# Responses of Soybean (*Glycine Max* L.) Varieties to Rhizobia Inoculation and Phosphorus Fertilizer Application at Metu, Southwestern Ethiopia

**M.Sc. THESIS**

**WONDE SAFO BOSERA**

**NOVEMBER 2019**
**JIMMA, ETHIOPIA**

# Responses of Soybean (*Glycine max* L.) Varieties to Rhizobia inoculation and Phosphorus Fertilizer Application at Mettu, Southwestern Ethiopia

**M.Sc. Thesis**

**Submitted to Jimma University College of Agriculture and Veterinary Medicine Department of Horticulture and Plant Sciences, in Partial Fulfillment of the Requirements for the Degree of Master of Science in Agronomy**

**Wonde Safo Bosera**

**Major Advisor: Abush Tesfaye (PhD)**

**Co- advisor: Amsalu Nebiyu (PhD)**

**November, 2019**
**Jimma, Ethiopia**

**Jimma University**

**College of Agriculture and Veterinary Medicine**

**Department of Horticulture and Plant Sciences**

**Thesis Submission Request Format (F-08)**

Name of student: **Wonde Safo Bosera** **ID.No: RM 1246/10**

Program of Study**: Degree of Master of Science (MSc) in Agronomy**

Title: - **Responses of Soybean (*Glycine max* L.) Varieties to Rhizobia Inoculation and Phosphorus Fertilizer Application at Mettu, Southwestern Ethiopia**

I have completed my thesis research work as par the approved proposal and it has been evaluated and accepted by my advisors. Hence, I hereby kindly request the Department to allow me to present the findings of my work and submit the thesis.

Name of the student: **Wonde Safo Bosera** Signature: __________Date: ____/___/____

We, the thesis advisors have evaluated the contents of this thesis and found to be satisfactory executed according to the approved proposal, written according to the standard and format of the University and the thesis is ready to be submitted .Hence, we recommend the thesis to be submitted.

Major Advisor: Abush Tesfaye (PhD) Signature: ___________ Date: __________

Co- advisor: Amsalu Nebiyu (PhD) Signature: ___________ Date: __________

Internal Examiner (if Depends on the Verdict)

Name: _______________________________Signature: _______________ Date: __________

Decision / suggestion of Department Graduate Council/ DGC/

_______________________________________________________________________________

| | | |
|---|---|---|
| Chairperson, DGC: | Signature: | Date: |
| ______________________ | __________________ | ____________________ |
| Chairperson, CGS: | Signature: | Date: |
| ______________________ | __________________ | ____________________ |

# DEDICATION

This thesis work is dedicated to Almighty God ,Who gave me all the strength and fortitude to my wife, Misgane Melkamu who have sown the interest of my post-graduate study in me and wishing me a great career throughout my life.

# STATEMENT OF THE AUTHOR

First I declare that this thesis is my work and that all sources the materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the equipment for M.Sc. degree at Jimma University, College of Agriculture and Veterinary Medicine and is deposited at the University Library to be made available to borrower under the sale of Library. I solemnly declare that is thesis not submitted to any often institution anywhere for the awards of any academic degree, diploma and certificate.

Brief quotation for this thesis is allowable without special permission provided that accurate acknowledgement of source is made. Request for permission for extended quotation from our reproduction of this manuscript in the whole or any part may be grunted by Dean or Coordinator of the School of A graduate studies or Head of the Department of Horticulture and Plant Science where the proposed use of the material is for academic interest. In all other case, however, permission must be obtained for the author.

Name: Wonde Safo

Sign: ____________

Jimma University, Jimma

Date: _____________________

# BIOGRAPHICAL SKETECH

The author Mr. Wonde Safo was born from his father Mr. Safo Bosera and his mother Mrs. Anbesse Kitessa in 1982 in Babo Kebele, Darimu District, Ilu Aba Bor zone, of the Oromia Regional National State. He attended his elementary education at Abuna Gali Elementary School from 1990-1997 and his Secondary School at Dupa from 1998-1999 and High School from 2000-2001. After completion of High School in 2001, he joined Bako ATVET College and graduated 2004 with a diploma in Plant Sciences. Following his graduation, he was employed by the Oromia Regional National State, Buerau of Agriculture in Ilu Aba Bor zone, and Chewaka District Department of Agriculture, where he served as Development Agent from September 2005 and he joined Jimma University, College of Agriculture and Veterinary Medicine in 2007 and graduated in June, 2008 with a Bachelor of Science in Horticulture. Then, in 2017 he joined Jimma University College of Agriculture, and Veterinary Medicine to pursue his study leading to a degree in Master of Science in Agronomy.

# ACKNOWLEDGEMENTS

Above all, I thank my helper and protector, the Almighty God, for His immeasurable help, and blessings, and provision of stamina required to successfully complete this piece of work. I, sincerely, thank my major advisor, Dr. Abush Tesfaye, for his keen interest, kindness, valuable guidance, direction and assistance in all the time to complete this journey. I am also greatly indebted to my co- advisor Dr. Amsalu Nebiyu for his valuable comments, supervision and supports during the course of the thesis research work. Special gratitude goes to Jimma Agricultural Research Center, Metu Sub-Center, for the administrative and technical support provided in conducting my thesis research. I would like also to thank Ilu Aba Bora Zone, Agriculture and Natural Resource Office for the permition to pursue the M.Sc. study. My wife, Misgane Melkamu deserves special thanks for encouraging, understanding and supporting me throughout the study. I thank my brother Tefari safo, my colleagues/friends Fantahun Tessema, Masresha Yirga, Hirpho Morkata, Idilu Tafesse, for their constant encouragement and support throughout the study.

## ABBREVATIONS AND ACRONMYS

| | |
|---|---|
| ANOVA | Analysis of Variance |
| BFN | Biological nitrogen fixation |
| CDI WUR | Center for Development Innovation, Wageningen University and Research |
| ENI | Ethiopia Nutrition Institute |
| EIAR | Ethiopian Institute of Agricultural Research |
| FAO | Food and Agricultural Organization of the United Nations |
| IAR | Institute of Agricultural Research |
| JARC | Jimma Agricultural Research Center |
| MAP | Mono ammonium phosphate |
| OM | Organic matter |
| TSP | Triple super phosphate |
| USDA | United State Development Agriculture |
| US$ | US dollar |

# TABLE OF CONTENTS

## TABLE OF CONTENTS (*Continued*)

# TABLE OF CONTENTS (*Continued*)

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF APPENDICES

# LIST OF FIGURE IN THE APPENDICES

# RESPONSES OF SOYBEAN (*Glycine max* L.) VARIETIES TO RHIZOBIA INOCULATION AND PHOSPHORUS FERTILIZER APPLICATION AT METU, SOUTHWEST ETHIOPIA

## ABSTRACT

*Soybean (Glycine max (*L.*) Merril) is an important legume crop in terms of production and global trade. Soybean is an inexpensive and high quality source of protein for resource poor families and can be used for edible oil production in Ethiopia. Due to limited availability of phosphorus in the soil of Metu area, as a result of soil acidity and lack of compatible rhizobia, yield of soybean in this area was very low. Therefore, this study was initiated to determine the responses of soybean varieties to rhizobia inoculation and phosphate fertilizer application rates on some important growth, root and nodulation and yield and yield components at Metu, southwestern Ethiopia. The experiment was conducted at Metu sub-center, maremiya bet site of the Jimma Agricultural Research Center in the 2018 main cropping season. The experiment consisted of eighteen treatment combinations of three factors: i.e., two inoculation treatments (inoculation with the Rhizobia strain of SB-MAR-1495 and non-inoculated), three levels of phosphate fertilizer (0, 20, 40 kg P ha$^{-1}$ in the form of TSP) and three soybean varieties (Afgat, Clark 63K and SCS-1) using Randomized Complete Block Design in factorial arrangement with three replications. The total nitrogen and available phosphorus after harvest showed slight increase from the initial soil result although; it is categorized in the different ratings. Significant ($p<0.01$) interaction effects of variety, rhizobia inoculation and phosphate rate for all growth, nodulation characteristic and yield and yield components. Varieties showed significant ($p<0.01$) influence for all Phenological, growth, root, nodulation and yield and yield components. Phosphorus rates had significant ($p<0.01$) influence for all growth, root, nodulation and yield and yield components, phenologically on days to 90% physiological maturity. Also seed inoculation with rhizobia strains had significant ($p<0.01$) influence for phenologically on days to 90% physiological maturity, growth, root, nodulation and yield and yield components. Significant influence on plant height, leaf area, number of nodules per plant, nodule effectiveness, nodule dry weight, taproot length, and number of pods per plant, number of seed per pods, grain yield, hundred seed weight, dry biomass, harvest index and phosphorus uptake efficiency were recorded from the interaction of rhizobia inoculation and 40 kg P ha$^{-1}$fertilizer application rate . Significant and positive correlations were found for all growth, root and nodulation parameters with grain yield. Yield was highly significantly and positively correlated with dry biomass ($r=0.81$), plant height ($r=0.67$) and hundred seed weight ($r=0.65$).The combined application of the Rhizobia strain (SB-MARK 1495) and 40 kg p ha$^{-1}$ produced significantly better nodulation and yield than the rest of the treatments. Afgat variety should better results on growth, nodulation and yields of plant height, number of nodules and grain yields. Therefore, the use of rhizobia SB-MARK 1495 and 40 kg p ha$^{-1}$ found important in increasing nodulation, P uptake, dry biomass and yield of soybean in the study area. The application of inoculated rhizobia and 40 kg P ha$^{-1}$ produced the highest net benefit 34098ETB ha$^{-1}$. The highest marginal rate of return (3641%) was obtained from yield at inoculated rhizobia and 40 kg P ha$^{-1}$ of Afgat soybean variety was the most profitable treatment .However, as the study was conducted only for one season and one location, it needs to be repeated over seasons and locations for valid recommendations.*

*Keywords: Inoculation, phosphorus, symbiotic nitrogen fixation, SB-MARK-1495*

# 1. INTRODUCTION

Soybean (*Glycine max* L*)* is an important leguminous crops worldwide (Plahar, 2006). It is the first ranking source of edible vegetable oil and protein to feed humans worldwide in the near and distant future (Hartman *et al*, 2011). Soybean is believed to be a crop with the highest level of protein than any other crops, and it is estimated to have 42-45% protein. It is also an important oil grain legume, only second to ground nut interms of oil content, containing 20-25% oil on dry grain weight (Alam *et al*., 2009; Gurmu *et al.*, 2009). According to El-Aground *et al.* (2012) soybean may contain up to 30% oil that is cholesterol free and 40% protein as well as important vitamins required for healthy growth of humans. Among all the legumes, soybean has the greatest potential of producing the cheapest source of food protein as well as other essential nutrients for smallholder farmers (Rao and Reddy, 2010).Soybean is an important oil crop, and one of the most cultivated grain legumes in Sub-Sahara Africa. It also serves as supplementary feed for poultry, fish and livestock (Masuda and Goldsmith, 2009).

Soybean is non-host plant to striga, but it produces chemical substances that stimulate the germination of striga seeds. Germinated seeds subsequently, die off within a few days, because they cannot attach their root system to that of the soybean plant to draw food substances and water (MoFA and CSIR, 2005). Ugwu (2010) also shows that soybean has advantages over other legumes, such as groundnut and cowpea, interms of tolerance to diseases and pests, better storage quality and fertility improvement through biological nitrogen fixation (BNF).

South Africa is the leading country in soybean production in Africa, followed by Nigeria in 2017 (Khojely *et al*., 2018). Other countries, such as Ethiopia, Zambia, Zimbabwe, Seychelles, Botswana, Tanzania and Gabon also export significant amount of soybean (Abate *et al.*, 2012*).* In Ethiopia, there is enormous potential to intensify soybean production through mono-cropping and intercropping (Fassil, 2010). There are many favorable locations for the production of soybean, particularly in southwest and eastern part of the country, including Jimma, Bedele, Chewaka, Assosa, Pawe, Harar, Shashemane and Arsi (Wijnands *et al*., 2011). The average productivity of soybean is about 2.24 t ha$^{-1}$ while, the productivity around

Mettu area is 1.3 t $ha^{-1}$, which is by far below the national average (2.24 t $ha^{-1}$) while the potential productivity of soybean in the research plots may reach up to 3.5 t $ha^{-1}$ (Abush *et al*., 2017), and much lower than its potential yield of 6-8 metric t $ha^{-1}$ (Cooper, 2003).

Deficiency of phosphorus and nitrogen are the most important factors that limit the productivity of soybean in Ethiopia (Argaw, 2012). Phosphorus has been classified as a macronutrient, which is required by crops in a relatively large amount. In addition, phosphorus is one of the most unavailable and immobile macronutrient in the soil (Vance *et al*., 2003), and frequently limits plant growth. It is well known that symbiotic nitrogen fixation is a high phosphorus requiring process. Hence, nodule formation and nitrogen fixation are generally, limited by low P availability, which contains effective nodule number and mass as well as nitrogenase activities (Schulze *et al.*, 2006). The main soybean production areas in Southwestern Ethiopia have been known for high rainfall and soil acidity, which is associated with high p fixation and low P availability (Tesfaye, 2007). Furthermore, Abera (2013) reported increased yield of soybean as a result of application of P in Western Ethiopia.

Nitrogen deficiency in the soil might be amended by the application of chemical fertilizer, which are not affordable to farmers and negatively affects the environments. This emphasizes the importance of utilizing alternative ways of satisfying the nitrogen requirements of soybean through the use of symbiotic nitrogen fixation, using beneficial bacteria. Nitrogen fixation was becoming more attractive and economically viable nitrogen inputs that might substitute inorganic fertilizers for resource poor farmers, and it is an environmentally friendly nitrogen source (Bekere *et al*., 2012).

The awareness on the importance and use of integrated soil fertility management system is increasing, which includes the use of a combination of microbial inoculation and phosphorus fertilization to maintain the productive potential of acid soil, and maintain soil health and biodiversity sustainably (Ellafi *et al.,* 2012). Therefore, there is a need to investigate the combined effects of rhizobia inoculation and phosphate fertilizer application on the productivity of soybean. Studies conducted in Ethiopia showed that soybean nodulating rhizobia are persistent in a soil that has been inoculated before (Argaw, 2014). This is, especially, important for developing countries like Ethiopia, where farming will continue to be in the hands of smallholder farmers. Most tropical soils, including Ethiopia, do not contain the appropriate strains of bacteria (Giller, 2001). It is therefore necessary to inoculate the soybean seeds with the specific strains of rhizobia, in order to promote better growth of the host plant and effective nitrogen fixation by the nodule, especially, if legume crop has never or not recently been grown in that fields (Erker *et al*., 2014). In the absence of rhizobia bacteria inoculation, the leguminous crops may deplete the soil nitrogen, like any other non-leguminous plants, instead of replenishing the soil nitrogen store (Chandrasekaran *et al*., 2010).

The importance of inoculation with efficient strain of rhizobia in improving soybean yield and biological nitrogen fixation has already been proved in several countries (Tairo and Ndakidami, 2014). Experiment conducted at Jimma and Mettu in Southwestern Ethiopia indicated that rhizobia strain SB-MAR 1495 and SB-MOROK revealed better grain yield, nodule number and plant height compared to the rest of the strains (*i.e*., SB-6 IA2, SB-12,SB-6 I B 2) (JARC, 2015). Similarly, Tesfaye (2007) reported increased soybean yield in response to phosphorus application. Argaw (2011) estimated that the utilization of phosphate fertilizer by the plant is only 20 to 25%, which is very low and this low utilization efficiency is largely due to its chemical fixation on acidic soils.

However, improving soybean yields and biological nitrogen fixation through the identification of the right soybean variety, inoculation with efficient strains of rhizobia along with different rates of phosphorus fertilizer have not been studied widely in soil acidity prone major soybean production areas of Southwestern Ethiopia. Therefore, it is paramount important to determine the best soybean varieties that responds well to the different rate of phosphate fertilizer combined with Rhizobia inoculation that can enhance optimum productivity of the crop on soil acidity prone major soybean producing agro-ecologies of Southwestern Ethiopia.

## General Objective

- To improve soybean yields through optimization of variety, rhizobia inoculation and phosphorus fertilization application.

## Specific Objectives

- To determine the responses of soybean varieties to rhizobia inoculation and application of phosphorus fertilizer.
- To identify soybean varieties that best responds to rhizobia and phosphorus application.

# 2. LITERATURE REVIEW

## 2.1. Agro-Ecology and Growth Habit of Soybean

Soybean (*Glycine max* L.) in Ethiopia could be grown from sea level up to 2200m altitude, but performs best between 1300m and 1800m altitude with optimum temperature for growth ,nodulation, and nitrogen fixation of the crop ranges from 25-30 $^{0}$C with soil pH of 5.5 to 7.0 (Stefan *et al*., 2009; Gurmu, 2007) as cited by Tigist (2017). The ideal rain fall for the crop is between 500 to 1000mm, but it can grow in areas with as little as 180mm of rain, due to its extensive root system (Belfield *et al*., 2011) as cited by Owusu (2017).

Soybean possesses two growth phases and two growth habits (Purcell *et al*., 2014). The growth phases are vegetative phases, a phases prior to the onset of flowering and reproductive phase, a phase from the beginning of flowering onwards (Purcell *et al*., 2014). Soybean fixes atmospheric nitrogen symbiotically in its root nodule. The presence of rhizobia bacteria in the roots of soybean enables the crops to fix nitrogen in the soil, contributing to improved soil fertility (Kasasa *et al*., 2000).

## 2.2. Soybean Production and Productivity in Ethiopia

Soybean is one of the very important leguminous and oil crops, cultivated globally and believed first domesticated in China, between 2500-2300 B.C. The crop has had its habitation within South and South East Asia following migration routes among local populations within that region until late 18th century when it was introduced in Europe, being grown as an ornamental crop in England and France (Hartman *et al*., 2011). Soybean quickly gained more relevance in the early 19th century, when it was grown as an animal feed in Yugoslavia. From Europe, the crop spread to other parts of the world through, mostly, British and French imperialism, missionary work and trade (Hartman *et al*., 2011). Soybean were first grown in Ethiopia in the 1950's as a trial and the trial was discontinued because of the yields were very low, and then the trials began again in the late 1960s and with the introduction of new high-yielding cultivars in the 1970s, new interest was generated (Amare, 1987).

Throughout the 1970s, Ethiopia produced 6,000 tons of soybeans in a year, making it one of the top four African soybean producing countries. The current national production of soybean in the country is estimated at 36,635.79 ha (CSA, 2017). Both the production and the area of production for soybean are expected to increase due to growing demand of domestic processing industries and feed (both livestock and poultry) industries. Ethiopia has suitable agro-ecological conditions and vast land for investment in soybean farming or production. There are many favorable locations, particularly in West, South west and East parts of the country including Jimma, Bedele, Chawaka, Assossa, Pawe, Harar, Shashemene, and Arsi (Wijnands *et al.*, 2011) while, the actual yield productivity of the crop has been 2.24 t ha$^{-1}$ (CSA, 2017). However, it is possible to achieve the potential yield of 3500kg ha$^{-1}$ (Abush *et al*, .2017). Soybean is important, for production in rotation, especially with cereals, such as maize and sorghum, and counters effects the depletion of plant nutrients in the soil due to continuous mono cropping of cereals (CDI-WUR, 2011).

Among food legumes grown in Ethiopia, soybean is gaining more importance in recent following extensive works done to incorporate soybean as a food legume in people’s diets, either directly in seed form or processed into value-added foods (e.g., soynuts, soymilk, soy pulp) or added as a blends in traditional foods though it was introduced to the country in the 1950s (Zinaw *et al*. (2013) as cited by Bezabeh (2016).

Recently, the increasing trend of soybean in area under the crop is mainly triggered by the rising demand from domestic processing industries (Hailu and Kelemu, 2014). Large scale production of the crop may, therefore, enhance the income of small scale farmers. The country can also earn a substantial amount of foreign currency from the export of soybean grain owing to the strategic location of the country to the world's consumers. In Ethiopia, soybean productivity is far from world average, which is less than 2.24 metric ton ha$^{-1}$(CSA, 2016), mainly due to lack of appropriate production packages and promotional activities suitable for the different cropping systems and agro ecologies.

Jimma Agricultural Research Center (JARC), in collaboration with other research partners started adaptation, demonstration and popularization of soybean to coffee based farming system of Southwestern Ethiopia. Yearly a number of soybean field days organized, soybean farm visits paid and recipes exhibited to enhance knowledge exchange on soybean production and use. However, the level of soybean production, productivity, adoption, expansion patterns and consumption behaviors are either not studied or up to date information are not available. Thus, this survey was conducted in three districts viz. Kersa, Omo-Nada and Tiro-Afeta, where soybean production technologies have been demonstrated and promoted by different actors. Information about soybean production and productivity under the circumstance of the three districts uncovered and synthesized to the context of similar agro ecology of southwest Ethiopia.

## 2.3. Importance of Soybean

Soybean is a major oil crops in the world and called as a golden bean or miracle bean because of its versatile nutritional quality having 20% oil and 38 and 43% protein which has biological value as meat and fish protein and rich amino acids, like lysine and tryptophan (Reni and Rao, 2013). Bezner Kerr *et al.* (2008) reported that edible legumes which are an excellent source of dietary proteins and oils that can play an important role in meeting food needs in present situation of food shortage and widespread malnutrition. In light of these circumstances soybean has thus been variously described as a “miracle bean” or a “golden bean” because it is a relatively cheap and protein rich grain (Sanginga *et al*., 1999). It contains 40% high quality protein, 20% edible vegetable oil and a good balance of amino acids (Fekadu *et al.*, 2009; Mahamood *et al.*, 2009).Therefore, tremendous potential to improve the nutritional status and welfare of the resource poor families. Many leguminous crops provide some protein, but soybean is the only available crop that provides an inexpensive and high quality source of protein comparable to meat, poultry and eggs. Soybean protein has a great potential as a major source of dietary protein. The oil produced from soybean is highly digestible and contains no cholesterol. Soybean cake, a byproduct from the oil production is used as a high protein animal feed in many countries (FAO, 2008).

