

**GROWTH, SYMBIOTIC AND YIELD PERFORMANCE OF SOYBEAN
(*Glycine max* L.) VARIETIES IN RESPONSE TO *Bradyrhizobium*
INOCULATION AND PHOSPHORUS APPLICATION AT ASSOSA,
WESTERN ETHIOPIA**

MSc. THESIS

TESFAYE TEKOLA SEIFU

HAWASSA UNIVERSITY, HAWASSA, ETHIOPIA

NOVEMBER, 2017

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TESFAYE TEKOLA SEIFU

**ATHESIS SUBMITTED TO SCHOOL OF PLANT AND
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(Submission sheet -2)

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DEDICATION

I dedicate this thesis manuscript to my families for their unconditional love.

STATEMENT OF AUTHOR

First, I declare that this thesis is my genuine work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree at the Hawassa University and is deposited at the university library to be made available to borrowers under rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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LIST OF ABBREVIATIONS

AARC	Assosa Agricultural Research Center
ANOVA	Analysis of variance
BNF	Biological nitrogen fixation
CSA	Central Statistical Agency
HGW	Hundred grain weight
HI	Harvest index
LA	Leaf area
LAI	Leaf area index
LSD	Least significant difference
MoA	Ministry of Agriculture
PARC	Pawe Agricultural Research Center
SAS	Statistical analysis software
SRARI	South Region Agricultural Research Institute
TSP	Triple super phosphate
ATVET	Agricultural Technical and Vocational Education Training

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Growth, Symbiotic and Yield Performance of Soybean (*Glycine max* L.) Varieties in Response to *Bradyrhizobium* Inoculation and Phosphorus Application at Assosa, Western Ethiopia

TESFAYE TEKOLA (B.Sc.), Debre Birhan University
TAREKEGN YOSEPH (PhD): Major-advisor, Hawassa University
WALELIGN WORKU (PhD): Co-advisor, Hawassa University

ABSTRACT

*This research was conducted during 2016 main cropping season on field at Assosa, in Benishangul Gumuz Regional State with the objectives of investigating growth, symbiotic and yield performance of three soybean (*Glycine max* L.) varieties in response to *Bradyrhizobium* inoculation and P fertilizer application. The treatments studied included three P levels (0, 10 and 20 kg P ha⁻¹), two inoculation levels (uninoculated and inoculated with *Bradyrhizobium* strain; MAR-1495) and three soybean varieties (Gizo, Belessa-95 and Local) in randomized complete block design with factorial arrangement using three replications. The results revealed that *Bradyrhizobium* inoculation with MAR-1495 significantly ($P \leq 0.01$) increased most of the parameters studied as compared to the control. Inoculation with strain MAR-1495 significantly ($P \leq 0.01$) increased nodule number, nodule dry weight, shoot dry weight, root dry weight, leaf area, grain yield, and above ground total biomass as compared to the control. Application of P at 20 and 10 kg ha⁻¹ significantly increased nodule number, nodule dry weight, and root dry weight, shoot dry weight, leaf area, above ground total biomass and grain yield. There was significantly ($P \leq 0.01$) cultivars effect for most of studied parameters; Belessa-95 variety had better responded on nodule number, nodule dry weight, grain yield and above ground total biomass as compared to Gizo and Local varieties. The highest grain yield was recorded from Belessa-95 variety 3.29 ton ha⁻¹ from combined application of 20 kg P and *Bradyrhizobium* inoculation. Nodule number plant⁻¹ was positively and significantly correlated with most of growth parameters, yield and yield components. It could, thus, be deduced that use of MAR-1495 *Bradyrhizobium* strain and Belessa-95 variety with application of 20 kg P markedly increased the productivity of the crop at Assosa area, however economic analysis revealed that the highest net return was obtained from application of 10 kg P and *Bradyrhizobium* inoculation with strain MAR-1495. The use of MAR-1495 strain and Belessa-95 variety with application P at 10 kg ha⁻¹ could be recommended, but verification of the results on different farmers' field could be required before wide use of this study.*

Keywords: *Bradyrhizobium*, fertilizer, nodulation, soybean, growth, yield components

1. INTRODUCTION

Soybean (*Glycine max* L.) is a small erect and branching annual leguminous plant classified under the family *Leguminosae* and sub-family Papilionodeae (Maphumo, 2011). It is a short day self-pollinated C₃ plant that grows well in tropical, sub-tropical and temperate climates. Soybean grows from sea level up to 2000 meters above sea level latitudes ranging from the equator up to 55° north and 55° south. The optimum temperature for growth and development is 23-25°C. The crop requires 500-850 mm water during the growing season (Hymowitz, 2010).

Soybean has quantitatively highest protein content (40%) of all food crops and is second in terms of oil content (20%) among food legumes. These two products are used in thousands of ways for food, feed and industrial uses. It accounts for about 50% of the oil seed production across the globe and its oil consists of up to 85% of unsaturated fatty acids which is free from cholesterol. Thus, soybean can contribute significantly to the overall human nutrition in terms of both calorie and protein intake to meet the fast growing demand for vegetable oil in most developing countries (Abdullahi *et al.*, 2013). On the other hands, the crop provides balanced combination of protein, fat and carbohydrate to serve as valuable bio-feed stocks (Abate *et al.*, 2012). Hence, production of soybean in Ethiopia is very essential to overcome malnutrition and partially compensate the expensive sources of animal protein. Besides its high nutritional values, soybean can be encouraged in smallholder farmers based production systems in the tropics and sub-tropics to diversify production, generate income, and capture positive rotation effects that improve productivity of other crops being grown (Rusike *et al.*, 2013). In Ethiopia, soybean is an important food crops widely produce in west and south western parts of the country. Now a day,

its production is highly concentrated in high rain fall areas of Ethiopia like Assosa and it is a legume being recently integrated in to cropping system of smallholder farmers (CDI, 2014).

Currently soybean production is increasing rapidly all over the globe as a result of numerous benefits derived from the crop (Vanlauwe *et al.*, 2014). It is grown on more than 10,000 million ha of land worldwide with more than annual total production of 241 million ton and productivity of 2 ton ha⁻¹ (FAO, 2015). In Ethiopia, it has been noted that from 31,854.75 ha of land 63,653.1 ton of soybean was produced and the average productivity was 1.9 ton ha⁻¹ during 2012/13 (Bekabi, 2015). In Benishangul Gumuz Regional State, from 13,946.74 ha⁻¹ of land 29,227.4 ton of soybean was produced and the average productivity was 2 ton ha⁻¹ in 2016/17 cropping season (CSA, 2017).

Declining of soil fertility and high fertilizer cost are the major limitations to crop production of smallholder farmers in developing countries (Matusso *et al.*, 2014). Requirement for nitrogen (N) exceeds any other major nutrients and rarely do soils have enough of this nutrient to produce high sustainable yields. The quality of N needed for agriculture is projected to increase and this would lead to greater environmental degradation. Reduced dependence on N fertilizer and adapting farming practices that favor the more economically viable and environmentally prudent N₂-fixation will benefit both agriculture and the environment (Matusso *et al.*, 2014). There are several options that are available to manage the N in farmers' fields and chemical fertilizers are often considered to be an immediate answer current nutrient deficiency in soil. Unfortunately, commercial N fertilizers are expensive and out of reach of most small scale farmers (Abaidoo *et al.*, 2013). As a result, cheaper source of N need to be sought if yields are to be sustained to attain food security. The expensive fertilizer cost cut down through the process of N₂-fixation by

legume crops to acquire their own N needs and also provide some nutrients left over to succeeding crops through decomposition of their nodules, shoots and roots (Vanlauwe *et al.*, 2014). For instance, the *Bradyrhizobium* strain (MAR-1495) used for this experiment has shown better agronomic and symbiotic performance in soybean growing area in Ethiopia (COMPRO, 2013). Therefore, inoculating the seed of soybean with this and other compatible and appropriate strain/s may be a better solution for optimizing the economic yields of soybean in areas where a low population of native *rhizobia* strains predominant. This bio-fertilizer will offer immediate economic return to the resource poor farmers who cannot afford the full package of inorganic fertilizers. *Rhizobium* inoculants seem to be attractive and cost effective sources of N for legume cultivation in some countries and require little technical expertise (Vanlauwe *et al.*, 2014).

Phosphorus (P) is an important plant macronutrient, making up about 0.2-0.5% of a plant's dry weight and it is second only to N as the most limiting element for plant growth. Phosphorus is a major growth-limiting nutrient, and unlike the case for N, there is no large atmospheric source that can be made biologically available (Bashir *et al.*, 2011). Inadequate P restricts root growth, the process of photosynthesis, translocation of sugars and other such functions which directly influence N₂-fixation and yield of legume plants (Abdul-Aziz, 2013). Symbiotic N₂-fixation has a high P demand because the process consumes large amounts of energy and energy generating metabolism strongly depends upon the availability of P.

Assosa is one of the areas in Western Ethiopia where farmers grow soybean widely. They produce it as a source of food, cash and also rotate it with cereal crops. As is true for soils in most parts of Ethiopia, the area also faces N and P deficiencies during crop production. In around Assosa, improved soybean technologies are being promoted by the government. The

technologies promoted include improved varieties, recommended fertilizer rates and types, improved agronomic and weed control practices. However, most farmers of the area cannot afford the cost of inorganic fertilizer to overcome their N and P deficiencies. As a result, farmers apply below the recommended dose of fertilizer. Therefore, symbiotic N₂-fixation can be used as a cheaper alternative for improving soil fertility. There is also knowledge limitation to identify types of varieties which gives more yield and which variety give response favorably to the application of fertilizers even though different soybean varieties are easily available around Assosa. Currently, inoculation is exercised in the world as an alternative means to improve the N nutrition of legumes, because it is sustainable, cheaper and has ecological benefit. Even if, there was identified inoculant strain to be used as N source for soybean production in study area, no study is tasted its effectiveness on the taste soybean varieties. Therefore, the overall aim of this study was to evaluate the effect of *Bradyrhizobium* inoculation and P application on growth, symbiotic and yield performance of soybean (*Glycine max* L.) varieties at Assosa, Western Ethiopia.

1.1. Specific objectives;

- To assess the effect of *Bradyrhizobium* inoculation and P application on growth and symbiotic and yield performance of soybean at Assosa.
- To determine interaction effect of *Bradyrhizobium*, P and varieties on growth and symbiotic and yield performance of soybean at Assosa.
- To evaluate the economic feasibility of the *Bradyrhizobium* inoculation and P application for soybean production at Assosa.

2. LITERATURE REVIEW

2.1. Origin and Description of Soybean

The soybean (*Glycine max* L.) belongs to the family *Leguminosae*, sub-family *Papilionoidae* is originated in Eastern Asia, probably in North and Central China around 1700-1100 B.C. (Laswa *et al.*, 2005). It is believed that cultivated varieties were introduced into Korea and later into Japan some 2000 years ago. Soybean have been grown as a food crop for thousands of years in China and other countries of East and South East Asia and constitute to this day, an important component of the traditional popular diet in these regions (FAO, 2015). Soybean was first introduced to Africa in the early 19th century, through Southern Africa and it is now widespread across the continent (Wikipedia, 2009). From the early 1960s until 1976, soybean production in Africa had increased slowly but steadily, but in 1977 take off began, fuelled by large increases in production in Egypt and Zimbabwe (Shurtleff and Aoyagi, 2007).

Soybean is a self-pollinated C₃ plant that grows well in tropical, sub-tropical and temperate climates. Its germination is epigeal and the crop has a tap root system (Hymowitz, 2010). It is an annual, erect hairy herbaceous plant, ranging in height of between 30 and 183 cm, depending on the genotype (Hymowitz, 2010). The stem, leaves and pods are covered with fine brown or gray hairs. The leaves are trifoliate, having three to four leaflets leaf¹. The fruit is a hairy pod that grows in clusters of three to five, each of which is five to eight centimeters long and usually contains two to four seeds. Soybean seeds occur in various sizes, the seed coat color ranges from cream, black, brown to yellow. The hull of the mature bean is hard and protects the cotyledons and hypocotyls from damage (Wikipedia, 2009).

2.2. Importance of Soybean

Soybean grows for its edible bean, an important source of inexpensive and high quality protein (40%) and oil (20%) around the world (Abdullahi *et al.*, 2013). It has the highest protein content of all food crops and second only to groundnut in term of oil content among food legumes (Abdullahi, 2013). It is an essential source of protein, oil and micronutrients in human and animal diets and become an increasingly important agricultural commodity, with a steady increase in worldwide annual production due to its excellent nutritional value and health benefit. It has various medicinal properties as well as industrial and commercial uses; and agronomic values such as soil conservation, green manure, compost and N₂-fixation (Rusike *et al.*, 2013). It can therefore be an excellent substitute for meat in developing countries, where animal protein-rich foods such as meat, fish, eggs and milk are often scarce and expensive resources for poor families to afford. The cake obtained from soybean after oil extraction is also an important source of protein feed for livestock such as poultry, pig and fish. The expansion of soybean production has led to significant growth of the poultry, pig and fish farming (FAO, 2010). Therefore, products from soybean are exceedingly required for the populations in Ethiopia who are often affected by protein-energy malnutrition and for those who have constraints to include animal sources of foods in their diets.

