



**GROWTH, SYMBIOTIC AND YIELD RESPONSES OF SOYBEAN
(*Glycine max* L.) VARIETIES TO *BRADYRHIZOBIUM* INOCULATION
AND PHOSPHORUS APPLICATION AT ALAGE, CENTRAL RIFT
VALLEY OF ETHIOPIA**

M.Sc. THESIS

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HAWASSA UNIVERSITY, HAWASSA, ETHIOPIA

FEBRUARY, 2021

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VALLEY OF ETHIOPIA**

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DEDICATION

I dedicate this thesis manuscript to my loved father Yadeta Gemedo who was not lucky to see my success. I also dedicate this piece of work to my beloved mother Yeshe Jiru who had always been supporting me towards and sought my success.

STATEMENT OF AUTHOR

By my signature below, I declare and affirm that this thesis is my own work. I have followed all ethical and technical principle of scholarship in the preparation, data collection, data analysis and compilation of this thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation and that all sources of materials used for this thesis have been duly acknowledged.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADP	Adenosine Diammonium Phosphate
ANOVA	Analysis of Variance
ARC	Agricultural Research Center
ASHC	Africa Soil Health Consortium
ATP	Adenosine Triphosphate
ATVET	Agricultural Technical and Vocational Education Training
BNF	Biological Nitrogen Fixation
CEC	Cation Exchange Capacity
CIMMYT	International Maize and Wheat Improvement Center
CSA	Central Statistics Agency
DAP	Diamonium phosphate
DNA	Deoxyribonucleic Acids
EIAR	Ethiopian Institute of Agricultural Research
ETB	Ethiopian Birr
FAO	Food and Agricultural Organization
IAR	Institute of Agricultural Research
IITA	International Institute Tropical Agriculture
RCBD	Randomized Complete Block Design
RNA	Ribonucleic Acids
TSP	Triple Super Phosphate

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**Growth, Symbiotic and Yield Responses of Soybean (*Glycine max* L.)
Varieties to *Bradyrhizobium* Inoculation and Phosphorus Application at
Alage, Central Rift Valley of Ethiopia**

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ABSTRACT

*Soybean (*Glycine max* L.) is one of the most important food legumes of great nutritional value that has the highest protein content (40%) of all food crops and is equivalent to the protein of animal product. However, in Ethiopia its productivity is constrained mainly by low soil fertility and lack of improved varieties. A field experiment was conducted during 2020 belg cropping season at Alage Central Rift Valley of Ethiopia with the objective of evaluating the effects of *Bradyrhizobium japonicum* strain inoculation and P fertilizer rates on growth, symbiotic and yield response of soybean varieties. The treatments studied includes four P levels (0, 10, 20 and 30 kg P ha⁻¹), two inoculation levels (un-inoculated and inoculated with *Bradyrhizobium* strain; TAL-379) and two soybean varieties (Afgat and Nova) in randomized complete block design with factorial arrangement with three replications. The results revealed that the main effect of varieties were significantly ($P \leq 0.01$) influenced most of measured parameters. The results also revealed that inoculation of soybean varieties with *Bradyrhizobium* strain TAL-379 significantly ($P \leq 0.01$) influenced days to maturity, nodule number, nodule dry weight, shoot dry weight, root dry weight, pod number plant⁻¹, hundred seed weight, grain yield, above ground biological yield and harvest index. Conversely, days to emergence, days to flowering, plant height and seed number pod⁻¹ was not statistically affected by inoculation of strain TAL-379. The effect of P fertilizer was also significant for most of studied parameters except branch number and harvest index. The interaction effect of varieties x strain, strain x P, and varieties x P caused significant variation on days to maturity, number of nodule, nodule dry weight, shoot dry weight, root dry weight, pod plant⁻¹, hundred seed weight, grain yield and above ground biological yield. Hence, the use of Afgat variety with *Bradyrhizobium* strain TAL-379 inoculation significantly gave the highest grain yield (2.99 t ha⁻¹) followed by the interaction of Afgat variety with the supply of 20 kg P ha⁻¹ (2.89 t ha⁻¹). Grain yield was positively and significantly correlated with most of studied parameters. From the economic point of view the partial budget analysis of the study treatments revealed that the highest net return (63750 ETB ha⁻¹) was gained from *Bradyrhizobium* strain TAL-379 inoculation followed by application of 20 kg P ha⁻¹ and Afgat variety. Thus, it could be recommended that the use of Afgat variety, *Bradyrhizobium* strain TAL-379 and application of 20 kg P ha⁻¹ to get higher yield and profit of this crop at Alage Central Rift Valley of Ethiopia. However, verification of the result on farmers' fields across season and areas could be required before wide use of this study to put the recommendation in firm ground.*

Keywords: Afgat, Growth parameter, Nodulation, Nova, Strain TAL-379, soybean, variety

1. INTRODUCTION

1.1. Background of the Study

Soybean (*Glycine max* L.) is a member of leguminosae family, rich in nutrients and it is regarded as a food security crop. Nutritionally, it contains 40% protein compared to 20% and 13% protein content in meat and egg, respectively (FAO, 2010). It is the second only to groundnut in terms of oil content (20%) among food legumes (Hailemarim and Tsigie, 2006). It can be used directly for food in the household, or processed in the food industry for flour, cooking oil, cookies, candy, soy milk, vegetable cheese, and lecithin (ASHC, 2014). Furthermore, it can be used to produce a range of other products, including infant weaning food and also the poultry industry uses soybean for feed production. The crop residues are also rich in protein and are good feed for livestock or form a good basis for compost manure (Rusike *et al.*, 2013).

According to FAO (2015), the three major soybean producers in the world are USA (29 million hectares), Brazil (23 million hectares) and Argentina (14 million hectares); with productivity of 83, 53 and 38 million tons, respectively (FAO, 2015). In Africa, it is grown on an average of 1.2 million ha with an average production of 1.3 million tons and 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 more than 22 Africa countries, that allocating 1.3 million ha for soybean production were 1.4 million tons of grain is obtained (FAO, 2008).

In Ethiopia, soybean research started in the 1950s by introduced genotypes with the main emphasis on identifying adapted lines for the potential areas of the country, with the aim of replacing imported soybean flour, compromising into the existing cropping systems, and improving the diet of the poor peoples' life (Addisu and Erimias, 2017). Though soybean is a recently introduced crop in Ethiopia, records obtained for the period 2008 to 2016 indicated that in area coverage, total production and yield of soybean have been grown very rapid at a rate of 30.8%, 45.4% and, 11.2% per annum respectively, and reached 36,635.79 ha of land to produce 81,234.7 tons of soybean with a national average yield of 2.21 t ha⁻¹ (Birhanu *et al.*, 2018). Currently in Ethiopia, soybean covers about 38072.70 ha of land and 86467.9 tons of soybean is produced annually with average productivity of 2.27t ha⁻¹ (CSA, 2017).

Nitrogen (N) is one of the most abundant elements in the atmosphere. Despite its abundance in the atmosphere as a gas, it cannot be used directly by plants. Most plants utilize N in its ionic forms of ammonium (NH₄⁺) and nitrate (NO₃⁻) from soil (Solomon *et al.*, 2012). However, it is one of the most limiting factors of the growth and production of crops. N can be utilized when it is reduced to ammonia by N₂-fixation. It can be reduced by chemical fixation through industrial production and/or biological fixation involving microorganisms. Even in the presence of such process called biological nitrogen fixation (BNF); N is one of the usually deficient plant nutrients in soils. The increasing cost of fertilizers and their impact on the environment have forced people to look for other possible sources of plant nutrients. In this regard, N₂-fixation which is a process by which elemental atmospheric N₂ is changed to organic forms by BNF both by symbiotic and asymbiotic microorganisms in soil has drawn much attention. The symbiotic N₂-fixation is used to maximum advantage in the case of leguminous crops. There is no doubt that specificity exists between rhizobial strain and the

legume, and compatibility between the two is essential for successful nodulation (Melchiorre *et al.*, 2011). This necessitates using specific cultures for different legumes. When growing a new legume species on a soil, it is necessary that the appropriate rhizobial culture be applied (Abaidoo *et al.*, 2006). However, the use of inoculants as a means of improving crop productivity has not yet received due attention in Ethiopia, but in recent year's researchers from various disciplines have shown interest in the field. Some researchers have evaluated the response of introduced bacteria in the different agro-climatic zone of the country and others have brought inoculants from abroad and have got good results in their trials (Tarekegn *et al.*, 2017).

Cultivar variation affects levels of N₂-fixation in many legume crop species, and in some crops, particular combinations of strain and cultivar have been shown to be especially efficient at fixing N (Graham, 2000). There were varying reports on the interaction between variety and strain in soybean. Thao *et al.* (2002) found a significant interaction between variety and strain on different parameters, whereas Munyinda *et al.* (1988) reported a non-significant interaction.

Phosphorus (P) is the second most important plant nutrient but for legumes, it presumes primary significance, which plays important role in root proliferation and thereby atmospheric N₂-fixation. Singh *et al.* (2008) reported that the yield and nutritional quality of legumes are greatly influenced by the application of P and biofertilizers. It is crucial in the production of protein, phospholipids and phytin in legume grains (Rahman *et al.*, 2008). Its application also plays a vital role in increasing legume yield through its effect on the plant itself and also on the fixation process by *Rhizobium*. P stress reduces N₂-fixation due to decreased nodule

formation and reduced nodule sizes and finally affecting the yield and grain quality and quantity (Sadeghipour and Abbasi, 2012).

1.2. Statement of the Problem

Soybeans grown in most parts of the world and in some parts of the country, but a lot of constraints have contributed to poor growth performance and the yield potential of the crop. The use of optimum plant nutrients is considered to be one of the most important agronomic practices to increase growth and crop yield. However, both over and under application of P could adversely affect the growth, yield and yield components of soybean. Application of excess P resulted in reduced plant nodulation, acetylene, the emergence of seedling by 23% since it causes the burning effects on the seed of soybean and create weak (longer height and thinner stem diameter) plant that is easier to lodging, which resulted in the lower components of yield and seed yield (Da-bing *et al.*, 2012).

On the other hands P deficiency followed by N is the major constraint in pulse production since it affects growth, nodule formation and development and N₂-fixation (Yohanes and Richer, 1999). It 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). It has important effects on flowering, seed formation, fruiting and improvement of crop quality (Brady, 2002). Inadequate P availability restricts root growth, photosynthesis, translocation of sugars, and other such functions that 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 adequate P availability (Tang *et al.*, 2001).

In the study area, the 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 nutrient deficiencies during crop production. In the study area, improved soybean technologies are being supported 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 N and P deficiencies in the soil. As a result, farmers apply below the recommended dose of fertilizer which makes the improvements of crop productivity hardly possible. Therefore, symbiotic N₂-fixation can be used as a cheaper alternative for improving soil N-content. There is also a knowledge limitation to identify soybean varieties that give high yield as well as respond favorably to the application of P fertilizer and growing areas. In addition, there is currently no documented information regarding the response of soybean to *Bradyrhizobium* inoculation and P fertilizer application under Alage conditions at the Central Rift Valley of Ethiopia. Thus, identifying appropriate P fertilizer rates in combination with the use of *Bradyrhizobium* inoculation would be useful in developing effective nutrient management for low N and P soils. Similarly, using effective *Bradyrhizobium* inoculants as N sources for improving soybean growth, symbiotic and yield response is very important for sustainable crop production under Alage conditions.