Soybean is an important raw material for food and oil processing industries; in crop rotation, due to its nitrogen fixing capacity that is important in improving soil fertility and health benefits of its consumption (Tesfaye, 2007). It is also considered as a strategic crop in fight against the world’s food shortage and malnutrition, because of its high nutritional value (Thoenes, 2014).

## 2.4. Soybean Production Constraints in Ethiopia

Soybean was introduced to Ethiopia in the 1950's (Abush *et al*., 2018). Despite this several decades since its introduction, it was not easy to achieve wider dissemination and production of soybean; especially among the small scale farmers. The main limitations for this were: lack of knowledge of the local farmers on how to utilize the crop, unavailability of attractive market for the produce and lack of systematic approach in popularizing the crop through training female farmers on how to prepare different meals from soybean. Consequently, the proportion of land in the country on which soybean was grown remained low for several years.

In spite of the importance of the crop and efforts made to enhance its production, the productivity of soybean on farmer's field has been very low *i.e*., 920-1410 kg $ha^{-1}$ relative to its potential productivity 2000-3500 kg $ha^{-1}$ (Tesfaye, 2007). The low productivity has been attributed to several constraints among which include: lack of application of the right type and amount of fertilizers, poor soil fertility management, and poor crop management practices and lack of appropriate rhizobia to inoculate.

Poor soil fertility management practices i.e., lack of use of commercial fertilizers, and lack of use of improved varieties with high yield and nutrient use efficiency are among the key factors responsible for the low productivity of soybean, especially on soils of low fertility (Bationo *et al.*, 2006). Besides, most farmers spare fertile soils for the production of cereals, especially maize, while pulses, including soybean are grown on marginal soils, usually, for crop rotation. This is due to nitrogen fixing capacity of legume crops which used to improve soil fertility for high productivity. In addition; farmers sometimes apply below the optimum level, but usually do not apply commercial fertilizers on pulse crops in general, and soybean in particular. The main reasons for low use of commercial fertilizers by subsistence farmers have been poor financial capacity, and fertilizer infrastructure (Vance *et al*., 2003), high price of commercial fertilizers, poorly developed rural transportation and distribution systems particularly, during rainy seasons. Consequently, subsistence farmers grow most crops, including soybean under very low levels of soil nutrients.

In soils of low pH, containing high amounts of Al and Fe oxides, P is deficient in the soil solution because, it is precipitated or surface adsorbed with Al and Fe as insoluble compounds (Kanyanjua *et al*., 2002). Several other essential plant nutrients which are present in the soil solution as cations are deficient. In acid soils, the productivity of soybean is affected directly or indirectly which might include injury on plant roots therefore, reducing water and nutrient uptake, reduced availability of essential plant nutrients, toxicity of Al and Manganese (Mn); and survival of microorganisms in the soil (Crawford *et al.,* 2008; Onwonga *et al*., 2008).

Ensuring good crop yields on acid soils might require several amendment measures that need to be adopted to correct nutrient deficiency these include liming, addition of organic matter, and fertilization with mineral fertilizers (Onwonga *et al.,* 2010; Masarirambi *et al*., 2012). Liming reduces $Al^{3+}$ and $H^+$ ions, as it reacts with water leading to the production of OH- ions, which react with $Al^{3+}$ and $H^+$ in the acid soil to form $Al(OH)_3$ and $H_2O$. The precipitation of $Al^{3+}$ and $H^+$ by lime causes the pH to increase, enhances microbial activity and nutrient availability (Onwonga *et al.,* 2008). Soybean as a leguminous crop relies on microbial nitrogen fixation, as source of N. However, under acid soils, the population of rhizobia bacteria is reduced and consequently nodulation and N fixation is impaired. This affects, negatively, on crop nutrition and yields. Therefore, liming acid soils for soybean production improves soils condition for microorganism development. Mineral fertilizers increase and nutrient availability in the soil solution, since they are readily available, and the addition of organic matter supply food for the microorganisms, enhancing their population, and therefore, mineralization (Crawford *et al*., 2008).

## 2.5 Factors Affecting Rhizobia Inoculation

A symbiotic association between the host plant and their nitrogen fixing bacteria is dependent upon many environmental factors and can highly affect farmer's management or agronomic practice (Hungria and Vargas, 2000). Some of the adverse environmental factors, such as, salinity, unfavorable soil pH, nutrient deficiency, mineral toxicity, extreme temperature condition, low or extremely high levels of soil moisture, inadequate photosynthesis and disease conditions might affect plant growth and development. In addition, the indigenous rhizobia populations affect root infection and nitrogen fixation by limiting the capacity of the host plant (Panchali, 2011). Intensive rainfall has a profound effect on soybean by suppressing plant growth, nodulation and yield (Matsumani *et al*, 2007) and this causes water logging on the process of nitrogen fixation (Jung *et al*., 2008). Oxygen limiting environment can reduce rhizobia activity. The bacteria are the living organism and require ample oxygen availability to be active. Biological nitrogen fixation of soybean is very sensitive to soil moisture stress compared to other physiological processes, such as photosynthesis and transpiration. The moisture stresses can adversely affect the nodule function. After exposure to the moisture stress for ten days the nodule cell wall starts to degrade resulting in senescence of bacteriods (Naya *et al.*, 2007). The drought condition can also reduce nodule weight and nitrogenase activity. The accumulation of $Na^+$ can reduce the plant growth, nodule formation and symbiotic N fixation capacity under salinity conditions (Kous *et al.*, 2010). High salt level can directly affect the early interaction between rhizobium in legume nodule formation.

Higher soil moisture is considered as one of the main problem for legume growth through its effect on nitrogen fixation in both tropical and subtropical area. Rhizobia strain ability to live in soil long period as influenced by soil temperature (Mohammad *et al*., 2012). The effectiveness and efficiency of different legumes in nitrogen fixing capacity is different. For instance, the critical temperature for nitrogen fixation is between 5 and 30$^o$C for common bean; while that of cowpea, soybean and peanut ranges between 35 and 40$^o$c (Long, 2001). Nodule formation and nitrogen fixation depends on rhizobia strain and the nodulating legume, as well as, the plant genotype. The survival of rhizobia in a well-aggregated and high temperature exposed soil is higher than non-aggregated soil and also better in dry rather than moist condition. For example, the susceptibility of *R. leguminosarum By, Trifoliithan* bradyrhizobium species is high on soil temperature remediated in sandy soil (Mohammadi *et al*., 2012). Activity and health of bacteria can deteriorate in storage as well. It is important that the rhizobia inoculum is stored in a cool dry area to avoid heat or water damage.

Rhizobia colonization in a legume rhizosphere was reduced by extreme soil PH. Nitrogen fixation inhabited by low soil pH (Van Jaarsveld *et at*., 2002). The characteristics of high acidic soil (pH<4) includes: low level of phosphorus, calcium, molybdenum, along with aluminum and manganese toxicity, which affects both the plant and rhizobia. As a result, under low soil pH conditions, nodulation and N fixation is more affected than plant growth.

High alkaline (pH>8) soils tends to be high in sodium, Chlorine, bicarbonate, Borate, which reduces N fixation.

In addition to environmental factors, agricultural management factors influence the percentage of nitrogen derived from the atmosphere as well. Management factors includes: inoculation, P-fertilization, nitrogen, choice of variety and plant density that affects the plant growth and development (Roner and Franke, 2012) as cited by Abdul-Aziz(2013). According to Peoples *et al* (2009), a symbiotic relationship between legume and soil bacteria established, when there is nodule formation, because of soil rhizobia infecting roots of legume. The potential soybean production areas of Africa that contains low nitrogen fixation activities might not produce optimum soybean yields, unless inorganic fertilizer or rhizobia inoculants are applied (Abaidoo, *et al*., 2007). Inoculation of legume depends on the presence of compatible rhizobia in the soil and their effectiveness.

Phosphorus supply has a direct effect on processes, such as specific nodule activity and nitrogen fixation (Nkaa *et al.,* 2014). Phosphorus (P) is an essential nutrient for plants participating as a structural components of nucleic acid, phospholipids and adenosine triphosphate (ATP), as a key elements of metabolic and biochemistry pathways, particularly for biological nitrogen fixation and photosynthesis (Rechardsea and Simpso, 2011). Plants absorb P in two soluble forms: monobasic ($H_2PO_4$) and dibasic ($HPO_4^2$). However, the large proportion of P is present in insoluble forms, and it is, consequently, not available for plant nutrition. Low levels of P reflects the reactivity of phosphate with other soluble components, such as aluminum in acid soils (pH<5) and calcium in alkaline soils (pH>7) (Mclaughlin *et al.*, 2011).

Phosphorus can add to the soil, naturally, being released from clay minerals, as a key weather over time. However, for agricultural use, P levels are typically mineralized artificially through the use of fertilizers. Phosphate fertilizer comes to in many forms, such as livestock manure, diammonium phosphate (DAP), triple super phosphate (TSP) and mono-ammonium phosphate (MAP). Phosphorus was immobile in the soil profile. Therefore, some growers incorporate P in to the soil profile with tillage, or apply it directly into the soil at the time of planting, so that it might be taken up and utilized by the plant root system more easily. In no tillage situation, P becomes stratified in the soil layer, due to the lack of incorporation (Hankinson, 2015).

Nitrogen availability in the soil might also reduce the rhizobia-soybean symbiotic a relationships. The plant may not initially needs the bacteria, due to excess residual nitrogen in the soil. In such cases, soybean will not recognize the bacteria reaction, and thus will not initiate nodular tissue formation. According to Salvagiotti *et al*. (2008), nitrogen fixation reduced because of the supplementation of 200-300kg N ha$^{-1}$.

## 2.6. Economic benefits of Rhizobia Inoculation and Phosphate Fertilizer in Soybean

Production of grain-legumes is increasing significantly, due to their vast use in different situations, including human food, animal feed, as well as industrial demands. Considering the increasing needs for human consumption of plant products and the economic constraints of applying fertilizer in legumes, there is a greater role for grain legumes in cropping systems especially in regions, where affordability of fertilizer is difficult (Ndakidemi *et al*., 2006). Grain legumes, such as soybean, cowpea and common bean have many potential uses and are grown in different agro-ecological zones (Yagoub *et al*., 2012). They are economically important crops used in a wide range of products (Tahir *et al*., 2009). They play a very important role in sustaining the agricultural systems. Biological Nitrogen fixation is becoming more attractive and economically viable nitrogen inputs, substitute of inorganic fertilizers for resource poor farmers, and an environmentally friendly agricultural input (Bekere *et al*., 2012). For economically viable and environmentally sensible farming practices, nitrogen inputs should be managed properly through symbiotic nitrogen fixation (Sharma *et al*., 2011).

The nutrient supply in crop production is one of the key components to higher yields (Gehl *et al*., 2005). Increased crop yields, due to mineral nutrient supplementation in the developed world are widely documented (Giller *et al*., 1998). However, Africa is reported to have the lowest use of fertilizer in the world. The per capital consumption of fertilizer in Tanzania is standing at 8 kg ha$^{-1}$ as compared with 52 kg ha$^{-1}$ for South Africa and Zimbabwe and 27 kg ha$^{-1}$ for Malawi (Walter, 2007). In Ethiopia, per capital consumption of fertilizer is 26 kg ha$^{-1}$N (World Bank, 2016). Nitrogen (N) is the most limiting nutrient for crop yields, and nitrogen fertilizers is a very expensive input in agriculture costing more than US$45 billion per year globally (Gyaneshwar *et al*., 2002). Biological nitrogen fixation can reduced the need for N fertilizers, resulting in an economy estimated in US dollar 3 billion per crop season (Nicolas *et al*., 2006).

## 2.7. Response of Soybean to Rhizobia Inoculation

The aim of legume inoculation is to ensure high, viable and effective rhizobia in the rhizosphare to allow rapid colonization, nodulation and nitrogen fixation by selected inoculant strain in order to maximize legume yield potential (Deaker *et al*., 2004). In order to obtain optimum growth of plants, the nutrients necessary for their growth need to be available in sufficient and balanced quantities. The most important constrains limiting crop yield in developing nations, especially among resource poor farmers, is the decline in soil fertility. Sustainable agriculture based on the use of microbial products is an effective option for overcoming problem of soil fertility. Commercial microbial products are containing the living cell of different types of microorganisms, which when applied to seeds, plant surface or soil, colonize the rhizosphare of the interior of the plant and promote growth (Chen *et al*., 2002).

For decades, supposed that soybean could form nodules only in association with *Bradyrhizobium japonicum* (Rodríguez-Navarro *et al*., 2011). However, in time, it was reported that soybean can also be nodulated by different species of bradyrhizobium, as well as Rhizobium, Mesorhizobium, and Sinorhizobiumfredii (Biate *et al*., 2014). Soybean nodulators includes: both slow growing rhizobia and fast growing rhizobia (e.g. R. tropici, R. oryzae and M. tianshanense (Biate *et al*., 2014).

The success of an introduced inoculant depends on the quality of the inoculant (Ronner *et al*., 2016), and the number of viable rhizobia per unit of inoculant and the number of introduced rhizobia that result in root infection are critically important for successful nodulation. Since the mobility of rhizobia in the soil is limited under real field conditions, inoculation methods must ensure that sufficient rhizobia are present around the seeds for successful nodulation (Giller, 2001). However, the plant demand for N is determined by the yield potential of a crop in a given environment. If the N demand of soybean can be matched by the indigenous rhizobia population, inoculation with even efficient rhizobia strains may not show any improvement in yield.

The survival and persistence of rhizobia are affected by soil and environmental factors (Hungria and Vargas, 2000). The survival of rhizobia in soil has been shown to be affected by extreme soil pH, desiccation, nutrient deficiencies, salinity, alkalinity, extreme temperatures, toxicities (Giller, 2001), and predation by protozoa.

## 2.8. Soybean Response to Phosphate Fertilizer

Phosphorus is an essential element for growth, development and yield of soybean and has beneficial effects on both nodulation and nitrogen fixation capacity of soybean. Phosphorus is important for optimum nodule, flower and seed formation and advancing crop maturity (Acharya *et al*., 2007). Phosphorus application significantly increased dry matter production, as well as, yield and yield contributing characters of soybean.

Agri-fax reported that the application of 60 kg $P_2O_5$ $ha^{-1}$ increased seed yield, hastened flowering and reduced plant height compared to both an unfertilized check and a rate of 30 kg $P_2O_5$ $ha^{-1}$. Adequate P enhances a fundamental process of photosynthesis, N fixation, flowering, seed production (quality and quantity) and maturation and root growth (Brady and Weil, 2002). The different responses reported by the authors may be due to the fact that the availability of inorganic phosphorus is determined by various factors, such as soil pH, soluble iron, aluminum and manganese, presence of iron, aluminum and manganese containing minerals, available calcium and calcium minerals, amount of decomposition of organic matter, and activities of microorganisms. The number of branches per plant was significantly affected by different rates of phosphorus application and this might probably be due to the cumulative effect of phosphorus on the process of cell division and balanced nutrition (Zafar *et al.*, 2003). Phosphorus application also significantly increased plant height over the control. Accordingly, maximum plant height might be due to stimulated biological activities in the presence of balanced nutrient supply.

## 2.9 .The Need to Use Phosphorus Efficiently in Soybean

Now a day, there is concern about scarcity of future P source for agriculture, since phosphate rock could be depleted in the next 50 to 100 years (Cordell *et al*., 2009). Phosphorus is an essential nutrient for soybean yield, but rock phosphate as fertilizer is a non-renewable resource and an important limiting factor in achieving optimal yields in agriculture (Smit *et* *al*., 2009). The application of phosphorus fertilizer has a direct effect on processes, such as nitrogen fixation, specific nodule activity and nodulation (Nkaa *et al*., 2014). For leguminous plants developing mechanisms that will lead to enhanced P uptake and utilization, due to high P requirement for legume symbiosis is very important. There are several mechanisms by which leguminous plants employ as adaptation to acquire P under low soil P conditions (Graham and Vance, 2001). There adaptive mechanisms includes changes in root morphology, enhanced P acquisition via mycorrhizal symbiosis, acidification of the rhizosphere by releasing $H^+$ ions, production and release of acid phosphatase (Vance *et al*., 2003). High P input levels in agriculture have led to environment problems. Phosphorus present in the soil is also not available for the plant, due to the phosphorus binding capacity of most soils (Syers *et al*., 2008). The amount of P in the soil is very low, which ranges 100 to 200 mg P per kg of soil, representing approximately 350 to 7000 kg p $ha^{-1}$ in the 25cm soil surface, although only small portion of P is immediately available for crop uptake (Grant *et al*., 2005). About 30% of the soil worldwide shows a high phosphate fixing capacity, e.g. in Southern China, Brazil and Sub-Sahara Africa (Vance and Gerard, 2016). To improve the capacity of phosphorus nutrient the application of Pi-containing fertilizer is very important, but such phosphorus is basically from non-renewable resource (Vaccari, 2009).

# 3. MATERIAL AND METHODS

## 3.1. Description of Experimental Site

The study was carried out at Mettu Agricultural Research Sub Center of the Jimma Agricultural Research Center, under the Ethiopian Institute of Agricultural Research, during the 2018 main cropping season. The sub-center is located at 600 km away from Addis Ababa in Iluabbabor Zone of the Oromia Regional National State. Geographically, it is located at latitude 8°19' 0'' N and longitude 35°35' 0''E at an altitude of 1550 m.a.s.l. Agro-climatically, it has been characterized as Tepid (slightly warm) to cool humid mid-highlands with mean  an annual rainfall of 1835 mm. The mean annual temperature ranges from 12 to 27$^{0}$C. The predominant soil type is Nitisol, which is dark red brown, and characterized by very strong to moderately acidic soil, and low soil P, specifically around experimental sites with pH of 4.5, and phosphorus level of 1.16 ppm and exchangeable acidity of 2.48 meq/100g of soil (Abush *et al*., 2017).

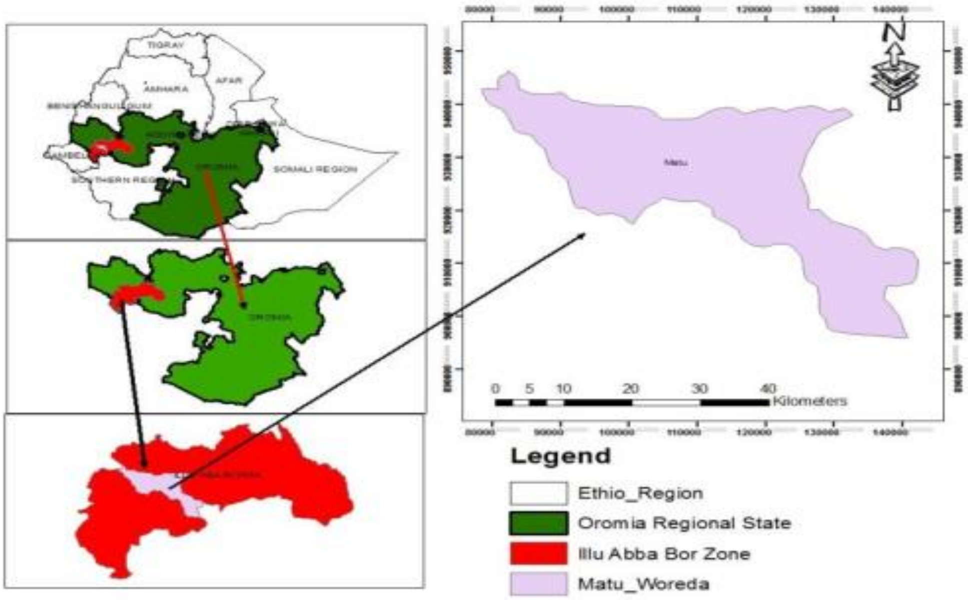


Figure 1. Location map of the study area

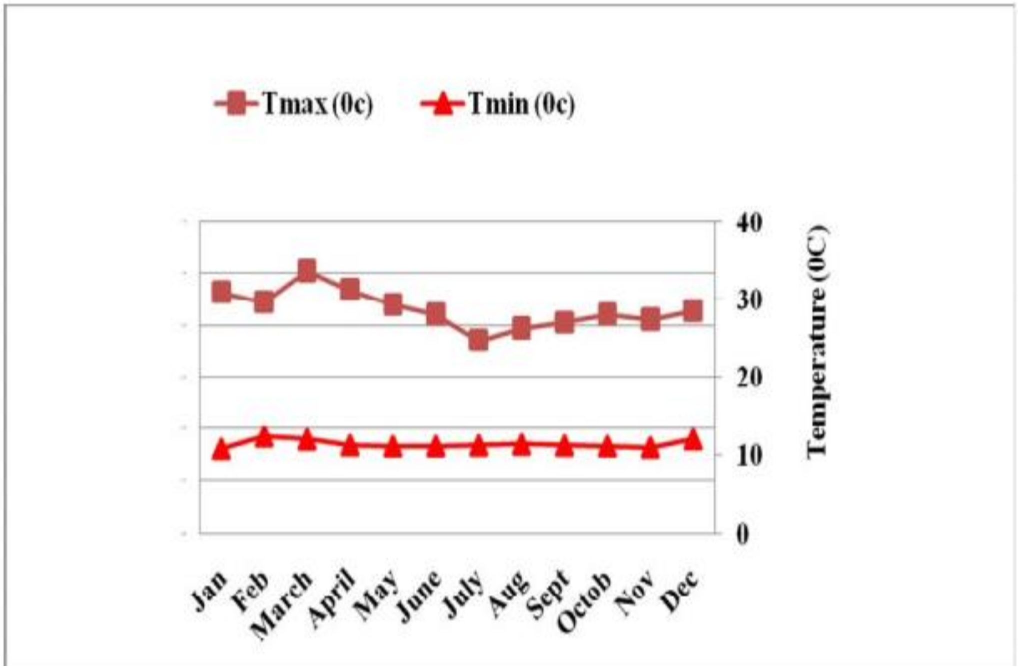
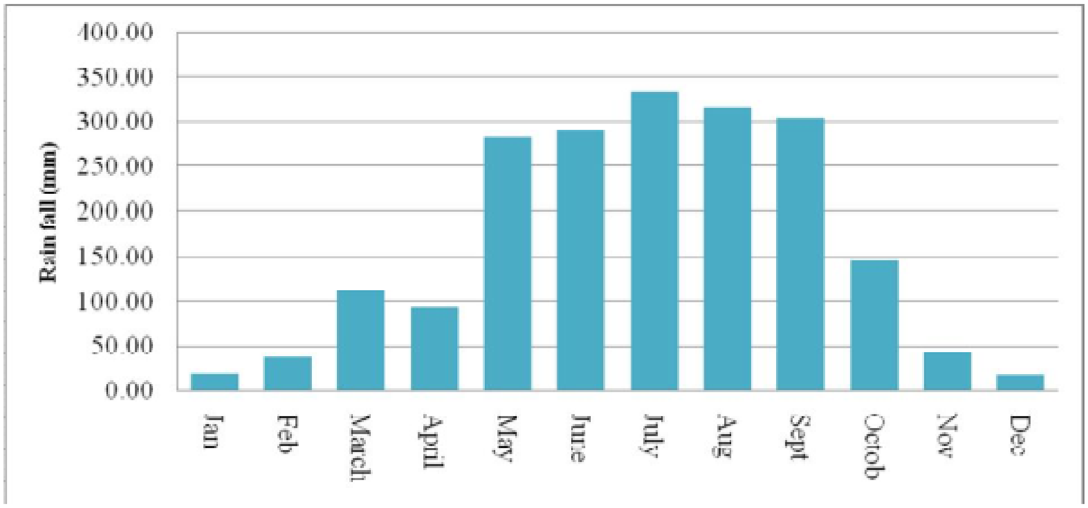


Figure 2.Mean minimum and maximum temperatures (°C) of Mettu during crop growth period in 2018



Appendix figure 1.Monthly total rainfall (mm) of Mettu during crop growth period in 2018

Source: Jimma Agricultural Research, Mettu Sub center Agro meteorology Department

### 3.2. Experimental Materials

Soybean varieties that are well adapted in the Southwestern Ethiopia, which includes: Afgat, Clark 63k, and SCS-1 were obtained from Jimma Agricultural Research Center. The varieties selected for this study were medium maturing within 120-134 days to maturity.