2.3. Environmental Requirement of Soybean

Soybean is grown from the equator to latitude 55° N or 55° S and from below sea level to altitudes close to 2000 meter. Rusike *et al.* (2013) described that soybean as being typically a short day plant, physiologically adapted to temperate climatic conditions. However, some have

been adapted to tropical, subtropical and temperate climates. In the tropics, the growth duration of adapted genotypes is commonly 90-110 days, and up to 140 days for the late maturing ones (Rusike *et al.*, 2013). Most legumes require an optimum temperature of between 17.5⁰C and 27.5⁰C for development. For soybean the optimum temperature for growth is 23-25⁰C and well distributed rainfall throughout the growth cycle is required. Soybean can grow on all types of soil, but deep fertile loam with good drainage is most suitable for growth.

2.4. Soybean Production in Ethiopia

According to FAO (2015) the three major soybean-producing countries in the globe were USA (29 million ha), Brazil (23 million ha) and Argentina (14 million ha). Seed productions from the cultivated ha of land were 83, 51 and 38 million tons for USA, Brazil and Argentina respectively (FAO, 2015).

In Africa, soybean was grown on an average of 1.2 million ha with an average production of 1.3 million tons and the major producers are Kenya, Nigeria, Zimbabwe, Egypt, South Africa, Zambia, Malawi and Uganda (Nassiuma and Wasike, 2008). Soybean is grown as a commercial crop in over 22 countries of Africa and 1.3 million ha was allocated to soybean production and 1.4 million ton of grain was obtained (FAO, 2008).

Soybean was first introduced in Ethiopia as a trial in 1950, but it was soon abandoned due to low yield. The real production was started later in the 1970s with introduced high yielding varieties from Europe and USA (Aserse *et al.*, 2013). It grows from sea level up to 2200m altitude and with minimum annual rain fall 500-700mm, but performs best between 1300 and 1800m altitude with annual rain falls of 700-900mm. In Ethiopia, it has been noted that from 31,854.75 ha of

land 63,653.1 ton of soybean was produce and the average productivity was 1.9 ton ha⁻¹ during 2012/13 (Bekabi, 2015). In Benishangul Gumuz Regional State, from 13,946.74 ha⁻¹ of land 29,227.4 ton of soybean was produce and the average productivity was 2 ton ha⁻¹ in 2016/17 cropping season (CSA, 2017). Soybean widely produce in west and south western parts of the country, mostly in Hawassa, Bako, Jimma, Ilubabor, Ambo, Assosa, Pawe and other similar areas (CDI, 2014). Currently, in Ethiopia soybean gaining importance due to increased demands from domestic processing industries and increased demand for use in animal feed. In Ethiopia, particularly in the capital city, Addis Ababa, Faffa Food Share Company, East African Flour Factory, and Health Care Food Manufacturing private limited companies and others are using local and imported soybeans in the preparation of enriched food products for children and adults (WHO, 2015).

Even if it's growing demand in national and international markets, there is shortage of yield supply and the productivity is quite low as compared to developed countries. Increasing soybean production and productivities will contribute substantial for development of the country is economies and for well-being of its inhabitants. The cultivation of soybean will open a new way of earning income for soybean producers in the country (Nigussie *et al.*, 2009). Therefore, enhancing N₂-fixation of soybean by inoculation and improving its protein quality through P application is the best option in Ethiopia soybean production.

2.5. Nitrogen Fertilization and Its Role

Nitrogen (N) is an essential component of DNA, RNA, and proteins, the building blocks of life. All organisms require N to live and grow. Even though N is among the most abundant elements

on earth, it is the critical limiting element for growth of most plants due to its unavailability (Javaid and Mahmood, 2010). No other element has such an effect on promoting vigorous plant growth as has N. Abundant protein tends to increase the size of the leaves, and accordingly, brings about an increase in carbohydrate synthesis. Nitrogen application of 11.5 and 23 kg ha⁻¹ improved the mean plant height of soybean by 17.4 and 7.6%, respectively, over the zero application (Tahir *et al.*, 2009). Application of reduced amount of N as starter fertilizer could improve growth of soybean. Nitrogen fixation by *Bradyrhizobium* enhanced the vegetative growth of soybean, which resulted in substantial increase in its biomass yield by 62.8% over the control. The maximum mean biomass yield plant¹ was obtained after application of 23 kg ha⁻¹ N compared to zero application (Tahir *et al.*, 2009). A deficiency of N limits cell division and expansion, chloroplast development, chlorophyll concentration and enzyme activity (Van Cleemput *et al.*, 2008).

Plants absorb N in its cationic form (NH₄⁺) or the anionic form (NO₃⁻), to less extent as urea and NH₃. Production of high-quality, protein-rich food is extremely dependent upon availability of sufficient N. Plants acquire N from two principal sources: (a) the soil, through commercial fertilizer, manure, and/or mineralization of organic matter; and (b) the atmosphere through symbiotic N₂-fixation (Tairo and Ndakidemi, 2014).

2.6. Phosphorus Fertilization and Its Role

Phosphorus is the second major plant growth-limiting nutrients in most agriculture soil (Shahid *et al.*, 2009). Phosphorus is an important plant macronutrient, making up about 0.2-0.5% of a plant's dry weight and it is second only to N as the most limiting element for plant growth.

Unlike the case for N, there is no large atmospheric source that can be made biologically available for P (Bashir *et al.*, 2011). Phosphorus has important effects on photosynthesis, N fixation, root development, flowering, seed formation, fruiting and improvement of crop quality (Brady, 2002). Phosphorus deficiency occurs in many soils of East Africa due not only to P depletion through crop harvest and erosion but mainly to the prevalence of high P fixing soils in the region (Schulze *et al.*, 2009). Unlike N, P replenishment, particularly in smallholder agriculture, remains a challenge as it is mainly fertilizer dependent. While soluble mineral P fertilizers are the obvious best means to combat P deficiency, even though, its use is limited by high cost at the farmers' level. Several researchers have focused on developing strategies adapted to smallholder farming system for replenishing P and improving its availability to crops (Bashir *et al.*, 2011). Seed yield in legumes in general is a more intricate process than in cereals in that the development of generative organs is relatively gradual and could be prolonged if the external conditions like soil nutrients and other environmental factors are not conducive (Tilahun, 2006). Phosphorus is an essential element for growth, development and yield of soybean and has beneficial effects on nodulation, N₂-fixation capacity, flower and seed formation and advancing crop maturity (Sara *et al.*, 2013). During various chemical reactions, P is integrated into organic compounds, including nucleic acids (DNA and RNA), phosphoproteins, phospholipids and sugar phosphate compounds like adenosine tri-phosphate (Bashir *et al.*, 2011).

Phosphorus application of 23 and 46 kg ha⁻¹ P₂O₅ significantly improved the shoot growth of soybean crop by 14.7 and 18.9%, respectively compared to zero phosphorus application (Tahir *et al.*, 2009). Soybean plant requires an application of 20-30 kg P₂O₅ ha⁻¹ during the growing season to sustain a high crop yield in low soil P. Soil phosphorus availability during plant

seedling development is an important determinant of plant growth, N₂-fixation and grain formation of soybean (Vance, 2001).

2.7. The Role of Phosphorus in Biological Nitrogen Fixation

Phosphorus is one of the essential nutrients for legume growth and biological nitrogen fixation (BNF) (Mhango *et al.*, 2008). Symbiotic N₂- fixation has a high P demand because the process consumes large amounts of energy (Schulze *et al.*, 2009). Soybean plant requires an application of 20-30 kg P₂O₅ ha⁻¹ during the growing season to enhance N₂-fixation and grain formation of soybean (Vance, 2001). Root hairs, root tips and the outer most layers of root cells are the most pathways of P entering in the plants (Rotaru, 2010). During various chemical reactions, P is integrated into organic compounds, including nucleic acids (DNA and RNA), phosphorproteins, phospholipids and sugar phosphate compounds like adenosine triphosphate. A study conducted by Kamanga *et al.* (2010) reported that P fertilizer treatments recorded the greater nodule number, nodule dry weight, and vegetative growth and grain yields of soybean, pigeon pea, cowpea and groundnuts compared to unfertilized treatments. Soybean showed response to P application (20 kg ha⁻¹) with 0.5 t/ha extra grain yield than unfertilized plots. Fertilizer application increased biomass of these legumes 1.5 t/ha of biomass on top of the unfertilized treatment showed that best performance nodulation due to adequate availability of P fertilization. Phosphorus is an essential ingredient for *rhizobium* bacteria to convert atmospheric N₂ in to available form useable by plants (Togay *et al.*, 2008). Thus, adding P fertilizer may reduce stress in the symbiotic relation between root bacteria and legume plant by providing this energy (Bashir *et al.*, 2011).

2.8. BNF and Its Role in the Agricultural System

Legume plants are unique for their ability to fix nitrogen from atmosphere by symbiotic relationship with *rhizobium* bacteria. The bacteria capable of nodulating and fixing nitrogen in association with legumes by forming nodule; meet nitrogen requirement of itself and the host plants are referred as generically as rhizobia and currently divided in to six genera, *Bradyrhizobium*, *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium*, and *Azorhizobium* with 38-40 species. The bacteria that have nitrogenase enzyme can reduce molecular nitrogen to ammonia (Hardarson and Atkins, 2012). It is a vital biological process which allows atmospheric molecular dinitrogen (N_2) to be converted into mineral N (NH_3) that can be assimilated by living organisms (Haque *et al.*, 2012). The amount of N that a plant can fix depends on the variety, the productivity of *rhizobia* bacteria, the soil and the climatic conditions. BNF is an effective and efficient source of N supply to plants under favorable atmospheric and environmental conditions (Matusso *et al.*, 2014). By symbiosis of bacteria and host plant gets their own N needs and also provides some nutrients left over to succeeding crops through decomposition of their nodules, shoots and roots. The practice appeared to be very useful for smallholder farmers as it is accost effective to improve N requirements of legumes and succeeding crops (Vanlauwe *et al.*, 2014). N_2 -fixing bacteria present in soybean root nodules will fix N from the atmosphere supplying most or all N needed by the plant. Nodules are organs that consist mainly of plant cells infected with bacteria that provide the host plant with fixed N. The bacteria live in compartments of up to 10,000 in a nodule, called bacteroids. The N_2 -fixation is aided by an enzyme, nitrogenase which takes place in an environment without oxygen, through a transfer compound, leghemoglobin (Rienke and Joke, 2010). The process of N_2 -fixation requires the presence of the right species of

the N₂-fixing bacteria in the soil, and they are often attracted to the roots by chemical signals from the soybean root (Rienke and Joke, 2010). Once in contact with the root hairs, a root compound binds the bacteria to the root hair cell wall. The bacteria release a chemical that causes curling and cracking of the root hair, allowing the bacteria to invade the interior of the cells, and begin to change the plant cell structure to form nodules.

Next to plant photosynthesis, BNF is probably the most important biochemical process for life on earth. Due to *rhizobial* inoculation, the higher amount of net N can be added to soil system from the inoculated legumes (Haque *et al.*, 2012). But the use of *rhizobia* inoculants for improvements in N₂-fixation and productivity of grain legumes is still in developing stage in most part of sub-Saharan Africa including Ethiopia (Vanlauwe *et al.*, 2014). Because of N fertilizers are expensive inputs for crop production, decline soil fertility without replenishing nutrients due to low household income (Abdullahi *et al.*, 2013). The total annual terrestrial inputs of N from BNF range from 139 million to 175 million ton of N with symbiotic associations growing, in the arable land accounting for 25 to 30% (35 million to 44 million ton of N) and permanent pasture accounting for another 30% (45 million ton of N) (Affholder *et al.*, 2013). Soybean is capable of fixing between 60 -168 kg of nitrogen ha⁻¹ year⁻¹ under suitable conditions (Rienke and Joke, 2010). Greater use of BNF will reduce society's current dependence on synthetic N fertilizers. The production of widely used synthetic N fertilizers such as anhydrous ammonia requires the use of relatively large amounts of energy from non-renewable energy sources such as natural gas. Distribution and application of these fertilizers also requires relatively large amounts of non-renewable energy sources such as diesel fuel (Affholder *et al.*, 2013).

2.9. The Role of *Rhizobia* in Biological Nitrogen Fixation

Rhizobia are symbiotic bacteria that facilitate formation of nodules on the roots of legume hosts, within which the bacteria fix atmospheric N into ammonia. Symbiotic N₂-fixation is the main route for sustainable input of N into ecosystems (Lindström *et al.*, 2010). N₂-fixation in agriculture can be improved by inoculation of legume crops with suitable *rhizobia*. Knowledge of the biodiversity of *rhizobia* and local populations is important for the design of successful inoculation strategies (Lindström *et al.*, 2010). A fully functional symbiosis requires successful completion of numerous steps, beginning with the exchange of recognition signals between the plant and bacteria. The signalling process is started by the plant that releases root exudates, including flavonoids and nutrients. These compounds, which are known as nod factors, trigger the early stages of nodule development, including root hair deformation, parenchyma cell division and nodule morphogenesis. *Rhizobia* are either indigenous to the soil or inoculated for a particular legume (Zengeni *et al.*, 2006). Unfortunately, adaptation of indigenous *rhizobia* populations to local environments, which is a big advantage for selection of inoculants strains, poses a challenge to productivity. Adaptability of indigenous *rhizobia* to their environment results in high levels of saprophytic competence (Abaidoo *et al.*, 2007). Sometimes indigenous *rhizobia* may be found in greater numbers than those of the inoculated strains which are also limited in mobility. This challenge must be overcome by the inoculants strains, and it raises the standard of the inoculants required with regard to competitiveness with the native *rhizobia* and N₂-fixation. *Rhizobia* may also be an indicator of soil properties as they are affected by soil acidity, temperature, moisture and other factors and therefore, the diversity may be an indicator

of the soil condition. Fields which receive consistent fertility management and legume cropping host higher *rhizobia* numbers and diversity (Zengeni *et al.*, 2006).