1.3. Objective of the Study

1.3.1. General objective

To evaluate the effect of *Bradyrhizobium japonicum* strain inoculation and P application on growth, symbiotic and yield response of soybean varieties at Alage, Central Rift Valley of Ethiopia.

1.3.2. Specific objective

- To assess the effect of *Bradyrhizobium* inoculation and P fertilizer rates on growth, symbiotic and yield response of soybean varieties at Alage.
- To examine the interactive effect of *Bradyrhizobium* inoculation and P applications on growth, symbiotic and yield response of soybean varieties at Alage.
- To determine the economic feasibility of the *Bradyrhizobium* inoculation and P application for soybean production at Alage.

2. LITERATURE REVIEW

2.1. Origin and Distribution of Soybean

Soybean (*Glycine max* L.) is native to Eastern Asia, mainly China, Korea, and Japan, from where it spread to Europe, and America and other parts of the world in the 18th century (Ngeze, 1993). Evidence in Chinese history indicates its existence more than 5,000 years ago, being used as food and a component of drugs (Norman *et al.*, 1995). Some researchers have suggested Australia and Eastern Africa as other possible centers of origin of the genus *Glycine* (Addo-Quaye *et al.*, 1993). It is broadly grown on a large scale in both the temperate and tropical regions such as China, Thailand, Indonesia, Brazil, the USA and Japan; where it has become a major agricultural crop and a significant export commodity (Evans, 1996).

According to Burkill (1935), soybean cultivation reached Africa in the late 1800s, although little is known of the countries to which it was first introduced. It is possible, perhaps likely, that soybeans were cultivated at an early date on the eastern coast of Africa since that region had long trade with the Chinese. The same report indicates that soybean has been under cultivation in Tanzania in 1907 and Malawi in 1909 (IITA, 2009). In Ghana, the Portuguese missionaries were the first to introduce the soybean in 1909. This early introduction did not flourish because of the temperate origin of the crop (Mercer and Nsowah, 1975).

In Ethiopia, pulse crops rank second as food after cereals and occupy about 17.7% of the total cultivated areas, and contribute about 12% of the total production. The major food legumes in the country are faba bean, chickpea, field pea, haricot bean, fenugreek, lupin and soybean. However, the yield of most pulse crops in Ethiopia is low and ranges between 500 - 900 kg ha⁻¹, whereas the average yield potential of these crops is about 1760 kg ha⁻¹ (Desta, 1988 in

Asfaw and Angaw, 2003). This is evident that most fertile soils are occupied by cereals, while pulses are grown in marginal soils, usually as rotation crops (Asfaw and Solh, 1994). From these legumes, haricot bean and soybean are the two main lowland food legumes in the Rift Valley of Ethiopia. The productivity of soybean is between 377 kg ha⁻¹ in Gamo Gofa and 810 kg ha⁻¹ in Wolaita (CACC, 2002). Even though there is wide gap in productivity of the crop between on farm and research plots, the area coverage and production trend of the country showed an increase of cultivated land from 19,397.16 ha in 2011 to 31,854 ha in 2012 with an increase of 64%, in the same year the soybean production also increased with the same trend from 35,880.3 ton to 63,653.1 ton and an increase of 77.4% (CSA, 2013).

2.2. Economic Importance of Soybean

Soybean has been cultivated all over the world since ancient times for its high protein and lipid content. It has been cultivated for varying purposes during different periods of history in different parts of the world. Its earlier uses have varied from green manure, to a forage and N₂-fixing crop due to its ability to fix substantial quantities of atmospheric N in association with nodule-forming bacteria (*Bradyrhizobium*) (Singh and Shivakumar, 2010). Besides its stated purpose as an oilseed crop, soybean has several significant beneficial features. Its role in improving soil properties through its deep and proliferated tap root system, residue incorporation by way of shedding leaves as well as green manure crop, soil and moisture conservation due to its thick and dense foliage, contribution to soil N enrichment through BNF and improvement in the soil biological health have been recognized from the beginning (Singh and Shivakumar, 2010). Because of its potential for large-scale production, soybean has excelled in the world agricultural economy as a major oilseed crop. At present, soybeans are

grown primarily for oil extraction and for use as a high protein meal for animal feed (Singh and Shivakumar, 2010) because it constitutes approximately 40% of protein and 20% of oil (Li-Juan and Ru-Zhen, 2010).

Soybean production in Ethiopia was 31,854.20 ha from which 63653.1 tons produced with productivity 1.998 t ha⁻¹ and in Oromia region 14,117.85 ha was cultivated with 251, 84.05 t production and a productivity of 1.784 t ha⁻¹ (CSA, 2013). The crop has good potential in the study area and it is being widely used as supplementary meals, as bread combined with maize flour, wot with peas, teff (injera) and milk for babies as well as for adults. The crop has great potential for Ethiopia as it has been dully recognized by many researcher and research organizations for its economic importance (IAR, 1982) and its domestic demand for various uses. Indigenous food processing industries using locally produced soybeans are highly expected to satisfy the vast growing interest of soybean based food stuffs. In Ethiopia, particularly in the capital city, Addis Ababa, Faffa Food Share Company, East African Flour Factory, and Health Care Food Manufacturing Private Limited Company are using local and imported soybeans mainly in the preparation of enriched food products for children and adults. This indicates that the local demand is increasing steadily. Inadequacy of appropriate technologies including varieties, plant population, planting pattern and fertilizer recommendation are some of the cultural practices that are responsible for low yield in most soybean growing areas of Ethiopia (ARC, 2004).

2.3. Soybean Production Requirement

2.3.1. Soil requirements

Soybean is well adapted to a variety of soils and soil conditions. However, it does well on fertile workable, loose, well-drained loam soil, which will encourage air movement to roots and N for effective N₂-fixation. It was reported by Njeze (1993) that soybean can also do well in fertile sandy soils with pH within the range of 5.5 and 7.0, and that the plant can tolerate acidity better than other legumes but does not grow well in waterlogged, saline and alkaline conditions. Optimum soil pH range of 5.5 – 7.0 enhances nutrient availability, such as N and P, breakdown of residues and symbiotic N₂-fixation by microbes (Ferguson *et al.*, 2003). Rienke and Joke (2005) reported high yield in loamy textured soil and added that if the seeds are able to germinate they do better in clayey soils.

2.3.2. Temperature and photoperiod

Soybean is a legume species that grows well in the tropical, subtropical and temperate climates (IITA, 2007). Plant breeders have argued that within the soybean species, there are varieties that react differently to photoperiod, and classified them as long day, short day, and day neutral plants (Borget, 1992). Rienke and Joke (2005), described soybean as being typically a short-day plant, physiologically adapted to temperate climatic conditions. However, some have been adapted to the hot, humid, tropical climate. In the tropics, the growth duration of adapted genotypes is commonly 90 - 110 days, and up to 140 days for the late maturing ones (Osafu, 1997). The relatively short growth duration is primarily due to sensitivity to the day length. This affects the extent of vegetative growth, flower induction,

production of viable pollen, length of flowering, pod filling, and maturity characteristics (Norman *et al.*, 1995).

Most legumes require an optimum temperature of between 17.5°C and 27.5°C for development (Ngeze, 1993). For soybean, the minimum temperature at which it develops is 10°C, the optimum being 22°C and the maximum about 40°C. The seeds germinate well at temperatures between 15°C and 40°C, but the optimum is about 30°C (Rienke and Joke, 2005). Addo-Quaye *et al.* (1993) have suggested the optimum temperature for growth as between 23 - 25°C.

2.3.3. Moisture requirements

Soybean requires optimum moisture for seeds to germinate and grow well. The optimum rainfall amount is between 350 and 750mm, well distributed throughout the growth cycle (Ngeze, 1993). Rienke and Joke, (2005) and Addo-Quaye *et al.* (1993) have described two periods as being critical for soybean moisture requirement; from sowing to germination and flowering, and pod filling periods. During seed germination, the soil needs to be between 50% and 85% saturated with water, as the seed absorbs 50% of its weight in water before it can germinate. The amount of water needs increases, and peaks up at the vegetative stage, and then decreases to reproductive maturity.

According to Bohnert *et al.* (1995), there are two major roles of water in plants, as a solvent and transport medium of plant nutrients, and as an electron donor in the photosynthetic reaction processes. Troedson *et al.* (1985) reported that, soybean is quite susceptible to water stress, and usually respond to frequent watering by substantially increasing vegetative growth and yield. Jones and Jones (1989) defined water stress as the lack of the amount of soil water

needed for plant growth and development, and which in certain cells of the plant may affect various metabolic processes. Direct impacts of drought stress to the physiological development of soybean depend on its water use-efficiency (Earl, 2002). According to Passioura (1997), grain yield is a function of the amount of water transpired, water use-efficiency and harvest index. Soybean, as a C₃ plant, is less efficient in water use due to high evapotranspiration and low photosynthetic rates. Pandey *et al.* (1984) found that, increasing drought stress progressively reduced leaf area, leaf area duration, crop growth rate and shoot dry matter; hence, limits soybean yield. Drought stress, during flowering and early pod formation causes greatest reduction in number of pods and seeds at harvest (Sionit and Kramer, 1977).

2.4. Biological Nitrogen Fixation in Soybean

Biological nitrogen fixation (BNF) involves association of rhizobia and legumes. The *Rhizobium* legume symbiosis plays an important role in agriculture, because it offers the ability to convert atmospheric molecular N₂ into forms useable by the plant (Jensen and Nielsen, 2003). To improve soybean yield, biological N₂-fixation through inoculation with efficient strains of bradyrhizobia has already been tested in several countries (Afzal *et al.*, 2010; Hussain *et al.*, 2011; Tairo and Ndakidemi, 2014) and this has become a usual practice in sustainable soybean production. Improving BNF component has been identified as part of the overall strategy for increasing productivity of soybean. In soybean BNF will be improved by mastering the functioning system of rhizobia-soybean symbiosis (Keyser and Li, 1992). The symbiosis involving soybean and *Bradyrhizobium* is a well-organized system, beginning at root surface and resulting in N₂-fixing nodules. The host plant provides carbon substrates as

a source of energy to the bacteria, and the bacteria reduces atmospheric N_2 to NH_3 which is exported to plant tissues for eventual protein synthesis (Keyser and Li, 1992).

The process of N_2 -fixation requires the presence of the right species of the N_2 -fixing bacteria in the soil, and they are often attracted to the roots by chemical signals from the legume 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. 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 and Jutiz, 2012). The amount of N that a plant can fix depends on the variety, the effectiveness of rhizobia bacteria, the soil and the climatic conditions. Due to rhizobial inoculation, the higher amount of net N can be added to soil system from the inoculated legumes (Haque and Jutiz, 2012).

2.5. Function of Phosphorous Nutrition in Soybean

Phosphorus (P), classified as a macronutrient, is a component of certain enzymes and proteins; adenosine diammonium phosphate (ADP) and adenosine triphosphate (ATP), ribonucleic acids (RNA), deoxyribonucleic acids (DNA), and phytin. ADP and ATP are essential in energy storage and transfer reactions (FAO, 2008). They provide the energy required by all biological processes. Seeds are high in P which plays an important part in their development. P plays an important role in soybean for root health, early growth of canopy and the ability of plants to better tolerate soil borne diseases (Singh *et al.*, (2009). Moreover, P is a component

of the complex nucleic acid structure of plants, which regulates protein synthesis. It is, therefore, important in cell division and development of new tissue. Apart from growth, Ganga-Suresh *et al.* (2010) noted that, P is a crucial element in legume crop production which plays an important role for many characteristics such as sugar and starch utilization, photosynthesis, cell division and organization and nodule formation. Malik *et al.* (2006) stated that P is also associated with complex energy transformations in the plant, early root formation and growth, greater flowering, seed production, grain quality, better growth in cold temperatures, water use-efficiency, early maturation of fruit and grain. Sulieman and Tran (2015) reveals the fact that phosphorus is essential in most metabolic processes that happen above the ground including the processes of energy generation, nucleic acid synthesis, photosynthesis, respiration, glycolysis, membrane synthesis and integrity, enzymatic activation or inactivation, redox reactions, signaling and carbohydrate metabolism. Consequently, inadequate phosphorus in soil gravely affects the growth and developments of plants (Vance *et al.*, 2003).

