkert *et al*., 2001; Bekunda *et al*., 2004). The current study was carried out as an effort to improve farm productivity by using integrated soil management and different cropping systems. The effects of cropping systems, *Rhizobium* inoculation and fertilization with P and K on leaf chlorophyll content of soybean, growth responses of soybean and maize, yield responses of soybean and maize, Land Equivalent Ratios (LER), nitrogent fixation, nutrient uptake in soybean tissues and soybean rhizosphere mineral composition was assessed. The results from two years experiment have indicated that there were significant effects of the factors tested on the parameters measured in this study. The results from current study indicated the importance of mineral elements in chlorophyll formation in soybean leaves. It can be generalised that N, P and K are equally necessary for the formation of chlorophyll and nitrogen fixation in soybean thereby improving final yields of both soybean and maize. Mineral elements, P and K from mineral fertilizers and N from BNF were tested and found to significantly increase soybean leaf chlorophyll content. *Rhizobium* inoculation and P and K fertilizers have differently affected the root length, number of nodules and/or nitrogen fixation in soybean. It was very interesting to note that cropping systems influenced the formation of nodules whereby soybean grown under maize intercropping significantly increased the number of nodules relative to sole soybean. The increased number of nodules in intercropped soybean may have been cause by inter specific interactions between the two crops (soybean and maize). The inoculation of soybean with *Bradyrhizobium japonicum* significantly increased the soybean root length, number of nodules per plant and nitrogen fixation over un-inoculated soybean. P and K fertilization significantly increased the number of nodules per plant and nitrogen fixation over the control.

For the growth performance, the data presented in this study showed that cropping systems influenced plant height whereas intercropped soybeans were taller compared with mono cropped one indicating that there was significant completion for light. Similarly, the stem girths of soybean were greater in pure stand soybean than in intercrop indicating that there was stiff competition for nutrients which lowered the stem girths of intercropped soybean. *Rhizobium* inoculation significantly increased the plant height, stem girth, number of leaves and number of branches per plant, leaf area and finally plant vigour over un-inoculated treatments. P and K fertilization also significantly improved growth parameters of soybean that were assessed. Growth performance of maize was also influenced by cropping systems and P and K fertilization. Plant height was significantly higher in maize intercropped with inoculated soybean compared with other treatments. The increased plant height in maize intercropped with inoculated soybean is the results of nitrogen in soybean and that nitrogen has been made available for plant growth compared with other cropping systems. P and K fertilization significantly increased plant growth parameters such as plant height, number of leaves, stem girth, plant vigour and greenness over the control. This has been attributed by the enhanced uptake of these major mineral nutrients following their application in depleted soils. Interestingly, their combined application significantly increased growth traits than when they were singly applied and their combined doubled (40K+52P (kg ha^{-1})) improved plant growth over all other fertilizers treatments for the two cropping seasons.

Assessment of Land Equivalent Ratio (LER) showed that intercropping maize with soybean was advantageous because all the values of LER were above 1.0. The supply of inputs such as *Rhizobium* inoculants and P and K fertilizers significantly ($p<0.05$) increased the LERs over the control. The rhizobial inoculated plots yielded the highest LER of 1.73 and 1.61 grain and biological yield respectively compared with un-inoculated plots which yielded the lowest LER of 1.31 and 1.39 grain and biological yield respectively. However, even uninoculated plots produced LER values which are greater than 1 indicating the importance of intercropping for the increased yield. Fertilization with P and K also greatly increased the LER over the control. In this study, the wider spacing of soybean resulted to a greater LER compared with narrower spacing suggesting the use of wider spacing for legume-cereals intercropping.

Determination of yield responses in this study showed that cropping systems, Rhizobia inoculation and the supply P and K significantly increased the yield and yield attributes of soybean and maize for the two cropping seasons. Interestingly, it was found that intercropping maize with *Rhizobium* inoculated soybean significantly improved maize yield compared with intercrop without inoculation. The increased maiz yield in plots intercropped with *Rhizobium* inoculated soybean may have been attributed by nitrogen fixation in soybean which eventually improved maize yield relative to maize intercropped with un-inoculated soybean and the sole maize. Application of K alone produced lower yield attributes of soybean compared with that of P which produced more yield parameters tested in this study. However, doubling of P from 26 kg to 52 kg per hectare did not significantly change the yield parameter of soybean. Furthermore, doubling of K from 20 kg to 40 kg per hectare improved most of the yield parameters of soybean relative to control. The combined P and K at their lower dose of 20 kg K + 26 kg P was the best combination for this study. Doubling the combined fertilizers may not significantly increase the soybean yield but rather a burden of production costs to farmers.

Nutrient uptake was determined and found that there was synergistic and antagonistic interactions between the applied nutrients and those found in the soil. In this study, Fe uptake was increased is soybean intercropped with maize compared with the sole soybean. This finding indicates that inter specific facilitations occurred between maize and soybean which enhanced the uptake of Fe in intercropped soybean relative to soybean pure stand (Li *et al*., 2003). Macro and micro nutrients uptake were significantly increased with rhizobium inoculation compared with the uptake in un-inoculated soybean. Several researchers have proposed the mechanisms for increased uptake of nutrients in rhizobium inoculated legumes. Bambara and Ndakidemi (2010) and Ndakidemi *et al*. (2011) indicated microorganisms (*Rhizobium*) can change the soil pH to the level which favours the uptake of plant nutrients. Furthermore, *Rhizobium* inoculation releases to the soil dead cells which contain plant nutrients or chemical molecules that can mobilize unavailable nutrients and make them available for uptake by plants (Halder and Chakrabartty, 1993; Abd-alla, 1994; Saharan and Nehra, 2011; Makoi *et al*., 2013). In addition, *Rhizobium* inoculation produces iron carrier compound called siderophores which tent to increase the Fe in the soil and make it available for uptake by plants (White and Broadley, 2009). Fertilization of soybean with P and K significantly increased the uptake of N, P and K for both cropping seasons indicating the synergism of these nutrients. However, the uptake of calcium (Ca) and Magnesium (Mg) decreased with P and K fertilization indicating the antagonisms of P and K on Ca and Mg. The uptake micronutrients Fe and Cu were also decreased with the application of P and K. Antagonisms of plant nutrients occur when the concentration of one element increased in the soil decreases the uptake of other elements in plant tissues. The increased uptake of nutrients in crops indicates that these nutrients will be made available for human and animal bodies when feed on these crops.