2.10. Factors Affecting Biological Nitrogen Fixation

In the *rhizobium*-legume symbiosis, which is N₂-fixing system, the process of N₂-fixation is strongly related to the physiological state of the host plant. Therefore, the *rhizobium* species survival in soil is influenced by a combination of factors including; *rhizobial* competition, acidity, salinity, alkalinity, soil temperature, moisture and water (Sameh *et al.*, 2014).

2.10.1. *Rhizobial* competition

Naturally occurring *rhizobia* often exist at populations of 1.0×10^4 to 1.0×10^7 cells g⁻¹ of soil (Brockwell *et al.*, 1995). Where naturalized *rhizobia* are few or absent, the introduction of new strains by seed or soil inoculation is normally successful (Campo and Hungria, 2004). On the other hand, where large populations of naturally occurring *rhizobia* exist, inoculation is invariably futile. At intermediate levels of naturalized *rhizobia* (10–1000 g⁻¹ of soil) competition between naturalized and introduced *rhizobia* for nodule formation is of practical concern. These include ability to colonize the soil and tolerate environmental stresses, ability to compete for nodule formation with background population of *rhizobia* and capacity to form effective nodules that fix-N and to have no deleterious effects on non-target hosts. Strains of *rhizobia* differ widely in their ability to survive, nodulate and fix-N in the soil environment; thus strain selection plays a major part in its survival and infective ability (Campo and Hungria, 2004).

2.10.2. Soil acidity

Acidity related factors (high Al, low Ca and low P) have a direct impact on *rhizobia* growth and persistence, on nodule initiation and N fixation effectiveness. Soil acidity can restrict the survival, persistence and growth of *rhizobia* in soils and severely affect nodulation and nitrogen fixation processes. Most leguminous plants require a neutral or slightly acidic soil for growth, especially when they depend on symbiotic N₂ fixation. Nodulation range of pasture and crop legumes (e.g., white clover, subterranean clover, sweet clover, lucerne, pea, cowpea, bean, etc.) is reduced in acid soils, mainly because of sensitivity of early nodulation events, such as attachment, root hair curling and initiation of infection thread formation. Soil acidity affects all stages of the legume- rhizobium symbiosis, including strain survival in the soil, root hair infection, nodule initiation and nitrogen fixation (Graham *et al.*, 1982).

2.10.3. Temperature

The survival and multiplication of the bacteria in the soil is influenced by temperature. Excessively high and low temperatures depress bacterial growth and N₂- fixation. Reduction in the root zone temperature below optimal temperatures (17-25⁰C) reduced N₂-fixed and delayed the onset of N₂-fixation in soybean (Boddey *et al.*, 1995). High root temperature strongly affects bacterial infection and N₂-fixation in several legume species including soybean and cowpea. Temperature affects root hair infection, bacteroid differentiation, nodule structure and the functioning of the legume root nodule. At 39⁰C, the release of nod-gene inducers from soybean and common bean was decreased. High temperature also affects nodule function and nodule initiation (Hungria and Franco, 2010).

2.10.4. Soil moisture and water supply

Soil moisture status has a major impact on nodulation and N₂-fixation in legumes. Nodulation is affected by moisture stress through reduced *rhizobial* motility in the soil, insufficient *rhizobia* multiplication in the rhizosphere, reduced formation of infection threads and retarded nodule development (Hungria and Phillips, 2010). Increasing or decreasing soil water can greatly affect the rate of N₂-fixation because of inadequate aeration in saturated soil and reduced water availability in dry soils at critical times during development and growth. Growth rates of *rhizobia* may be slow in situations of higher early soil moisture (Hungria and Phillips, 2010).

2.10.5. Mineral nutrient status

Mineral nutrient inhibits the *rhizobium* infection process and N₂-fixation. Various microelements (Cu, Mo, Co, B) are necessary for N₂-fixation. Some of these are components of nitrogenase for example, Mo. N₂-fixation in the nodule requires more Mo than the host plant; thus, Mo deficiency is the most important micronutrient deficiency. Maximum N₂-fixation occurs only when available soil N is at a minimum. Excess NO₃⁻ concentration in the soil can reduce nitrogenous activity, *rhizobial* activity and N₂-fixation. The reduction in N₂-fixation is related to the competition for photosynthate between NO₃⁻ reduction and N₂-fixation reactions (Mengel, 2006).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

A field experiment was conducted during the 2016 cropping season at Asossa Agricultural Technical and Vocational Education Training College in Benishangul Gumuz Regional State. The site is located west of Addis Ababa about 653 km distance between (10° 02' 05" N latitude, and 34° 34' 09.9" E longitudes, at an altitudinal range of 1580 m above sea level. The area experiences a mono-modal rainfall pattern and has annual total rainfall of about 1275 mm. The rainy season occurs from May to October and the maximum rain is received in the months of July and August. The minimum and maximum temperatures are 16.75⁰C and 27.92⁰C respectively. The soil type of the area is Nitisols and is dark reddish brown to dark red in colour (AARC, 2015).

3.2. Source of Planting Material and *Bradyrhizobium* Strain

Seeds of *Glayscale max* L. varieties Belessa-95, Gizo and Local were obtained from the Pawe Agricultural Research Centre, Ethiopia. The three soybean varieties were chosen based on their high grain yield, acceptability by farmers and seed availability. The *Bradyrhizobium* inoculant MAR-1495 was obtained from the Assosa Agricultural Research Centre, Soil Laboratory Unit, Assosa, Ethiopia. The strain has been shown to enhance growth, nodulation and grain yield of various soybean varieties under a wide range of ecological conditions and are considered the best strains as far as symbiotic and agronomic performance of soybean is concerned.

3.3. Experimental Design and Procedure

The field experiment was laid down in three factorial randomized complete block design (RCBD) with three replications. There were three different levels of P (0, 10, and 20 kg ha⁻¹) and two levels of *Bradyrhizobium* (uninoculated and inoculated with MAR-1495) combined with three varieties and replicated three times. Thus, the experiment consists of 18 treatments with a total of 54 plots. The size of each experimental plot was 2.4m x 3.6m (8.64m²) and spacing between plants, rows, plots and blocks were 5cm, 60cm, 0.7m and 1.5m, respectively and the total area was 921.24 m². Each plot was made as a separate seedbed plots.

3.4. Seeds Inoculation

EIAR (2014) reported that several field demonstration have confirmed that leguminous crop showed remarkable growth and yield response to *rhizobium* inoculation in different agro-ecologies in Ethiopia. COMPRO (2013) also reported that a strain of *Bradyrhizobium* namely (MAR-1495) inoculant has done well in soybean growing area in Ethiopia. Then seeds were weighed on electronic balance and soaked in sugar solution. Then strain of *Bradyrhizobium* (MAR-1495) was applied on moistened seeds at the rate of 10 g kg⁻¹ of seed with peat-based method. The *Bradyrhizobium* inoculum was mixed thoroughly with the seed. Seeds were allowed to air dry for 15 minutes under shade to maintain the viability of cell and sown within an hour at the required rate and spacing (Beleachew and Hailemariam, 2010).

3.5. Management of the Experiment

Land was prepared in April 2016 using tractor. The plot was leveled manually. Soybean was sown on June 25, 2016 with 60cm spacing between rows and 5cm between plants with in the rows (AARC, 2016). As a precaution of avoiding cross-contamination; non-inoculated treatments were planted first followed by inoculated treatments. Inoculated seeds of each treatment were sown by a single person and different management practices (weeding, cultivation etc.) were carried out during the growing period. Ridges were also made to prevent movement of bacteria through rain water within plots and blocks. The experimental field was weeded three times during the growing season. The first weeding was done 25 days after planting to avoid competition during early stage of crop growth. The second weeding was under taken one month later and finally the third weeding was under taken one month later. After germination, thinning was done so as to maintain the population density of plants in each plot.

3.6. Soil Sampling and Analysis

About 1 kg pre-sowing surface soil sample was collected by means of auger from different spots of the experimental field at the depth of 0-30 cm and bulked together to get a representative composite soil sample. Then, air-dried and crushed soil samples were thoroughly mixed and packed in a polythene bag, labeled and stored in the laboratory for analysis. The composite sample was taken to Assosa soil laboratory center for analysis of the selected parameters. Parameters determined on the soil sample collected before planting were, textural analysis (percent sand, silt and clay), soil pH, total N, organic carbon, available P and cation exchangeable capacity (CEC). Analysis of organic carbon content of the soil in a laboratory was

determined by Walkley and wet oxidation method as described by Jackson (1958) and total N by Kjeldhal method as described by (Dewis and Freitas, 1975). The pH of the soil was measured in water at soil to water ratio of 1:2.5 Page (1982) and cation exchange capacity was determined using Kjeldhal procedure as described by (Ranist *et al.*, 1999). Available P was determined according to the methods of (Olsen and Dean, 1965). Soil texture analysis was performed by Bouyoucous hydrometer method (Day, 1965).

3.7. Economic Analysis

Mean grain yield of the treatments were used in partial budget analysis (CIMMTY, 1988). The field price of 1 kg of soybean that farmers receive from sale for the crop was taken as 7.00 Birr based on the market price of soybean at Assosa near the experimental site. P price was 18.55 Birr kg^{-1} and the daily laborer expense was 50.00 Birr. The gross benefit was calculated as 10% adjusted grain yield (kg ha^{-1}). The total variable cost included the cost of P, *Bradyrhizobium* strain and labor as the sum of all cost that was variable or specific to a treatment against the control. Dominance analysis and marginal rate return (%) were used to evaluate the economic performance of treatments and net return was calculated by subtracting total variable cost from the gross benefit.

3.8. Data Collected

Nodule number and dry weight plant⁻¹: nodulation assessment was undertaken at mid flowering stage of soybean from the net to boarder rows of each plot. Five plants were randomly sampled by careful uprooting and adhering soil particles were removed by washing the root with their nodules gently with water over a metal sieve. The nodules from each plant were removed and separately spread on the sieve for some minutes until the water was drained from the surface of the nodules. The total number of nodules was counted and their average was taken as number

of nodules plant⁻¹. After determination of their numbers, the nodules were oven dried at 50⁰C for 48 hour to determine nodule dry weight plant⁻¹. The average of five plants was taken as nodule dry weight plant⁻¹.

Plant height: five plants from the central rows of each plot were randomly selected for measuring plant height at mid flowering stage. Then the average values of these plants were recorded as plant height of the crop.

Leaf area determination: leaf area was measured from five randomly selected plants from the central rows of each plot at mid flowering stage. The average of the five plants leaf area was taken as the leaf area of the plant.

Shoot dry matter: shoot dry matter of plant was determined at mid flowering stage of the crop from plants that were sampled for nodulation. The plant samples were placed in a labeled perforated paper bags and oven dried for 48 hours at 70⁰C to a constant weight as described by Jones (2001) to determine the dry matter yield. The average shoot dry matter of five plants was recorded as shoot dry matter plant⁻¹.

Root dry matter: root dry matter of plant was determined at mid flowering stage of the crop from plants that were sampled for nodulation. The plant samples were placed in a labeled perforated paper bags and oven dried for 48 hours at 70⁰C to a constant weight as described by Jones (2001) to determine the root dry matter. The average root dry weight of five plants was recorded as root dry matter plant⁻¹.

Number of primary branches plant⁻¹: number of primary branches was determined by counting the average number of primary branches from the main stem from the five randomly

selected plants plot⁻¹ at physiological maturity. The average of the five branch number was taken as the number primary branch plant⁻¹.

Number of pods plant⁻¹: the total number of pods from the five randomly selected plants was counted at the time of harvest and recorded as the number of pods plant⁻¹.

Number of seeds pod⁻¹: from the above counted pods plant⁻¹ randomly selected plants, the total number of seeds was threshed and counted and determined as the average number of seeds pod⁻¹.

Hundred seed weight: hundred seeds were counted from the harvested bulk of seeds per net plot and their weight (g) was determined at seed moisture content of 10% by used a sensitive balance.

Grain yield: the three central rows were manually harvested and threshed to determine grain yield plot⁻¹ and the average yield was reported in ton ha⁻¹.

Above ground total biomass: at harvest, plants from three central rows were manually harvested. The harvested plants were sun-dried in an open air until constant weight attained and weighed to determine above ground total biomass yield and the average above ground total biomass was reported in ton ha⁻¹.

Harvest index: the harvest index was calculated as the ratio of grain yield to above ground total biomass yield.

3.9. Statistical Data Analysis

Data collected was subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) of the Statistical Analysis System software (SAS, 2002) version 9.0. Mean separation was done using Fisher's LSD Test at 5% probability level. Correlation analysis was done using Pearson's simple correlation coefficients for the intended parameters.

4. RESULTS AND DISCUSSION

4.1. Soil Physico-Chemical Properties of the Study Area

The results of pre-planting soil analysis revealed that the soil of the study area is clay loam in texture (29% sand, 30% silt and 41% clay). Soil texture is a fundamental soil property which in practice the farmer can do little to modify. It is also closely related to the water-holding capacity of soils, since loams and clays hold more water than do sandy soils (Brady, 2002). Thus, the soil of the study area has good water holding capacity, which creates a good growing media for soybean. Soybean is characteristically grown on such soils which holds sufficient amount of residual soil moisture. The soil was slightly acidic in reaction with the pH (H₂O 1:2.5) value of 5.9, which is within the range of optimum soil pH for legume production including soybean (Havlin *et al.*, 1999). The total N, Available P, OC and CEC of the soil before planting were 0.14%, 6 mg kg⁻¹, 1.5%, and 23.4 cmol (+) kg⁻¹, respectively (Table 1). According to Havlin *et al.* (1999) soils are classified depending on their total N content in percentage (%), as very low (<0.1), low (0.1-0.15), medium (0.15-0.25), and high (>0.25). Thus, the soil of the study site has low total N content. Olsen *et al.* (1954) classified available P content of the range < 5 as very low, 5 – 15 as low, 15 – 25 as medium and > 25 mg kg⁻¹ as high. According to Landon (1991) the soil organic carbon content ranges of 1 – 2, 2 – 4, and 4 – 6% are rated as low, medium and high respectively. Thus, the OC content of the soil is considered as low before planting. The CEC ranges of 5 – 15, 15 – 25 and 25 – 40 cmol kg⁻¹ are rated as low, medium and high respectively. Based on these ratings the cation exchange capacity (23.4 cmol kg⁻¹) before planting of the experimental field was in the medium range.