2.6. Absorption of Phosphorous Nutrition by Soybean

Phosphorous is a highly reactive element, and as such, does not exist in the elemental form in the soil. The majority of P in most soil is in essentially insoluble forms, and unavailable to plants. In fertile soil a significant portion of the total P is in moderately soluble forms, which act as a "ready reserve" to replenish the pool of soluble P as it is depleted by and other organisms (Mengel and Kirkiby, 1999). Soybean roots take up P mostly in the ionic form of either H_2PO_4^- or HPO_4^{2-} (orthophosphate). The ionic form that is predominantly absorbed depends on soil pH. H_2PO_4^- is more readily absorbed in low pH soils whereas HPO_4^{2-} is

preferentially absorbed in high pH soils (Mamo *et al.*, 2002). At a soil pH of 7.0 there is approximately equal amounts of the two phosphorus forms and as the soil pH increases above pH 7.0, the secondary orthophosphate ion becomes the dominant form of available phosphorus.

Phosphorus is a limiting factor to plant growth and productivity on 40% of the world's arable soil (Mengel and Kirkiby, 1999). Phosphorous uptake in plants is often constrained by the very low solubility of phosphorous in the soil. Phosphorous forms insoluble complexes with cations and is incorporated into organic matter due to microbial activity (Haynes and Naidu, 2006).

2.7. Source and Forms of Phosphorus Nutrition

Bone meal, fish bone meal and soy husks and rock phosphate are good source of P. Similarly, compost, composted yard waste and manures generally provided all P normally required by most plants in most soils (FAO, 2008). Some food sources have pretty high levels of P naturally - banana peels, crab shells, shrimp peelings, most grains and nuts - and these should all be added to compost when available. Phosphorus loading contributed by runoff from pastures and croplands is largest source of nonpoint P on a statewide basis. Mamo *et al.* (2002) stated that approximately 30% of the P load to Minnesota waters comes from point sources such as municipal and industrial wastewater treatment facilities. The magnitude of various sources of P varies greatly throughout the state due to the diverse nature.

Phosphorus in water exists in two main forms: dissolved (soluble) and particulate (attached to or a component of particulate matter) (Tisdale *et al.*, 1995). Ortho Phosphate is the primary

dissolved form of P and is readily available to algae and aquatic plants. Most of P discharged by waste water treatment facilities is in dissolved form.

Particulate phosphorus can change from one form to another (called cycling) in response to a variety of environmental conditions and portion of particulate phosphorus is contained in organic matter such as algae, plant and animal tissue, waste solids, or other organic matter (FAO, 2008). Microbial decomposition of organic compounds can convert organic particulate P to dissolved P. Some of the P in soil mineral particles can also be converted to dissolve P both in the water column and during chemical and physical changes in bottom sediment. Only the most tightly bound forms of particulate phosphorus such as aluminum bound phosphorus are not generally available for algal growth (Brady and Weil, 2002).

2.8. Effect of *Bradyrhizobium* Inoculation on Growth, Symbiotic and Yield components of Soybean Varieties

Soybean varieties, regardless of rhizobial strain inoculation, did not vary significantly in relation to nodulation rating. Nodulation rating was not significantly affected by the interaction effect of soybean variety and rhizobial strain (Solomon *et al.*, 2012). However, (Danso *et al.*, 1987) reported varietal differences in terms of nodule numbers due to inoculation of different rhizobial strains. Similarly, Solomon *et al.* (2012) reported that the interaction between soybean varieties and rhizobial strains was found significant in relation to number of nodules.

Nodule dry weight was significantly affected by cowpea variety and Bradyrhizobia inoculation (Tarekegn *et al.*, 2017). This is in agreement with the report of (Lindermann and

Ham, 1979) who observed that nodule dry weight was significantly dependent on *Bradyrhizobium japonicum* strain and soybean variety interaction. Interaction of variety and strain resulted in a non-significant influence on the number of pods plant⁻¹ (Solomon *et al.*, 2012). Number of seeds pod⁻¹ was markedly affected by variety, *Bradyrhizobium* inoculation and their interaction (Muhammad, 2002). Tarekegn *et al.* (2017) was also reported similar results, number of nodules plant⁻¹, number of seeds pod⁻¹ and hundred seed weight were significantly affected by the interaction effect of *Bradyrhizobium* inoculation and variety.

The symbiotic relationship between the soybean root and rhizobial root colonies and subsequent symbiotic N₂-fixation is one of the most important physiological processes which occurs in the growth and development of the soybean plant. Soybean seed inoculation by *Bradyrhizobium japonicum* bacteria has long been established as an important practice in improving soybean yield on fields that have not seen soybean production in recent years. Yield increases of 40% have been observed in soybean inoculated by *Bradyrhizobium japonicum* compared to non-inoculated in fields new to soybean production. However, with cost of rhizobial inoculation quite affordable, usually around \$3 per acre, many farmers have adopted the practice of inoculating their soybean seed each year, to achieve greater root colonization than that provided by the indigenous rhizobial population, potentially resulting in increased nitrogen fixation and yields. Such seed inoculations promote root nodulation around the primary root near the location where the inoculum was placed on the seed, as *Bradyrhizobium japonicum* is relatively immobile (Ciafardini, 1987).

2.9. Effects of P on Growth Performance and Yield of Soybean

The promoting effect of P on soybean growth might be due to the fact that P is an essential component of certain enzymes and proteins; adenosine diammonium phosphate (ADP) and adenosine triphosphate (ATP), ribonucleic acids (RNA), deoxyribonucleic acids (DNA), phytin and some amino acids and absorbed phosphorus helped a direct stimulation of cellular activity in roots and leave (Malik *et al.*, 2006). On the other hand Kakar (2008) stated that useful for the process of cell division and meristemetic growth and the net assimilation rate of phosphorus fed soybean were accelerated by their increased content and the absorbed phosphorus helped the formation of food reserves due to higher photosynthetic activity. On soils containing sub optimum levels of available phosphorus, soybean crops show economic responses to the application of phosphate nutrition. Asaminew (2007) reported that application of P increased the total yield and yield components of soybean like number of seed pod⁻¹, fresh weight, dry weight, and number of pod plant⁻¹. The effect of P application in increasing soybean yield and its yield components could be explained through its importance for crop (Abdel, 2008). However, according to Singh (2009) the excess application of phosphorus fertilizer had a major effect on the production and productivity of soybean crop, hence increased burning of vegetative parts, yield and its yield components also reduced.

2.10. Effect of *Bradyrhizobium* Inoculation and P Application on Nodulation and Yield of Soybean

Phosphorus (P) is the 2nd most plant growth limiting nutrient next to N despite being abundant in soils both as inorganic and organic forms. Many soils throughout the world are P deficient because low free P availability (Gyaneshwar *et al.*, 2002). Its deficiency has also been shown

to be an important fertility problem limiting legume production in the tropical Africa and reduces nodulation, N₂-fixation and plant growth (Crews, 1993). Similar to the other agricultural soils of the tropics, Ethiopian soils also are generally low in P (Beyene, 1982) which necessitate other alternate P source; as only 15 - 30% of applied P may be taken up by plants in the year of fertilization (Syers *et al.*, 2008). It is well known that symbiotic N₂-fixation is a high P demanding process; hence nodule formation and N₂-fixation are generally limited by low P availability which adversely affects nodule number and mass, as well as nitrogenase activity (Schulze *et al.*, 2006). Its application also plays a vital role in increasing legume yield through its effect on the plant and also on the fixation process by *Rhizobium* (Sara *et al.*, 2013). On the other hand, P deficiency reduces N₂-fixation due to decreased nodule formation and reduced nodule sizes and finally affecting the yield and grain quality and quantity (Sadeghipour and Abbasi, 2012). The reason for this reduction has been indicated by the fact that plants dependent on symbiotic N₂-fixation have high ATP requirements for nodule development and function (Ribet and Drevon, 1996) and need additional P for signal transduction and membrane biosynthesis. The combined application of P and inoculation with effective rhizobia had quite prominent effects on nodulation, growth and yield parameters (Kumaga and Ofori, 2004). Ndakidemi *et al.* (2006) reported that the combined application of bacterial inoculants and phosphorus (P) fertilizer to soybean and common bean significantly increased biomass production and grain yield compared with the single use of N and P or rhizobial strains alone.

The higher nodulation due to inoculation resulted in higher N₂-fixation by *Rhizobium* and eventually the number of pods plant⁻¹ which bring about higher grain yields as a whole Singh *et al.*, (2011). In other studies, Ibrahim *et al.* (2011) reported increase in yield and yield of

legumes by inoculating the seeds with specific strain of rhizobia. Solomon *et al.* (2012) has reported that *Bradyrhizobium* is increasing the number of pods plants⁻¹ and increase the number of seeds pod⁻¹. Inoculation with *Bradyrhizobium japonicum* increased soybean yield (Mary *et al.*, 2017). Furthermore, Mohamed and Hassan, (2015) reported that inoculated plants produced higher nodule dry weight, grain yield, number of pods, seed number than the un-inoculated plants.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

A field experiment was conducted during the 2020 belg cropping season at Alage Agricultural Technical and Vocational Educational Training College. The college is located 217 km south of Addis Ababa city and 32 km west of Bulbula town in the vicinity of Abidjata and Shalla lakes. It is situated between 7° 65' N latitude and 38° 56' E longitudes and at an altitude of 1600 meters above sea level in the agro-ecology of the dry plateau of the southern part of the Ethiopian rift valley system. The area is characterized by a bimodal rainfall pattern, where a short rainy season occurs during the months of March and April and the main rain starts in June and extends to September. The high amount of rainfall is in the month of July and August. While the mean annual rainfall is 800mm, the annual mean minimum and maximum temperatures are 11°C and 29°C, respectively. The soil textures of the Alage area range from sandy loam to sandy clay loam with some clay loam and few clay soils (Eylacfhew, 2004).