### 3.3. Experimental Design and Treatments

The experiment was laid out in a randomized complete block design in a factorial arrangement (3x2x3) with three replications. The treatments included: rhizobia inoculation (using SB-MARK 1495 strains) and non-inoculated, as a control, and three soybean varieties (Afgat (V1), Clark 63k (V2) and SCS-1 (V3)), and phosphorus rates (0, 20 and 40 kg P ha$^{-1}$), as rates of fertilizers. The plot size was 9.6m$^2$ (4 m x 2.4m) with the recommended spacing of 60cm between rows and 5cm between plants, and the spacing between plots and blocks were 0.6 m and 1 m, respectively.

Phosphorus was applied in the form of triple super phosphate (20% P) to each plot at the rate of either 20 or 40kg P/ha, and the P rate was determined considering the recent recommendation of 121 kg ha$^{-1}$ NPSB (NPS Boron) fertilizer rate, as a reference rate. Granulated lime (calcium carbonate) was applied to all experimental plots at the rate of 6.1tons/ha, after planting.

Table 1.Treatment combinations of the experiment

| Varieties | Rhizobia status | Phosphorus rates(kg P ha$^{-1}$) | | |
|---|---|---|---|---|
| | | P0 | P1 | P2 |
| V1 | R0 | V1R0P0 | V1R0P1 | V1R0P2 |
| | R1 | V1R1P0 | V1R1P1 | V1R1P2 |
| V2 | R0 | V2R0P0 | V2R0P1 | V2R0P2 |
| | R1 | V2R1P0 | V2R1P1 | V2R1P2 |
| V3 | R0 | V3R0P0 | V3R0P1 | V3R0P2 |
| | R1 | V3R1P0 | V3R1P1 | V3R1P2 |

V1= Afgat, V2= Clarck 63 K, V3= SCS-1, Fertilizer: P0= No P applied, P1=20 kg p ha$^{-1}$, P2=40 kg p ha$^{-1}$, R0= No Rhizobium and R1= inoculated with Rhizobia

### 3.4. Land preparation and planting

The field which was cropped with maize in the previous year was ploughed to a depth of 15 cm and harrowed to get a fine seed bed and leveled, manually, before the field layout was made. Having fine seed bed would be prepared and according to the design, field layouts would be made and each treatment would be assigned randomly to the experimental units within the blocks. Manually, sowing of the experimental treatments was done using the intra and inter row spacing of 60 cm x 5 cm. The three soybean varieties, namely: Afgat, Clark 63 K and SCS-1 were obtained from Metu Agricultural Research Sub-center of the Jimma Agricultural Research Center. This experiment was carried out under rain-fed conditions. Thinning out was done to approximately 5cm between plants in a row, 20 days after sowing, when the soil was moist and seedlings well established. This left a total of 20 plants per meter length of row. Weeding was done manually by hand using a hoe, on the 3rd and 6th week after sowing to control weeds. Each weeding operation was completed on the same day for all the blocks on the day of weeding. However, granulated lime (calcium carbonate) was applied to all experimental plots at the rate of 6.1tons/ha.

### 3.5. Experimental Procedures

Soybean seeds varieties were divided into three bowls with each one weighing 1 kg and inoculated with SB MARK 1495 rhizobia inoculants using the thick slurry methods and mixed with dry seeds, and to ensure the applied inoculants stick to the seed the required quantity of inoculants were suspended in a 1:1 ratio in 10% sugar solution for 15 minutes. The rhizobia strain SB MAR 1495 was applied at the rate of 10 g inoculant per 1kg of seed (Ajeigbe *et al.*, 2010). The inoculum was then poured onto a 1 kg seeds of each of the soybean varieties in the bowl, after sprinkling water over the seeds. The seeds and inoculum in the bowl were mixed carefully, until seeds were coated with black film of inoculants to maintain the viability of the bacteria, inoculation would be done under the shade and allowed to air dry for 30 minutes and the triple super phosphate (TSP) fertilizer was applied during planting and used as source of Phosphorus (P) fertilizer. This was drilled along the seed line in furrow and mixed with the soil before sowing the seed, then seeds sown at the recommended rate and spacing. A plot with un-inoculated seeds would be planted first to avoid contamination.

### 3.6. Soil Sampling and Analysis

Soil samples were collected before the field was ploughed. Seven soil samples were taken at the depth of 0–20 cm from each replication, and bulked to obtain one samples (Sahlemedhin and Taye, 2000). Soil samples were air-dried, ground and sieved through a 1mm sieve. Selected physical and chemical properties of the soil were analyzed using standard laboratory procedures. The composite sample was used for soil physiochemical analysis, and for the determination of lime requirement of the soil. The particle size analysis was done using the Bouyouncos hydrometer method. The soil pH was measured using a pH meter making use of the supernatant suspension of 1:2.5 soil and water mixture, respectively.

After harvesting, the soil samples were collected from each plots of replication from the surface 0-20 cm depth, and composite samples were made for selected soil chemical analysis, and then the soil samples were air dried, sieved to pass through 2 mm sieve, and placed in a labeled plastic bags and submitted to JARC soil laboratory for soil chemical properties analysis. Soil organic carbon was determined using Walkley and Black method (Walkley and Black, 1934). Whilst total nitrogen of the soil was determined by the Kjeldahl method (Bremner and Mulvaney, 1982). Available phosphorus was determined using Bray II method (Bray and Kurtz, 1945). Using Bray II methods it depends on the soil physical properties when the soil pH is more acidic and Olsen for Alkalinity of the soil. Cation Exchange Capacity (CEC) was determined by leaching the soil with neutral 1 N ammonium acetate (FAO, 2008. Soil sample after harvest was analyzed for available phosphorus and total nitrogen content for each plot to determine the effect of applied P and rhizobia strains. Similarly, the composite samples were analyzed for soil texture, Soil pH, available Phosphorus, total nitrogen, organic carbon and CEC before planting.

## 3.7. Data collected and methods of data collection

### 3.7.1. Phenological parameters

**Days to 50% flowering:** it was recorded as the number of days from emergence to the time when 50% of the plants in a plot produced at least one flower.

**Days to maturity**: It was recorded as the number of days from emergence to the time when 95% of the plants showed yellow color and their pods turned into yellow in each net plot before senescence (Fehr and Caviness, 2015).

### 3.7.2. Growth parameters

**Leaf area:** This is measured at physiological maturity from five randomly selected tagged plants, and five leaves were sampled randomly from each plant to represent the bottom, middle and upper part and measured the length and width with a ruler in centimeter, and multiplied the width by the length, and the correction factor (ck), and then we get the area of each leaf, and the average leaf area was calculated.

**The plant height**: Plant height was measured from the ground level to the highest tip of the stem on five randomly sampled plants per plot at physiological maturity, before harvest. The average plant height in cm was calculated for each treatment.

**Number of primary branches**: This was taken at the sampling periods and at physiological maturity, when all plants had ceased growth. Branches of five sampled plants from each plot were counted and the average was calculated.

### 3.7.3. Nodulation and root parameters

Nodule and root traits were measured by carefully uprooting five randomly selected plants along with the soil from each plot. Then the soil was removed by washing the root and nodules gently in a plastic container taking care not to damage the root and not to lose any nodule

**Root fresh weight**: is the weight of each roots of randomly selected five plants, and then the mean of the root weight of the five plants was computed for each plot

**Root dry weight**: the roots of five plants were dried in an oven at 70 $^{O}$c for 48 hrs to a constant weight. Dry roots were then weighted and the mean of root dry weight was recorded.

**Root volume**: the root samples of five plants were collected and immersed carefully in 1000 ml capacity plastic cylinder, which is filled up with water to a volume of 100ml. The volume of water displaced by the roots was recorded as root volume per plant

***Tap root length:*** the tap root length of five randomly selected plants was measured and the average of the root lengths was recorded

**Nodule count:** other sets of five randomly sampled plants from each plot were taken at flowering to assess nodulation. The samples were carefully dug out, retrieving detached nodules. The nodules were kept in labeled polythene bags and washed, counted and the fresh weight taken. After which, the nodules were cut opened to determine apparent effectiveness, using a blade.

**Effective nodules:** Nodules with deep dark red, slightly dark red and pink were considered effective and fixing nitrogen; while those with green or white might be considered ineffective. After this, the percentage (%) effective nodules were calculated.

**Number of nodule per plant**: which is the count of all the nodules formed by the roots of five plants were counted at flowering stage and averaged as number of nodules

**Nodule fresh weight**: The nodules were collected from five plants, and then weighed, and the average of nodule fresh weight was recorded

**Nodules dry weight**: The nodules were dried at 70 Oc for 48 hrs in an oven to a constant weight. The dry nodules were then weighted and the average of nodule dry weight was recorded.

### 3.7.4. Yield and yield components

**Number of pods per plant**: was counted from five randomly selected plants from two middle rows at maturity and expressed as an average of each plant

**Thousand seeds weight:** was counted from the harvested bulk of seeds per net plot, randomly, and their weight (g) was determined at 12.5% (Standard moisture content for soybean crops) seed moisture content by using a sensitive balance

**Number of seeds per pods**: was counted from five randomly selected plants from middle rows at maturity and expressed as an average of the five plants

**Grain yield:** was measured by harvesting the crop from the net plot area of the middle two rows. The moisture content of the grain was adjusted to 12.5%, and then, the weight was converted to kg $ha^{-1}$

Grain yield (at 12.5% moisture content) kg $ha^{-1}$ =GW *(100-MCA) /(100-MCD)... Equation 2

Where, GW= fresh grain weight, MCA= moisture content of fresh grain, MCD= moisture content of grains at 12.5% moisture

**Dry biomass**: the total above ground biomass of the net plot area of the middle two rows was determined by harvesting close to the soil surface at physiological maturity and sun-drying it to gain a constant weight. Finally the biomass yield of the net plot area of the plants was converted to per hectare and expressed in ton ha-1.

**Harvest index (%)**: harvest index per plot was recorded as the ratio of grain yield to above ground biomass yield per plot.

It was calculated using the flowing equation:

$$H.I=GY/BY*100$$

Where, H.I. = Harvest index, GY = Grain Yield (g), BY = Biological Yield (g) (Dagash, 2003).

### 3.8. Phosphorus uptake efficiency

Phosphorus uptake efficiency (PUE) was estimated using an equation by Albrizio *et al*. (2010).

$$PUE=\text{Concentration of P}*\text{Dry biomass yield}$$

**Agronomic efficiency (AE):** is defined as the quantity of grain yield per unit of nutrient applied.

$$AE\ (kg/kg) = \underline{Gf\text{-}Gu}/Na$$

Where Gf is the grain yield of the fertilized plot (kg), GU is the grain yield of the unfertilized plot (kg), and Na is the quantity of P applied (kg).

### 3.9. Partial Budget Analysis

Partial budget analysis would be done for economic evaluation, following the CIMMYT methodology. The cost of soybean yield was used for the benefit analysis. Marginal rate of return was calculated as change of benefit divided by change of cost. To assess the costs and benefits associated with different treatments the partial budget technique as described by (CIMMYT, 1988) which applied on the yield results. Economic analysis done using the prevailing market prices for variety, rhizobia and P rate fertilizer inputs and for outputs at the time the crop (soybean) harvested. All costs and benefits were calculated on hectare basis in Ethiopia birr (Birr ha$^{-1}$). Marginal Rate of Return (MRR) will be calculated by the following formula:

$$MRR(between\,Trts, a\ \&\ b) = \frac{Change\ in\ NB(NBb - NBa)}{Change\ in\ TCV(TCVb - TCVa)} X\ 100$$

MRR of 100% implies a return of one birr on every birr of expenditure in the given variable input.

### 3.10. Statistical Analysis

The all data were examined for normality distribution and subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS, 2012) 9.3 Version software, using proc GLM procedure. Treatment differences were separated using the Least Significant Difference (LSD) procedure at 5% level of probability. Correlation analysis was carried out among the parameters to determine magnitude and degree of their relation.

# 4. RESULTS AND DISCUSION

## 4.1. Soil physical and chemical properties of the Experimental site

Soil texture analysis after planting revealed that clay, sand and silt proportions of the experimental site were 45, 41 and 14%, respectively; and according to Van Reeuwijk (1992), such soil are categorized as clay loam texture. Soybean tolerates a wide range of soil texture and also performs well on clay loam texture. The pH of 4.62% was rated as very strong acidic (Table 1) (Walkley and Black, 1934). Soil pH is an important factor for plant growth, as it affects nutrient availability, nutrient toxicity and has a direct effect on the protoplasm of plant root cells .Soybean grows well in the soil pH of 6.0 or higher, but can also tolerate a pH of 4.3 to 4.8 (Munns *et al.*, 1981).

Soil organic carbon (2.80), and total nitrogen (0.28%) of the experimental site (Table 1), according to Nelson and Somers (1982); and Bruce and Rayment (1982) might be regarded as medium. The reason for the relatively low organic carbon and total nitrogen could be due to the extensive tillage for cereal crops that have been planted earlier which depleted soil nutrients and most of the soil nutrients were removed by crops, leaching, gaseous and soil erosion. Increased temperature and rainfall are found to lead in the reduction of soil organic matter contents. Soil organic carbon is the most important property of the soil that explains the variation in rhizobia population (Venkateswarlu *et al.*, 1997). Hence, there is a need to build up the population by additional organic matter as well as, inoculation of the rhizobia strains.

The available phosphorus was 1.93ppm and according to Bray and Kurtz, (1945) this level is considered very low (Table 1). Soil erosion and crop removal are the major ways by which soil phosphorus is lost. The low available P was probably ascribed to the high P fixation caused due to soil acidity and heavy rainfall in the area that aggravated leaching of the basic cations (Tesfaye, 2007). Therefore, adequate phosphorus fertilization is required for optimum crop production. As per the recommendation of Landon (1991), the Cation exchange capacity (CEC) of the soil might be considered medium. This implies that the medium the Cation exchange capacity, the medium the negative charge, and the medium the cations that can be held (Marschner, 1995). Soybean grows well in soils with more than 25 ppm available P, 0.1%N, 25 meq/100g CEC and 1.5% OM. Soil analysis results depicted that P nutrient was below the critical levels for soybean growth and development in the study area. Therefore, phosphate fertilization will have paramount importance in improving the soils to improve soybean growth and productivity.

Available P and total nitrogen of the soil showed slight increase after harvest from the initial soil analysis result. However; it was categorized in parallel rating. This might be due to the fact that more than 80% of the total shoot nitrogen at harvest was found in the grain. Thus, even if, all the soybean residues incorporated into the soil significantly contributes to the soil nitrogen pool (Myers, 1992); similarly, phosphorus might be utilized by crop shoot and grain biomass, through crop removal. As a result, both available P and total nitrogen were very low and medium, which were blow the critical level. According to Landon (1999) available phosphorus below 5 ppm is rated as low.

Table 2.Soil physical and chemical properties of Maremiya experimental study area before and after harvesting

| Properties | Value before planting | Value after harvesting | Rating | Methods |
|---|---|---|---|---|
| Sandy | - | 41 | | |
| Silt | - | 14 | | |
| Clay | - | 45 | | |
| Soil PH(1:2.5) | 4.3 | 4.68 | Strong acidic | Walkey and Black ,1994 |
| Organic carbon (%) | 2.61 | 2.80 | Medium | Nelson and Somers ,1982 |
| Total N (%) | 0.23 | 0.28 | Medium | Bruce and Rayment,1982 |
| Available P (ppm) | 1.17 | 1.93 | Very low | Bray and Kurtz,1945 |
| EC(cmol/kg) | 4.38 | 2.49 | Medium | Landon ,1991 |
| Soil texture Class | Clay loam | | | |

## 4.2. Responses of three Soybean varieties to interaction of Rhizobia Inoculation and Phosphorus Application for Different Traits

The varieties showed highly significant differences ($p < 0.01$) for growth, yield and yield components, root and nodulation parameters (Appendix Table 1).Rhizobia inoculation also showed highly significant difference ($p<0.01$) for the root parameter of taproot length. Phosphorus fertilizer application showed highly significant ($p<0.01$) effect on all the growth, yield and yield components, root and nodulation parameters (Appendix Table 1).The interaction of variety X rhizobia X phosphorus application also showed highly significant ($p<0.01$) effect on all growth, yield and yield components, root and nodulation parameters, *i.e.*, leaf area, nodule dry weight, number of nodules per plant, number of pods per plant, taproot length, grain yield, agronomic efficiency and harvest index( Appendix Table 2). Hence, all the results will be presented and discussed based on the three way interactions.

## 4.3. Phenological Parameters

### 4.3.1. Days to 50% flowering (No)

The latest days to flowering of 63 days was recorded for the soybean SCS-1 variety under inoculated condition and no phosphorus fertilizer application (Table 3). However, the earliest flowered soybean variety was Afgat, under Rhizobia inoculation and with 20 kg p $ha^{-1}$ application (Table 3).

The study revealed that rhizobia inoculation delayed the number of days to flowering which suggests that inoculation, had influence on days to 50% flowering. The prolonged days to flowering could be due to the rhizobia inoculation that facilitated nitrogen fixation and enhanced vegetative growth, which in turn extended the flowering date. Nitrogen produced by biological nitrogen fixation, as part of the protein compound, enzyme and effective compound in energy transfer takes part in the structure of DNA, in the structure of chlorophyll, and direct impact on vegetative growth (Abera, 2013). Soybean inoculation increased the leaf chlorophyll content and plant biomass, and the leaf chlorophyll content of the plant remained at high levels until, the pod filling stage, as a result of delays in days to flowering (Abera, 2013).

This result is in agreement with Tairo and Ndakidemi (2014), who reported that inoculation induced late flowering in soybean in both glasshouse and field experiments. This might be due to the fact that sufficient nitrogen produced by $N_2$ fixation promoted vegetative growth which in turn extended days to flowering.

Phosphorus application was non-significantly ($P<0.05$) affected days to flowering. This could be due to the presence of adequate P applied to enhance shoot and root growth and promote early flowering. Similarly, Gidago *et al.* (2011) reported that though the effect of P was not significant, P application has slightly reduced the days to 50% flowering on haricot bean as P rate increased. The three ways of interaction were non-significantly influenced on days 50% flowering. Argaw (2012) reported the non-significant effect of co-inoculation of Bradyrhizobium japonicum and Phosphate Solubilizing Pseudomonas spp on days to 50% flowering.

### 4.3.2. Days to 90% physiological maturity (No)

Afgat variety at the rate of 0 kg ha$^{-1}$ P application and with the inoculation of Rhizobium bacteria was the latest matured with 119 days to maturity; while the earliest matured (114 days to maturity) varieties were SCS-1, with Rhizobia inoculation and 40 kg ha$^{-1}$ P application, and Clarck 63 K and Afgat, with both no Rhizobia inoculation and 40 kg ha$^{-1}$ P fertilizer application (Table 3).