Generally the soil analysis result indicated that the area is N and P deficient to support the potential crop production. This may be associated with poor farm management practices and continuous cropping with little or no fertilizers input which resulted in a decline in soil fertility of the area. It may be because of this that growth; nodulation, yield and yield components responded to *Bradyrhizobium* inoculation and applied P fertilizer under this experiment.

Table . Physico-chemical properties of the experimental soil before planting

pH	Organic Carbon (%)	Total N (%)	Available P Mg kg ⁻¹	CEC cmol kg ¹	Sand (%)	Slit (%)	Clay (%)	Textural class
5.9	1.5	0.14	6	23.4	29	30	41	Clay loam

4.2. Effect of *Bradyrhizobium* Inoculation and P application on Nodulation and Growth of Soybean Varieties

4.2.1. Nodule number plant⁻¹

There was significant difference among soybean varieties for nodule number (Table 2). Variety Belessa-95 exhibited greater nodule number compared to others. However, the Local and Gizo varieties did not differ in nodule number. The observed differences in nodule number among the soybean varieties could be attributed to genotypic differences. In line with this result Ali *et al.* (2008) found that performance of three different varieties of garden pea (*Pisum sativum* L.)

varied widely for nodule number. The result is in agreement with the work of Yoseph *et al.* (2017) who reported that marked differences among the cowpea varieties on nodule number plant⁻¹.

Bradyrhizobium inoculation had significant effect on nodule number of soybean compared to the control (Table 2). The maximum mean nodule number (15.85) was recorded from the strain MAR-1495 and, zero application of treatments result the minimum nodule number plant⁻¹. The increased nodule number with *Bradyrhizobium* inoculation could be associated with the efficiency of introduced *rhizobia* to compete with indigenous bacteria dwelling in the soil. These results are in line with the findings of Argaw (2012) who revealed that the *Bradyrhizobium* inoculation significantly enhanced nodule number of field grown legumes. Similar results were reported by several authors (Argaw, 2014; Yoseph and Worku, 2014; Maphosa, 2015) who reported that inoculation of soybean with effective bacterial strains increased significantly nodule numbers when compared to uninoculated control. This was in line with the finding of Solomon *et al.* (2012) who found that the nodulation parameter nodule number plant⁻¹ was significantly influenced by the main effect of *Bradyrhizobium* inoculation strain alone. In the present study, the uninoculated control was resulted with poor nodulation status which was evidenced that indigenous rhizobia population was ineffective in fixing N. legumes could be grown

Significant difference ($P < 0.01$) was also observed on nodule number plant⁻¹ among different levels of applied P. Application of 20 kg P ha⁻¹ increased mean nodule number plant⁻¹ by 47.24 and 16.03% over zero P fertilized control and application of 10 kg P ha⁻¹, respectively (Table 2). The increased nodule number due to applied P might be related to the availability of adequate P

which possibly promotes early root growth and the formation of lateral fibrous and healthy roots. In line with these results Yoseph and Worku (2014) reported that increased nodule number plant⁻¹ due to application of P ha⁻¹ when compared to the control.

The variety x phosphorus interaction showed much greater nodule number was obtained in Belessa-95 variety over Gizo and Local varieties at 20 kg P ha⁻¹, but decreased nodule number at zero-P level for all the three varieties (Fig. 1a). The data from variety x *Bradyrhizobium* inoculation interaction showed that Belessa-95 variety recorded much greater nodule number over Gizo and Local regardless of inoculation with strain MAR-1495 (Fig. 1b). Nodule number generally increased with bacterial inoculation at all P levels compared to uninoculated plants (Fig. 1 C). But, there were no marked differences in nodule numbers for P levels 0 and 10 kg P ha⁻¹ for uninoculated plants. Phosphorus application resulted with increased nodule number due to P enhanced the strain to capable of effective in nitrogen fixation through increased nodule number and biomass (Graham *et al.*, 204). Nodule number plant⁻¹ was positively and significantly correlated with grain yield (r = 0.88), above ground total biomass yield (r = 0.88), shoot dry weight (r = 0.78) and leaf area (r = 0.71) (Appendix Table 1).

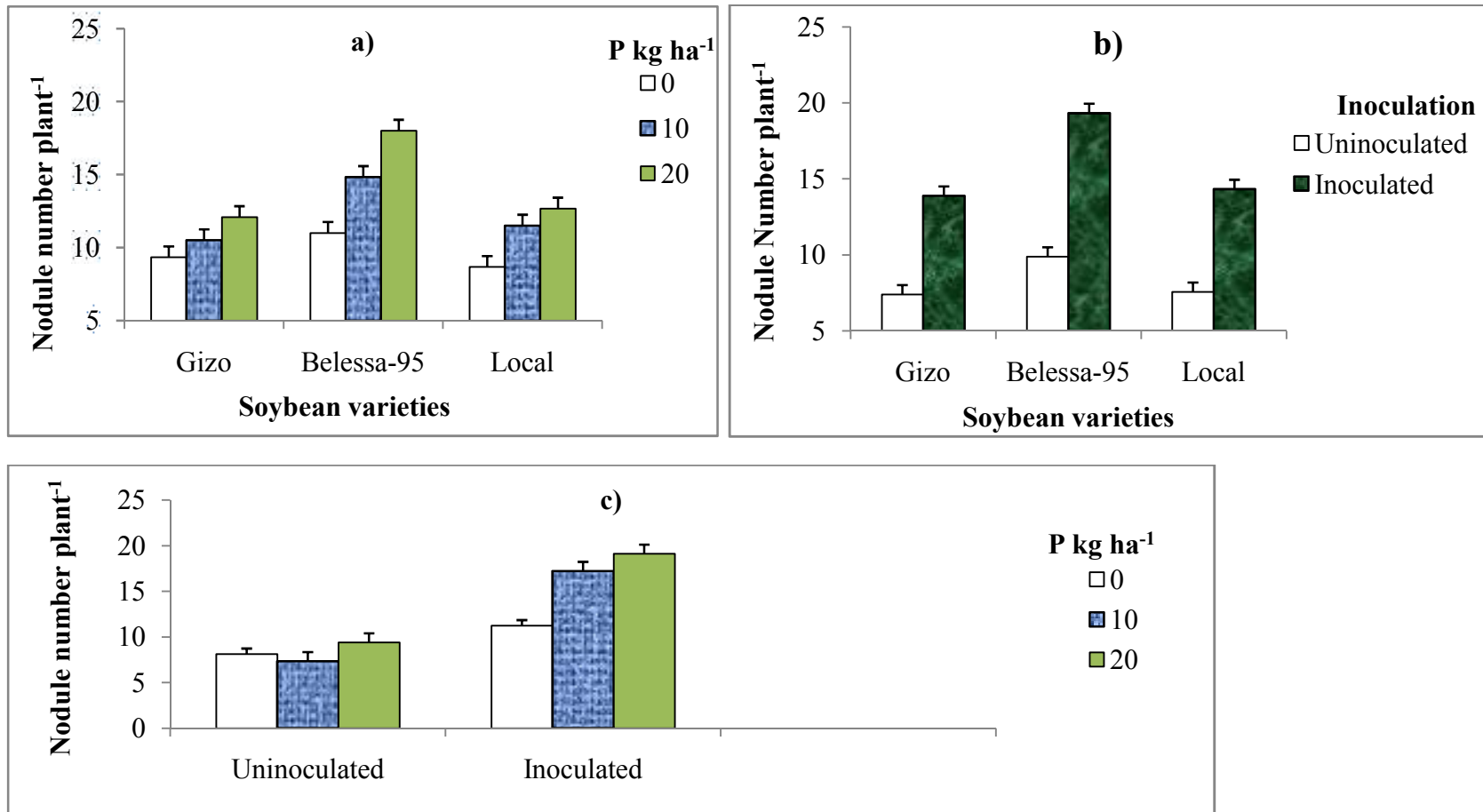


Figure . Interaction effects of; **a)** variety x phosphorus, **b)** variety x *Bradyrhizobium* inoculation and **c)** *Bradyrhizobium* inoculation x phosphorus on nodule number. Vertical lines on bars represent standard error of the statistical means.

4.2.2. Nodule dry weight plant⁻¹

The nodule dry weight of soybean was highly significantly ($P < 0.01$) influenced by the soybean varieties (Table 2). The highest nodule dry weight (0.18 g plant⁻¹) was recorded from variety Belessa-95, while the lowest (0.10 g plant⁻¹) nodule dry weight was recorded from variety Gizo reflecting inherent genetic differences among the varieties for nodule dry weight. The observed differences on nodule dry weight plant⁻¹ among the three soybean varieties could be attributed to genotypic differences. The result is in agreement with the work of Yoseph *et al.* (2017) who reported that marked differences among the cowpea varieties on nodule dry weight plant⁻¹.

Inoculation with *Bradyrhizobium* strain MAR-1495 resulted in significantly increased nodule dry weight compared to the uninoculated control (Table 2). The maximum nodule dry weight (0.20 g plant⁻¹) was recorded from *Bradyrhizobium* strain MAR-95, while the minimum nodule dry weight (0.08 g plant⁻¹) was recorded from the control treatment. The difference between the nodule dry weight obtained from inoculated and uninoculated plants may be attributed to the size of the nodules. Inoculated plants formed bigger nodules than uninoculated due to the effectiveness of the introduced *Bradyrhizobium* strain to initiate nodulation with soybean roots. This was in line with the finding of Solomon *et al.* (2012) who found that the nodulation parameter nodule dry weight plant⁻¹ was significantly influenced by the main effect of *Bradyrhizobium* inoculation strain alone. In the present study, the uninoculated control was resulted with poor nodulation status which was evidenced that indigenous rhizobia population was ineffective in fixing N. legumes could be A similar promoting effect of seed inoculation on dry weight of nodules plant⁻¹ have been reported by (Bejandi *et al.*, 2011; Nyoki and Ndakidemi, 2014; Chiamaka, 2014).

The applications of 10 and 20 kg P ha⁻¹ to soybean plants significantly increased nodule dry weight over zero-P control (Table 2). However, much greater values were obtained at higher P level (20 kg P ha⁻¹). The improved nodule dry weight due to higher P rates might be attributed due to higher nodule numbers. In line with this finding Azam (2002) also reported that greater nodule dry weight plant⁻¹ due to higher P rate. Nodule dry weight plant⁻¹ was positively and significantly correlated with leaf area (r = 0.67), shoot dry weight (r = 0.72), grain yield (r = 0.81) and above ground total biomass yield (r = 0.79) (Appendix Table 1).

4.2.3. Plant height

Analysis of variance revealed highly significant (P<0.01) effect of the varieties, but there was no significant difference on other main factors and their interactions on the plant height (Table 2). Regarding variety effect, the highest value for plant height was recorded with Belessa-95 variety (51.52 cm) followed by variety Gizo. Whereas, the lowest value of plant height was recorded from Local variety (44.45 cm), but statistically at par with variety Gizo. The observed difference in plant height among the three soybean varieties might be attributed to inherent genotypic difference (Magani and Kuchinda, 2009).

Inoculation with MAR-1495 strain didn't bring significant effect on plant height compared to the control (Table 2). The *Bradyrhizobium* inoculation does not affect plant height was earlier confirmed by Karikari *et al.* (2015) who found that lack of marked differences on plant growth. In contrary Abbasi *et al.* (2010) concluded that *rhizobium* inoculation increased soybean plant height up to 12%.

Table . Main effects of varieties, *Bradyrhizobium* inoculation and P levels on nodulation and growth of soybean

Treatments	Nodule number (plant ⁻¹)	Nodule dry weight (g plant ⁻¹)	Plant height (cm)	Leaf area (cm ² plant ⁻¹)	Shoot dry weight (g plant ⁻¹)	Root dry weight (g plant ⁻¹)
Varieties						
Gizo	10.64 ^B	0.10 ^C	46.93 ^B	79.16 ^B	11.95 ^B	3.77 ^A
Belessa-95	14.61 ^A	0.18 ^A	51.52 ^A	83.46 ^A	12.77 ^A	4.25 ^A
Local	10.94 ^B	0.13 ^B	44.45 ^B	82.46 ^{AB}	10.72 ^B	2.57 ^B
LSD.05	0.88	0.02	3.06	4.70	0.92	0.87
Inoculation						
Uninoculated	8.28 ^B	0.08 ^B	47.05	73.22 ^B	10.28 ^B	2.99 ^B
MAR-1495	15.85 ^A	0.20 ^A	48.22	90.17 ^A	13.35 ^A	4.06 ^A
LSD.05	0.72	0.02	NS	3.84	0.75	0.71
P (kg ha⁻¹)						
0	9.67 ^C	0.09 ^C	46.72	75.83 ^B	9.77 ^B	2.77 ^B
10	12.28 ^B	0.13 ^B	47.19	84.27 ^A	12.57 ^A	3.49 ^{AB}
20	14.25 ^A	0.17 ^A	48.99	84.98 ^A	13.11 ^A	4.32 ^A
LSD.05	0.88	0.02	NS	4.70	0.92	0.87
CV (%)	10.81	24.21	9.49	8.50	11.46	36.38

Mean values followed by dissimilar letters in a column are significantly different at *: p≤0.05; **: p≤0.01; ***: p≤0.001 and NS; Non-Significance.