The chemical properties of the rhizosphere soils were determined and found that *Rhizobium* inoculation altered most of the chemical properties. The rhizosphere soil chemical properties of such as pH, OC, EC, macro and micro nutrients (N, P, Ca, Mg, and Na) and (Fe, Cu, Mn, and Zn) respectively were significantly increased in the *Rhizobium* inoculated soybean over the control. The increased concentrations of macro and micro nutrients have been attributed by increased soil pH which favoured the availability of most plant nutrients (Bagayoko *et al*., 2000; Condron *et al*., 1993). These results strongly support the use of microorganism to improve soil chemical properties for improved plant growth, development and production. Fertilization of soybean with P and K fertilizers significantly increased the rhizosphere content of macro nutrients such as (P, K, Ca, and Mg) and also they altered the pH and EC of the rhizosphere soil relative to control. Increased concentration of macro nutrients such as Ca and Mg in the rhizosphere soil could be due to synergistic effect of P and K which made these nutrients to concentrate more in the rhizosphere soil. High concentration P and K in the rhizosphere soil could be due to P and K fertilization which increased the availability of these nutrients. Furthermore, root exudates may have contributed to the increased macro nutrients in the plots treated with P and K. It was also noted that the rhizosphere soil pH and electrical conductivity (EC) were significantly higher in P and K fertilised plots relative to control. The higher levels of EC are associated with the concentrations of ions such as N, P, K, Ca, Mg, Na, Mn, Zn, and Cu (Heiniger *et al*., 2003; Grisso *et al*., 2009; Hamzehpour and Abasiyan, 2016). Significant interactions were also reported by inoculating soybean with *Rhizobium*, and supplying P and K, in intercropping systems indicating the need for these inputs combination in the study area.

11.2. Conclusion

From this study, several contributions and discoveries have been made. Both of the factors tested (ie. Cropping systems, *Rhizobium* inoculation and the supply of P and K) significantly influenced (i) chlorophyll content in soybean, (ii) growth parameters of maize and soybean, (iii) yield and LER of both maize and soybean (iv) nitrogen fixation and (v) nutrients uptake in soybean. Soybean is known to grow better in tropical hot area. Interestingly, the current study was conducted at the base of Mount Kilimanjaro (Lyamungu) which is cold area and *Rhizobium* inoculation improved the performance of soybean in terms of growth, and yield compared with un-inoculated soybean. This opened our eyes that *Rhizobium* inoculation can enhance tolerance of soybean in cold areas and the use of rhizobia inoculation technique could safeguard nature from nitrogen pollution. It was also very interesting to note that soybean grown under maize intercropping significantly increased the number of nodules relative to sole soybean. The increased nodules in intercropped soybean were advantageous because they increased nitrogen fixation which was reflected in the improved maize yield. Since all the factors are important for normal growth and development of crops, and following the results from this study, it is concluded that intercropping systems and the supply of moderate mineral P and K fertilizers, and *Rhizobium* inoculation are important components for cereals and legume based production systems.

11.3. Recommendations and gaps

i. Generally, several parameters tested in this study have shown to perform better in combined lower rates (20 kg K ha^{-1}+26 kg P ha^{-1}) of P and K. It is therefore recommended that the combined lower rates of these fertilizers should be adopted and be used by farmers in areas with similar characteristics as that of study area. Doubling of these fertilizers may be costly and will not significantly change the performance of the crops.
ii. Assessment of LER have shown that intercropping is advantageous, suggests that farmers may be advised to intercrop maize with soybean at a recommended spacing (wider), and supplying with the tested inputs such as P and K and the rhizobium inoculation. However, application of P and K fertilizers will depend on the level of these nutrients in respective soil under consideration.
iii. This study has revealed that Nitrogen (N) fixation through legume-*Rhizobium* symbiosis is important for enhancing agricultural productivity and is therefore of great economic interest. *Rhizobium* inoculation in soybean showed a highly significant effect on nitrogen fixation over un-inoculated treatments with an increase of 63 and 55.16 (kg of nitrogen ha^{-1}) in 2015 and 2016 respectively. Therefore, government and nongovernmental organisation should consider the use of biofertilizers such as *Rhizobium* in their legume-cereal based agricultural planning in order to reduce the costs of production arising from nitrogenous fertilizers and safeguard the environment from nitrogen pollution.
iv. The findings of this study, suggest further studies to test different combination of K and P to see how they may influence the yield parameters.
v. This study was carried out in one location. Therefore, studies comprising multi location may come up with different results

vi. The mechanisms for decreased and increased uptake of Mg and Fe respectively are not clear, and hence further research may reveal the mechanisms behind their uptake in intercropping systems.

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