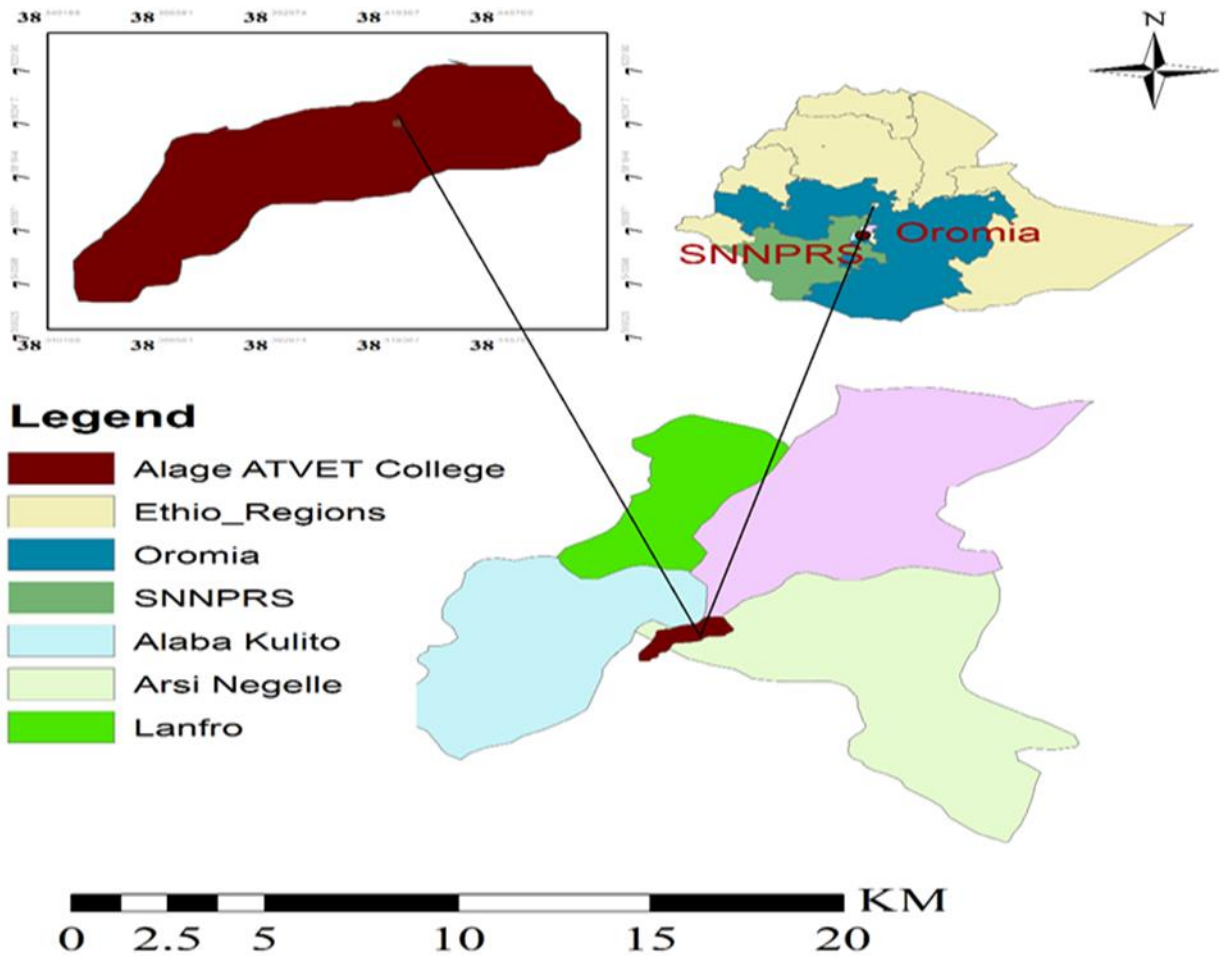


Figure 1: Geographical location of study area

Source: Google map from internet

3.2. Source of Planting Material, and Experimental Treatments

The two soybean varieties were used for the study, namely Afgat (TGX-1892-10F) and Nova were obtained from Hawassa Agricultural Research Center, Sidama Regional State, Ethiopia. The varieties were selected based on their high grain yield, early maturity, and well adapted to the experimental site (ASHC, 2014). Their maturity period varies from short (Nova) to

medium (Afgat) and recommended for short rainfall areas. The Triple Super Phosphate (TSP) fertilizer was obtained from Holeta Agricultural Research Centre, while *Bradyrhizobium japonicum* strains TAL 379 was obtained from Menagesha Biotech Industry P.L.C. (MBI), Addis Abeba, Ethiopia which is already known for its nodulation and agronomic performance for the selected agro-ecology (EIAR, 2003).

3.3. Treatments and Experimental Design

The treatment combinations consisted of four P- levels (0, 10, 20, and 30 kg ha⁻¹) in the form of TSP, two levels of *Bradyrhizobium japonicum* strains TAL 379 (un-inoculated and inoculated with strain TAL 379) and two soybean varieties namely Afgat (TGX-1892-10F) and Nova. Thus, the experiments consisted of 16 treatments replicated three times with a total of 48 plots. The size of each experimental plot was 2m x 2.8m (5.6m²) and spaces between plants, rows, plots and blocks were 0.1m, 0.4m, 0.8m and 1.5m, respectively. The experiment was laid out in Randomized Complete Block Design (RCBD) in a factorial arrangement. The total experimental area was 12m x 58.4m (700.8m²). There were 7 rows per plot and data were collected from the five central rows considering border effects.

3.4. Seeds Inoculation

Seeds were inoculated with lignite-based inoculants of *Bradyrhizobium* strain TAL 379 at the rate of 10g kg⁻¹ of seeds (Rice *et al.*, 2001). In order to ensure the inoculums sticks to the seed, the required amount of inoculant was suspended in 1:1 ratio in 10% sucrose solution and the dry seeds were thoroughly mixed with the thick slurry of sugar solution. The inoculation was done just before planting under shade to maintain the viability of the bacterial cells. Seeds

were allowed to air dry for 5 – 15 minutes before planting to avoid fungal growth (EIAR, 2003).

3.5. Method of Planting and Cultural Practices

The experimental field was prepared in January 2020 using a tractor and the plots were leveled manually. Soybean varieties were sown on March 10, 2020, on the prepared land that was uniformly applied supplementary irrigation before three days, and the seeds were planted in the rows at the rate of two seeds per hole. The treatments which received P as TSP were applied fully at the sowing in side banding application method. As a precaution of avoiding cross-contamination, the control treatment was planted first and followed by inoculated treatments (EIAR, 2003). The inoculated seeds of each treatment were sown by taking the maximum care. A ridge was made to prevent the movement of bacteria through water within plots and across blocks. Thinning was done after germination to maintain the intended population density of plants in each plot. The first weeding was done manually two weeks after the seedling emerged and the second weeding after three weeks. To avoid cross-contamination, weeding was done in the un-inoculated plots first and also different persons were assigned for each plot. Furthermore, all other necessary agronomic practices like, supplementary irrigation, cultivation and others were carried out uniformly at plant emergence and mid stage of vegetative growth for all plots.

3.6. Soil Sampling and Analysis

About 1 kg pre-sowing surface soil sample collected from different spots of the experimental field at the depth of 0 - 30 cm using Augur and was 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 sent to Oromia Agricultural Research, Institute of Batu Soil Research Center for the analysis of the selected parameters.

Similarly, a surface soil sample at the same depth was taken after harvest from each plot of the experimental site. The parameters were determined on the soil sample before planting were soil textural class (% sand, % silt, and % clay), soil pH, total N, available P, organic carbon and cation exchangeable capacity (CEC), and similarly after harvesting; soil pH, total N and available P were analyzed following the standard procedures. The soil texture was determined using the modified Bouyoucos hydrometer method (Day, 1965). Soil pH was determined potentiometrically in supernatant of 1:2.5 soils: water ratio using a combine glass electrode pH meter (Chopra, 1976). The total N content of the soil was determined using wet- oxidation procedure of the Kjeldahl method as described by Dewis and Freitas (1975). The available P content of the soils was determined by 0.5 M sodium bicarbonate extraction solution (pH 8.5) according to the procedure of Olsen method (Olsen and Sommers, 1982). The organic carbon content of the soil was determined using wet combustion procedure of Walkley and Black (1954). The CEC was determined using Kjeldhal procedure as described by (Ranist *et al.*, 1999) for planting.

3.7. Data Collected

3.7.1. Phenological parameters

Days to emergence: it was recorded as the number of days from the date of planting to the date when 50% of the seedling in a plot emerged above the ground through visual observation.

Days to flowering: this was determined by counting the number of days from the date of planting to the date on which 50% of the plants per plot had the first open-flower.

Days to physiological maturity: this was recorded as the numbers of days from planting to 90% of the plants in each plot showed matured. This was when the plants indicates yellowing of leaves and pods in each plot.

3.7.2. Nodulation and growth parameters

Number of nodule plant⁻¹: it was taken from five randomly selected plants at mid flowering stages. The plants were uprooted carefully with the bulk of root mass and nodule. The adhering soil particles were removed through washing the root with their nodules gently with water over a metal sieve. The nodules from each plant were then removed separately and spread on the sieve for some minutes until the water was drained from the surface of the nodules. The total numbers of nodules were counted and their average was taken as number of nodule plant⁻¹.

Nodule dry weight (g plant⁻¹): the five randomly selected plants from each plot for nodule count were used to determine nodule dry weight and dried using oven at 70°C for 48 hours and measured using sensitive balance and the average of the five plants were taken as nodule dry weight plant⁻¹.

Shoot dry matter (g plant⁻¹): dry matter of plant was also determined at mid flowering stage of the crop growth from the same plants which were sampled for nodulation. The sampled plants were placed in a labeled perforated paper bags and dried in oven for 48 hours at 70°C to a constant weight as described by Jones (2001) to determine the dry matter yield. The average shoot dry matter of the five plants was recorded as shoot dry matter plant⁻¹.

Plant height (cm): it was measured as the height of five randomly selected plants from the ground level to the apex of each plant at the time of physiological maturity from each plot and the mean was recorded as plant height.

Root dry weight (g plant⁻¹): dry matter of plant was determined at mid flowering stage of the crop from the plants that were used for nodulation. The plant samples were placed in a labeled perforated paper bags and oven dried for 48 hours at 70°C to a constant weight as described by Jones (2001) to determine the root dry matter yield. The average root dry matter of the five plants was recorded as root dry matter plant⁻¹.

3.7.3. Yield and yield components

Number of primary branches plant⁻¹: the numbers of primary branches arising on the main stem of the five randomly selected plants were counted and recorded at the final stages of harvesting. The primary branches plant⁻¹ was worked out and expressed in number.

Number of pod plant⁻¹: it was determined by counting the five randomly taken plants from the net plot area at harvest and the means was recorded as number of pods plant⁻¹.

Number of seeds pod⁻¹: the total numbers of pods per plant from five randomly taken plants were threshed and the seeds were counted and the total numbers of seeds were divided by total number of pods to compute average number of seeds pod⁻¹.

Hundred seed weight (g): it was recorded by weighting hundred randomly taken matured seeds from each plot using a digital balance and the weight was adjusted to 10% seed moisture content.

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

Above ground biological yield (t ha⁻¹): plants from the three central rows were harvested manually at harvesting time. The harvested plants were sun-dried in an open air until constant weight attained and weighed to determine above ground biological yield and the average above ground biological yield was reported in ton ha⁻¹.

Harvest index (%): the harvest index was calculated as the ratio of grain yield to above ground biological yield.

$$\text{Harvest index (\%)} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

3.8. Statistical Data Analysis

The collected data was subjected to analysis of variance (ANOVA) using stat-8 software to analyze the result. The significant level of treatment means were compared using least significant difference (LSD) test at 5% probability level. Correlation analysis was done using Pearson's simple correlation coefficients for the intended parameters.

3.9. Partial Budget (Economic) Analysis

To consolidate the analysis of variance of the agronomic data, economic analysis was done for each treatment. For economic evaluation, cost and return were calculated according the procedure given by CIMMTY (1988). Actual soybean yield was adjusted downward by 10% to reflect the difference between the experimental soybean yield and the soybean yield that farmers would expect to get from the same treatment (CIMMTY, 1988).

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 25.00 Birr based on the market price of soybean at Abba Koricho market near the experimental site. TSP price was 18.50 Birr kg⁻¹, *Bradyrhizobium* strain TAL-379 400.00 Birr kg⁻¹ and the daily laborer expense was 40.00 Birr.