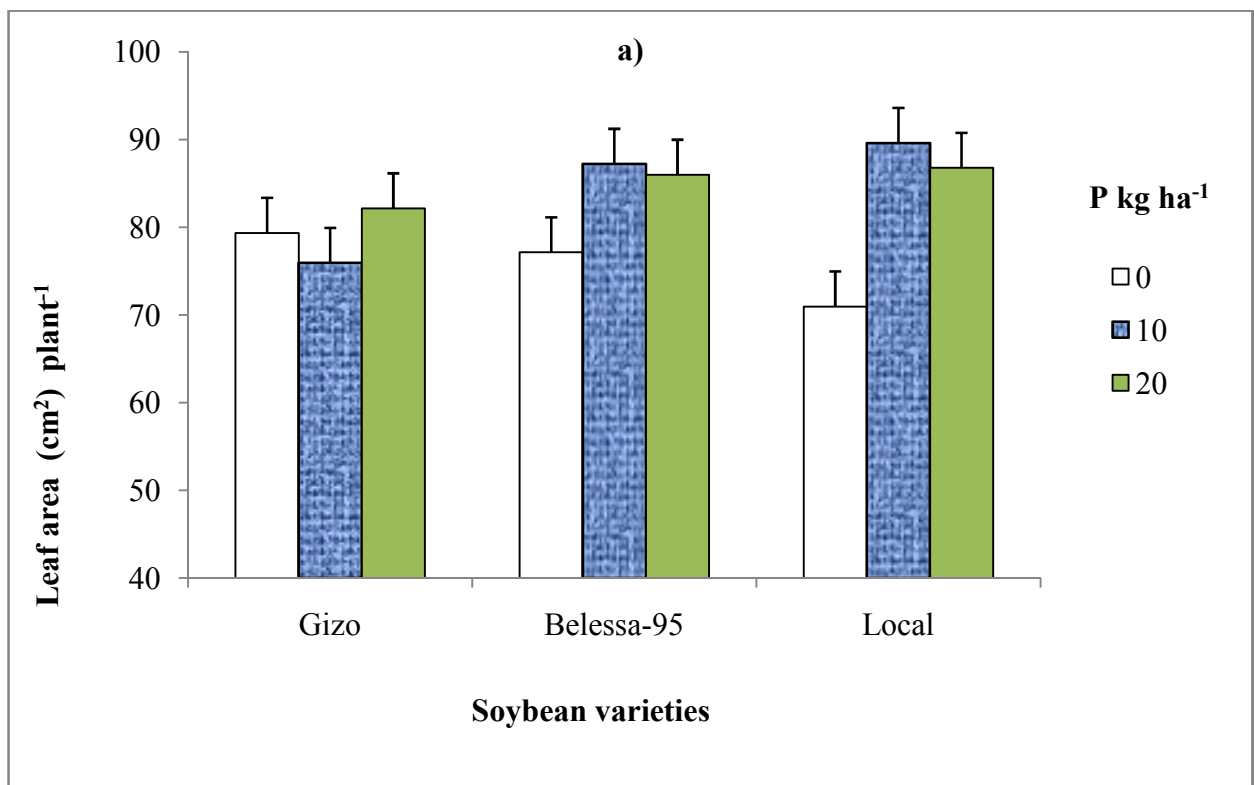
4.2.4. Leaf area

The analysis of variance revealed that varieties, *Bradyrhizobium* inoculation and application of P highly significantly ($P < 0.01$) affected the leaf area plant⁻¹. The interaction effect of inoculation and variety as well as P and variety were also found to be significant on the leaf area plant⁻¹ (Table 2). Among the three soybean varieties, Belessa-95 recorded the greater leaf area followed compared to Gizo variety, but there were not significance differences between Belessa-95 and Local varieties. The observed differences in leaf area among the three soybean varieties could be attributed to genotypic differences.

Bradyrhizobium inoculation with strain MAR-1495 resulted in 23.16% increased on leaf area plant⁻¹ over the control. This might be due to the fact that *Bradyrhizobium* strain was effective and compatible for fixing N through biological N₂-fixation to fulfill soybean N requirement. Similar finding was observed in previous finding of Tahir *et al.* (2009) where inoculation brought a significant effect on leaf area of legumes.

Significantly increased mean of leaf area (12.07%) was obtained from application of 20 kg P ha⁻¹ over the control (Table 2). However, significant differences were not detected between this level and application of 10 kg P ha⁻¹ while the minimum leaf area (75.83 cm²) was observed in the control. The increase in leaf area due to adequate P fertilization could be explainable in terms of possible increase in nutrient mining capacity of plant as a result of better root development and increased translocation of carbohydrates from source to growing points in adequate P fertilized plots. This result is in line with that of Shahid *et al.* (2009) who reported that application of adequate amount of P resulted in markedly increase on leaf area of soybean plants.

The variety x phosphorus interaction showed greater leaf area in Local over Gizo at higher level P kg ha⁻¹, but there was no marked difference between Local Belessa-95 varieties (Fig. 2a). The data from variety x *Bradyrhizobium* interaction showed that Local variety recorded greater leaf area over Gizo with inoculation strain MAR-1495, but there was no marked difference between Local and Belessa-95 varieties (Fig. 2b). Leaf area generally increased in all varieties with *Bradyrhizobium* inoculation compared to uninoculated plants (Fig. 2b). Leaf area plant⁻¹ showed positively and significantly correlated with shoot dry weight (r = 0.65), grain yield (r = 0.75) and above ground total biomass yield (r = 0.74) (Appendix Table 1).



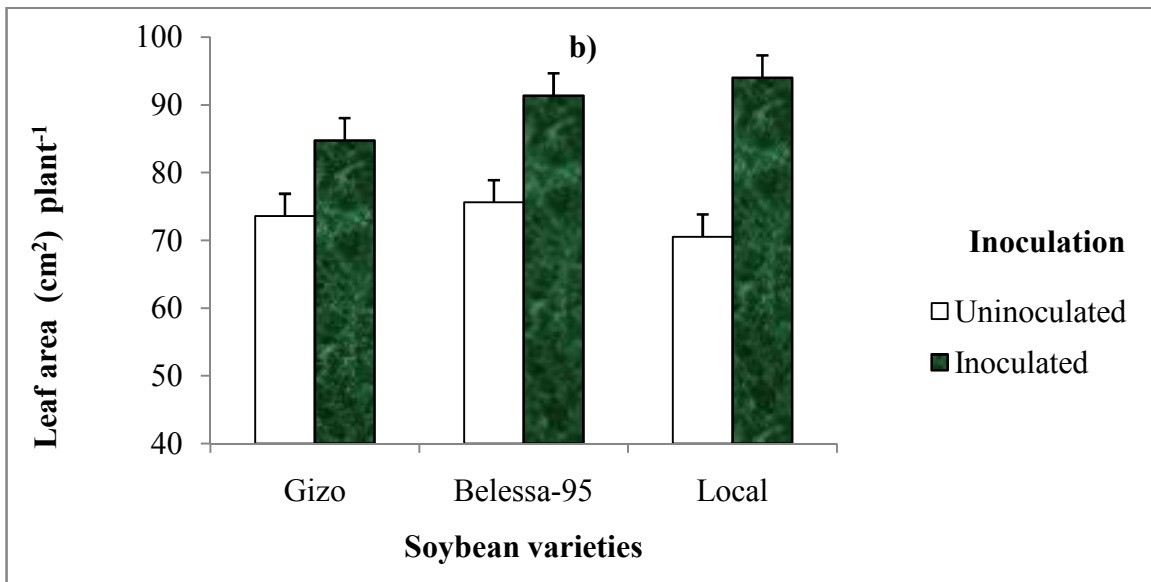


Figure . Interaction effects of; **a)** variety x phosphorus and **b)** variety x *Bradyrhizobium* inoculation on leaf area. Vertical lines on bars represent standard error of the statistical means.

4.2.5. Shoot dry matter

Plant growth measured as shoot dry matter was significantly ($P < 0.001$) affected by soybean variety, *Bradyrhizobium* inoculation and P application as well as their interactions (Table 2). The highest ($12.77 \text{ g plant}^{-1}$) and lowest ($10.72 \text{ g plant}^{-1}$) shoot dry matter was recorded from variety Belessa-95 and variety Local respectively, but statistically parity between variety Gizo and variety Local. The observed differences could be genetic or difference due to the ability of N_2 -fixing among the varieties. This is in line with finding of Singh *et al.* (2011) who reported that some varieties have the ability to out yield than the other varieties and exhibit superior plant growth. Addo-Quaye *et al.* (2011) also found that cowpea varieties have different capacities for shoot dry matter accumulation.

Bradyrhizobium inoculation with strain MAR-95 gave higher shoot dry matter that was greater by 29.9% over the control (Table 2). The observed benefits on soybean by *Bradyrhizobium*

inoculation seem to be to the supply of N to the crop through symbiotic N₂-fixation (Togay *et al.*, 2008). Yoseph and Shanko (2017) also reported that the significant effect of seed inoculation on shoot dry weight compared to the control treatments.

Application of P fertilizer at the level of 20 kg ha⁻¹ showed significantly increased mean shoot dry matter yield (34.15%) compared to the control. However, the shoot dry matter of plants grown at 10 and 20 kg P ha⁻¹ were in statistical parity (Table 2). The marked increase in shoot dry weight in response to the increased rates of P application might be ascribed to the increased availability of P in the soil for uptake by plant roots, which may have sufficiently enhanced vegetative growth through increasing cell division and elongation (Sara *et al.*, 2013). Similarly Togay *et al.* (2008) who reported that the greatest shoot dry weight on soybean due to higher P supply than the control.

From data variety x *Bradyrhizobium* inoculation interaction revealed that greater shoot dry matter was recorded by Belessa-95 variety compared to Local and Gizo varieties (Fig. 3a). However, without inoculation there was no marked difference between varieties Gizo and Belessa-95. The data from *Bradyrhizobium* inoculation x phosphorus application interaction showed that higher shoot dry weight was recorded from *Bradyrhizobium* inoculation with 20 kg P ha⁻¹ compared to zero-P but, statistically parity between 20 and 10 kg P ha⁻¹. Without *Bradyrhizobium* inoculation shoot dry matter was increased with increased P level (Fig. 3b). Shoot dry matter plant⁻¹ was positively and significantly correlated with pod number plant⁻¹ (r = 0.71), grain yield (r = 0.79) and above ground total biomass yield (r = 0.84) (Appendix Table 1).

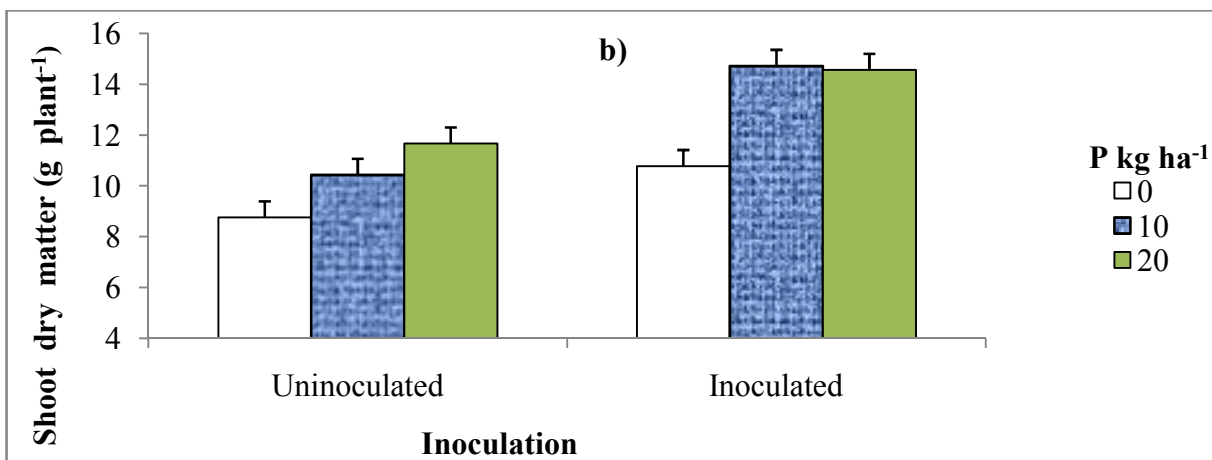
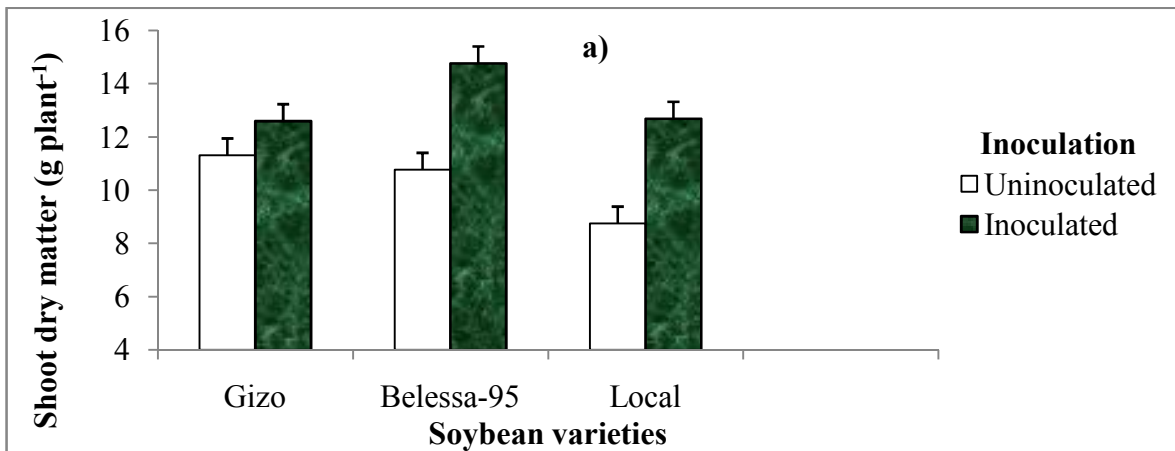


Figure . Interaction effects of; **a)** variety x *Bradyrhizobium* inoculation and **b)** *Bradyrhizobium* inoculation x phosphorus application on shoot dry matter. Vertical lines on bars represent standard error of the statistical means.