The result revealed that days to maturity delayed in response to phosphate fertilizer application relative to unfertilized plots (Table 3). This might be due to the important role of phosphorus, as the vital component of adenosine triphosphate (ATP). Phosphate application of 20 and 40 kg P ha$^{-1}$ significantly decreased days to maturity under both rhizobia inoculated and uninoculated conditions respectively, over the control (0 kg P ha$^{-1}$) (Table 3). Soybean needs phosphorus for adequate growth and nitrogen fixation. Sufficient phosphorus levels are also required to enhance different plant organs growth and promote nodulation and early maturity (Kamara *et al.*, 2010). Phosphorus is a very important nutrient for plant cell division, growth and root lengthening, seed and fruit development, and early ripening, as well. In line with this, Mengel and Kirkby (1987) observed that phosphorus is known for its effect to promote maturity. The other reason for hastening maturity could be due to the mobilization of leaf nitrogen to the reproductive organs for development (Alemu, 2013).

Soybean maturity was delayed by a result of inoculation rhizobia. The delay in maturity of soybean might be due to the fact that the $N_2$ fixed by inoculation promoted vegetative growth the crop. Similarly, Wafaa *et al*. (2002) reported that inoculation enabled soybean to display better growth and increase in days to flowering. The interaction between the varieties, inoculation and p application did not significantly (p=0.067) influenced days to physiological maturity.

Table 3.Responses of three soybean varieties to rhizobia treatment and rate of phosphorus application for flowering date and 90% days to physiological maturity

| Varieties | Inoculation Status | Number of days 50% Flowering | | | | Number of days 90% Physiological maturity | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Phosphorus application(Kg ha$^{-1}$) | | | Mean | Phosphorus application(Kg ha$^{-1}$) | | | Mean |
| | | 0 | 20 | 40 | | 0 | 20 | 40 | |
| Afgat | Inoculated | $53.6^{de}$ | $52^{e}$ | $53.8^{e}$ | 52.67 | $119^{a}$ | $116^{de}$ | $116^{cd}$ | 117 |
| | Uninoculated | $53^{e}$ | $53^{e}$ | $53^{e}$ | 53.00 | $114^{g}$ | $116^{c}$ | $114^{fg}$ | 115 |
| Clark 63K | Inoculated | $57^{cd}$ | $58^{b}$ | $55^{cd}$ | 56.67 | $115^{ef}$ | $117^{b}$ | $116^{c}$ | 116 |
| | Uninoculated | $56^{cd}$ | $58^{b}$ | $57^{cd}$ | 57.00 | $117^{bc}$ | $114^{fg}$ | $114^{g}$ | 115 |
| SCS-1 | Inoculated | $63^{a}$ | $62^{a}$ | $62.5^{a}$ | 62.33 | $117^{bc}$ | $115^{ef}$ | $114^{g}$ | 115 |
| | Uninoculated | $62^{a}$ | $61^{a}$ | $62^{a}$ | 62.00 | $114^{fg}$ | $116^{cd}$ | $115^{de}$ | 115 |
| | Mean | 57.33 | 57.50 | 57.00 | 0.00 | 116.00 | 115.67 | 114.83 | |
| LSD (5%) | | NS | | | | NS | | | |
| CV (%) | | 1.14 | | | | 0.51 | | | |

Mean value followed by the same letter within the same column is non-significant difference at 5% level of significance.

## 4.4. Growth Parameters

### 4.4.1. Plant height (cm)

The tallest plant height of 76.53cm was recorded on Afgat variety under rhizobia inoculated and the application of 40 kg $ha^{-1}$ P fertilizer conditions, followed by the same variety at the rate of 20 kg $ha^{-1}$ P application and inoculated with rhizobia, and with no Rhizobia inoculation and 40 kg $ha^{-1}$ P application, and the variety SCS-1 with Rhizobia inoculation, and no application of P fertilizer. The shortest plant height of 56.8 cm was found with Clarck 63 K variety under no Rhizobia inoculation and application of P at the rate of 20 kg $ha^{-1}$ (Table 4).

A linear increase in plant height was observed with the advancement in age of all the soybean varieties. The plant height became slow till maturity stage, which might be due to the fact that the plant converted from the vegetative to reproductive phase of growth and development. Thus, it was also observed from the data that the increase in plant height continued up to maturity stage. Amongst the varieties, Afgat produced significantly the highest plant height of 76.53cm at physiological maturity stage; whereas, Clark 63K produced the shortest plant height of 56.8cm.

Phosphorus application affected the height of soybean. It was found that P application at the rate of 20 and 40 kg P $ha^{-1}$ significantly increased plant height of rhizobia inoculated soybean by 6.27 and 8.42 % and 20 kg P $ha^{-1}$ 5.15 % with uninoculated respectively over the control (0 kg P $ha^{-1}$). Also, El-Azouni (2008) stated that phosphate solubilizing fungi inoculated soybean plants scored significantly higher plant heights, which was 81% over the non-inoculated treatment.

This finding is in agreement with the reports of Bekere *et al.* (2012) who reported that phosphorus application of 60, 120 and 180 mg P/kg significantly influenced soybean height. The increase in plant height, due to increased phosphorus application, might be attributed to pronounced vegetative growth in response to the applied P.

Table 4. Responses of three soybean varieties to rhizobia treatment and rate of phosphorus application for plant height

| Varieties | Inoculation Status | Plant Height(cm) | | | |
|---|---|---|---|---|---|
| | | Phosphorus application((Kg ha$^{-1}$) | | | Mean |
| | | 0 | 20 | 40 | |
| Afgat | Inoculated | 59.20$^{fgh}$ | 71.60$^{b}$ | 76.53$^{a}$ | 69.11 |
| | Uninoculated | 59.86$^{efg}$ | 67.13$^{cd}$ | 71.46$^{b}$ | 66.15 |
| Clark 63K | Inoculated | 58.80$^{gh}$ | 62.06$^{e}$ | 65.83$^{d}$ | 62.23 |
| | Uninoculated | 59.20$^{fgh}$ | 56.80$^{h}$ | 61.13$^{efg}$ | 59.04 |
| SCS-1 | Inoculated | 69.46$^{bc}$ | 66.33$^{d}$ | 62.33$^{e}$ | 66.04 |
| | Uninoculated | 61.66$^{ef}$ | 66.60$^{d}$ | 62.20$^{e}$ | 63.49 |
| | Mean | 61.36 | 65.09 | 66.58 | 61.36 |
| LSD (5%) | | 2.71 | | | |
| CV (%) | | 2.60 | | | |

Mean value followed by the same letter within the same column is non-significant difference at 5% level of significance.

### 4.4.2. Leaf area (cm$^2$)

The highest leaf area of 136.5, 135.86 and 131.82 cm$^2$ were produced by the variety Clark 63K under rhizobia inoculation and application of 40 kg P ha$^{-1}$ and Afgat variety at the rate of 20 kg ha$^{-1}$ P application and inoculation of Rhizobia, and the same variety at the rate of 40 kg ha$^{-1}$ P and with Rhizobia inoculation (Table 5). The lowest leaf area was produced by the variety Afgat (86.04cm) under the control (Zero) P application and no rhizobia inoculation, SCS-1 (87.43cm) at the control (zero) application of P and with rhizobia inoculation, and the same variety (90.73cm) with rhizobia inoculation and 40 kg ha$^{-1}$ P application. The top variety showed 36.99 % leaf area increase over the genotype that produced the lowest leaf area.

All the three varieties exceeded the minimum recommended leaf area index value of 3.5 – 4.0 by 10WAP, when the plants had 100% flowered (Westgate, 1999; and Board and Harville, 1992). This is important at their productive stages for improved soybean yield. This finding is in agreement with the report of Malone *et al.* (2002) that early maturing soybean genotypes achieve minimum leaf area values required for maximum potential yield at the early reproductive growth stage. Phosphate is also very important for leaf expansion, increase in leaf surface area and higher number of leaves. Phosphate application has been reported to increase shoot growth and leaf growth of soybean and other leguminous crops (Cadish *et al*., 1992). Reduction in phosphate supplementation resulted in decrease in shoot growth, specifically in leaf size. A reduction in leaf area with decrease phosphate fertilizer implied changes in leaf initiation rates and activity of shoot apical meristem. Decreased size of individual leaves could be related to changes in either cell division or cell expansion of both (Chiera, 2002).

### 4.4.3. Number of primary branches (No)

The highest number of primary branch of 5.066 was produced by each of Clark 63 k and SCS-1 varieties under rhizobia inoculation and with respective P application rate of 40 and 20 kg ha$^{-1}$ (Table 5). The variety that produced the highest number of primary branch showed more than 24.9 % increase over the lowest variety (Table 5). There was no significant effect on number of primary branches because of Bradyrhizobium inoculation or phosphorus application and their interaction (p=0.39). Similarly, Bekere *et al*. (2012) reported that application of nitrogen in limed and unlimed soil had no significant effect on soybean number of pod bearing primary branches. The number of branches per plant was significantly affected by different rates of phosphorus application and this might probably be due to the cumulative effect of phosphorus on the process of cell division and balanced nutrition (Zafar *et al.,* 2003).

Table 5. Responses of three soybean varieties to rhizobia treatment and rate of phosphorus application for leaf area and number of primary branches

| Varieties | Inoculation Status | Leaf Area(cm$^{2}$) | | | | Number of Primary Branches(No) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Phosphorus application(Kg ha$^{-1}$) | | | Mean | Phosphorus application(Kg ha$^{-1}$) | | | Mean |
| | | 0 | 20 | 40 | | 0 | 20 | 40 | |
| Afgat | Inoculated | $127.16^{b}$ | $135.86^{a}$ | $131.82^{ab}$ | 131.61 | $4.00^{bcd}$ | $4.40^{abcd}$ | $4.66^{abcd}$ | 4.35 |
| | Uninoculated | $86.04^{f}$ | $107.37^{d}$ | $128.97^{b}$ | 107.46 | $3.80^{d}$ | $4.33^{abcd}$ | $4.33^{abcd}$ | 4.15 |
| Clark 63K | Inoculated | $102.17^{d}$ | $127.89^{b}$ | $136.50^{a}$ | 122.19 | $4.33^{abcd}$ | $5.00^{ab}$ | $5.066^{a}$ | 4.80 |
| | Uninoculated | $126.86^{b}$ | $127.04^{b}$ | $121.34^{c}$ | 125.08 | $3.93^{cd}$ | $4.13^{abcd}$ | $4.40^{abcd}$ | 4.15 |
| SCS-1 | Inoculated | $87.43^{f}$ | $93.87^{e}$ | $90.73^{ef}$ | 90.68 | $4.33^{abcd}$ | $5.066^{a}$ | $4.86^{abc}$ | 4.75 |
| | Uninoculated | $106.66^{d}$ | $102.23^{d}$ | $93.11^{e}$ | 100.67 | $4.80^{abcd}$ | $4.40^{abcd}$ | $4.46^{abcd}$ | 4.55 |
| | Mean | 106.06 | 115.71 | 117.08 | 0.00 | 4.20 | 4.55 | 4.63 | |
| LSD (5%) | | 5.33 | | | | NS | | | |
| CV (%) | | 2.92 | | | | 10.72 | | | |

Mean value followed by the same letter within the same column is non-significant difference at 5% level of significance.

## 4.5. Root and Nodulation Parameters

### 4.5.1. Root fresh weight (g)

The highest root fresh weight of 50.11 and 49.79 g plant$^{-1}$ was produced by variety Clark 63k and SCS-1, respectively under rhizobia inoculation and 40 kg ha$^{-1}$ P application; and variety SCS-1 produced among the highest (49.3g plant$^{-1}$) root fresh weight under no rhizobia inoculation and 40 kg ha$^{-1}$ P application (Table 6); whereas the lowest root fresh weight (20.64 g plant$^{-1}$) was recorded on Afgat variety under rhizobia inoculation and application of Zero P. The variety that produced the highest root fresh weight showed more than 58.81% increase over the lowest variety (Table 6).

The mean performance of Clark 63K and SCS-1 varieties under combined P application and rhizobia inoculated conditions showed higher performance compared to their performance under no rhizobia inoculated conditions. This indicated that varieties responded to P and rhizobia, which might be attributed to genetic factors responsible for tolerance to soil condition. In line with this result, Belachew and Stoddard (2017) reported root fresh weight of tolerant accessions, including GLA 1103 and NC 58.

Progressive increase in root fresh weight was found with increasing the rate of phosphate fertilizer. It was found that phosphorus rate at 40 and 20 kg ha$^{-1}$ application increased root fresh weight by 44.72 and 21.99, 32.48 and 15.06% over unfertilized plot of rhizobia inoculated and no rhizobia inoculated conditions, respectively (Table 6). This might be due to the role of phosphate in the initial root development and increased population of free living rhizobia in the rhizosphere, and therefore, it enhances nodulation and nitrogen fixation of the study area. The specific growth factors that have been associated with phosphorus includes: stimulated root development, increased stalk and stem strength, improved flower formation and seed production more uniform and earlier crop maturity (Tahir *et al*., 2009).This finding is in agreement with Tahir *et al*. (2009), who reported significant effect of the application of P fertilizer on soybean root fresh weight and nodule development.

### 4.5.2. Root dry weight (g)

Varieties Clark 63k with Rhizobia inoculation and variety SCS-1 with no Rhizobia inoculation and variety SCS-1 with the inoculation of Rhizobia produced the highest respective root dry weights of 13.93, 13.76 and 13.33g plant$^{-1}$ all at the rate of 40 kg ha$^{-1}$ P fertilizer application (Table 6). The lowest root dry weight per plant (6.2 g) was obtained on the variety Afgat under Rhizobia inoculated condition and no application of P fertilizer (Table 6). However, this variety produced the highest root dry weight values at 40 kg ha$^{-1}$ P application, indicating that the cultivar performs less efficiently at low levels of P and is least tolerant to phosphorus stress. A similar cultivar difference to phosphorus has been reported by Howel (1954). The significant difference for the root dry weight might be attributed to phosphorus fertilization, which enhances root proliferation, and consequently, improving the P uptake capacity of the soybean, which facilitates root growth, and then increased root diameter or root thickness of the varieties, and root dry weight is the result of root growth and development, including root length and number of lateral roots (Haling *et al*., 2011).

Higher value of root dry weight in the fertilizer treatment suggests that soybean responded to phosphate fertilizer application, and the application of P influenced root growth and development. This might be due to the role of phosphate in enhancing root growth through increasing the efficiency of biological nitrogen fixation. The fact that phosphate fertilization increased root dry weight underline the importance of phosphorus for root development through its basic function, as an energy source. The growth might also be attributed to increased cell dry matter accumulation with well- developed taproot, during the growth of the plants. In agreement Bekere *et al*. (2012) reported the increase in root dry weight in three p-application rates (60, 120 and 180 mg kg$^{-1}$).Also Tahir *et al*. (2009) reported that application of P significantly increased root length and root dry weight by 33 and 64% over the control.

In line with this result Abush *et al*. (2017) reported reductions of root parameters i.e. root volume, root dry and fresh weight of soybean genotypes on phosphorus untreated acid soil. Except genetic factor that increased or maintained number of lateral roots, the other factor that increases root growth and development includes: concentration of Calcium (Murata *et al*., 2003) or P concentration, which increased the root dry weight.

Table 6. Responses of three soybean varieties to rhizobia treatment and rate of phosphorus application for root fresh and dry weight

| Varieties | Inoculation Status | Root Fresh Weight(g plant $^{-1}$) | | | | Root Dry Weight(g plant $^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Phosphorus application (Kg ha$^{-1}$) | | | Mean | Phosphorus application (Kg ha$^{-1}$) | | | Mean |
| | | 0 | 20 | 40 | | 0 | 20 | 40 | |
| Afgat | Inoculated | 20.64$^{f}$ | 25.08$^{ef}$ | 37.52$^{b}$ | 27.75 | 6.20$^{h}$ | 8.70$^{defg}$ | 10.46$^{cd}$ | 8.45 |
| | Uninoculated | 24.66$^{ef}$ | 33.84$^{bcd}$ | 36.96$^{bc}$ | 31.82 | 7.30$^{fgh}$ | 6.81$^{gh}$ | 10.06$^{cde}$ | 8.06 |
| Clark 63K | Inoculated | 24.02$^{ef}$ | 34.09$^{bcd}$ | 50.11$^{a}$ | 36.07 | 6.96$^{fgh}$ | 8.83$^{defg}$ | 13.93$^{a}$ | 9.91 |
| | Uninoculated | 28.29$^{def}$ | 27.57$^{def}$ | 35.04$^{bcd}$ | 30.30 | 7.46$^{fgh}$ | 8.30$^{efgh}$ | 9.63$^{cde}$ | 8.46 |
| SCS-1 | Inoculated | 31.30$^{bcde}$ | 38.29$^{b}$ | 49.79$^{a}$ | 39.79 | 9.66$^{cde}$ | 11.23$^{bc}$ | 13.33$^{ab}$ | 11.41 |
| | Uninoculated | 28.95$^{cde}$ | 35.04$^{bcd}$ | 49.30$^{a}$ | 37.76 | 8.80$^{defg}$ | 9.20$^{cdef}$ | 13.76a | 10.59 |
| | Mean | 26.31 | 32.32 | 43.12 | 0.00 | 7.73 | 8.85 | 11.86 | |
| LSD (5%) | | NS | | | | NS | | | |
| CV (%) | | 15.05 | | | | 14.00 | | | |

Mean value followed by the same letter within the same column is non-significant difference at 5% level of significance.

### 4.5.3. Number of nodule per plant (N<u>o</u>)

The highest number of nodules per plant (52.0) was obtained from the variety Afgat under rhizobia inoculation and application of 40 kg $ha^{-1}$ P; followed by Afgat variety (38.4) and Clarck 63 K variety (36.5) under un-inoculated condition, and Clarck 63K (33.5), under inoculated condition, and all at the rate of 40 kg P $ha^{-1}$ application. The lowest number of nodules per plant (5.06) was recorded on the variety Clark 63 K with no inoculation of rhizobia, and the control P application (no application of P) (Table 7), which is due to rhizobia inoculation and phosphorus application increases the number of nodule per plants by attributing nodule formation and nitrogen fixation of basic energy transfer of phosphorus. which showed about 927.66% decrease over the variety that produced the highest number of nodules.

Likewise Ogoke *et al*. (2004) reported that application of P fertilizer at the rates of 30 and 60 kg $P_2O_5$ $ha^{-1}$ significantly increased number of nodules. Ahiabor *et al*. (2014) also reported an increased number of nodules per plant under the highest rate of phosphorus (45 kg $P_2O_5$ $ha^{-1}$) plus liming or Rhizobium inoculation. Phosphate application increased the activity of rhizobia, which might have intern increased the formation of root nodule. Plant tissue develops around the infected area, forming the nodule and site for the bacterial growth, which creates environment for the fixation of elemental nitrogen from the soil.

In line with Zoundji *et al. (*2015*)* reported that a combination of 50kg $P_2O_5$ $ha^{-1}$ with STM3043 strain of Rhizobia gave the best nodule number (39 nodule per plant), which is lower relative to the number of nodules, when compared to the present study. Correspondingly, Masresha and Kibebew (2010) found that the application of 46kg $ha^{-1}$ $P_2O_5$ and inoculation of *Rhizobium japonicum* significantly increased the number of nodules per plant by 240.7% and 123%, respectively. This result was in corroborate with Tran *et al*. (2007),who reported that the application of *Bradyrhizobium japonicum* strain USDA110 and 60kg of $P_2O_5$ enhanced the number of nodule per plant.

### 4.5.4. Effective nodule (No)

Nodule effectiveness on the variety SCS-1 was highest (4.0) under rhizobia inoculated and the application 40 kg ha$^{-1}$ P conditions, followed by varieties SCS-1 and Afgat under rhizobia inoculated and application of 20 and 40 kg P ha$^{-1}$ conditions respectively; while the lowest number of effective nodules was produced by the variety SCS-1 (1.33) under uninoculated and the control (no) application of P conditions (Table 7). Nodules with deep dark red, slightly dark red and pink were considered effective and fixing nitrogen and typically of healthy and effective was due to presence of leghemoglobin protein (Iron and Molybdenum) responsible for oxygen binding (Gottschalk,2012). Also by Adjei and Chambeiss (2002), Butler and Evers (2004) reported legume nodules having dark pink or red centers (due to leg hemoglobin presence) are an indication for effectiveness of the strain used and also positively correlated with higher $N_2$ fixation. This is due to compatibility between rhizobia inoculation and host plant. This is due to the deficiency of phosphorus and fixation of availability of P in the soil condition of the acidity (Tesfaye, 2007). Larger response to inoculation and higher number of nodules per plant in comparison to un-inoculated treatments in a field that has no soybean cropping history was reported by (Bekere *et al.*, 2012). This indicates suggested that inoculation does not always elicit or enhance nodulation. Okogun *et al*. (2004) reported that improved soybean varieties (TGx 1448-2E) did not respond to inoculation in terms of nodule production in the Nigeria's moist savanna zone.

However, P rates and their interaction ($p=0.0687$) with inoculation did not significantly ($P<0.05$) affect the number of effective nodules. The non-responsiveness of the number of effective nodules due to P application might be ascribed to the high P fixation of the soil. Besides, iron which is deficient in alkaline soils plays vital role in leg hemoglobin formations which render pink or red color to nodules.