The gross benefit was calculated as 10% adjusted grain yield (kg ha⁻¹). The total variable cost was included the cost of P, *Bradyrhizobium* strains and labor as the sum of all cost that was variable or specific to a treatment against the control. Dominance analysis and marginal rate of return (%) was used to evaluate the economic performance of treatments and net return was calculated by subtracting total variable cost from the gross benefit. The economic analysis was based on the formula developed by (CIMMTY, 1988) and given as follows.

Gross average yield (kg ha⁻¹) (AvY): is an average yield of each treatment

Adjusted yield (AjY): is the average yield which was adjusted downward by a 10%

$$\text{AjY} = \text{AvY} - (\text{AvY} \times 0.1).$$

Gross field benefit (GFB): was computed by multiplying field/farm gate price that farmers receive for the crop when they sale it as adjusted yield.

GFB = AjY x field/farmer gate price of a crop.

Total variable cost (TVC): is the cost of input that were used for the experiment as mean current prices of TSP, strain and labor for application were considered per hectare. The costs of other inputs and production practices such as labor cost for land preparation, planting, weeding, crop protection and harvesting were assumed to remain the same among treatments.

Net benefit (NB): was calculated by subtracting the total costs from gross field benefits for each treatment.

NB = GFB - TVC

Marginal rate of return (%): is percent marginal rate of return was calculated as changes in net benefit (raised benefit) dividing by changes in cost (raised cost).

MRR (%) = (change NB / change TVC) x 100

Where = NB = Change in net benefit that was obtained on experiment

TVC = Change in total cost that was invested on experiment

MRR = Marginal Rate of Return expressed in percent

The minimum acceptable rate of return was set at 100% (CIMMYT, 1988).

4. RESULTS AND DISCUSSION

4.1. Soil Physico-Chemical Properties of the Study Area

The laboratory results of some of the selected pre-planting soil physical and chemical properties are presented in Table 1. The results revealed that the soil is silt clay loam in texture with a proportion of 18.0% sand, 50.5% silt, and 31.5% clay. The soil of the study area was slightly alkaline in reaction with a pH (H₂O 1:2.5) value of 7.82 which is within the range of ideal soil pH for soybean production and rhizobial growth (Graham *et al.*, 2004). The total N, available P, organic carbon (OC) and CEC of the soil before planting were 0.12%, 11.48 mg kg⁻¹, 1.39% and 31.74 cmol (+) kg⁻¹, respectively (Table 1).

The total N content of the soil was within the range of low according to Havlin *et al.* (1999) who classified the range of total N <0.1, 0.1 – 0.15, 0.15 – 0.25 and >0.25% as very low, low, medium and high, respectively. According to Olsen *et al.* (1954), P content (mg kg⁻¹) <5 was very low, 5 – 15 as low, 15 – 25 was medium, and >25 mg kg⁻¹ was high, based on this the study site had low available P in the soil. Landon (1991) classified the soil OC content as 1 – 2, 2 – 4, and 4 – 6% which were rated as low, medium and high, respectively. Thus, the soil at the experimental site had low OC content before planting. The CEC is an important parameter of soil, because it gives an indication of the type of clay mineral present in the soil and its capacity to retain nutrients against leaching (Tadesse, 2011). According to Landon (1991), the soil CEC ranges of 5 – 15, 15 – 25 and 25 – 40 cmol (+) kg⁻¹ were rated as low, medium and high, respectively. Based on these rating the CEC value of (31.74 cmol (+) kg⁻¹) before planting of the study area was within the high range.

Generally, the soil analysis result indicated that the area is nutrient 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, symbiotic, yield and yield components responded to *Bradyrhizobium* inoculation and applied P fertilizer under this research.

Table 1: Physico-chemical properties of the experimental soil before planting

pH (H ₂ O 1:2.5)	Total N (%)	Available P (mg kg ⁻¹)	Organic C (%)	CEC (cmol (+) kg ⁻¹)	Soil textural percentage			Textural class
					Sand	Silt	Clay	
7.82	0.12	11.48	1.39	31.74	18.0	50.5	31.5	Silt clay loam

4.2. Phenological Response of Soybean varieties to *Bradyrhizobium* inoculation and P application

4.2.1. Days to emergence

Analysis of variance indicated that varieties and P application had significantly ($P \leq 0.01$) affected days to 50% emergence. However, the main effect of *Bradyrhizobium* inoculation and their interactions did not affect days to 50% emergence (Appendix Table 1).

Among the varieties, the earlier days to emergence (6.54 days) were recorded from the variety Nova, while the longer days to emergence (7.25 days) were recorded in Afgat (Table 2). The observed differences between the two varieties for days required to emerge might be due to seed size. In fact, the large seed size took longer days than small-sized seed, because small-seeded variety have the ability to germinate and multiply their seed weight more rapidly than

larger sized seed (Kaydar and Yagmur, 2008). Aikins and Afuakwa (2008) showed that uniform and complete emergence of vigorous seedlings positively affect the overall output of an annual crop by allowing the establishment of better canopy structure and providing time and spatial advantages to compete with weeds. This result is in line with the finding of Aid, (2013) who reported that significant difference ($P < 0.001$) between common bean varieties on days to emergence.

Inoculation with *Bradyrhizobium* strain TAL-379 didn't bring significant effect on days to emergence compared to the control (Table 2). This is because N_2 -fixation period only starts two weeks after bacterial infection and has no effect on the germination process (Dupont *et al.*, 2012).

Application of P resulted in differences on time of emergence in soybean plants. Higher P rate (30 kg ha^{-1}) led to earlier seedling emergence followed by 20 and 10 kg P ha^{-1} . The latest seedlings emergence (7.75 days) was observed from the control. However, the application of 10 kg P ha^{-1} was in par with unfertilized control. This result is in line with the finding of Snyder (2000) who indicated that P fertilizer application enhances seed germination, root development, uptake, and transfer of nutrients and increases photosynthesis.

Table 2: Effect of *Bradyrhizobium* inoculation and P application on phenological performance of Soybean varieties at Alage, during 2020 belg cropping season

Treatments	Days to emergence	Days to flowering	Days to maturity
Varieties			
Nova	6.54 ^b	43.08 ^b	90.22 ^b
Afgat	7.25 ^a	49.58 ^a	112.50 ^a
LSD0.05	0.42	1.11	2.10
Inoculation			
Un-inoculated	7.04	45.87	97.52 ^b
TAL-379	6.75	46.79	105.21 ^a
LSD0.05	ns	ns	2.10
P levels (kg ha⁻¹)			
0	7.75 ^a	48.58 ^a	105.23 ^a
10	7.33 ^a	46.92 ^b	102.25 ^b
20	6.58 ^b	45.17 ^c	99.20 ^c
30	5.92 ^c	44.67 ^c	98.77 ^c
LSD0.05	0.59	1.57	2.97
CV (%)	10.52	4.10	3.55

Mean values followed by dissimilar letters in a column are significantly different at * : $P \leq 0.05$;

** : $P \leq 0.01$; *** : $P \leq 0.001$; NS: Non-significant; LSD: Least significant difference and CV:

Coefficient of variation.

4.2.2. Days to flowering

Analysis of variance revealed that the varieties and P rates had highly significant ($P < 0.001$) effect on days to flowering. However, the main effect of *Bradyrhizobium* inoculation, and all the interactions did not show a significant effect on days to flowering (Appendix Table 1).

Variety Afgat took the longer days to flowering (49.58 days) than Nova variety (43.08 days) (Table 2). The difference of 6.5 days for flowering was recorded between the longer and shorter days to flowering, which might be due to inherent genotypic differences. This result indicated that variety Nova produce flowers earlier than the Afgat variety. This result is in agreement with the finding of Aida (2013) who stated significant variation among common bean varieties to reach 50% flowering.

Among the P rates, the earlier flowering (44.67 days) was produced by plants supplied with 30 kg P ha⁻¹, although there was no significant difference between the 30 kg P ha⁻¹ and 20 kg P ha⁻¹. However, the latest days to flowering (48.58 days) was taken for plants grown without P fertilizer (Table 2). The observed differences on days to flowering might be attributed due to the availability of adequate P to increase shoot and root growth and promote early flowering. The current study agreed with the previous work by Brady and Weil (2002) who stated that P is helpful in initiating flowering and hastens the maturity of crops if in a single application without N. The results also supported by the findings of Neenu *et al.* (2014) and Saedullah (2015), who reported that flowering was delayed in control plots as compared to P- fertilized plots. This is in contrary with findings of Tewari and Singh (2000) who reported no significant effects of P application in number of days to 50% flowering on French bean. Likewise, Gifole

et al. (2011) reported that though the effect of P was not significant, P application has slightly reduced the days to 50% flowering on haricot bean as P rate increased.

4.2.3. Days to maturity

The result of analysis of variance on days to maturity revealed that there was a highly significant ($P < 0.001$) difference in the effect of varieties, *Bradyrhizobium* inoculation, P application, and varieties by inoculation interaction (Appendix Table 1).

Regarding the main effect of varieties, the longer days to maturity (112.50 days) was recorded from Afgat, while the shorter days to maturity (90.22 days) was obtained from Nova (Table 2). The observed differences on days to maturity between the two soybean varieties may be attributed to inherent genotypic differences. In agreement with this result, Kilasi (2010) reported that highly significant variation was observed ($P \leq 0.001$) among common bean varieties for day to maturity.

Inoculation with *Bradyrhizobium* strain TAL-379 significantly increased days to maturity compared to the control (Table 2). The longer days to maturity (105.21 days) was recorded in plants inoculated with strain TAL-379, while the shorter days to maturity (97.52 days) was obtained from the control. The delay in maturity of the inoculated soybean might be due to the fact that the N_2 -fixed by inoculation improved the vegetative growth of the crop. In line with this result, Wafaa *et al.* (2002) reported that *Rhizobium japonicum* inoculum enabled the soybean to display better growth and hence increase days to maturity. Also, the current finding was similar with the findings of Iqbal *et al.* (2014) and Wondwosen *et al.* (2016) who reported that extended days to physiological maturity by the applied soil fertility treatments

(inoculation and P application). Conversely, Bejandi *et al.* (2012) reported that inoculation of seeds with *Rhizobium* reduced days to 70% physiological maturity in chickpea as compared to un-inoculated treatment.

The application of P-fertilizer showed significant effect on days to maturity compared to the control (Table 2). Soybean that received 30 kg P ha⁻¹ were the earliest to reach physiological maturity (98.77 days) followed by 20 kg P ha⁻¹ (99.20 days) and 10 kg P ha⁻¹ (102.25 days). The latest physiological maturity (105.23 days) was observed where 0 kg P ha⁻¹ was applied. However, there was no marked difference between the supply of 30 kg P ha⁻¹ and 20 kg P ha⁻¹. Hastening crop maturity due to increasing P supply was also reported by Brady and Weil (2002). This result is in agreement with the study by Gifole *et al.* (2011) who found that P application significantly shortened days to physiological maturity as compared to the control treatment in haricot bean. Similarly, Marschner (2002) also reported that P could reduce the days to physiological maturity by controlling some key enzyme reactions that involve in hastening crop maturity.