4.2.6. Root dry matter

The analysis of variance showed that a highly significant ($P < 0.01$) effect of the main effects; varieties, *Bradyrhizobium* inoculation and P application, but their interaction had no significant effect on the root dry matter (Table 2). Of the three soybean varieties, Belessa-95 and Gizo showed greater root dry matter compared to Local. Independent of treatment effects, these

differences could be attributed to genetic variability which is common among soybean varieties (Addo-Quaye *et al.*, 2011).

Bradyrhizobium inoculation with strain MAR-1495 significantly increased the root dry matter compared to uninoculated control (Table 2). Increased root dry matter might be due to that *Bradyrhizobium* inoculation gave N nutrition to soybean crop for root growth through N₂-fixation. Similar to this result Togay *et al.* (2008) also reported that *Brdayrhizobium* inoculation on soybean crop increased root growth and root dry matter.

Application of 20 kg P ha⁻¹ to soybean crop markedly increased root dry matter over zero-P control (Table 2). However, plants fertilized with 10 and 20 kg P ha⁻¹ as well as 10 and zero kg P ha⁻¹ did not differ statistically with each other (Table 2). Increased root dry weight due to supplied P rates might be attributed due to promoting effects of root growth and the formation of lateral fibrous roots when supplied with adequate P levels. In line to this finding Gentili (2008) reported that adequate amount of P to soybean improved the whole plant fresh and dry mass, nodule dry weight and root dry matter. Root dry matter plant⁻¹ was positively and significantly correlated with pod number plant⁻¹ (r = 0.62) (Appendix Table 1).

4.3. Effect of *Bradyrhizobium* and P application on Yield and Yield Components of Soybean Varieties

4.3.1. Number of branches plant⁻¹

Analysis of variance revealed that *Bradyrhizobium* inoculation had significant ($P < 0.05$) effect on the number of primary branches plant⁻¹, but the other main factors and their interaction had no significant effect on the number of primary branches plant⁻¹ (Table 3).

Bradyrhizobium inoculation with MAR-1495 was significantly increased number of primary branches plant⁻¹ by (4.85%) compared to the control (Table 3). Similar to this result Tairo and Ndakidemi (2014) reported that increased number of branches plant⁻¹ due to *Bradyrhizobium* inoculation in the field experiment.

4.3.2. Number of pods plant⁻¹

Analysis of variance revealed that varieties exerted significant influence on number of pods plant⁻¹ (Table 3). Among the three soybean varieties, Gizo had highest pod number plant⁻¹ (78.33) followed by Belessa-95 variety. Whereas, the lowest pod number plant⁻¹ was recorded from Local variety (71.55), but there was lack of marked difference statistically between Belessa-95 and Gizo varieties as well as Belessa-95 and Local varieties. The observed differences in pods plant⁻¹ among the three soybean varieties could be attributed to genotypic differences. The result is in agreement with the work of Yoseph *et al.* (2017) who reported that marked differences among the cowpea varieties on pod numbers plant⁻¹.

Bradyrhizobium inoculation with strain MAR-1495 showed that significantly increased pod number plant⁻¹ compared to the uninoculated (Table 3). The minimum pod number plant⁻¹ (68.33) was recorded from the control treatment. This increased pod number plant⁻¹ with *Bradyrhizobium* inoculation could be associated with enhanced growth and higher assimilate accumulation which is resulted due to better N nourishment from BNF. The result is in agreement with the work of Dereje (2007) also reported that increased number of pods plant⁻¹ with inoculation in green gram and soybean.

Significantly increased mean of pod numbers plant⁻¹(16.56 and 8.81%) was obtained with application of 20 kg P ha⁻¹ compared to the zero-P and 10 kg P ha⁻¹respectively (Table 3). Highest number of pods plant⁻¹ in sufficient P treated plots was obtained possibly due to improved reproductive performance of the plants because of improved P nutrition. Number of pods plant⁻¹ was positively and significantly correlated with above ground total biomass yield (r =0.72) and grain yield (r =0.69) (Appendix Table 1).

4.3.3. Number of seed pod⁻¹

The analysis of variance revealed that number of seeds pod⁻¹ was significantly affected by varieties (Table 3). The highest number of seeds pod⁻¹ was recorded from Belessa-95 variety (3 seeds pod⁻¹); whereas the lowest was recorded from Gizo variety (2.5 seeds pod⁻¹). This might be due to inherent genetic difference among the soybean varieties for seed production pod⁻¹. However, the seeds pod⁻¹ is less affected by introduced *Bradyrhizobium* strain. This result was in conformity with Patra *et al.* (2012) who stated that *rhizobium* inoculation did not affect seed numbers pod⁻¹ of different genotypes of soybean.

Application of 20 kg P ha⁻¹ to soybean crop markedly increased numbers of seed pod⁻¹ over 10 kg P ha⁻¹ and zero-P (Table 3). However, plants fertilized with 10 kg P ha⁻¹ and zero kg P ha⁻¹ did not differ statistically with each other (Table 3). The increased in numbers of seed pod⁻¹ due to adequate P fertilization could be explainable in terms of possible increase in nutrient mining capacity of plant as a result of increased translocation of carbohydrates from source to growing points in adequate P fertilized plots. This result is in line with that of Shahid *et al.* (2009) who reported that application of adequate amount of P resulted in markedly increase yield of soybean plants.

4.3.4. Hundred seed weight

The result of analysis of variance on hundred seed weight showed that there was highly significant difference ($P \leq 0.01$) on effect of varieties and phosphorus application, but there was no significance difference on *Bradyrhizobium* inoculation and all interactions (Table 3).

Regarding the variety, the heavier and lighter seed weight was recorded due to variety Belessa-95 (16.35 g) and variety Local (15.86 g) respectively. The significant difference in hundred seed weight among the soybean varieties may be due to the difference in translocation and partitioning efficiency of assimilates from source to sink. Similar El Naim and Jabereldar (2010) who reported that observed a significant variation in hundred seed weight of soybean crop.

Application of 20 kg P ha⁻¹ to soybean crop markedly increased numbers of hundred seed weight (Table 3). However, plants fertilized with 10 kg P ha⁻¹ and zero kg P ha⁻¹ did not differ statistically with each other (Table 3). The heavier seed weight due to adequate P fertilization could be increase translocation and partitioning of assimilates from source to grain.

4.3.5. Grain yield

The grain yield was affected significantly by soybean varieties (Table 3). Among the three soybean varieties tested in this experiment, Belessa-95 variety gave highest grain yield (2.31 ton ha⁻¹), whereas the lowest grain yield was recorded from Gizo varieties (2.12 ton ha⁻¹) (Table 3). Belessa-95 variety was showed increased grain yield by (9.1 and 8.1%) compared to Gizo and Local varieties respectively, but the differences in grain yield between Gizo and Local varieties were statistically at par. The greater grain yield recorded for the variety Belessa-95 was due to its ability to produce more and longer pods, as well as higher seed number pod⁻¹, which increased its economic yield and profitability as a crop. The higher grain yield could also be attributed to the better plant growth of Belessa-95 variety. By contrast, varieties Gizo and Local proved to be low-yielding varieties regardless of agronomic practices such as *Bradyrhizobium* inoculation and P supply. Similar to this result Haruna and Usman (2013) observed a significant variation in grain yield of some improved varieties of cowpea at the same location and attributed it to genetic makeup of the varieties examined.

Similar to other yield components, grain yield was also highly affected by *Bradyrhizobium* inoculation (Table 3). *Bradyrhizobium* inoculation with strain MAR-1495 resulted in significantly increased grain yield compared to the uninoculated control. The highest grain yield (2.7 ton ha⁻¹) was recorded from plants inoculated with strain MAR-1495 and the lowest grain yield (1.67 ton ha⁻¹) was recorded from the control (Table 3). The significant increase in grain yield in response to *Bradyrhizobium* inoculation with stain MAR-1495 might be attributed to the increased availability of N in the soil for uptake by plant roots, through fixed N. Increased in grain yield due to *Bradyrhizobium* inoculation might be attributed to the effectiveness of the

inoculant in fixing N thereby meeting the nutrient requirement of the plant (Nyoki and Ndakidemi, 2013). Ulzen *et al.* (2016) also observed that significantly increased in grain yield of cowpea after inoculation with *Bradyrhizobium* inoculant. A similar promotive effect of *Bradyrhizobium* inoculation on grain yield of soybean has also been reported by (Abbasi *et al.*, 2010). Similar to this finding Shahid *et al.* (2009) who reported that grain production of soybean crop can increase by 70-75% when the proper *rhizobia* isolates are inoculated.

A similar trend of increased in grain yield was also noticed with the increasing levels of P (Table 3). Application of 20 kg P ha⁻¹ brought higher grain yield over the control (42.77%) and application 10 kg P ha⁻¹ (12.1%). Increased grain yield is due to increased of P availability which is known to help developing a more extensive root system and thus enabling plants to extract water and nutrients from more depth. This could enhance the plants to produce more assimilates, which was reflected in higher biomass. Moreover, the positive response of soybean to P application in this study was obviously due to low available P of the experimental soil. In line with this result sufficient available P is also required by legumes to enhance plant growth, promote nodulation, early maturity and grain formation (Shahid *et al.*, 2009).

The variety x *Bradyrhizobium* inoculation interaction showed that much greater grain yield was obtained from Belessa-95 variety with *Bradyrhizobium* inoculation compared to Gizo and Local varieties, but without *Bradyrhizobium* inoculation the three soybean varieties almost equally responded for grain yield (Fig. 4a). The data from *Bradyrhizobium* inoculation x phosphorus application interaction showed that *Bradyrhizobium* inoculation with highest level of P (20 kg ha⁻¹) recorded much greater grain yield than zero-P application, but statistically parity between this level and 10 kg ha⁻¹ with *Bradyrhizobium* inoculation. Without *Bradyrhizobium* inoculation

grain yield was increased with increased P levels (Fig. 4b). Grain yield was positively and significantly correlated with above ground total biomass yield ($r = 0.98$) (Appendix Table 1).

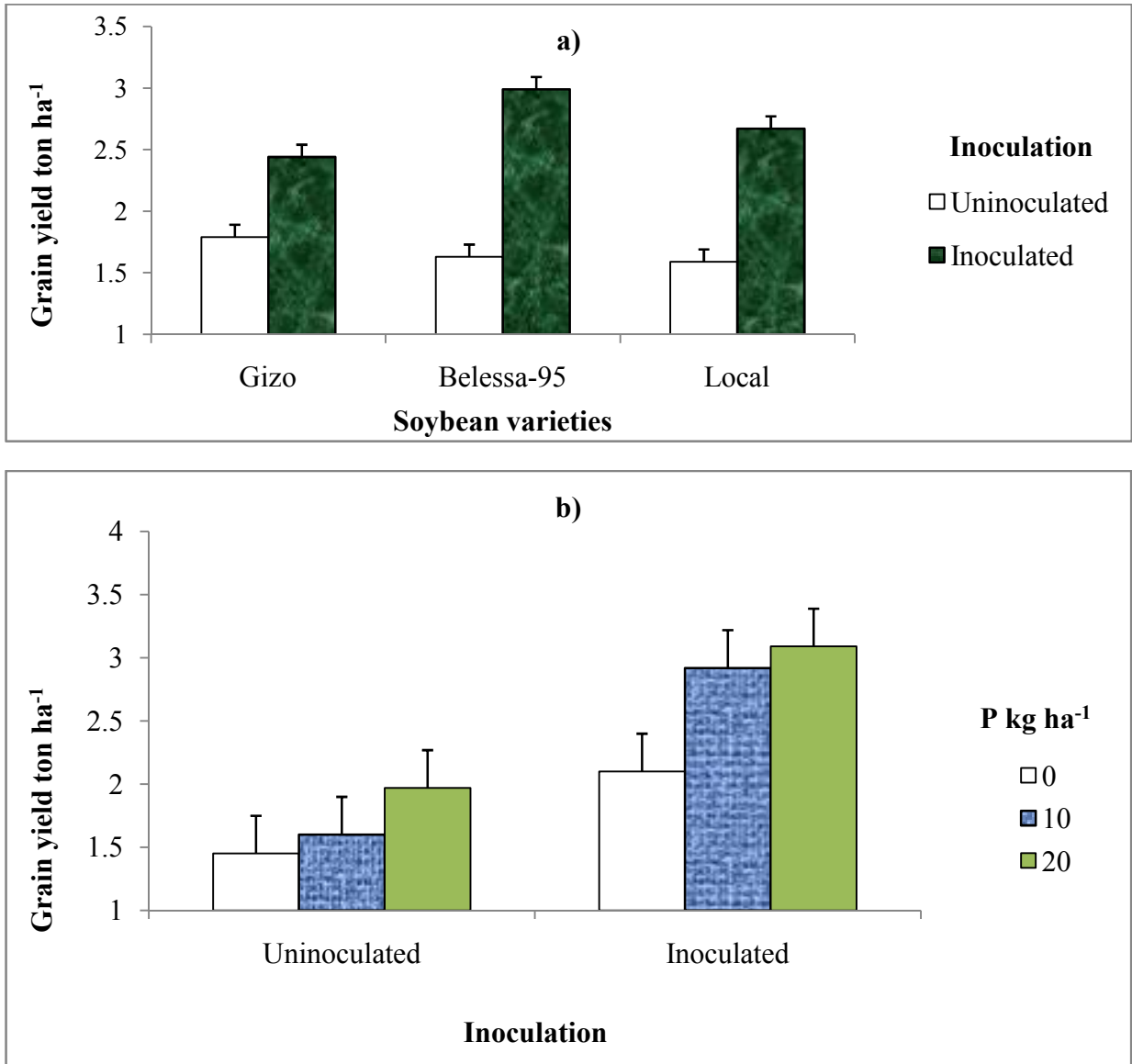


Figure . Interaction effects of; **a)** variety x *Bradyrhizobium* inoculation, **b)** *Bradyrhizobium* inoculation x phosphorus on grain yield of soybean. Vertical lines on bars represent standard error of the statistical means.