Table 7. Responses of three soybean varieties to rhizobia treatment and rate of phosphorus application for number of nodule per plant and nodule effectiveness

| Varieties | Inoculation Status | Number of Nodule per Plant(No) | | | | Nodule Effectiveness(No) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Phosphorus application (Kg ha$^{-1}$) | | | Mean | Phosphorus application (Kg ha$^{-1}$) | | | Mean |
| | | 0 | 20 | 40 | | 0 | 20 | 40 | |
| Afgat | Inoculated | 15.20$^{gh}$ | 16.80$^{gh}$ | 52$^{a}$ | 28.48 | 2.00$^{cde}$ | 3.00$^{abc}$ | 3.00$^{abc}$ | 2.67 |
| | Uninoculated | 23.86$^{ef}$ | 23.2$^{ef}$ | 38.4$^{b}$ | 28.00 | 2.33$^{bcde}$ | 2.00$^{cde}$ | 3.00$^{abc}$ | 2.44 |
| Clark 63K | Inoculated | 24.53$^{de}$ | 19.06$^{fg}$ | 33.46$^{bc}$ | 25.68 | 2.66$^{bcd}$ | 2.66$^{bcd}$ | 3.33$^{ab}$ | 2.88 |
| | Uninoculated | 5.06$^{i}$ | 22.6$^{ef}$ | 36.53$^{b}$ | 21.40 | 1.66$^{de}$ | 3.00$^{abc}$ | 2.33$^{bcde}$ | 2.33 |
| SCS-1 | Inoculated | 25.73$^{de}$ | 17$^{gh}$ | 27.26$^{de}$ | 23.33 | 1.66$^{de}$ | 3.33$^{ab}$ | 4.0$^{a}$ | 2.25 |
| | Uninoculated | 29.40$^{cd}$ | 29.46$^{cd}$ | 12.66$^{h}$ | 23.84 | 1.33$^{e}$ | 2.00$^{cde}$ | 2.66$^{bcd}$ | 1.99 |
| | | 20.63 | 21.35 | 33.39 | 0.00 | 1.94 | 2.67 | 3.05 | |
| LSD (5%) | | 5.11 | | | | 1.12 | | | |
| CV (%) | | 12.48 | | | | 27.30 | | | |

Mean value followed by the same letter within the same column is non-significant difference at 5% level of significance.

### 4.5.5. Nodule fresh weight (g)

The highest nodule fresh weight (14.75g plant$^{-1}$) was recorded by Afgat variety under rhizobia inoculation and application of 20 kg  P ha$^{-1}$, followed by the same variety that produced 12.55 and 12.13g fresh nodule weight at the rate of 40 kg P ha$^{-1}$ and Zero P application (the control), respectively, and both with the inoculation of rhizobia (Table 8); whereas the lowest nodule fresh weight of 2.12, and 3.86g plant$^{-1}$ were found on variety Clark 63k and SCS-1, respectively, both under no inoculated and 40 kg  P ha$^{-1}$ application conditions. The variety that produced the highest nodule fresh weight showed more than 72.6% increase over the variety that produced the lowest nodule fresh weight (Table 8).

The application of 20 kg P ha$^{-1}$ showed 21.6 % increase nodule fresh weight of Afgat variety over the control (Zero P) both under rhizobia inoculated conditions (Table 8).

In line with  Masiresha and Kibebew (2010) reported that number and fresh weight of nodules are commonly used as the criteria of effective complementary between macro and micro with the rate of atmospheric nitrogen fixation. Similarly, Kumah (2016) reported that a simple relationship arises between nodule number and nodule fresh weight and they are indices of nitrogen fixation.

### 4.5.6. Nodule dry weight (g)

Variety  Afgat  gave the highest nodule dry weight of 8.24 and 7.25g plant$^{-1}$ with the application of 40 and 20 kg  P ha$^{-1}$ P applications, respectively, both under rhizobia inoculated conditions, followed by the  same variety which produced 6.47g plant$^{-1}$ nodule dry weight under rhizobia inoculation and Zero (the control) P application; while, the lowest nodule dry weight per plant (0.89g) was produced by the variety  Clark 63k under no rhizobia inoculation and application of 40 kg P ha$^{-1}$  (Table 8), which was about 89.2% of nodule dry weight reduction over the variety that produced the highest nodule dry weight. The reason might be due to the overall improvement in the performance of the plant in response to phosphorus fertilizer and inoculation, leading to better nodulation underlines phosphorus has on nodule development through its basic function of as an energy source.

In soybean production, phosphorus and inoculation with the appropriate rhizobia strain have quite prominent effects on nodule number, dry weight and yield components. Similarly, Zoundji *et al*. (2015) indicated that a combination of 50 kg $P_2O_5$ ha$^{-1}$ and STM3043 rhizobia strain gave the best nodule dry weight of 4.97g plant$^{-1}$. In the present study, however, nodule dry weight ranged between 0.89-8.24 g; where Afgat variety produced the highest nodule dry weight with the application of 40 kg P ha$^{-1}$ and inoculation of Rhizobia bacteria, which is high relative to what is reported by Zoundji *et al*. (2015). In agreement with Bekere and Hailemariam (2012) reported that phosphorus application levels with inoculation influence nodule dry weight. Fatima *et al*. (2017) also reported that *B. japonicum* strains with P fertilizer increased the nodule dry weight. Bekere *et al*. (2012); Bekere and Hailemariam (2012) revealed that phosphorus application levels, without inoculation, did not influence nodule dry weight.

Table 8. Responses of three soybean varieties to rhizobia treatment and rate of phosphorus application for nodule fresh and dry weight

| Varieties | Inoculation Status | Nodule fresh weight (g plant$^{-1}$) | | | | Nodule dry weight (g plant$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Phosphorus application (Kg ha$^{-1}$) | | | Mean | Phosphorus application(Kg ha$^{-1}$) | | | Mean |
| | | 0 | 20 | 40 | | 0 | 20 | 40 | |
| Afgat | Inoculated | $12.13^{bc}$ | $14.75^{a}$ | $12.55^{ab}$ | 13.14 | $6.47^{b}$ | $7.25^{ab}$ | $8.24^{a}$ | 7.32 |
| | Uninoculated | $6.73^{efg}$ | $7.54^{def}$ | $9.88^{cd}$ | 8.05 | $3.47^{defg}$ | $4.14^{de}$ | $5.24^{c}$ | 4.28 |
| Clark 63K | Inoculated | $5.03^{gh}$ | $6.05^{fgh}$ | 8.06def | 6.38 | $2.48^{gh}$ | $3.30^{efg}$ | $3.99^{def}$ | 3.26 |
| | Uninoculated | $2.12^{i}$ | $8.65^{de}$ | $8.73^{de}$ | 6.50 | $0.89^{i}$ | $4.29^{cde}$ | $4.34^{cd}$ | 3.17 |
| SCS-1 | Inoculated | $4.04^{ghi}$ | $7.90^{def}$ | $4.44^{hi}$ | 5.45 | $1.85^{hi}$ | $4.19^{de}$ | $2.01^{h}$ | 2.68 |
| | Uninoculated | $6.50^{efg}$ | $8.35^{def}$ | $3.86^{hi}$ | 6.24 | $3.09^{fg}$ | $4.33^{cd}$ | $1.90^{hi}$ | 3.11 |
| | Mean | 6.09 | 8.87 | 7.92 | 0.00 | 3.04 | 4.58 | 4.29 | |
| LSD (5%) | | 2.40 | | | | 1.02 | | | |
| CV (%) | | 17.13 | | | | 15.45 | | | |

Mean value followed by the same letter within the same column is non-significant difference at 5% level of significance.

### 4.5.7. Root volume (ml)

Variety: Clark 63K (58.0ml), with the inoculation of Rhizobia, and SCS-1 (56.6ml), without the inoculation of the rhizobia, gave the highest root volume under the application of 40 kg ha$^{-1}$ P, followed by Afgat, which produced 48.33ml of root volume with the application of 40 kg ha$^{-1}$ P and inoculation of Rhizobium bacteria (Table 9). The lowest root volume of 28.33ml was produced by the variety Afgat, with the inoculation of Rhizobium bacteria and at both the control (zero) and 20 kg P ha$^{-1}$ application.

The variety that produced the highest root volume showed 51.15% increase over the variety that produced the lowest root volume. Phosphate application and inoculation of soybean increased root volume and root biomass, due to the increased rate of nitrogen fixation. Similarly, Abdul *et al. (*2007) reported that increasing $P_2O_5$ application up to 50 kg ha$^{-1}$ increases root volume. The application of 40 kg $P_2O_5$ ha$^{-1}$ has increased the root volume by 64.1 and 39.0% under inoculated and uninoculated conditions, respectively; while the application of 20 kg $P_2O_5$ ha$^{-1}$ has increased root volume by 15.8 and 12.77 %, respectively over the control (Zero P) application (Table 9).

### 4.5.8. Taproot length (cm)

The variety SCS-1 gave the longest taproot length of 27.29cm under rhizobia inoculation and application of 40 kg P ha$^{-1}$ while the next longest root length was produced by the same variety (25.35cm) and Afgat (24.84cm) with the inoculation of Rhizobia bacteria, and the control (Zero) P application, and the variety SCS-1 (24.98cm) under no Rhizobia inoculation and the application of 40 kg P ha$^{-1}$(Table 9). The shortest taproot lengths of 21.06, 21.20 and 21.30cm were produced by the variety Clarck 63 K with the application of 20kg P ha$^{-1}$, and Afgat variety with the control (Zero) P application and 20 kg P ha$^{-1}$ applications and no rhizobia inoculated, respectively (Table 9). Increasing Phosphate fertilizer could be due to P treatment which causes root cells to grow. There was a significant difference level of phosphate rate for taproot length in this study, and other studies also reported that systems with large root surface length and density ensures P uptake efficiency, on soils of low P availability (Fernandes, 2012). Tahir (2009) reported the average increase in root length by 41% as compared to uninoculated control, due to rhizobia inoculation and phosphorus application. Similarly, Abbas *et al.* (2011) reported that phosphate stimulates root growth, and it is associated with early crop maturity. The highest taproot length of

Clark 63K (23.61cm) was recorded on uninoculated rhizobia and 40kg P ha$^{-1}$ and the shortest taproot length (21.65cm) of the same variety with inoculated rhizobia and 40 kg P ha$^{-1}$.This is due to uninoculated rhizobia strain around the infected roots airs which is not nutrient availability for plants is deep taproot length for nutrient absorption and the inoculated rhizobia and phosphorus application is fixation of nitrogen and nutrient availability which is used for vegetative growth and developments rather than taproot length.

Table 9. Responses of three soybean varieties to rhizobia treatment and rate of phosphorus application for root volume and taproot length.

| | | Root volume (ml) | | | | Tap root length(cm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Varieties | Inoculation Status | Phosphorus application (Kg ha$^{-1}$) | | | Mean | Phosphorus application (Kg ha$^{-1}$) | | | Mean |
| | | 0 | 20 | 40 | | 0 | 20 | 40 | |
| Afgat | Inoculated | $28.33^{h}$ | $28.33^{h}$ | $48.33^{b}$ | 35.00 | $24.84^{b}$ | $24.24^{bcd}$ | $22.68^{efg}$ | 23.92 |
| | Uninoculated | $33.33^{fgh}$ | $40.00^{cdef}$ | $45.00^{bc}$ | 39.44 | $21.20^{h}$ | $21.30^{h}$ | $22.24^{fgh}$ | 21.58 |
| Clark 63K | Inoculated | $31.66^{gh}$ | $41.66^{bcde}$ | $58.00^{a}$ | 43.77 | $24.50^{bc}$ | $23.26^{def}$ | $21.65^{gh}$ | 23.14 |
| | Uninoculated | $34.33^{efgh}$ | $37.66^{cdefg}$ | $43.33^{bcd}$ | 38.44 | $21.70^{gh}$ | $21.06^{h}$ | $23.61^{cde}$ | 22.12 |
| SCS-1 | Inoculated | $39.33^{cdefg}$ | $45.00^{bc}$ | $56.66^{a}$ | 47.00 | $25.35^{b}$ | $24.28^{bcd}$ | $27.29^{a}$ | 25.64 |
| | Uninoculated | $36.66^{defg}$ | $40.00^{cdef}$ | $56.66^{a}$ | 44.44 | $24.62^{bc}$ | $24.30^{bcd}$ | $24.98^{b}$ | 24.63 |
| | Mean | 33.94 | 38.78 | 51.33 | 0.00 | 23.70 | 23.07 | 23.74 | 33.94 |
| LSD (5%) | | NS | | | | 1.19 | | | |
| CV (%) | | 11.94 | | | | 3.10 | | | |

Mean value followed by the same letter within the same column is non-significant difference at 5% level of significance.

## 4.6. Yield and Yield Components

### 4.6.1. Number of pods per plant (No)

The varieties that produced the highest number of pods per plant included: SCS-1 (51.46) and Clarck 63 K (50.60) both with the inoculation of Rhizobia and the application of 40 kg P ha$^{-1}$ (Table 10). The lowest number of pods per plant (32.2) was produced by the variety Clark 63K with rhizobia inoculated and the control (zero) P level, which showed about 59.81% of pod number decrease over the variety and amendment that produced the highest pod number.

Phosphorus applications at the rate of 20 and 40 kg P ha$^{-1}$ increased number of pods per plant by 18.17 and 24.60 % respectively, over the unfertilized control. This result support with Mohan and Rao (1997) that higher numbers of pods per plant were produced in response to higher doses of phosphorus. This result revealed increased number of pods per plant with increased rate of phosphorus application. This result is similar to Bekere *et al*. (2012) who reported increased number of pods per plant in soybean following the application of $P_20_5$ at the rate of 20, 40 and 60 kg ha$^{-1}$.

In line with Masresha and Kibebew (2010) reported that the application of 23 and 46 kg ha$^{-1}$ $P_2O_5$ with *B.Japonicum* resulted in 59.7 and 167.7% increase of mean pod number over the control treatment, respectively. Similarly, significant increase in the number of pods/plant, due to the interaction effect of rhizobia strain IRJ 2180A with the application of 50 kg of $P_2O_5$ ha$^{-1}$ over the control was reported by Muhammad (2010). In general, mean pod number plant $^{-1}$ enhanced due to the combined effect of rhizobia inoculation and phosphorus application.

### 4.6.2. Number of seeds per pod (No)

The highest number of seeds per pod was recorded SCS-1(2.62) and Clarck 63 K (2.58) varieties under inoculated rhizobia and 40 kg P ha$^{-1}$ application (Table 10).The lowest number of seeds per pod (2.37) under uninoculated rhizobia and 0 kg P ha$^{-1}$( zero P) application of SCS-1 variety (Table 10). The results of analysis of variance (Table 10) showed that the variations among treatments due to phosphorus application, *Bradyrhizobium* inoculation and their interaction were not significant on number of seeds per pod.

The number of seeds per pods perceived as a significant constituent that directly contributes to the recovery of potential yield in leguminous crops, like soybean (Devi *et al*., 2012). The number of seeds pod$^{-1}$ non-significantly varied between genotypes. In agreement with Guareschi *et al*. (2011) reported non-significant difference in number of seeds per pods after varying p rates. Solomon (2007) reported that inoculation with TAL 379 had significant effect on number of seeds per pod of soybean even though TAL 378 was at par with the control.

Table 10.Responses of three soybean varieties to rhizobia treatment and rate of phosphorus application for number of pods per plant and number of seed per pods

| Varieties | Inoculation Status | Number of pods per plant(No) | | | | Number of seeds per pod(No) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Phosphorus application (Kg ha$^{-1}$) | | | Mean | Phosphorus application (Kg ha$^{-1}$) | | | Mean |
| | | 0 | 20 | 40 | | 0 | 20 | 40 | |
| Afgat | Inoculated | 42.06$^{g}$ | 49.00$^{bc}$ | 46.46$^{de}$ | 45.84 | 2.54$^{abc}$ | 2.56$^{abc}$ | 2.49$^{bcdef}$ | 2.53 |
| | Uninoculated | 34.80$^{j}$ | 47.66$^{cd}$ | 43.60$^{fg}$ | 42.02 | 2.52$^{bcd}$ | 2.50$^{bcde}$ | 2.46$^{cdefg}$ | 2.49 |
| Clark 63K | Inoculated | 32.20$^{k}$ | 43.20$^{fg}$ | 50.60$^{ab}$ | 42.00 | 2.41$^{efg}$ | 2.53$^{abcd}$ | 2.58$^{ab}$ | 2.51 |
| | Uninoculated | 36.46$^{ij}$ | 36.40$^{ij}$ | 50.26$^{ab}$ | 41.04 | 2.48$^{cdef}$ | 2.53$^{abcd}$ | 2.50$^{bcde}$ | 2.50 |
| SCS-1 | Inoculated | 37.73$^{hi}$ | 44.66$^{ef}$ | 51.46$^{a}$ | 44.62 | 2.40$^{fg}$ | 2.48$^{cdef}$ | 2.62$^{a}$ | 2.50 |
| | Uninoculated | 38.86$^{h}$ | 43.80$^{fg}$ | 44.46$^{f}$ | 42.37 | 2.37$^{g}$ | 2.45$^{defg}$ | 2.56$^{abc}$ | 2.46 |
| Mean | | 37.02 | 44.12 | 47.81 | 0.00 | 2.45 | 2.51 | 2.54 | 37.02 |
| LSD (5%) | | 1.94 | | | | NS | | | |
| CV (%) | | 2.77 | | | | 2.50 | | | |

Mean value followed by the same letter within the same column is non-significant difference at 5% level of significance.

### 4.6.3 .Grain yield (kg $ha^{-1}$)

The grain yield had highly significantly ($P<0.01$) affected by the three way interaction ($p=<.0001$) (Table 11). The highest grain yield was obtained by Afgat variety (3117.8 kg $ha^{-1}$) and SCS-1 variety (2825.7 kg $ha^{-1}$) both under rhizobia inoculation and application of 40 kg P $ha^{-1}$ (Table 11). At the rate of 40 kg P $ha^{-1}$ application, the yield of the highest yielding variety Afgat with rhizobia inoculation showed 17.96% increase over the uninoculated control. Though, the difference in percentage increase was high, this finding supports Shahid *et al*. (2009) who reported that grain yield can increase by 70-75%, when the proper rhizobia bacteria is inoculated. The highest grain yield (3117.8 kg $ha^{-1}$) obtained from study was very high relative to the national average (2217 kg $ha^{-1}$) and the productivity in the study area i.e., in Illuababor zone, which is 1300kg $ha^{-1}$, which indicates that the production and productivity of soybean can be increased significantly through rhizobia inoculation and application of P. The result is also in agreement with Alam *et al*. (2009) and Rahman *et al*. (2012) who reported varietal difference in rice and soybean with rhizobia inoculation and phosphorus application, respectively.

Afgat variety showed grain yield increase of 13.03 and 24.94 % at P application rates of 20 and 40 kg P $ha^{-1}$, respectively over the control (no P application). Similarly, Aziz *et al*. (2016) reported that P applications at the rate of 22.5 and 45.0 kg $P_2O_5$ $ha^{-1}$ increased grain yield by 33.9 and 35.4%, respectively, over the unfertilized control in Ghana. Bekere and Hailemariam (2012) also reported that seed yield of soybean increased in response to the application of phosphorus fertilizer at the rates of 20, 40 and 60 kg $P_2O_5$ $ha^{-1}$.

In line with Tahir *et al. (*2009) reported that combined application of rhizobia inoculation and p application resulted in 21% increased grain yield. Adequate phosphorus application and rhizobia will lead to effective pod dry matter accumulation and subsequently to higher grain yield. Similarly Amos *et al* (2004) who reported that p application with rhizobia inoculation significantly increased pod number, grain yield and dry matter yield, compared to uninoculated treatment. The average grain yields recorded from the present study were lower, when compared to the yield of 7610 kg $ha^{-1}$ obtained by Akpalu *et al*. (2014). However, the yield obtained from this study were comparable with the average soybean yields (770-3380 kg $ha^{-1}$) reported by Zoundji *et al*. (2015).

### 4.6.4. Thousand Seed weight (g)

Afgat variety had the highest hundred seed weight (17.11g ) under rhizobia inoculated and 40 kg P $ha^{-1}$ application, followed by (17.06g) under rhizobia inoculated and 20 kg P $ha^{-1}$ of SCS-1 soybean variety, while the lowest thousand seed weight (13.86g) was recorded on Clark 63 K variety under rhizobia inoculated and 40 kg P $ha^{-1}$ application (Table 13). The interaction effect of inoculation and P rates on thousand seeds weight was significant increased. This could be due to combined application of rhizobia and $N_2$ fixation which supplied by biological compounds that play major roles in photosynthesis which eventually increased seeds weight.

Interaction effect of the factors under study 50 (I x P) on 1000-seed weight was significantly reported (Shahid *et al*., 2009). Also increasing P levels from 0 to 69 kg $P_2O_5$ $ha^{-1}$ showed increase in thousand seeds weight, the highest thousand seeds weight (20.5 and 20.4 g) were scored at 23 and 69 kg $P_2O_5$ $ha_{-1}$ application, respectively. Tairo and Ndakidemisi (2013) found a significant increase of thousand seeds weight by 5-18% due to P application from 20 to 80 kg P $ha_{-1}$ over the control treatment.

The Afgat variety that produced the highest thousand seed weight showed thousand seed weight increase of 18.99% p application over the variety that produced the lowest seed weight. In agreement with this result, Habtamu (2017) reported significant difference among soybean varieties for hundred seed weight, in which the highest hundred seed weight was produced by the variety BFS 39 and the lowest hundred seed weight was recorded by the variety Roba.