Regarding the interaction effect of variety by inoculation, the longest days to maturity was recorded by Afgat variety with *Bradyrhizobium* inoculation. Also, without inoculation Afgat variety resulted in longest days to maturity than the Nova variety. However, the shortest days to maturity was recorded by Nova variety with both inoculated and un-inoculated treatment (Figure. 2). This shows that early-maturing crop varieties (Nova) most probably have shorter periods of vegetative growth than the medium maturing crop varieties (Afgat). This result also indicates, maturity period was more genetically controlled rather than inoculation.

The other reason for the faster senescence of leaves and pods could be due to the mobilization of leaf nitrogen and rubisco to the reproductive organs for development (Pandey *et al.*, 2001).

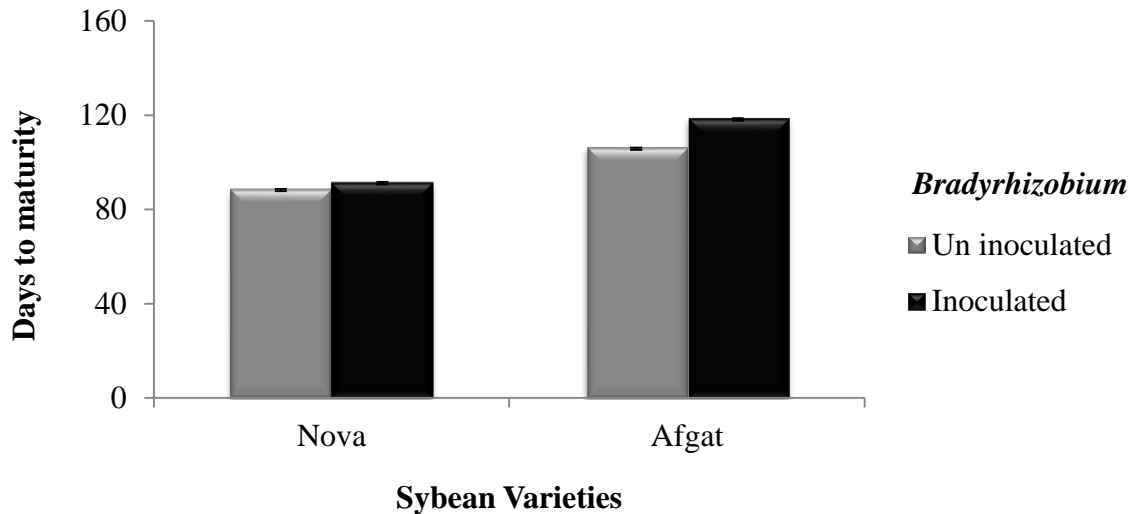


Figure 2: Interaction effects of variety x *Bradyrhizobium* inoculation on days to maturity.

Vertical lines on bars represent standard error of the statistical means.

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

4.3.1. Nodule number plant⁻¹

Number of nodule plant⁻¹ was highly significantly ($P < 0.001$) affected by the main effect of varieties, *Bradyrhizobium* inoculation and P application. Similarly, the interaction effect of inoculation x variety and inoculation x P were highly significantly ($P \leq 0.001$) and significantly ($P \leq 0.05$) affected nodule number plant⁻¹, respectively (Appendix Table 2).

Concerning the main effect of variety, the maximum and minimum number of nodules was recorded from the variety Afgat and Nova, respectively (Table 3). The variation in the number

of nodule plant⁻¹ between the soybean varieties might be related to the genotypic variation of the cultivars in producing nodules. The result is in agreement with the work of Tarekegn *et al.* (2017) who found that the performance of five different varieties of cowpea varied significantly for nodule number. A similar result was also observed by Tesfaye *et al.* (2018); Samago *et al.* (2018) who found a highly significant variation among soybean and common bean varieties for a number of nodule plant⁻¹. However, the current finding contradicts with the results of Solomon *et al.* (2012) who reported that non-significant differences among the soybean varieties on nodule number plant⁻¹.

Inoculation with *Bradyrhizobium* strain TAL-379 had significant effect on nodule number plant⁻¹ compared to the control (Table 3). The higher number of nodule plant⁻¹ (15.80) was recorded from plants inoculated with strain TAL-379, while the lower number of nodule plant⁻¹ (8.98) was recorded from un-inoculated control. The increased nodule number with *Bradyrhizobium* inoculation over the control could be associated with the effectiveness of introduced rhizobia to compete with indigenous bacteria dwelling in the soil. In line with this result, Tesfaye *et al.* (2018) reported that *Bradyrhizobium* inoculation with strain MAR-1495 significantly enhanced nodule number of soybean varieties. These results are also in agreement with the work of Argaw (2012) who reported that the *Bradyrhizobium* inoculation significantly enhanced nodule number of field grown legumes. Similar results were reported by several authors (Neveen, 2008; Argaw, 2014; Yoseph and Worku, 2014) who reported that inoculation significantly increased soybean nodule number over un-inoculated treatments. Lamptey *et al.* (2014) also reported that the improvement of nodulation by *Rhizobium* inoculation resulted in higher N₂-fixation and consequently enhanced vegetative and dry matter yield of soybean compared to un-inoculated. However, this result was in contrary with

the finding of Semira *et al.* (2008) who reported a non-significant effect of *Rhizobium* strain inoculation on number of nodules compared to the control treatment at Kindo Koye, Southern Ethiopia.

On the other hand, the application of 20 kg P ha⁻¹ recorded highest mean nodule number plant⁻¹ followed by 30 kg P ha⁻¹. Whereas, the lowest mean nodule number plant⁻¹ was recorded from the control. The enhanced nodule number due to applied P may be related to the presence of sufficient P to promote early root growth and the formation of lateral fibrous and healthy roots. This is in agreement with reports from earlier workers who showed significant influence of P fertilizer application on soybean root and nodule development (Kumaga and Ofori, 2004; Aduloju *et al.*, 2009; Tahir *et al.*, 2009). Similarly, Yoseph and Worku (2014) reported that application of P resulted in a significant increase in nodule number plant⁻¹. Also, other study reported that application of phosphorus influenced nodulation and N₂-fixation of faba bean (Kiros *et al.*, 2015).

The variety x *Bradyrhizobium* inoculation interaction showed that Afgat variety recorded greater nodule number over Nova regardless of inoculation with strain TAL-379 (Fig. 3A). Generally, inoculating soybean varieties with strains TAL-379 produced greater nodule numbers than the un-inoculated treatments. Such improvement on nodulation due to inoculation also indicates the effectiveness of introduced strains in fixing N than the bacteria dwelling on the soil. In line with this finding, positive interaction effect and response of legume host and *Rhizobium* strain in *Vicia faba* for nodulation (Mytton and Skot, 1993). Similarly, Tahir *et al.* (2009) reported that the highest number of nodules in the inoculation of seeds with *Rhizobium* + 25 kg N ha⁻¹ + 90 kg P ha⁻¹. A similar finding was also reported by

Okereke and Unaegbu (1992); Tesfaye *et al.*, (2018) who found interaction effect of variety and rhizobial strain on nodule number in soybean.

Bradyrhizobium x P interaction resulted in the highest nodule number with a combination of 20 kg P ha⁻¹ with inoculation with *Bradyrhizobium* strain. However, the lowest values were obtained from the combination of 0 kg P ha⁻¹ levels with the un-inoculated seed of soybean (Fig. 3B). P application resulted in increased nodule number due to P enhanced the strain to capable of effective in N₂-fixation through increased nodule number and biomass (Graham *et al.*, 2004). In line with this result, seed inoculation with *Rhizobium* and application of 40 kg P₂O₅ ha⁻¹ in chickpea (*Cicer arietinum*) either alone or in combination enhanced nodulation over un-inoculated control (Chowdhury *et al.*, 1998). This result also in agreement with the finding of Tesfaye *et al.* (2018).

The correlation analysis showed that number of nodule plant⁻¹ was highly significant and positively correlated with days to maturity (r = 0.56^{***}), nodule dry weight (r = 0.91^{***}), root dry weight (r = 0.61^{***}), shoot dry weight (r = 0.88^{***}), number of primary branch plant⁻¹ (r = 0.62^{***}), number of pod plant⁻¹ (r = 0.89^{***}), hundred seed weight (r = 0.57^{***}), grain yield (r = 0.95^{***}), above ground biomass yield (r = 0.85^{***}), harvest index (r = 0.49^{***}) and also significant and positively correlated with days to flowering (r = 0.30^{*}) and number of seed pod⁻¹ (r = 0.32^{*}) (Appendix Table 4). This is because it has a positive relationship with those parameters and contributes a great growth performances and yield parameters for the plants. This has also indicated the positive effect of nodulation on growth and yield of Nova and Afgat through biological N₂-fixation.

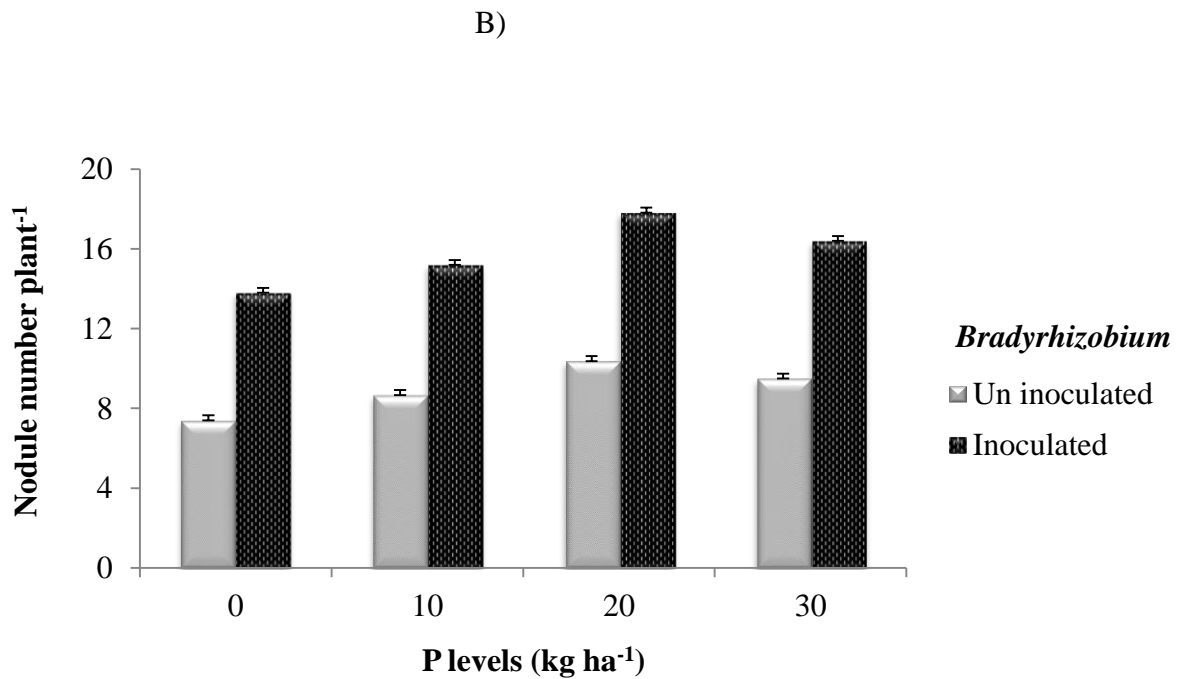
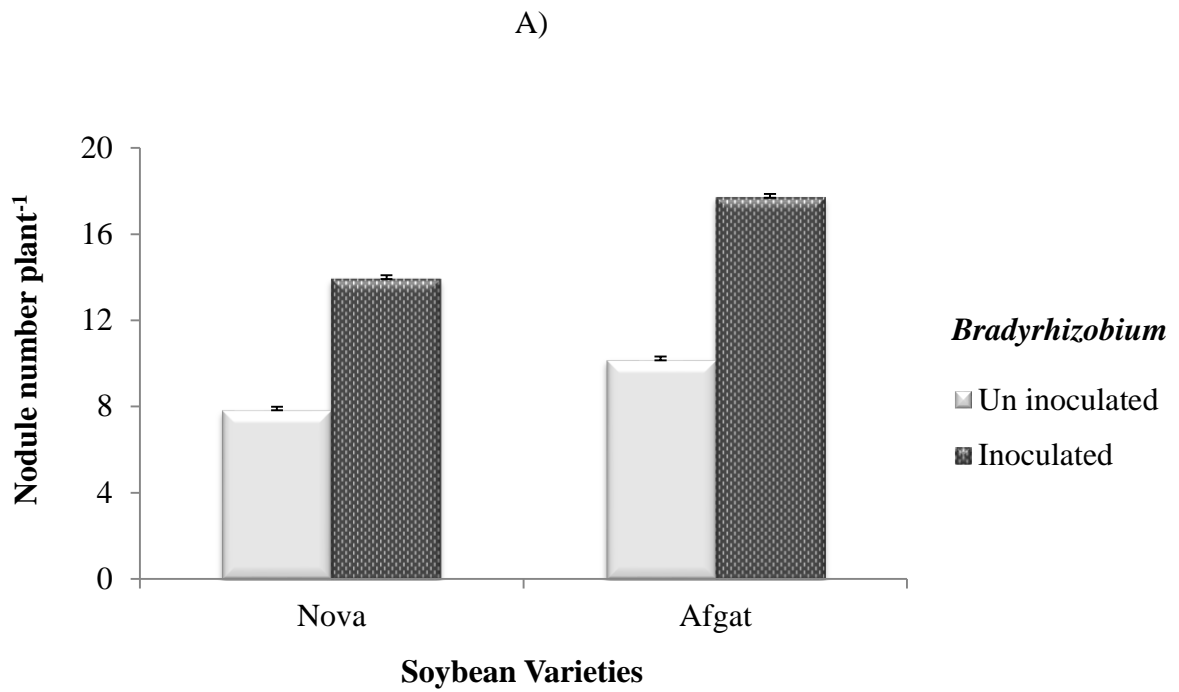