4.3.6. Above ground total biomass yield

Above ground total biomass yield was significantly affected by variety, *Bradyrhizobium* inoculation and P application (Table 3). There was variety inoculation as well as inoculation phosphorus interaction effect on above ground total biomass yield.

The highest above ground total biomass yield was recorded (3.06 ton ha⁻¹) from Belessa-95 variety, while the lowest above ground total biomass yield was recorded (2.80 ton ha⁻¹) from Local variety, but statistically parity between variety Gizo and variety Local as well as variety Belessa-95 and variety Gizo (Table 3). The higher above ground total biomass yield could also be attributed to the better plant shoot growth and grain formation ability of Belessa-95 variety.

Bradyrhizobium inoculation with strain MAR-1495 resulted in significantly increased above ground total biomass yield compared to the uninoculated treatment. The highest above ground total biomass yield (3.54 ton ha⁻¹) was recorded from plants inoculated with strain MAR-1495 and the lowest above ground total biomass yield (2.29 ton ha⁻¹) was recorded from the control (Table 3). Inoculation with *Bradyrhizobium* was significantly increased above ground total biomass yield of soybean by (53.98%) compared to the control treatments. The increased above ground total biomass due to inoculation could be attributed to the effectiveness of the *Bradyrhizobium* inoculant. In line with this result Abbasi *et al.* (2010) who pointed out that above ground total biomass yield of soybean was increased ranging from 39 to 75% by the inoculation of different strains of *rhizobia*.

Significantly ($P \leq 0.01$) highest above ground total biomass yield was achieved from application of 20 kg P ha⁻¹ by (36.86 and 8.63%) compared to zero-P and 10 kg P ha⁻¹ respectively, while 25.99 % above ground total biomass yield obtained from application of 10 kg P ha⁻¹ compared to the control (Table 3). This might be due to that P fertilization enhanced shoots biomass and grain production in the soybean crops as the result increased above ground total biomass yield was obtained. Similar to these results Lamptey *et al.* (2014) who reported that increased in above ground biomass yield of soybean as a result of increased levels of P fertilizer.

The variety x *Bradyrhizobium* inoculation interaction showed much greater above ground total biomass yield was obtained from Belessa-95 variety compared to Gizo and Local varieties with *Bradyrhizobium* inoculation, but without *Bradyrhizobium* inoculation the three soybean varieties almost equally responded for above ground total biomass yield (Fig. 5a). The data from *Bradyrhizobium* inoculation x phosphorus application interaction showed that 20 kg ha⁻¹P level with *Bradyrhizobium* inoculation gave greater above ground total biomass yield compared to zero-P application, but statistically parity between this level and 10 kg ha⁻¹ P. Without *Bradyrhizobium* inoculation above ground total biomass yield was increased with increased P levels (Fig. 5b).

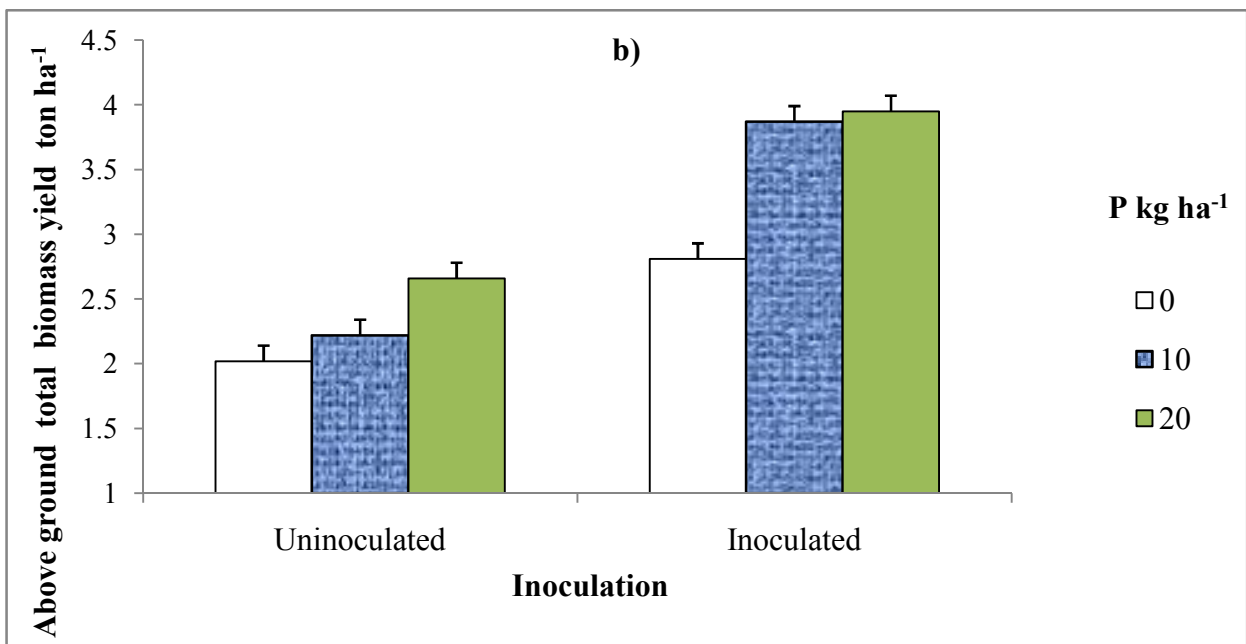
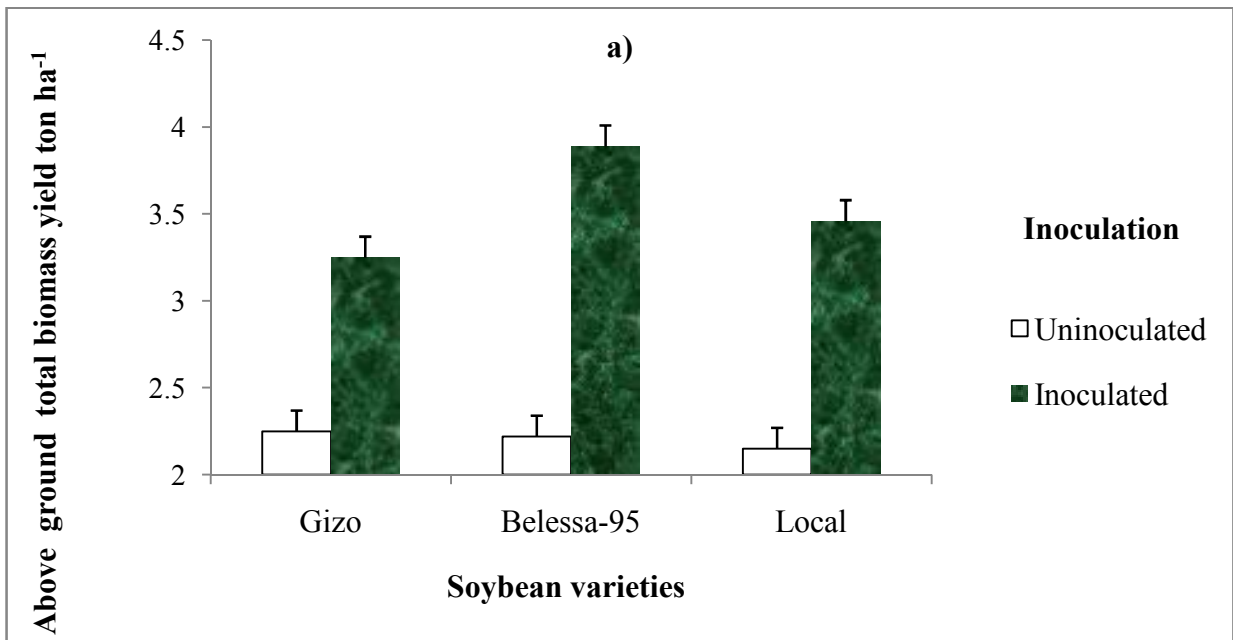


Figure . Interaction effects of; **a)** variety x *Bradyrhizobium* inoculation and **b)** *Bradyrhizobium* inoculation x phosphorus on above ground total biomass yield of soybean. Vertical lines on bars represent standard error of the statistical means.

4.3.7. Harvest index

Analysis of variance indicated that *Bradyrhizobium* inoculation had significantly effect on harvest index. But varieties, P application and their interactions failed to responded significant effect on harvest index (Table3).

Bradyrhizobium inoculation increased the harvest index of soybean crop by (5.36%) compared to over the control (Table3). The physiological ability of a crop plant to convert proportion of dry matter into economic yield is measured in terms of harvest index. The results indicated that adequate supply of N through biological N₂-fixation enhanced dry matter partitioning in favor of grain shown a greater harvest index. Similar Roy *et al.* (1995) reported that soybean seeds inoculation increased harvest index.

Table . Main effects of varieties, *Bradyrhizobium* inoculation and P levels on yield and yield components of soybean

Treatments	Branch Number plant ⁻¹	Pod Number plant ⁻¹	Seed Number pod ⁻¹	Number Hundred Weight (g)	Seed Grain (t ha ⁻¹)	Yield Total Biomass (t ha ⁻¹)	Harvest Index
Varieties							
Gizo	11.78	78.33 ^A	2.50 ^B	16.05 ^B	2.12 ^B	2.89 ^{AB}	0.73
Belessa-95	11.78	74.88 ^{AB}	3.00 ^A	16.35 ^A	2.31 ^A	3.06 ^A	0.75
Local	11.72	71.55 ^B	2.88 ^A	15.86 ^B	2.14 ^B	2.80 ^B	0.76
LSD.05	NS	4.65	0.24	0.26	0.15	0.17	NS
Inoculation							
Uninoculated	11.48 ^B	68.33 ^B	2.77	15.98	1.67 ^B	2.29 ^B	0.72 ^B
MAR-1495	12.04 ^A	81.51 ^A	2.81	16.19	2.70 ^A	3.54 ^A	0.76 ^A
LSD.05	0.42	3.79	NS	NS	0.12	0.14	0.02
P (kgha⁻¹)							
0	11.78	69.44 ^C	2.72 ^B	16.02 ^{AB}	1.77 ^C	2.41 ^C	0.73
10	11.78	74.39 ^B	2.72 ^B	15.97 ^B	2.26 ^B	3.04 ^B	0.74
20	11.72	80.94 ^A	2.94 ^A	16.27 ^A	2.53 ^A	3.30 ^A	0.76
LSD.05	NS	4.65	0.24	0.26	0.15	0.17	NS
CV (%)	6.44	9.16	12.55	2.42	9.93	8.71	7.10

Mean Values followed by dissimilar letters in a column are significantly different at *: p≤0.05; **: p≤0.01; ***: p≤0.001 NS; Non-Significance difference.

4.4. Partial Budget (Economic) Analysis of Treatment Effects

Partial budget is a method of organizing experimental data and information about the cost and benefits of various alternative treatments. Getting maximum profitability lies not only in reducing use of P per unit area but also in lowering costs per unit crop production through higher yields. Therefore, economic analysis is required for making recommendation for farmers from such agronomic experiments. To assess the cost and benefit associated with different treatments, the partial budget analysis technique of CIMMYT (1988) was applied.

Dominance analysis and percentage of marginal return rate were used to evaluate the economic performance of treatments. Dominance analysis is carried out by listing the treatments in order of increasing costs that vary, and any treatments that has net benefits that are less than or equal to those of a treatment with lower costs that vary is dominated and as such would not be considered for investment by the farmers (CIMMYT, 1988). The marginal rate analysis reveals just how the net benefits from an investment increase as the amount invested increase and which was carried out on the undominated treatments by calculating change in net benefits divided by change in cost and expressed as a percentage. The minimum acceptance rate of return is fixed as baseline for acceptance of an option in order to account the cost of capital, inflation, and risk. The minimum rate of return acceptable to farmers is between 50 and 100% marginal rate return (CIMMYT, 1988).