Table 11.Responses of three soybean varieties to rhizobia treatment and rate of phosphorus application for grain yield and hundred seed weight

| | | Grain Yield(kg ha$^{-1}$) | | | | Thousand(1000 )seed weight(g) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Varieties | Inoculation Status | Phosphorus application(kg ha$^{-1}$) | | | Mean | Phosphorus application(kg ha$^{-1}$) | | | Mean |
| | | 0 | 20 | 40 | | 0 | 20 | 40 | |
| Afgat | Inoculated | 2326.18$^{gh}$ | 2690.46$^{cd}$ | 3117.8$^{a}$ | 2716.13 | 16.80$^{ab}$ | 17.04$^{ab}$ | 17.11$^{a}$ | 16.9 |
| | Uninoculated | 2129.71$^{i}$ | 2359.48$^{g}$ | 2542.26$^{ef}$ | 2344.37 | 16.40$^{abc}$ | 15.97$^{abcd}$ | 15.54$^{abcde}$ | 15.9 |
| Clark 63K | Inoculated | 2000.30$^{j}$ | 2295.16$^{h}$ | 2645.42$^{d}$ | 2326.63 | 14.41de | 15.38$^{bcde}$ | 13.86$^{e}$ | 14.5 |
| | Uninoculated | 2163.05$^{i}$ | 2353.2$^{g}$ | 2732.55c | 2427.87 | 14.36$^{de}$ | 14.14$^{e}$ | 16.03$^{abcd}$ | 14.8 |
| SCS-1 | Inoculated | 2120.28$^{i}$ | 2569.60$^{e}$ | 2825.72$^{b}$ | 2510.27 | 16.42$^{abc}$ | 17.06$^{ab}$ | 16.81$^{ab}$ | 16.7 |
| | Uninoculated | 2285.50$^{h}$ | 2511.12$^{f}$ | 2743.33$^{c}$ | 2508.50 | 14.94$^{cde}$ | 16.91$^{ab}$ | 16.75$^{ab}$ | 16.2 |
| | Mean | 2170.84 | 2463.17 | 2767.85 | | 15.56 | 16.08 | 16.02 | |
| LSD (5%) | | 53.58 | | | | 1.11 | | | |
| CV (%) | | 1.34 | | | | 4.29 | | | |

Mean value followed by the same letter within the same column is non-significant difference at 5% level of significance.

### 4.6.5. Dry biomass (kg ha$^{-1}$)

The highest dry biomass (7430.7kg ha$^{-1}$) was obtained under the rhizobia inoculated and 40 kg ha$^{-1}$ P application of Clark 63k variety (Table 12); while the lowest dry biomass yield (4438.2kg ha$^{-1}$) was recorded on variety SCS-1 under uninoculated and the control main plots (no phosphorus), which was lower by about 40.27 % over the variety that produced the highest above ground dry biomass yield. The phosphorus application rate had significantly ($P<0.05$) affected above ground biomass of soybean. Phosphorus application of 20 and 40 kg P ha$^{-1}$ increased the above ground dry biomass yields by 29.42 and 39.31%, respectively, over the control plots of inoculated Clark 63K variety (Table 12). Phiri *et al*. (2016) reported that the application of 25 and 35 kg P ha$^{-1}$ increased the soybean dry biomass yields by 54 and 70%, respectively, over the control plots.

The dry biomass increased, due to the three way interaction effects of 40 kg P ha$^{-1}$ application and inoculated rhizobia strain of Clark 63 K variety. This might be due to the development of root system that might have increased water and nutrient uptake, and consequently, increased photosynthesis, and production of photosynthetic material that might have resulted in increased biological yield. In line with Olivera *et al*. (2004) also reported that phosphate application and inoculation of *B .Japonicum* to legumes, like soybean increased plant biomass, due to increased rate of nitrogen fixation.

### 4.6.6. Harvest index (%)

Afgat variety resulted in significantly higher harvest index (47.62%) under rhizobia inoculated and 40 kg ha$^{-1}$ P application and lowest values of harvest index (37.28%) was obtained under uninoculated and zero P application of Clark 63 K variety (Table 12). Phosphate fertilizer application increases the number of nodule and size and grain formation due to increased nitrogen fixation. This can be explained by a good symbiotic activity induced by the strain of rhizobia and the host plant. The highest harvest index implies the higher partitioning of dry matter in grain. Whereas, the lowest harvest index (37.61%) gained from unfertilized plot (Table 12). In line with Zafar *et al*. (2003) found that the values of harvest index showed an increasing trend in the harvest index values with application of phosphorus

Rhizobia inoculation is known to increase the yields of soybean of harvest index by way of increasing the nodulation and biomass of root and shoot. In agreement with Abera (2013) reported that inoculation of SB6 B1 and legume fix recorded significantly higher harvest indices of 54 and 53% over uninoculated plot. Furthermore, Roy *et al*. (1995) reported soybean seeds inoculation increased the nodule number per plant and thus increasing harvest index.

Table 12 .Response of three soybean varieties to rhizobia treatment and rate of phosphorus application for dry biomass and harvesting index

| Varieties | **Inoculation Status** | **Dry biomass(kg ha$^{-1}$)** | | | | **Harvest index (%)** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Phosphorus application(kg ha$^{-1}$) | | | Mean | Phosphorus application (kg ha$^{-1}$) | | | Mean |
| | | 0 | 20 | 40 | | 0 | 20 | 40 | |
| Afgat | Inoculated | $6111.9^{abcde}$ | $6946.0^{abc}$ | $7226.7^{ab}$ | 6761.53 | $42.75^{gh}$ | $44.94^{de}$ | $47.62^{a}$ | 45.1 |
| | Uninoculated | $4652.8^{fgh}$ | $5798.8^{cdefg}$ | $5973.3^{bcdef}$ | 5474.96 | $44.07^{ef}$ | $41.49^{ij}$ | $42.52^{h}$ | 42.7 |
| Clark 63K | Inoculated | $4509.4^{gh}$ | $6389.7^{abc}$ | $7430.7^{a}$ | 6109.93 | $37.62^{k}$ | $41.33^{j}$ | $43.44^{fg}$ | 40.8 |
| | Uninoculated | $4440.7^{h}$ | $5002.3^{efgh}$ | $4492.5^{gh}$ | 4645.16 | $37.28^{k}$ | $42.33^{hi}$ | $42.17^{hij}$ | 40.6 |
| SCS-1 | Inoculated | $6492.8^{abcd}$ | $6042.2^{bcde}$ | $6459.5^{abcd}$ | 6331.5 | $44.22^{ef}$ | $45.12^{d}$ | $46.12^{bc}$ | 45.2 |
| | Uninoculated | $4438.2^{h}$ | $5973.7^{bcdef}$ | $5243.9^{defgh}$ | 5218.6 | $42.18^{hij}$ | $45.31^{cd}$ | $46.69^{b}$ | 44.7 |
| | Mean | 5107.63 | 6025.45 | 6137.77 | | 41.35 | 43.42 | 44.76 | |
| LSD (5%) | | 1332.8 | | | | 0.87 | | | |
| CV (%) | | 14.05 | | | | 1.23 | | | |

Mean value followed by the same letter within the same column is non-significant difference at 5% level of significance.

### 4.6.7. Phosphorus uptake efficiency (ppm)

The estimation of grain phosphorus uptake efficiency (PUE) revealed Afgat variety gave significantly high PUE of 21.3 and 20.2ppm, at the rate of 40 and 20 kg P ha$^{-1}$ application and both with  the inoculation rhizobia(Table 13). The lowest GUP of 15.40 was produced under uninoculated and the control main plot (no phosphorus) by the variety Clark 63 K.

The increased PUE due to added supply of nutrients might enhance absorption of water and nutrients. In agreement with the findings, Masresh *et al.* (2017) reported that 46 kg P ha$^{-1}$ and inoculation with *B. Japonicum* increased P uptake by 18.78% compared to the uninoculated (control) treatment. Increased in P content of the seed and P uptakes in soybean, in response to the combined application of *B. japonicum* inoculation was also reported by Moharram *et al*. (1994).

In addition, rhizobia inoculation improved grain phosphorus uptake, due to rhizobia inoculation, which might be attributed to the fact that some isolates of rhizobium have the ability to solubilize precipitated P components and thereby, increase P uptake in plants (Tesfaye, 2015). The result of this study, clearly demonstrates that Rhizobium inoculation improves the P uptake efficiency of soybean which might also contributes ton increased productivity of the crop.

Significant differences was noted in PUE among the varieties between the inoculated and uninoculated treatments, when the rate of P application increased to 20 and 40 kg P ha$^{-1}$, showing that Rhizobia inoculation enhanced the GPU efficiency of the responsive varieties, indicating the importance of combined application of Rhizobia inoculation together with P fertilization for better response.  Higher rate of P application (40 kg ha$^{-1}$) resulted in higher phosphorus uptake efficiency. This indicates that increasing the rate of phosphorus application to the optimum level increases phosphorus uptake efficiency. Similarly, Egamberdiyeva *et al*. (2004) also reported that P uptake by soybean was increased with increasing levels of P.

### 4.6.8 Agronomic efficiency (kg kg$^{-1}$)

The highest agronomic efficiency (22.89kg kg$^{-1}$) was recorded on SCS-1 variety in response to the combination of rhizobia inoculation and application of 20 kg ha$^{-1}$ P (Table 13). The lowest agronomic efficiency (9.52) was obtained on Clark 63K variety under uninoculated and application of 40 kg ha$^{-1}$ P conditions (Table 13). The efficiency of applied phosphorus in the soil reduces, mainly due to soil erosion, leaching and gaseous losses. The utilization of nutrient decreases, when increasing the rate of nutrient application by the law of limiting factors (Hussein, 2009).

This finding is in line with Khair *et al.* (2002) who reported that P agronomic efficiency decrease in uninoculated plots, but increased in the inoculated plots. The efficiency of the applied P in this study was within the range of 22.89-9.52kg kg$^{-1}$ at the rates of 20 to 40 kg P ha$^{-1}$ application, respectively (Table 13). This is lower than the one reported by Gidago *et al*. (2011) who reported declining trend of agronomic efficiency on common bean from 69.8 to 9.3 kg kg$^{-1}$, when the rates of P increased from 10 to 60 kg P ha$^{-1}$. With the corresponding application of single super phosphate and inoculation, Shah *et al*. (2001) also reported increased phosphorus uptake efficiency and yield with phosphorus application and rhizobia inoculation.

Abdul-Aziz (2013) reported that under tropical conditions, the efficiency of applied nutrient has been estimated to be 10-30% for P. Estimates of agronomic efficiency showed that the lower level of P relatively responded better to grain yield, which is in agreement with Bationo and Buerker (2001). That small amount of applied fertilizer optimized nutrient uptake efficiency.

Table 13.Response of three soybean varieties to Rhizobia treatment and rate of phosphorus application for phosphorus uptake efficiency and agronomic efficiency

| | | Phosphorus uptake efficiency(ppm) | | | | Agronomic efficiency(kg kg$^{-1}$ ) | | |
|---|---|---|---|---|---|---|---|---|
| Varieties | Inoculation Status | Phosphorus application (Kg ha$^{-1}$) | | | Mean | Phosphorus application(Kg ha$^{-1}$) | | Mean |
| | | 0 | 20 | 40 | | 20 | 40 | |
| Afgat | Inoculated | $17.69^{bc}$ | $20.16^{a}$ | $21.30^{a}$ | 19.72 | $18.18^{c}$ | $19.44^{b}$ | 18.81 |
| | Uninoculated | $17.93^{bc}$ | $18.00^{bc}$ | $18.13^{b}$ | 18.02 | $12.18^{e}$ | $11.05^{ef}$ | 11.62 |
| Clark 63K | Inoculated | $17.28^{bc}$ | $18.33^{b}$ | $17.39^{bc}$ | 17.67 | $14.71^{d}$ | $17.07^{c}$ | 15.89 |
| | Uninoculated | $15.40^{cd}$ | $16.49^{cd}$ | $18.03^{bc}$ | 16.64 | $15.10^{d}$ | $9.52^{g}$ | 12.31 |
| SCS-1 | Inoculated | $16.53^{cd}$ | $17.73^{bc}$ | $18.53^{b}$ | 17.60 | $22.89^{a}$ | $17.63^{c}$ | 20.26 |
| | Uninoculated | 17.46bc | 18.31b | 17.83bc | 17.87 | 10.49fg | 11.42ef | 10.96 |
| | Mean | 17.05 | 18.17 | 18.54 | 0.00 | 15.59 | 14.36 | 17.05 |
| LSD (5%) | 1.58 | | | | | 1.19 | | |
| CV (%) | 5.44 | | | | | 4.24 | | |

Mean value followed by the same letter within the same column is non-significant difference at 5% level of significance.

## 4.7. Pearson correlation of root and nodulations with yield and yield components of soybean

Grain yield was significantly (P<0.01) and positively correlated with its components viz., grain yield, number of pods per plants, number of seeds per pods and growth parameters, like days to physiological maturity, leaf area and number of primary branches and root parameters viz., root fresh weight, root dry weight, root volume and also with all nodule parameters viz., number of nodule, nodule fresh and dry weight (Table 14). Grain yield was strongly correlated with above ground biomass (0.81), followed by thousand seed weight (r=0.65), among the yield components, respectively. Similarly Ortiz *et al*. (2002); Abeledo *et al*. (2003) reported that the significant associations of barley grain yield with its yield components. Results obtained in this study on rhizobia inoculated and phosphorus fertilizer application clearly showed that the remarkable increase in plant height and hundred seed weight, and greatly contributed to increase in grain yield of soybean.

There was positive and highly significant (p<0.01) association of above ground dry biomass with plant height and number of pods per plant (Table 14), in which, above ground biomass was strongly correlated with plant height (0.53), and number of pods per plant (0.52), respectively. Growth parameters, such as plant height was positively and significantly (P<0.01) associated with grain yield of soybean (Table 14). However, the grain yield was also strongly correlated with plant height (0.67). Similarly, Temasgen *et al*. (2017) reported positive and significant correlation of biomass yield with thousand seeds weight and number of seeds per spike of barley. Strong positive and highly significant (p<0.01) correlation was found between root dry weight and root fresh weight (0.941). Positive correlation was observed between, nodule dry weights and nodule fresh weights (0.938), Root volume and root fresh weights (0.83), root volume and root dry weight (0.79), respectively (Table 14).

Table 14 Pearson correlation of growth, root, nodulations traits with the same yield and yield components

| | GY | DPM | PH | LA | NPB | RFW | RDW | NNP | NFW | NDW | RV | TRL | NPP | NSP | DABM | HSW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GY | | | | | | | | | | | | | | | | |
| DPM | 0.33* | | | | | | | | | | | | | | | |
| PH | 0.67** | 0.38* | | | | | | | | | | | | | | |
| LA | 0.34* | 0.14NS | 0.35* | | | | | | | | | | | | | |
| NPB | 0.27* | 0.28* | 0.15NS | 0.04NS | | | | | | | | | | | | |
| RFW | 0.37* | 0.40* | 0.28* | 0.12NS | 0.27* | | | | | | | | | | | |
| RDW | 0.30* | 0.38* | 0.21NS | 0.047NS | 0.36* | 0.941** | | | | | | | | | | |
| NNP | 0.12NS | -0.101NS | 0.35* | 0.39* | 0.06NS | 0.003NS | -0.032NS | | | | | | | | | |
| NFW | 0.30* | -0.020NS | 0.43* | 0.55** | 0.031NS | -0.13NS | -0.170NS | 0.74** | | | | | | | | |
| NDW | 0.30* | 0.04NS | 0.414* | 0.54** | -0.03NS | -0.13NS | -0.174NS | 0.72** | 0.938** | | | | | | | |
| RV | 0.28* | 0.38* | 0.23NS | 0.16NS | 0.25NS | 0.83** | 0.79** | 0.02NS | -0.019NS | -0.04NS | | | | | | |
| TRL | 0.06NS | -0.282NS | -0.027NS | -0.266NS | -0.153NS | 0.33* | 0.35* | 0.007NS | -0.173NS | -0.155NS | 0.27* | | | | | |
| NPP | 0.48* | 0.401* | 0.38* | 0.221* | 0.260* | 0.59** | 0.54** | -0.014NS | 0.06NS | 0.108NS | 0.53** | 0.235NS | | | | |
| NSP | 0.261* | -0.001NS | 0.15NS | 0.20NS | -0.010NS | -0.018NS | -0.027NS | -0.093NS | 0.095NS | 0.08NS | 0.027NS | -0.041NS | 0.28* | | | |
| DABM | 0.81** | 0.35* | 0.53** | 0.49* | 0.22NS | 0.43* | 0.35* | 0.113NS | 0.24NS | 0.23NS | 0.37* | -0.013NS | 0.52** | 0.07NS | | |
| HSW | 0.65** | 0.16NS | 0.401* | -0.189NS | 0.002NS | 0.172NS | 0.14NS | -0.131NS | 0.082NS | 0.136NS | 0.008NS | 0.24NS | 0.27* | 0.05NS | 0.37* | |

Where,GY: Grain yield, DFM : Days to physiological maturity ,PH: Plant height, LA: Leaf area, NPB: Number of primary branch, RFW: Root fresh weight,RDW: Root dry weight, NFW : Nodule fresh weight, NDW: Nodule dry weight, RV: Root volume, TRL: Tap root length, NPP: Number of pods/plant,NSP: Number of seeds /pod,DABM;Dry above biomass, HSW: Hundred(100) seed weight,*: Correlation significant at 0.05 level, and **:Correlation highly significant at 0.01 level.

## 4.8. Economic Analysis

The partial budget analysis was used to identify treatments with the optimum return for the farmer's investment. The highest gross farm gate benefit ET 34098ETB ha$^{-1}$ was gained from yield obtained at combined effect of Afgat variety inoculated rhizobia and 40kg P ha$^{-1}$ and the second and third gross benefit 27480, 27398 ETB were obtained from the interaction effect of SCS-1 variety of uninoculated and 20kg P ha$^{-1}$ application and the same variety with inoculated rhizobia and 20kg P ha$^{-1}$ respectively having above 100% greater value over the lower gross farm benefit obtained at 23027ETB from SCS-1 inoculated rhizobia and 0 kg p ha$^{-1}$(Zero P application).Therefore , the marginal rate of returns was done based on a treatment to be considered as worthwhile to farmers, that is 50% and 100% marginal rate of return (MRR) is the minimum acceptable rate of return(CIMMYT,1988).

The highest net benefit was 34098ETB ha$^{-1}$ obtained a plot treated with combined application the three way interaction of Afgat variety with inoculated rhizobia and 40kg P ha$^{-1}$(Table 15) had maximum marginal rate of return. The adoption of this treatment would give an additional gain of 3641% from every one Birr invested in soybean production. Therefore, the interaction effect of inoculated rhizobia and 40 kg P ha$^{-1}$ was more economically attractive than all the other treatment (Table 15). The gross net benefit of 34098, 27480, 27398 ETB ha$^{-1}$ from inoculated rhizobia and 40 kg P ha$^{-1}$ were economically viable and had positive marginal rate of return except gross farm benefit obtained at 23027ETB ha$^{-1}$ from SCS-1 inoculated rhizobia and 0 kg p ha$^{-1}$(Zero P application) of negative marginal rate return (Table 15) which could be removed from the recommendation.

Table 15.**Partial budget analysis for the three soybean varieties to rhizobia and rate of phosphorus application at different yield levels**

| Treatment | Variety rate ha$^{-1}$ | Rhizobia rate ha$^{-1}$ | Phosphorus rate ha$^{-1}$ | Grain yield | Gross benefit | Net benefit | Total variable cost | MRR | MRR (%) |
|---|---|---|---|---|---|---|---|---|---|
| V3 R0 P0 | 1600 | 0 | 0 | 2056.95 | 26740.35 | 25140.35 | 1600 | 0 | 0 |
| V2 R0 P0 | 1600 | 0 | 0 | 1946.745 | 25307.69 | 23707.69 | 1600 | D | |
| V1 R0 P0 | 1600 | 0 | 0 | 1916.739 | 24917.61 | 23317.61 | 1600 | D | |
| V3 R1 P0 | 1600 | 180 | 0 | 1908.252 | 24807.28 | 23027.28 | 1780 | -1.68 | -168.11 |
| V2 R1 P0 | 1600 | 180 | 0 | 1800.27 | 23403.51 | 21623.51 | 1780 | D | |
| V1 R1 P0 | 1600 | 180 | 0 | 2093.562 | 27216.31 | 25436.31 | 1780 | D | |
| V3 R0 P1 | 1600 | 0 | 300 | 2260.008 | 29380.1 | 27480.1 | 1900 | 17.03 | 1703.00 |
| V2 R0 P1 | 1600 | 0 | 300 | 2118.1 | 27535.3 | 25635.3 | 1900 | D | |
| V1 R0 P1 | 1600 | 0 | 300 | 2123.53 | 27605.89 | 25705.89 | 1900 | D | |
| V3 R1 P1 | 1600 | 180 | 300 | 2312.64 | 30064.32 | 27398.38 | 2080 | 9.40 | 940.00 |
| V2 R1 P1 | 1600 | 180 | 300 | 2065.644 | 26853.37 | 24773.37 | 2080 | D | |
| V1 R1 P1 | 1600 | 180 | 300 | 2421.414 | 31478.38 | 29398.38 | 2080 | D | |
| V3 R0 P2 | 1600 | 0 | 600 | 2468.997 | 32096.96 | 29896.96 | 2200 | 4.73 | 472.76 |
| V2 R0 P2 | 1600 | 0 | 600 | 2459.295 | 31970.84 | 29770.84 | 2200 | D | |
| V1 R0 P2 | 1600 | 0 | 600 | 2288.034 | 29744.44 | 27544.44 | 2200 | D | |
| V1 R1 P2 | 1600 | 180 | 600 | 2806.02 | 36478.26 | 34098.26 | 2380 | 36.4 | 3641.00 |
| V2 R1 P2 | 1600 | 180 | 600 | 2380.878 | 30951.41 | 28571.41 | 2380 | D | |
| V3 R1 P2 | 1600 | 180 | 600 | 2543.148 | 33060.92 | 30680.92 | 2380 | D | |

Variety price =20birr kg ha$^{-1}$, Rhizobia =180birr kg ha$^{-1}$, Phosphorus (TSP) = 15birr kg ha$^{-1}$and Grain yield=15 birr kg$^{-1}$.