Figure 3: Interaction effects of; A) variety x *Bradyrhizobium*, B) *Bradyrhizobium* x Phosphorus on number of nodule plant⁻¹. Vertical lines on bars represent standard error of the statistical means.

4.3.2. Nodule dry weight

The result of the present study showed that varieties, *Bradyrhizobium* inoculation, and P rates significantly ($P < 0.001$) affected nodule dry weight. The interaction effect of inoculation and P was also found to be significant on the nodule dry weight (Appendix Table 2).

The higher nodule dry weight ($0.17 \text{ g plant}^{-1}$) was recorded from variety Afgat, while the lower ($0.12 \text{ g plant}^{-1}$) nodule dry weight was recorded from variety Nova reflecting inherent genetic differences between the two soybean varieties for nodule dry weight plant^{-1} . These results are consistent with the work of Karadavut and Özdemir (2001) who reported that the genetic differences among soybean varieties in nodule growth and dry weight. Similarly, Tarekegn *et al.* (2017) also reported that the marked differences among the cowpea varieties on nodule dry weight plant^{-1} . However, Gebrekidan (2003) reported non-significant differences in chickpea varieties grown at two locations.

Regarding the main effect of *Bradyrhizobium* strain TAL-379 inoculation, the maximum nodule dry weight ($0.19 \text{ g plant}^{-1}$) was recorded from *Bradyrhizobium* strain TAL-379, while the minimum nodule dry weight ($0.09 \text{ g plant}^{-1}$) was obtained from the control (Table 3). The difference between the nodule dry weight obtained from inoculated and un-inoculated plants might be attributed to the size of the nodules. Inoculated plants formed bigger nodules than un-inoculated due to the effectiveness of the introduced *Bradyrhizobium* strain to initiate nodulation with soybean roots. In the current study, the un-inoculated control resulted in poor nodulation status which was evidenced that the indigenous rhizobia population was ineffective in fixing N. A similar promoting effect of seed inoculation on the dry weight of nodules plant^{-1} have been reported by (Nyoki and Ndakidemi, 2014; Tesfaye *et al.*, 2018).

The application of P at the rate of 20 kg ha⁻¹ to soybean plants resulted in significantly higher nodule dry weight plant⁻¹ followed by the application of 30 kg P ha⁻¹ (0.16 g plant⁻¹), while the lower nodule dry weight plant⁻¹ was obtained from the control. Furthermore, the results revealed that the three P rates (10, 20 and 30 kg ha⁻¹) significantly improved nodule dry weight of the crop over the control (Table 3). The improved nodule dry weight plant⁻¹ due to higher P rates might be attributed due to higher nodule numbers. In line with this finding, Wondwosen *et al.* (2016) reported that greater nodule dry weight plant⁻¹ due to higher P supply. The result also agreed with the finding of Tesfaye *et al.* (2018) on soybean.

As shown in Figure 4, the combined application of *Bradyrhizobium* strain TAL-379 with 20 kg P ha⁻¹ recorded much greater nodule dry weight than the control. However, the values of *Bradyrhizobium* inoculation with 20 kg P ha⁻¹ and 30 kg P ha⁻¹ were statistically at par. The result also accords with the findings of Fatima *et al.* (2007) that using *Bradyrhizobium japonicum* strains with P fertilizer increased the nodule dry weight as compared with the control.

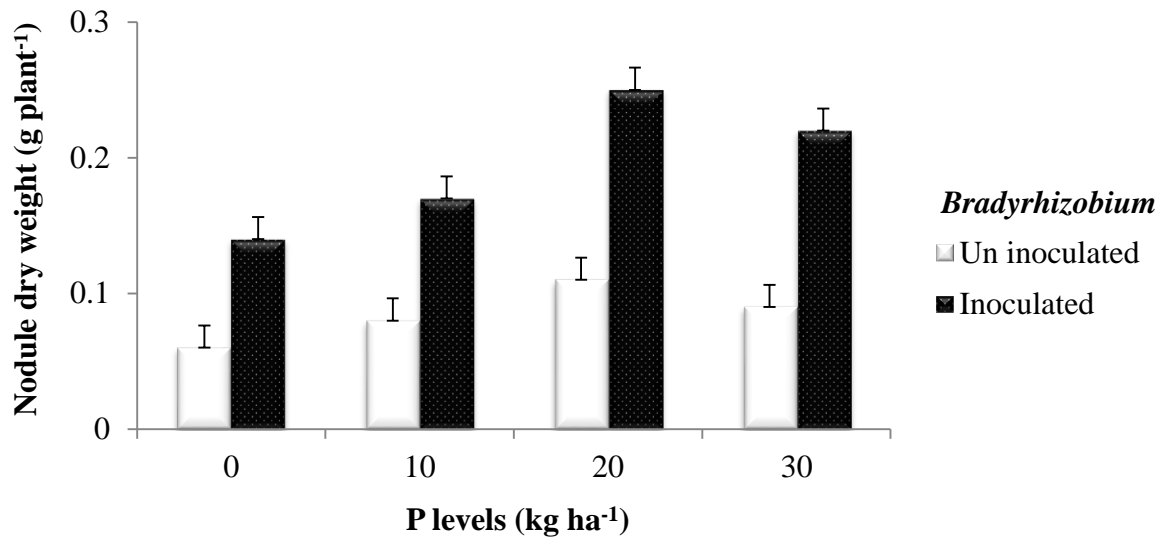


Figure 4: Interaction effects of *Bradyrhizobium* x Phosphorus on nodule dry weight. Vertical lines on bars represent standard error of the statistical means.

4.3.3. Plant height

Analysis of variance revealed a highly significant ($P < 0.001$) difference between the two soybean varieties and P application in plant height. However, the main effect of *Bradyrhizobium* inoculation and all their interactions did not show a significant effect on plant height (Appendix Table 2).

In terms of the main effect of variety, the tallest plants were recorded from Nova variety, while the shortest plant was recorded from Afgat. The observed difference in plant height between soybean varieties could be attributed to inherent genotypic differences (Magani and Kuchinda, 2009).

Soybean plants supplied with 20 kg P ha⁻¹ resulted in the tallest plant height than the other P rates. In addition, plants supplied with 20 and 30 kg P ha⁻¹ also gave a significantly ($P < 0.05$) higher plant height than 10 kg P ha⁻¹ and the control (Table 3). Plant height was declined at the highest rate of P application (30 kg P ha⁻¹). This might be due to the higher dose of P fertilizer tends to form nutrient interaction and may affect the availability of other nutrients that are essential for the growth of soybean. This result was in agreement with the finding of Shahid *et al.* (2009) who reported that P application significantly improved soybean plant height in soils which contain low available P. Correspondingly, Shakori and Sharifi (2016) showed that an increase in plant height of faba bean as a level of P application increased. However, this result was contrary to Abbasi *et al.* (2010) who reported the non-significant response of plant height to P application on soybean where the soil was slightly alkaline in reaction.

Table 3: Nodulation and growth performance of soybean varieties in response to *Bradyrhizobium* inoculation and P application at Alage, during 2020 belg cropping season

Treatments	Nodule number plant ⁻¹	Nodule dry weight (g plant ⁻¹)	Plant height(cm)	Shoot dry weight (g plant ⁻¹)	Root dry weight (g plant ⁻¹)
Varieties					
Nova	10.87 ^b	0.12 ^b	86.72 ^a	10.77 ^b	2.98 ^b
Afgat	13.92 ^a	0.17 ^a	82.69 ^b	12.27 ^a	3.40 ^a
LSD0.05	0.36	0.01	1.18	0.53	0.18
Inoculation					
Un-inoculated	8.98 ^b	0.09 ^b	84.39	9.80 ^b	3.05 ^b
TAL-379	15.80 ^a	0.19 ^a	85.03	13.24 ^a	3.33 ^a
LSD0.05	0.36	0.02	ns	0.53	0.18
P levels (kg ha⁻¹)					
0	10.60 ^d	0.09 ^d	82.46 ^c	9.57 ^d	2.58 ^c
10	11.93 ^c	0.13 ^c	83.28 ^c	11.25 ^c	3.14 ^b
20	14.09 ^a	0.18 ^a	87.53 ^a	13.25 ^a	3.61 ^a
30	12.95 ^b	0.16 ^b	85.57 ^b	12.02 ^b	3.43 ^a
LSD0.05	0.51	0.03	1.67	0.75	0.25
CV (%)	4.98	21.65	2.38	7.92	9.49

Mean values followed by dissimilar letters in a column are significantly different at * : $P \leq 0.05$; ** : $P \leq 0.01$; *** : $P \leq 0.001$; NS: Non-significant; LSD: Least significance difference and CV: Coefficient of variation.

4.3.4. Shoot dry weight

The analysis of variance revealed that shoot dry weight was significantly ($P < 0.001$) affected by soybean varieties, *Bradyrhizobium* inoculation, and P application as well as their two-way interactions (Appendix Table 2).