According to the economic analysis data (Table 4) both marginal rates were above 100% that is in a range of acceptance. Although Belessa-95 variety with *Bradyrhizobim* inoculation ranked among the treatments with the highest marginal rate return in percent, the fact that the

benefit lower than Belessa-95 variety with *Bradyrhizobim* inoculation and 10 kg P application. Therefore, from the budget summary of economic analysis, the highest net return (19984 Birr ha⁻¹) was obtained from Belessa-95 variety with *Bradyrhizobim* inoculation and 10 kg P ha⁻¹ application, while the lowest net economic return was recorded in the control treatment Belessa-95 with zero-P application with (8540 Birr ha⁻¹) (Table 4). Therefore, use of Belessa-95 varieties with *Bradyrhizobim* inoculation and 10 kg P ha⁻¹ application found to be economically feasible at Assosa soil.

Table . Cost benefits analysis of *Bradyrhizobium* inoculation and P application for soybean production at Assosa soil

Treatments	Yield (t ha ⁻¹)	Adjusted yield (t ha ⁻¹)	GB (birr)	P cost (Birr ha ⁻¹)	STC (Birr ha ⁻¹)	LC (Birr)	TVC (Birr ha ⁻¹)	Net benefit	Dominance	MRR %
LX 0P	1.47	1.32	9240	0	0		0	9240	D	
GX 0P	1.5	1.35	9450	0	0		0	9450		
BX 0P	1.37	1.22	8540	0	0		0	8540	D	
L XBR	2.12	1.91	13370	0	56		56	133014	D	
GXBR	1.77	1.59	11130	0	56		56	11074	D	
BXBR	2.39	2.15	15050	0	56		56	14994		9900
L X10 P	1.57	1.41	9870	409.78	0	200	609	9260	D	
GX10 P	1.77	1.59	11130	409.78	0	200	609	10520	D	
BX 10P	1.46	1.31	9170	409.78	0	200	609	8560	D	
L X BRX10P	2.9	2.61	18270	409.78	56	200	666	17604	D	
GX BRX10P	2.57	2.31	16170	409.78	56	200	666	15504	D	
BX BRX10P	3.28	2.95	20650	409.78	56	200	666	19984		818
L X20 P	1.75	1.57	10990	819.56	0	200	1019	9970	D	
GX20 P	2.1	1.89	13230	819.56	0	200	1019	12210	D	
BX20P	2.06	1.85	12950	819.56	0	200	1019	11930	D	
LXBRX20P	2.99	2.69	18830	819.56	56	200	1075	17754	D	
GXBRX20P	2.98	2.68	18760	819.56	56	200	1075	17684	D	
BXBRX20P	3.29	2.91	20370	819.56	56	200	1075	19294	D	

GB=Gross benefit, STC=Strain cost, LC=Labor cost, TVC=Total variable cost, MRR= Marginal rate return, L= Local variety, B= Belessa-9 5 variety, BR= Inoculation and P= Phosphorus

5. SUMMARY AND CONCLUSION

This research was conducted during the 2016 main cropping season at Asossa Agricultural Technical and Vocational Education Training College in Benishangul Gumuz Regional State to investigate growth, symbiotic and yield performance of soybean varieties in response to *Bradyrhizobium* inoculation and P fertilizer application. The experiment consisted of three soybean varieties (Gizo, Belessa-95 and Local), two inoculation levels (uninoculated, and inoculated with strain MAR-1495) and three levels of P fertilizer (0, 10 and 20 kg P ha⁻¹) in the form of TSP and in randomized complete block design with factorial arrangement using three replications.

There was highly significant ($P \leq 0.01$) varietal effect for most of studied parameters; Belessa-95 variety had better responded on nodule number, nodule dry weigh, grain yield and above ground total biomass as compared to Gizo and Local varieties. The interaction effect of strain-P, strain-varieties and P-varieties caused significant variation in numbers of nodules; shoots dry weight, leaf area, above ground total biomass and grain yield. The highest grain yield was recorded from Belessa-95 variety 3.29 ton ha⁻¹ from combined application of 20 kg P and *Bradyrhizobium* inoculation. It could be deduced that use of strain MAR-1495 *Bradyrhizobium* and Belessa-95 variety with application of 20 kg P markedly increases the productivity of soybean crop at Assosa. Results obtained from this experiment indicated that most of the parameters tested responded significantly to the treatments studied. For nodulation and growth parameters, *Bradyrhizobium* inoculation with strain MAR-1495 showed highly significant differences on nodule number, nodule dry weight, shoot dry weight, leaf area and root dry weight. However,

plant height was not affected by inoculation of strain MAR-1495. Similarly, appreciable increments in nodulation and growth parameters were obtained due to application of higher P rate. Significantly increased mean (47.24 and 79.4%) of nodule number and nodule dry weight plant⁻¹ were obtained from application of 20 kg P ha⁻¹ compared to the zero-P control, respectively. The application of P fertilizer at the rate of 20 kg ha⁻¹ showed significant increased mean by (34.15, 55.92, 12.07 and 15.68%) on shoots dry matter, roots dry matter and leaf area compared to the control zero-P application respectively.

In the case of yield and yield components, *Bradyrhizobium* inoculation with strain MAR-1495 showed significant effects on most parameters of yield and yield components. In this study, *Bradyrhizobium* inoculation with strain MAR-1495 increased number of pods, grain yield and above ground total biomass yields by (19.29, 53.98 and 61.63%) over the control respectively. Significantly increased in yield and yield components were also attained with application of P. Phosphorus application at level of 20 kg ha⁻¹ increased number pods plant⁻¹, grain yield and above ground total biomass yield by (16.56, 42.77 and 36.86%) compared to over the control treatment respectively.

Partial budget analysis indicated that using Belessa-95 variety with *Bradyrhizobim* inoculation and 10 kg P ha⁻¹ application is more profitable than the other treatments with a marginal rate of return of (810%). The partial budget analysis identified 10 kg P ha⁻¹ to be superior in economic return. Thus, the use of Belessa-95 variety with strain MAR-1495 inoculation and P fertilizer application at the level of 10 kg ha⁻¹ could be recommended to soybean producers in Assosa area to achieve superior yield and better economic return. However, verification of this result on different farmers' field could be required in order to put the recommendation in a firm ground.

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7. APPENDICES TABLES

Appendix Table . Correlation results among parameters

	NN	NDW	PH	LA	SDW	RDW	BNPP	PNPP	SNPP	HSW	GY	AGTB	HI
NN	1	0.90**	0.32*	0.71**	0.78**	0.57**	0.32*	0.63**	0.26	0.36**	0.88**	0.88**	0.30*
NDW		1	0.30*	0.67**	0.72**	0.51**	0.31*	0.58**	0.23	0.31*	0.81**	0.79**	0.29*
PH			1	0.26	0.27*	0.24	0.17	0.11	0.26	0.16	0.17	0.20	-0.16
LA				1	0.65**	0.24	0.22	0.55**	0.13	0.2	0.75**	0.74**	0.26
SDW					1	0.58**	0.29*	0.71**	0.16	0.21	0.79**	0.84**	0.11
RDW						1	0.38**	0.62**	-0.03	0.36**	0.44**	0.46**	-0.03
BNPP							1	0.25	-0.04	-0.04	0.24	0.24	0.07
PNPP								1	-0.17	0.32*	0.69**	0.72	0.14
SNPP									1	0.20	0.23	0.22	0.17
HSW										1	0.33*	0.30*	0.15
GY											1	0.98**	0.42**
AGTB												1	0.35*
HI													1

** And * significant at 1% and 5% levels respectively; NN=Nodule number, NDW =Nodule dry weight, PH=Plant height, LA=Leaf area, SDW=Shoot dry weight, RDW= Root dry weight, BNPP= Brach number plant⁻¹, PNPP= Pod number of plant⁻¹, SNPP=Seed Number pod⁻¹, HSW= Hundred seed weight, GY=Grain yield, AGTB= Aabove ground total biomass, and HI=Harvest index.

Appendix Table . Mean squares of ANOVA growth and nodulation of soybean varieties in response to *Bradyrhizobium* inoculation and P supply at Assosa, during 2016 cropping season

Source	DF	Mean squares					
		NN plant ⁻¹	NDW	PH	LA plant ⁻¹	SDW gram plant ⁻¹	RDW gram plant ⁻¹
Block	2	3.64	0.0009	19.87	27.93	6.59	5.43
Br	1	774.45 ^{**}	0.19 ^{**}	18.61 ^{NS}	3874.46 ^{**}	127.57 ^{**}	15.45 ^{**}
P	2	95.14 ^{**}	0.03 ^{**}	25.74 ^{NS}	466.27 ^{**}	57.97 ^{**}	10.81 ^{**}
V	2	87.95 ^{**}	0.03 ^{**}	231.72 ^{**}	91.26 ^{NS}	19.31 ^{**}	13.44 ^{**}
Br x P	2	67.26 ^{**}	0.00052 ^{NS}	30.47 ^{NS}	24.72 ^{NS}	6.01 [*]	4.02 ^{NS}
P x V	4	7.70 ^{**}	0.0021 ^{NS}	36.55 ^{NS}	189.49 [*]	1.30 ^{NS}	4.36 ^{NS}
Br x V	2	11.89 ^{**}	0.0035 ^{NS}	0.16 ^{NS}	186.77 [*]	10.72 ^{**}	5.94 ^{NS}
Br x P x V	4	1.28 ^{NS}	0.0008 ^{NS}	48.76 ^{NS}	69.48 ^{NS}	2.27 ^{NS}	2.56 ^{NS}
Error	34	1.70	0.001	20.42	48.23	1.83	1.65
CV (%)		10.81	24.21	9.49	8.50	11.46	36.38

^{**} Highly Significant at $P \leq 0.01$ level; ^{*} significant at $P \leq 0.05$ level; NS = Non significance; NN=Nodule number, NDW=Nodule dry weight, PH=Plant height, LA=Leaf area, SDW=Shoot dry weight, RDW= Root dry weight, BR= *Bradyrhizobium*, V=Varieties and DF= Degree of freedom.

Appendix Table . Mean squares of ANOVA effect of *Bradyrhizobium* inoculation and P application on yield and yields components of soybean

Source	DF	Mean squares						
		BNP ⁻¹	PN plant ⁻¹	SN pod ⁻¹	HSW	GY(t ha ⁻¹)	AGTB (t ha ⁻¹)	HI
Block	2	1.91	222.69	0.24	0.06	0.12	0.03	0.007
Br	1	4.17*	2346.96**	0.02 ^{NS}	0.60 ^{NS}	14.33**	20.77**	0.01**
P	2	0.019 ^{NS}	599.02**	0.29*	0.47 ^{NS}	2.65**	3.76**	0.003 ^{NS}
V	2	0.019 ^{NS}	206.74*	1.24**	1.09**	0.20*	0.29 ^{NS}	0.0007 ^{NS}
Br x P	2	1.06 ^{NS}	70.91 ^{NS}	0.07 ^{NS}	0.02 ^{NS}	0.52**	0.83**	0.0009 ^{NS}
P x V	4	0.82 ^{NS}	134.55 ^{NS}	0.13 ^{NS}	0.09 ^{NS}	0.05 ^{NS}	0.07 ^{NS}	0.0006 ^{NS}
Br x V	2	0.72 ^{NS}	33.19 ^{NS}	0.02 ^{NS}	0.04 ^{NS}	0.58**	1.03**	0.004 ^{NS}
Br x P x V	4	1.36 ^{NS}	115.05 ^{NS}	0.07 ^{NS}	0.29 ^{NS}	0.05 ^{NS}	0.04 ^{NS}	0.001 ^{NS}
Error	34	0.57	47.10	0.12	0.15	0.05	0.06	0.002
CV (%)		6.44	9.16	12.55	2.42	9.93	8.71	7.10

**Significant at $P \leq 0.01$ level; * significant at $P \leq 0.05$ level; NS = Non significant; BNP⁻¹ = Branch number per plant, SNP = Seed number per pod, GY= grain yield, AGTB = above ground total biomass, HI=harvest index, V=Varieties, Br= *Bradyrhizobium* and DF= Degree of freedom.

Appendix Table . Meteorological data of Assosa district during the 2016 cropping season

Month	Monthly Temperature	Max. Temperature	Monthly Temperature	Min. Temperature	Total Rain fall	Relative humidity	Sun-shine hours
January	28.4		13.1		0.0	50.9	5.2
February	33.4		12.7		0.0	56.7	5.9
March	34.5		14.2		0.0	52.5	5.6
April	31.9		14.1		0.0	55.2	8.3
May	26.3		17.3		125.1	55.2	5.9
June	30.1		16.6		131.8	63.6	5.6
July	22.6		17.9		170.7	72.8	3.2
August	23.4		20.7		226.7	71.4	3.7
September	24.8		15.5		258.1	57.4	4.9
October	26.1		13.3		134.5	61.9	4.6
November	26.3		12.3		17.7	46.3	7.7
December	30.2		11.4		0.0	31.0	9.8

Source: Assosa meteorological station (2016)

8. BIOGRAPHICAL SKETCH

The author, Tesfaye Tekola, was born on November 12, 1988 in Ankober, Amhara National Regional State from his father Mr. Tekola Seifu and his mother Mrs. Lakech Mekit. He attended elementary and junior secondary education at Alyu Amba Elementary and Junior Secondary School. He completed Secondary school at Debre Birehan secondary school. He completed Preparatory School at Hailemarima Mamo preparatory school in Debre Birehan. After passing the Ethiopian School Leaving Certificate Examination, he joined Debre Birehan University in 2008 and graduated with Bsc degree in Plant science and Protection on July 14, 2010.

Upon graduation, he was employed by Ministry of Agriculture and Rural Development as junior instructor at Assosa Agricultural Technical and vocational Educational Training College in November, 2010. He joined the School of Graduate Studies at Hawassa University in July, 2014 to pursue a study leading to the Degree of Master of Science in Agronomy.