# 5. SUMMARY AND CONCLUSION

Soybean (*Glycine max* L.) is one of the very important leguminous and oil crops, grown for its oil and protein source. Now a day there is a need to improve the yields of soybean through biological nitrogen fixation in the Ethiopian agriculture. The effect of phosphate nutrition on the rhizobia legume symbiosis has received considerable attention as plant phosphate status increase nodulation; nitrogen fixation and grain yield. Thus, the use of soybean variety with rhizobia inoculation and Phosphate fertilizer application produced reasonable good yield on soils of low fertility, especially on acidic soils, where the level of available P is very low. Therefore, this study was conducted to identify soybean varieties which tolerates low pH and low P soil by determining the responses of combined application of rhizobia inoculation and phosphate fertilizer application and to assess the interaction effect of variety, inoculation and phosphorus fertilizer application on growth and yield of soybean variety on acidic Nitisol soil of Metu condition. The treatments were laid out in a RCBD with three replications. The treatments included: rhizobia inoculation (using SB-MARK 1495 strains) and non-inoculated as a control, soybean varieties (Afgat (V1), Clark 63k (V2) and SCS-1(V3)) and phosphorus rates (0, 20 and 40 kg P ha$^{-1}$) as rates of fertilizers.

The physical and chemical properties of soil were analyzed before planting and after harvest. After harvest the available phosphorus and total nitrogen indicated a minor increase from initial soil analysis. Analysis of variance indicated that the combined application of rhizobia strain with phosphate fertilizer resulted in highly significant and significant improvement in the studied traits *i.e*. leaf area, taproot length, number of nodules per plant, number of pods per plant, harvest index, agronomic efficiency and grain yields and plant height, number of effective nodule, nodule fresh weight, number of seeds per pods and above ground dry biomass of soybean respectively. These improvements have resulted in increased grain yield and yield related parameters of soybean grown on the Nitisol of Metu.

The combination of inoculated rhizobia and phosphorus fertilizer application gave significantly the highest Plant height (76.53cm), number of Nodule per plant (52.0),nodule dry weight(8.24g plant$^{-1}$ ), grain yield (3.12 t ha$^{-1}$),harvest index (47.62%) and grain phosphorus uptake(21.3ppm), were recorded from the interaction of inoculated rhizobia and 40 kg p $ha^{-1}$of Afgat variety.

The application of inoculated rhizobia and phosphorus fertilizer significantly increased the parameters measured. For most of the parameters, supplying soybean with 40 kg P $ha^{-1}$ increased growth and yield more than application of 20 kg P/ ha. Phosphorus fertilizer application of 40 kg P $ha^{-1}$ with inoculated rhizobia were resulted in higher production and recommended for further evaluation.

Pearson correlation analysis revealed that nodule traits, and yield and yield related traits had a highly significant ($P<0.01$) and significant ($p<0.01$) positive correlation with each other. In conclusion on the combined application of rhizobia inoculation and 40 kg p ha-1 gave better nodulation and yield than the rest of the treatment and Afgat variety was tends better responds to growth parameters, nodulation and yield components on plant height, number nodule and grain yields. Therefore, use of rhizobia strain and 40kg P $ha^{-1}$ increase nodulation, grain yield and phosphorus uptake of soybean in the study area is recommended. The application of inoculated rhizobia and 40 kg P $ha^{-1}$ produced the highest net benefit 34098ETB $ha^{-1}$. The highest marginal rate of return (3641%) was obtained from yield at inoculated rhizobia and 40 kg P $ha^{-1}$ of Afgat soybean variety was the most profitable treatment. It has the highest return to the money invested in its production. However, the experiment was conducted for one season and one location. It is realistic to repeat similar experiment across wider ranges of agro ecology to give further recommendation. Furthermore, assessing different types of effective and compatible rhizobia strain along with different source of phosphate to increase phosphorus utilization efficiency and grain yield of soybean should require further investigation in the future.

# 6. REFERENCES

Abaidoo, R.C., Keyser, H.H., Singleton, P.W., Dashiell, K.E. and Sanginga, N., 2007. Population size, distribution, and symbiotic characteristics of indigenous Bradyrhizobium spp. that nodulate TGx soybean genotypes in Africa. *Applied Soil Ecology*, ***35*(1**):57-67.

Abate, T., Arega, D.,Alene, A.D., Bergvinson, D., Bekere Sh., Silim, S., Orr, A. and Solomon, A., 2012. *Tropical grain legumes in Africa and south Asia: knowledge and opportunities*. International Crops Research Institute for the Semi-Arid Tropics.

Abbas, G., Abbas, Z., Aslam, M., Malik, A.U., Ishaque, M. and Hussain, F., 2011. Effects of organic and inorganic fertilizers on mungbean (Vigna radiata (L.)) yield under arid climate. *International Research Journal of Plant Science*, ***2*(4**): 094-098.

Abdul Khaliq , M.Kaleem Abbasi A.and Tahir,H.,2007.effects of integrated use of organic and inorganic nutrient sources with effective microorganisms on seed cotton yield in Pakistan.Bioresource Technology **97**:967-972.

Abdul-Aziz, A.L., 2013. *Contribution of Rhizobium and phosphorus fertilizer to biological nitrogen fixation and grain yield of soybean in the tolon district* (Doctoral dissertation, MSC Thesis (unpublished). Faculty of Agriculture, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana).

Abebe, A.T., 2013. *Genetic analysis of quantitative traits in soybean (Glycine max L. Merril) under low and high phosphorus conditions* (Doctoral dissertation).

Abeledo G., F. Calderini and A.Slafer, 2003. Genetic improvement of barley yield potential and its physiological determinants in Argentina (1944–1998). *Euphytica*, **130**:325-334.

Abera, Z.,Dedefo, T. and Fred, K., 2013. Maize yield response to crop rotation, farmyard manure and inorganic fertilizer application in Western Ethiopia. *African Journal of Agricultural Research*, ***8*(46**):588-589.

Abush T., 2007. A terminal report submitted to Ethiopian institute of agricultural research and Jimma agricultural research center upon leave for further studies. January 2007. Jimma, Ethiopia

Abush,T., Mwangi, G., John, D. and Tolessa D., 2017. Genetic variability in soybean (*Glycine max L.*) for low soil phosphorus tolerance. *Ethiopian Journal of Agricultural Sciences*, **27**:1- 15.

Acharya, S.N., Thomas, J.E. and Basu, S.K., 2006. Fenugreek: an “old world” crop for the “new world”. *Biodiversity*, ***7*(3-4):**27-30.

Ahiabor, B.D.K., Lamptey, S., Yeboah, S. and Bahari, V., 2014. Application of phosphorus fertilizer on soybean [(Glycine max L.(Merril)] inoculated with rhizobium and its economic implication to farmers. *Journal of Experimental Agriculture International*, 1420-1434.

Ahlijah, K., 2017. Response of soybean to rhzobial inoculation and nitrogen management options in the southern guinea savannah zone of Ghana (Doctoral dissertation).

Ajeigbe, H.A., Dashiell, K. and Woomer, P.L., 2010. *Biological Nitrogen Fixation and Grain Legume Enterprise: Guidelines for N2Africa Lead farmers.* Tropical Soil Biology and Fertility Institute. IITA, Malawi.

Akpalu, M.M., Siewobr, H., Oppong-Sekyere, D. and Akpalu, S.E., 2014. Phosphorus application and Rhizobia inoculation on growth and yield of soybean (Glycine max L. Merrill). *American journal of experimental agriculture*, ***4*(6):**674.

Alam, M.A., Siddiqua, A., Chowdhury, M.A.H. and Prodhan, M.Y., 2009. Nodulation, yield and quality of soybean as influenced by integrated nutrient management. *Journal of the Bangladesh Agricultural University*, ***7*(2**): 229-234.

Alemu Abera,2013.Response of soybean varieties to dap rates in yayu district,western ethiopia . M.Sc Thesis submitted to the department of plant sciences , school of graduate studies , Haramaya University.

Amos, A.O., Ogendo M. and Joshua, O., 2004. Response of common bean to Rhizobium inoculation and fertilizers. *Journal of Food Technology in Africa*, **6(4):**121-125.

Argaw, A.,2014.Symbiotic effectiveness of inoculation with Bradyrhizobium isolates on soybean [Glycine max (L.) Merrill] genotypes with different maturities. *SpringerPlus*, ***3*(1**):753

Argaw,A.,2011. Evaluation of co-inoculation of Bradyrhizobium japonicum and Phosphate solubilizing Pseudomonas spp. effect on soybean (Glycine max L. Merr.) in Assossa Area. *Journal of Agricultural Science and Technology*, ***14*(1**):213-224.

Argaw,A.,2012. Evaluation of co-inoculation of Bradyrhizobium japonicum and phosphate solubilizing Pseudomonas spp. effect on soybean (Glycine max L.(Merr.)) in Assossa area.

Aziz, A., Ahiabor, K., Opoku,A. and Abaidoo,R., 2016. Contributions of Rhizobium Inoculants and Phosphorus Fertilizer to Biological Nitrogen Fixation, Growth and GrainYield of Three Soybean Varieties on a Fluvic Luvisol. *American Journal of Experimental Agriculture*, **10:** 1-11.

Bationo, A. and Bürkert, A., 2001. Soil organic carbon management for sustainable land use in Sudano-Sahelian West Africa. In *Managing Organic Matter in Tropical Soils: Scope and Limitations* (131-142). Springer, Dordrecht.

Bationo, A., Waswa, B., Kihara, J. and Kimetu, J., 2006. Advances in integrated soil fertility management in sub Saharan Africa: challenges and opportunities. *Nutrient cycling in agroecosystems*, 1-2P.

Bekere Workneh and Hailemariam Asfaw,2012. Influences of inoculation methods and phosphorus levels on nitrogen fixation attributes and yield of soybean (Glycine max L.) at Haru, western Ethiopia. *American Journal of Plant Nutrition and Fertilization Technology*, ***2*****(2**): 45-55.

Bekere Workneh,Endalkachew Wolde-Meskel and Kebede Tesfu, 2012. Growth and nodulation response of soybean (Glycine max L) to Bradyrhizobium inoculation and phosphorus levels under controlled condition in South Western Ethiopia. *African Journal of Agricultural Research*, ***7*****(30**):4266-4270.

Belachew, K.Y. and Stoddard, F.L., 2017. Screening of faba bean (Vicia faba L.) accessions to acidity and aluminium stresses. *PeerJ*, *5*:2963.

Bezabeh, A., Asfaw, E. and Anteneh, M., 2016. Trends of soybean production and productivity: evidence from three districts of southwest Ethiopia. *Sky Journal of Agricultural Research*, ***5*****(4):** 069-075.

Bezner-Kerr, R., Snapp, S., Chirwa, M., Shumba, L. and Msachi, R., 2008. Participatory Research on Legume Diversification with Malawian Smallholder Farmers for Improved Human Nutrition and Soil Fertility. *Experimental Agriculture* **43 (4**): 1–17.

Biate, D.L., Kumar, L.V., Ramadoss, D., Kumari, A., Naik, S., Reddy, K.K. and Annapurna, K., 2014. Genetic diversity of soybean root nodulating bacteria. In *Bacterial Diversity in Sustainable Agriculture* (131-145). Springer, Cham.

Board, J.E. and Harville, B.G., 1992. Explanations for greater light interception in narrow-vs. wide-row. *Crop Science, **32***(**1**):198-202.

Brady, N.C. and Weil, R.R., 2002. Phosphorus and potassium. *The nature and properties of soils. Prentice-Hall of India, Delhi, 352.*

Bray, R.H. and Kurtz, L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. *Soil science, **59***(**1**):39-46.

Bremner, J.M. and Mulvaney, C.S., 1982. Nitrogen-Total 1. *Methods of soil analysis. Part 2. Chemical and microbiological properties*, (methodsofsoilan2):595-624.

Bruce, R.C. and Rayment, G.E., 1982. *Analytical methods and interpretations used by the Agricultural Chemistry Branch for soil and land use surveys*. Queensland Department of Primary Industries.

Cadisch, G., Sylvester-Bradley, R. and Nosberger, J., 1992. Variation in growth and N~ 2 fixation within and among Centrosema spp. in response to phosphorus supply. *Tropical Grasslands*, ***26*:**226-226.

CDI (Center for Development Innovation), WUR (Wageningen University and Research), 20 11. Developing new value chains for soybean in Ethiopia. Accessed on November 29: 2014

Chandrasekaran,B., Annadurai, K. and Somasundaram, E., 2010.*A Text Book of Agronomy*. Published by New Age International (P) Ltd., Publishers, New Delhi, India.

Chen, L., Figueredo, A., Villani, H., Michajluk, J. and Hungria, M., 2002. Diversity and symbiotic effectiveness of rhizobia isolated from field-grown soybean nodules in Paraguay. *Biology and Fertility of Soils*, ***35*(6**):448-457.

Chiera, J., Thomas, J. and Rufty, T., 2002. Leaf initiation and development in soybean under phosphorus stress. *Journal of Experimental Botany*, ***53*(368**): 473-481.

Cooper, R.L., 2003. A delayed flowering barrier to higher soybean yields. *Field Crops Research*, ***82*(1):**27-35.

Cordell, D., Drangert, J.O. and White, S., 2009. The story of phosphorus: global food security and food for thought. *Global environmental change*, ***19*(2):** 292-305.

Crawford Jr, T.W., Singh, U. and Breman, H., 2008. Solving agricultural problems related to soil acidity in central Africa's great lakes region. CATALIST Project Report. *International Center for Soil Fertility and Agricultural Development (IFDC), Kigali, Rwanda*,1-133.

CSA (Central Stastical Agency) 2015/16. Report on the preliminary results of area, production and yield of temporary crops. Centeral Stastical Authority, Ethiop.

CSA , 2012. Crop Production Forecast Sample Survey. Report on Area and Crop Production Forecast for Major Crops (For Private Peasant Holding, Meher Season).

Date, R.A., 2001. Advances in inoculant technology: a brief review. *Australian Journal of Experimental Agriculture*, ***41*(3**):321-325.

Deaker, R., Roughley, R.J. and Kennedy, I.R., 2004. Legume seed inoculation technology—a review. *Soil Biology and Biochemistry*, ***36*(8):** 1275-1288.

Devi, N.K., Singh, N.K.L., Devi, S.T., Devi, N.H., Singh, B.T., Singh, K.K. and Singh, M.W., 2012. Response of Soybean [*Glycine max* (L.) Merrill] to Sources and Levels of Phosphorus. *Journal of Agricultural Science*, **4(6**):44-53.

Egamberdiyeva, D., Qarshieva, D. and Davranov, K., 2004. Growth and yield of soybean varieties inoculated with Bradyrhizobium spp in N-deficient calcareous soils. *Biology and Fertility of Soils*, ***40*(2):**144-146.

El-Agroudy, N., Mokhtar, S., Awad-Zaghol, E. and El-Gebaly, M., 2011. An economic study of the production of soybean in Egypt. Agric Biol JN Am, **2(2):** 221-225.

El-Azouni, I.M., 2008. Effect of phosphate solubilizing fungi on growth and nutrient uptake of soybean (Glycine max L.) plants. *J Appl Sci Res*, ***4*(6**):592-598.

Ellafi, A.M., Gadalla, A. and Galal, Y.G.M., 2011. Biofertilizers in action: contributions of BNF in sustainable agricultural ecosystems. *Int. Sci. Res. J,* ***3*(2**):108-116.

Erker, B., Brick, M.A. and Schwartz, H., 2014. Legume Seed Inoculants, Colorado State University Extension. 5/96. Revised 9/14. Crop Series Soil, Fact Sheet No. 0.305.

F. A. O., 2008**.**Fertilizer and plant nutrition bulletin. FAO, Rome, Itally, page:220.

Fassil Kebede ,Amir Seid Ahmed and Mitiku Haile, 2010. Land capability classification and growing period for Guila Abena watershed in Sassie Tseda Emba District in Eastern Tigray, Ethiopia. *Nature Sci*, ***8*(9**): 237-243.

Fassil Kebede, 2010. Some Legume Crops Effect on Soil Nitrogen Recovery in the Degraded Soils of North Ethiopia. Journal of American Science, **6(10):** 867-870.

Fatima, Z., Zia, M. and Chaudhary, M.F., 2017. Interactive effect of Rhizobium strains and P on soybean yield, nitrogen fixation and soil fertility. *Pakistan Journal of Botany*, ***39*(1**) :255.

Fekadu Gurmu ,2007. Participatory varietal selection of soybean [(Glycine max (L.) Merrill] varieties in umbullo wacho and beresa watersheds in the Southern region. In *Operational research and capacity building for food security and sustainable livelihoods: Proceedings of Irish Aid Supported Operational Research Project Review Workshop*.

Fekadu Gurmu, Hussein Mohammed and Getinet Alemaw, 2009. Genotype x environment interactions and stability of soybean for grain yield and nutrition quality. *African Crop Science Journal*, ***17*(2**).

Fernandes, A.M. and Soratto, R.P., 2012. Nutrition, dry matter accumulation and partitioning and phosphorus use efficiency of potato grown at different phosphorus levels in nutrient solution. *Revista Brasileira de Ciência do Solo*, ***36*(5**):1528-1537.

Gehl, R.J., Schmidt, J.P., Maddux, L.D. and Gordon, W.B., 2005. Corn yield response to nitrogen rate and timing in sandy irrigated soils. *Agronomy Journal*, **_97_**(**4**):1230-1238.

Gidago, G., Beyene, S. and Worku, W. 2011. The Response of Haricot Bean (*Phaseolus vulgaris* L.) to Phosphorus Application on Ultisols at Areka, Southern Ethiopia. *Journal of Biology Agriculture and Healthcare*, **1(3)**:38-49.

Gidago, G., Beyene, S. and Worku, W. 2011. The Response of Haricot Bean (*Phaseolus vulgaris* L.) to Phosphorus Application on Ultisols at Areka, Southern Ethiopia. *Journal of Biology Agriculture and Healthcare*, **1(3**) :38-49.

Giller, K.E., 2001. *Nitrogen fixation in tropical cropping systems*. Cabi.

Giller, K.E., Amijee, F., Brodrick, S.J. and Edje, O.T., 1998. Environmental constraints to nodulation and nitrogen fixation of Phaseolus vulgaris L in Tanzania II. Response to N and P fertilizers and inoculation with Rhizobium. *African Crop Science Journal*, **_6_(2)**:171-178.

Graham, P.H. and Vance, C.P., 2000. Nitrogen fixation in perspective: an overview of research and extension needs. *Field Crops Research*, **_65_(2-3):**93-106.

Grant, C., Bittman, S., Montreal, M., Plenchette, C. and Morel, C., 2005. Soil and fertilizer phosphorus: Effects on plant P supply and mycorrhizal development. *Canadian Journal of Plant Science*, **_85_(1):** 3-14.

Guareschi, R.F., Gazolla, P.R., Perin, A. and Santini, J.M.K., 2011. Antecipated fertilization on soybean with triple superphosphate and potassium chloride coated with polymers. *Ciência e Agrotecnologia*, **_35_(4):**643-648.

Gyaneshwar, P., Kumar, G.N., Parekh, L.J. and Poole, P.S., 2002. Role of soil microorganisms in improving P nutrition of plants. *Plant and soil*, **_245_**(**1**): 83-93.

Habtamu Alemu Keba, 2017. Genotype x Environment x Management Interaction of Common Bean (*Phaseolus vulgaris L.*) on Acidic Soils of Western Ethiopia. . An MSc Thesis Presented to the School of Graduate studies of Haramaya University.

Hailu Mekonnen . and Kaleb Kelemu , 2014. Trends in Soy Bean Trade in Ethiopia. *Research Journal of Agriculture and Environmental Management*, **_3_(9):**477-484.

Haling, R.E., Simpson, R.J., Culvenor, R.A., Lambers, H. and Richardson, A.E., 2011. Effect of soil acidity, soil strength and macropores on root growth and morphology of perennial grass species differing in acid-soil resistance. *Plant, Cell & Environment*, **_34_(3):**444-456.

Hankinson, M.W., 2015. *Planting Date and Starter Fertilizer Effects on Soybean Growth and Yield* (Doctoral dissertation, The Ohio State University).

Hartman, G.L., West, E.D. and Herman, T.K., 2011. Crops that feed the World 2. Soybean—worldwide production, use, and constraints caused by pathogens and pests. *Food Security*, ***3*****(1**), 5-17.

Howell, R. W., 1954. Phosphorus nutrition of soybeans. Plant Physiol. **29**:477-483.

Hungria, M. and Vargas, M.A., 2000. Environmental factors affecting N2 fixation in grain legumes in the tropics, with an emphasis on Brazil. *Field crops research*, ***65*****(2-3**):151-164.

Hussein, A.H.A.,2009. Phophorus use efficiency by two varieties of corn at different phosphorus fertilizer application rates. Research journal of applied sciences **4(2):**85-93.

IAR., 1982. Progress report of field crops research for the 1981/82 cropping season. Ethiopian Institute of Agricultural Research.

Jimma agricultural research center ,2015.Soybean breeding program .Report on soybean innovation lab project activities for the period 2015.

Jung, G., Matsunami, T., Oki, Y. and Kokubun, M., 2008. Effects of waterlogging on nitrogen fixation and photosynthesis in supernodulating soybean cultivar Kanto 100. *Plant production science*, ***11*****(3):**291-297.

Kamara, A.Y., Kwari, J., Ekeleme, F., Omoigui, L. and Abaidoo, R., 2008. Effect of phosphorus application and soybean cultivar on grain and dry matter yield of subsequent maize in the tropical savannas of north-eastern Nigeria. *African Journal of Biotechnology*, ***7*****(15).**

Kanyanjua, S.M., Ireri, L., Wambua, S. and Nandwa, S.M., 2002. Acidic soils in Kenya: Constraints and remedial options.

Kasasa, P., Mpepereki, S. and Giller, K.E., 2000. Quantification of nitrogen fixed by promiscuous soybean varieties under Zimbabwean field conditions. *Field Crop Research*, ***65***:137-149.

Khair, M.K., Moammed, T., Faisal, H.Tand Khalid, N.,2002. Phosphorus use efficiency as affected by phosphorus application and inoculation. Parkistan Journal of Agronomy **1(1)** :49-50.

Khojely, D.M., Ibrahim, S.E., Sapey, E. and Han, T., 2018. History, current status, and prospects of soybean production and research in sub-Saharan Africa. *The Crop Journal*, ***6*****(3**):226-235.

Kous, K., 2010, May. Comparative analysis versions of BPMN and its support with Control-flow patterns. In *The 33rd International Convention MIPRO* (315-319). IEEE.

Kumah, F.J.A., 2016. Influence of P sources and rhizobium inoculation on growth, nodulation, N & P uptake and yield of three soybean genotypes in Tanchera soil series of the northern Guinea Savannah zone of Ghana (Doctoral dissertation, Department of Soil Science, School of Agriculture, College of Basic and Applied Sciences, University of Ghana, Legon).

Landon, 1991. Tropical book of soil manual. 2nd edition

Long, S.R., 2001. Genes and signals in the Rhizobium-legume symbiosis. *Plant physiology*, ***125*(1):** 69-72.

Mahamood, J., Abayomi, Y.A. and Aduloju, M.O., 2009. Comparative growth and grain yield responses of soybean genotypes to phosphorous fertilizer application. *African Journal of Biotechnology*, ***8*(6**).

Malone, S., Ames Herbert Jr, D. and Holshouser, D.L., 2002. Relationship between leaf area index and yield in double-crop and full-season soybean systems. *Journal of economic entomology*, ***95*(5**):945-951.

Marschner, H., 1995. Mineral nutrition of higher plants. 2nd. *Edn. Academic Pres*.

Masarirambi, M.T., Mbokazi, B.M., Wahome, P.K. and Oseni, T.O., 2012. Effects of kraal manure, chicken manure and inorganic fertilizer on growth and yield of lettuce (Lactuca sativa L. var Commander) in a semi-arid environment. *Asian Journal of Agricultural Sciences*, ***4*(1**) :58-64.

Masiresha Abitew and Kibebew Kibret, 2017. Effects of Bradyrhizobium japonicum, Nitrogen and Phosphorus Fertilizers on Growth, Nodulation, Yield and Yield Components of Soybean at Pawe Northwestern Ethiopia. *World Journal of Agricultural Sciences*, ***13*(1**):53-62.

Masresha Abitew Tarekegn and Kibebew Kibret , 2017. Effects of rhizobium, nitrogen and phosphorus fertilizers on growth, nodulation, yield and yield attributes of soybean at Pawe Northwestern Ethiopia. *World Scientific News*, *67*(2),201-218.

Masuda, T. and Goldsmith, P.D., 2009. World soybean production: area harvested, yield, and long-term projections. *International food and agribusiness management review*, ***12* :**1-20.

Matsunami, T., Jung, G.H., Oki, Y. and Kokubun, M., 2007. Effect of waterlogging during vegetative stage on growth and yield in supernodulating soybean cultivar Sakukei 4. *Plant production science*, ***10*(1):** 112-121.

Mengel, K. and Kirkby, E.A., 1987. Principles of plant nutrition. Ed. *Worblaufen: International Potash Institute*,Bern,Switzerland ,**347-420.**

MoFA and CSIR, 2005**.** Soybean Production Guide. Food crops development project. Ghana's Ministry of Food and Agriculture. **38.**

Mohammadi, K. and Sohrabi, Y., 2012. Bacterial biofertilizers for sustainable crop production: a review. *J Agric Biol Sci*, ***7***:307-316.

Mohammadi, K., Sohrabi, Y., Heidari, G., Khalesro, S. and Majidi, M., 2012. Effective factors on biological nitrogen fixation. *African Journal of Agricultural Research*, *7*(12):1782-1788.

Moharram M., Safwat, A., Farghaly, M., 1994. Effect of inoculation rate and phosphorus fertilization on Nitrogen fixation in soybean. *African Crop Science Journal* **2**:125-129.

Muhammad, A., 2010. Response of a promiscuous soybean cultivar to Rhizobial inoculation and phosphorus in Nigeria's Southern Guinea Savanna Alfisol. *Nigerian Journal of Basic and Applied Sciences*, ***18*****(1):** 79-82.

Munns, D.N., Hohenberg, J.S., Righetti, T.L. and Lauter, D.J., 1981. Soil Acidity Tolerance of Symbiotic and Nitrogen-Fertilized Soybeans 1. *Agronomy Journal*, ***73*****(3**): 407-410.

Murata, M.R., Hammes, P.S. and Zharare, G.E., 2003. Effect of solution pH and calcium concentration on germination and early growth of groundnut. *Journal of plant nutrition*, ***26*****(6**):1247-1262.

Naya, L., Ladrera, R., Ramos, J., González, E.M., Arrese-Igor, C., Minchin, F.R. and Becana, M., 2007. The response of carbon metabolism and antioxidant defenses of alfalfa nodules to drought stress and to the subsequent recovery of plants. *Plant Physiology*, ***144*****(2**),1104-1114.

Ndakidemi, P.A., Dakora, F.D., Nkonya, E.M., Ringo, D. and Mansoor, H., 2006. Yield and economic benefits of common bean (Phaseolus vulgaris) and soybean (Glycine max) inoculation in northern Tanzania. *Australian Journal of Experimental Agriculture*, ***46*****(4**): pp.571-577.

Nelson, D.W. and Somers, L.E., 1982. Methods of soil analysis, Part 2: Chemical and Microbiological Properties, Page AL, Miller RH, Keeney DR (eds) Am. Soc. Agronomy, Inc. and Soil Sci. Soc. Am. *Inc. Madison, Wis.*565-571.

Nicolas, P., Chiara, P., Karine, M., Gilles I. and Alexandre J., 2006. Reactive oxygen and nitrogen species and glutathione: key players in the legume – *Rhizobium* symbiosis. Journal of Experimental Botany, Vol. **57**, No. 8,1769 – 1776

Nkaa, F., Nwokeocha, O.W. and Ihuoma, O., 2014. Effect of phosphorus fertilizer on growth and yield of cowpea (Vigna unguiculata). *IOSR J. Pharm. Biol. Sci*, ***9*****(5)**: 74-82.

Ogoke J., Togun, O., Carsky, J.and Dashiell, E.,2004. Phosphorus uptake and balance in a soybean-maize rotation in the moist savanna of West Africa. *Journal of Agriculture and Rural Development in the Tropics and Subtropics (JARTS)*, **105**:15-27.

Okogun, J.A., Otuyemi, B.T. and Sanginga, N., 2004. Soybean yield determinants and response to rhizobial inoculation in an on-farm trial in the Nothern Guinea Savanna of Nigeria. *West African journal of applied ecology*, ***6*****(1).**

Olivera, M., Tejera, N., Iribarne, C., Ocana, A. and Lluch, C., 2004. Growth, nitrogen fixation and ammonium assimilation in common bean (Phaseolus vulgaris): effect of phosphorus. *Physiologia Plantarum*, ***121*****(3)**:498-505.

Onwonga, R.N., Lelei, J.J., Freyer, B., Friedel, J.K., Mwonga, S.M. and Wandhawa, P., 2008. Low cost technologies for enhancing N and P availability and maize (Zea mays L.) performance on acid soils. *World Journal of Agricultural Sciences*, ***4*****(5)**:862-873.

Ortiz-Monasterio P.,H. Pfeiffer and H. Hede, 2002. Phosphorus use efficiency, grain yield and quality of triticale and durum wheat under irrigated conditions In: Proceedings of the 5th International Symposium, Annex, Radizkow, Poland.

Owusu-Mensah, P., 2017. *Growth and yield response of soybean varieties to inoculation and nitrogen levels* (Doctoral dissertation).

Pancheli, K., 2011. Symbiotic nitrogen fixation and seed development of genetically modified soybean in relation to bradyrhizobium inoculation and nitrogen use.P:**72**.

Peoples, M.B., Brockwell, J., Herridge, D.F., Rochester, I.J., Alves, B.J.R., Urquiaga, S., Boddey, R.M., Dakora, F.D., Bhattarai, S., Maskey, S.L. and Sampet, C., 2009. The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. *Symbiosis*, ***48*****(1-3)**:1-17.

Phiri T., Muindi, M., Omollo,O., Yegon,R. and Kausiwa, D.,2016. Inoculated Soybean Yields Response to Nitrogen and Phosphorus Application. *International Journal of Plant and Soil Science*, **10:** 1- 7.

Plahar, W.A., 2006, September. Overview of the soybean industry in Ghana. In *workshop on soybean for human nutrition and health, Accra, Ghana* (Vol. 28).

Purcell, L.C., Salmeron, M. and Ashlock, L., 2014. Soybean growth and development. *Arkansas Soybean Handbook. Fayetteville: University of Arkansas*.

Rahman, M., Imran, M. and Ashrafuzza, M., 2012. Effect of inoculant on yield and yield contributing characters of summer mungbean cultivars. Dept. of Crop Botany, Bangladesh Agricultural University, Mymensingh Journal of Environmental Science & Natural Resources, **5(1):** 211 – 215.

Rao, A.S. and Reddy, K.S., 2010. Nutrient management in soybean. *The soybean: botany, production and uses*, pp.161-190.

Rao, A.S. and Reddy, K.S., 2010. Nutrient management in soybean. *The soybean: botany, production and uses*, 161-190.

Reni, Y.P. and Rao, Y.K., 2013. Genetic variability in soybean [Glycine max (L) Merrill]. *International Journal of Plant, Animal and Environmental Sciences*, ***3*****(4)**:35-38.

Richardson, A.E. and Simpson, R.J., 2011. Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant physiology*, ***156*****(3),** 989-996.

Rodríguez-Navarro, D.N., Oliver, I.M., Contreras, M.A. and Ruiz-Sainz, J.E., 2011. Soybean interactions with soil microbes, agronomical and molecular aspects. *Agronomy for Sustainable Development*, ***31*****(1):**173-190.

Ronner, E., Franke, A.C., Vanlauwe, B., Dianda, M., Edeh, E., Ukem , B., Bala, A., Van Heerwaarden, J. and Giller, K.E., 2016. Understanding variability in soybean yield and response to P-fertilizer and rhizobium inoculants on farmers' fields in northern Nigeria. *Field Crops Research*, ***186***:133-145.

Roy, S.K., Rahaman, S.M.L. and Salahuddin, A.B.M.,1995. Effect of *Rhizobium* inoculum and nitrogen on nodulation, growth and grains yield of gram (*Cicer arietinum* L.). *Indian J.Agron .,* **65(12**):853-857

Sahlemedhin, S. and Taye, B., 2000. Procedures for soil and plant analysis. *Technical paper*, (**74**):110.

Salvagiotti, F., Cassman, K.G., Specht, J.E., Walters, D.T., Weiss, A. and Dobermann, A., 2008. Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. *Field Crops Research*, ***108*****(1**):1-13.

Sanginga, P.C., Adesina, A.A., Manyong, V.M., Otite, O. and Dashiell, K.E., 1999. *Social impact of soybean in Nigeria's southern Guinea Savannah*. Ibadan: IITA.

Schroder, J.J., Cordell, D., Smit, A.L. and Rosemarin, A., 2010. *Sustainable use of phosphorus: EU tender ENV. B1/ETU/2009/0025* (No. 357). Plant Research International.

Schulze, J., Temple, G., Temple, S.J., Beschow, H. and Vance, C.P., 2006. Nitrogen fixation by white lupin under phosphorus deficiency. *Annals of Botany*, ***98*****(4**):731-740.

Shah, P., Kakar, K.M. and Zada, K., 2001. Phosphorus use-efficiency of soybean as affected by phosphorus application and inoculation. In *Plant Nutrition* ( 670-671). Springer, Dordrecht.

Shahid, M.Q., Saleem, M.F., Khan, H.Z. and Anjum, S.A., 2009. Performance of Soybean (*Glycine max* L.) under Different Phosphorus Levels and Inoculation. *Pakistan Journal of Agricultural Sciences***, 46(4):**237-241.

Sharma, M.K. and Kumawat, D.M., 2011. Co-inoculation study of Bradyrhizobium japonicum and Aspergillus niger in soybean for nitrogen fixation. *The Journal of Microbiology, Biotechnology and Food Sciences*, ***1*****(3**):383.

Singh, G. and Shivakumar, B.G., 2010. The Role of Soybean in Agriculture Division of Agronomy. *Indian Agricultural Research Institute, New Delhi, India*.

Syers, J.K., Johnston, A.E. and Curtin, D., 2008. Efficiency of soil and fertilizer phosphorus use. *FAO Fertilizer and plant nutrition bulletin*, ***18*****(108).**

Tahir, M.M., Abbasi, M.K., Rahim, N., Khaliq, A. and Kazmi, M.H., 2009. Effect of Rhizobium inoculation and NP fertilization on growth, yield and nodulation of soybean (Glycine max L.) in the sub-humid hilly region of Rawalakot Azad Jammu and Kashmir, Pakistan. *African Journal of Biotechnology*, ***8*****(22**):6191-6200.

Tairo, E.V. and Ndakidemi, P.A., 2013. Possible benefits of rhizobial inoculation and phosphorus supplementation on nutrition, growth and economic sustainability in grain legumes. *Am. J. Res. Commun*, ***1*****(12**):532-556.

Tairo, E.V.and Ndakidemi, P.A.,2014. Macronutrients Uptake in Soybean as Affected by Bradyrhizobium japonicum inoculation and phosphorus (P) supplements. *American Journal of Plant Sciences*, ***5*****(04):** 488.

Temasgen Desalegn, Getachew Alemu, Ayalew Adella and Tolessa Debele, 2017. Effect of lime and phosphorus fertilizer on Acid soils and barley (*Hordeum vulgare l.*) performance in the central highlands of Ethiopia. *Experimental Agriculture*, **53**: 432-444.

Tesfaye Abebe, 2007 a terminal report submitted to Ethiopian institute of agricultural research and Jimma agricultural research center upon leave for further studies. January 2007. Jimma, Ethiopia.

Thoenes, P., 2014. Soybean International CommoditynFood Pro and Agriculture Organization-25. of the United.

Tran .T.N.S., Diep, C.N., Giang, T.T.M. and Thu, T.T., 2007. Effect of co-inoculants (Bradyrhizobia and phosphate solubilizing bacteria) liquid on soybean under rice based cropping system in the mekong delta. *Omon Rice*, ***15*****:**135-143.

Ugwu,D.and Ugwu ,H., 2010.Soybean production ,processing and marketing in Nigeria .Journal of applied sciences and development **1(1):**45-61.

Van Jaarsveld, C.M., Smit, M.A. and Krüger, G.H.J., 2002. Interaction amongst soybean [Glycine max (L.) Merrill] genotype, soil type and inoculant strain with regard to N2 fixation. *Journal of agronomy and crop science*, ***188*****(3)**: 206-211.

Vance, C.P., Uhde-Stone, C. and Allan, D.L., 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New phytologist*, ***157*****(3):**423-447.

Venkateswarlu, B., Hari, K. and Katyal, J.C., 1997. Influence of soil and crop factors on the native rhizobial populations in soils under dryland farming. *Applied Soil Ecology*, ***7*****(1)**:1-10.

Wafaa, M.H. and Haggag, M., 2002. Sustainable agriculture management of plant diseases. *Online J Biolog Sci*, ***2***:280-284.

Walkley, A. and Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil science*, ***37*****(1):**29-38.

Walter, D.,2007. Tanzania: the challenge of moving from subsistence to profit. Business for Development.

Westgate, M.E., 1999, August. Managing soybeans for photosynthetic efficiency. In *Crop, Soil and Water Managemnet. Proc. World Soybean Research Conference VI* (223-228).

Wijnands, J.H.M., Gurmesa, N.D., Lute, J.C.M. and Van Loo, E.N., 2011. *Ethiopian soya bean and sunflower value chains: Opportunities and challenges* (No. 2011-016). LEI, part of Wageningen UR..

World Bank group, 2016.http://data.worldbank.org/indicator/AG.CON.FERT.

Yagoub, S.O., Ahmed, W.M.A. and Mariod, A.A., 2012. Effect of urea, NPK and compost on growth and yield of soybean (Glycine max L.), in semi-arid region of Sudan. *ISRN Agronomy, 2012*.

Zafar, M., Maqsood, M., Ramzan, M., Amzan, A. and Zahid, A., 2003. Growth and yield of lentil as affected by phosphorus. *Journal of Agriculture and Biology*,1560-8530.

Zinaw,D., Seltene,A., Addisu,G., 2013. Genetic variability and heritability of soybean (*Glycinemax* (L.) Merrill) genotypes in Pawe district, Metekel zone, Benishangule Gumuz regional state, northwestern Ethiopia. Wudpecker J. Agric. Res., **2(9)**:240-245.

Zoundji, C.C., Houngnandan, P., Dedehouanou, H. and Toukourou, F., 2015. Determinants of soybean [glycine max (l.) merrill] production system in Benin. *Experimental Biology and Agricultural Sciences*, ***3***:430-439.

# 7. APPENDICES

Appendix Table 1.Mean squares of variety (V), rhizobia (R), phosphorus (P) and their interactions for the various traits studied at Mettu Southwestern Ethiopia in 2018

| Parameters | Mean Squares | | | | | | | | Ground mean |
|---|---|---|---|---|---|---|---|---|---|
| | V | R | P | V*R | V*P | R*P | V*R*P | Error | |
| Leaf area | 4104.56** | 1851.4** | 650.00** | 431.74** | 330.28** | 1266.98** | 111.17** | 10.91 | 112.9 |
| Nodule dry weight | 45.76** | 6.24* | 8.18** | 3.77* | 3.31** | 19.85** | 3.69** | 12.82 | 3.97 |
| Number of nodule per plant | 131.29** | 50.07** | 182.47** | 222.38** | 373.92** | 1116.3** | 272.63** | 9.84 | 25.12 |
| Number of pods per plant | 29.73** | 19.44* | 541.25** | 12.90* | 77.97** | 45.40** | 32.87** | 1.42 | 42.98 |
| Tap root length | 35.91** | 17.63** | 2.53* | 2.85* | 2.23* | 3.12* | 9.27** | 0.51 | 23.50 |
| Agronomic efficiency | 7.30** | 403.13** | 3.52* | 25.12** | 28.89** | 12.33** | 14.24** | 0.404 | 14.97 |
| Grain yield | 142835.7** | 98935.45** | 1604125.14** | 278564.43** | 6080.62** | 63725.16** | 12009.20** | 1095.34 | 2467.29 |
| Harvest index | 88.19** | 13.86** | 24.51** | 6.63** | 19.50** | 9.22** | 9.78** | 0.28 | 43.18 |

V=Variety, R=Rhizobia, P=Phosphorus, V*R=Variety interaction with Rhizobia, V*P=Variety interaction with Phosphorus, R*P=Interaction of Rhizobia with* Phosphorus and V*R*P=three way interaction of Variety, Rhizobia and Phosphorus

Appendix Table 2. Significance of mean squares of variety (V), rhizobia (R), phosphorus (P) and their interaction for the various parameters at Mettu Southwestern Ethiopia in 2018

| Parameters | Mean squares | | | | | | | Ground mean |
|---|---|---|---|---|---|---|---|---|
| | V | R | P | V*R | V*P | R*P | Error | |
| Number of nodules per plant | 131.29** | 50.07* | 182.47** | 222.38** | 373.93** | 1116.28** | 9.84 | 25.12 |
| Nodule fresh weight | 120.76** | 26.82* | 22.12** | 12.76* | 9.69* | 63.67** | 1.70 | 7.63 |
| Taproot length | 35.91** | 17.63** | 2.53* | 2.85* | 2.32* | 3.12* | 0.51 | 23.50 |
| Number of pods per plant | 29.73** | 19.44* | 541.25** | 12.91* | 77.98** | 45.41** | 1.42 | 42.98 |
| Phosphorus uptake efficiency | 13.70** | 9.028* | 10.82* | 4.5* | NS | NS | 0.95 | 17.92 |
| Plant height | 222.53** | NS | 129.90** | 38.41** | 131.78** | 44.02** | 64.34 | 2.80 |
| Root fresh weight | 372.16** | NS | 1296.68** | 113.26* | NS | 82.06* | 26.02 | 33.90 |
| Root dry weight | 34.98** | NS | 82.3** | 7.00** | NS | 7.55* | 1.76 | 9.48 |
| Root volume | 325.90** | NS | 1450.12** | 121.06* | NS | 145.72* | 24.39 | 41.35 |
| Thousand seeds weight | 19.10** | NS | 1.17* | 2.61* | NS | 1.57* | 1.11 | 15.88 |
| Days to 50% flowering | 413.13** | 2.66* | NS | NS | 2.4* | NS | 0.55 | 57.62 |
| Days 90% physio. maturity | 1.72** | 14.52** | 26.00** | 4.3* | 2.4* | NS | 0.35 | 115.88 |
| Number of primary branch | 0.72* | NS | 0.96 | NS | NS | 1.03* | 0.204 | 4.43 |
| Nodule effectiveness | NS | NS | NS | 2.17* | NS | 4.67* | 0.49 | 2.55 |
| Number of seeds per pods | NS | 0.017* | NS | 0.03* | 0.011* | 0.013* | 0.004 | 2.50 |
| Dry biomass | 2473235* | NS | 5748564* | NS | NS | NS | 654243 | |

V=Variety, R=Rhizobia, P=Phosphorus*R=variety and rhizobia, V*P= Variety and Phosphorus and R*P= rhizobia and phosphorus interaction.

Appendix figure 2. Field experiment performance at Metu Maremiya Bet in 2018

Appendix figure 2. Field experiment performance at Metu Maremiya Bet in 2018