The highest ($12.27 \text{ g plant}^{-1}$) shoot dry weight was demonstrated from Afgat variety, but the lowest ($10.77 \text{ g plant}^{-1}$) shoot dry weight was recorded from Nova variety (Table 3). The observed differences might be a genetic difference in the ability of N_2 -fixing and biomass accumulation between the varieties. In line with this result, Singh *et al.* (2011) reported that some varieties have the capacity 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 TAL-379 gave significantly higher shoot dry weight plant^{-1} over the control (Table 3). The observed differences on soybean in shoot dry weight by *Bradyrhizobium* inoculation seem to be to the supply of N to the crop through symbiotic N_2 -fixation and applied N (Togay *et al.*, 2008). A similar result was reported by Salih (2002) and Zuffo *et al.* (2015) who observed increased shoot dry weight in soybean plants inoculated with *Bradyrhizobium* strains. Yoseph and Shanko (2017) also reported that a significant effect of seed inoculation on shoot dry weight compared to the control treatments.

Application of P-fertilizer at the rate of 20 kg ha^{-1} showed a significant increase in shoot dry weight plant^{-1} which was followed by the P rate of 30 kg ha^{-1} , while the lowest value of this parameter was recorded from the control treatment (Table 3). This is an indication that

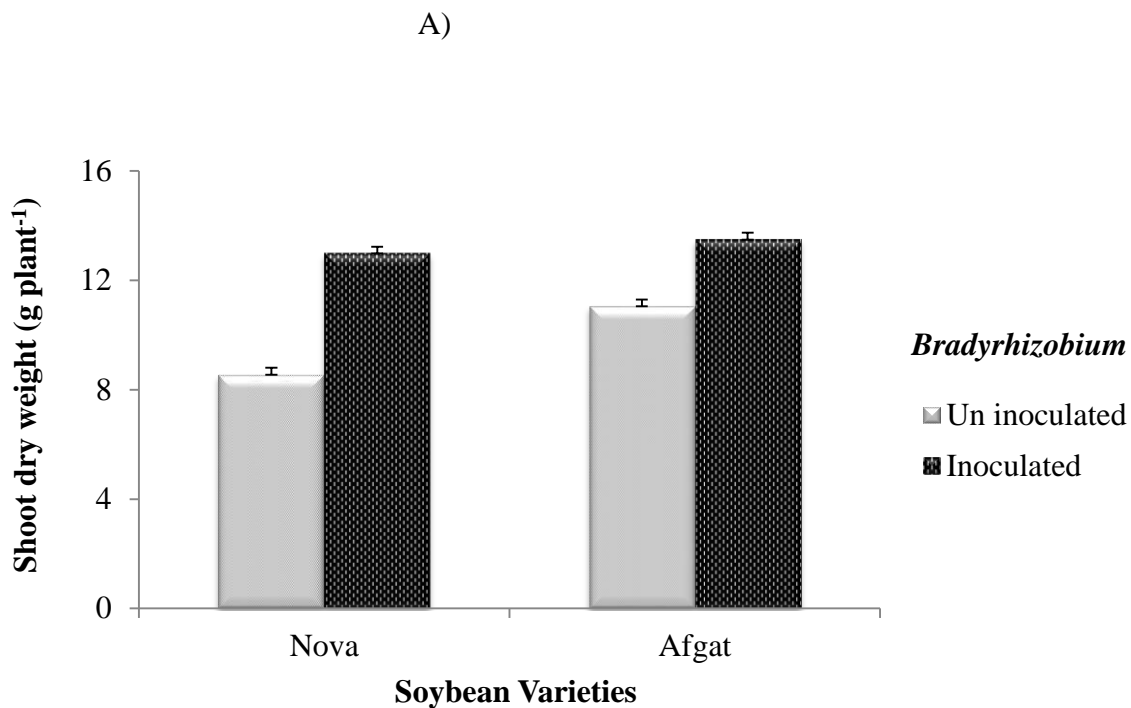
omission of P from soybean nutrition can drastically reduce shoot dry matter yield of soybean (Bekere *et al.*, 2012). The marked increase in shoot dry weight in response to the increased rates of P application could be ascribed to the increased availability of P in the soil for uptake by plant roots, which may have sufficiently improved vegetative growth through increasing cell division and elongation (Sara *et al.*, 2013; Togay *et al.* 2008). Similarly, Tesfaye *et al.* (2018) also reported that the highest shoot dry weight on soybean due to higher P supply than the control.

The results presented in Fig. 5A revealed that the variety Afgat produced significantly higher shoot dry weight than Nova varieties when inoculated with *Bradyrhizobium* strain TAL 379. The observed difference between the two soybean varieties for *Bradyrhizobium* inoculation indicates their differences in forming a symbiosis with the inoculant. This result was contrary to the finding of Solomon *et al.* (2012) who reported that no-significant variety and strain interaction with respect to shoot dry weight of soybean varieties on Nitisols of Bako.

Similarly, the interaction effect of inoculation x P rates showed the higher shoot dry weight when 20 kg P ha⁻¹ combined with *Bradyrhizobium* strain TAL-379, followed by 30 kg P ha⁻¹ over the combination of *Bradyrhizobium* inoculation and application of 10 kg P ha⁻¹ and 0 kg P ha⁻¹ (Fig. 5B). Without inoculation plants supplied with 20 kg P ha⁻¹ resulted in higher shoot dry weight than the other P rates. However, supply of 10 and 30 kg P ha⁻¹ without inoculation were statistically at par (Fig. 5B). In line with this result, Fatima *et al.* (2006) concluded that combined application of P and *Rhizobium* inoculation increased nitrogenase activity, growth, and grain yield as well as improved soil fertility for sustainable agriculture. The variety x P interaction resulted in greater shoot dry weight when the variety Afgat was grown by

supplying 20 and 30 kg P ha⁻¹ compared to Nova varieties with the same level of supplied P, but both Afgat and Nova variety was statistically at par when they are grown without the P-fertilizer (Fig. 5C). This result was in disagreement with the work of Habtamu *et al.* (2019) who reported that P rates with common bean varieties interaction had a significant effect on shoot dry weight.

As it is indicated in Appendix Table 4, the correlation analysis showed that shoot dry weight was very highly significant and positively correlated with nodule number plant⁻¹ ($r = 0.88^{***}$), nodule dry weight ($r = 0.89^{***}$), root dry weight ($r = 0.72^{***}$), number of primary branches plant⁻¹ ($r = 0.46^{***}$), number of pod plant⁻¹ ($r = 0.81^{***}$), hundred seed weight ($r = 0.48^{***}$), grain yield ($r = 0.92^{***}$), above ground biomass yield ($r = 0.83^{***}$) and harvest index ($r = 0.50^{***}$).



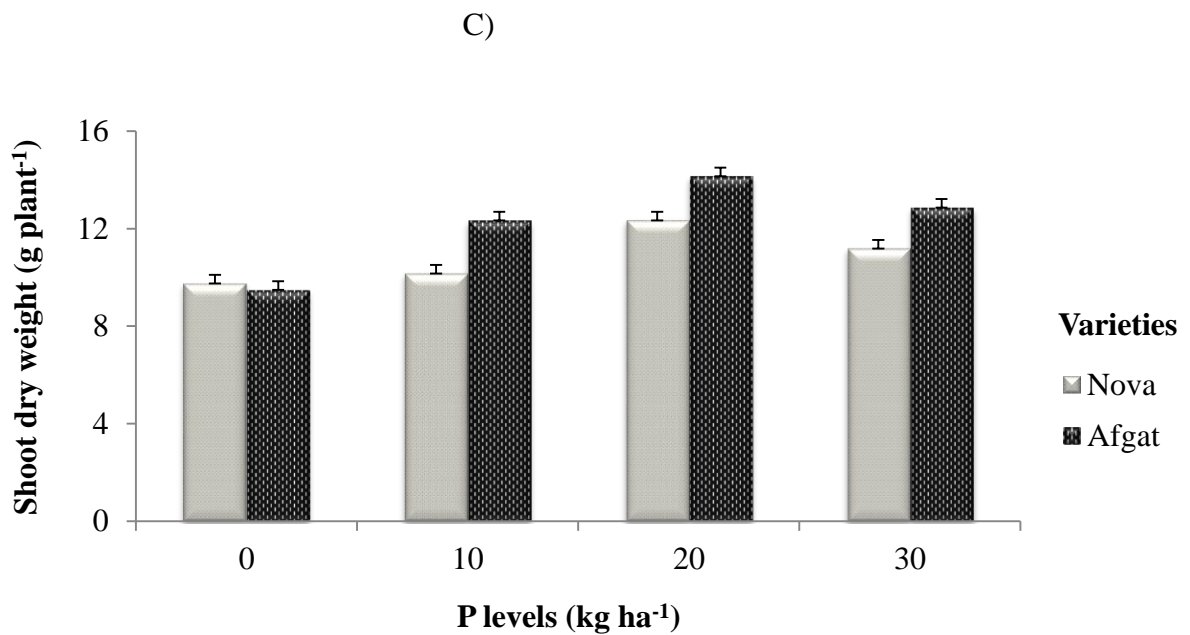
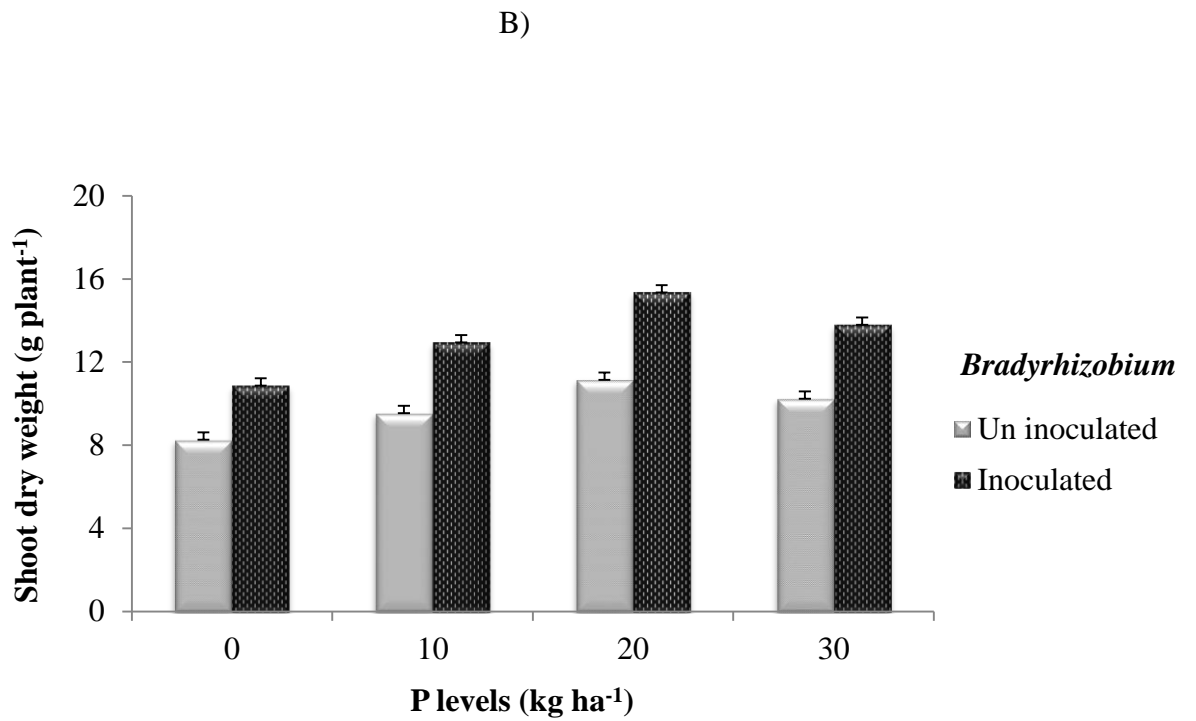


Figure 5: Interaction effects of; A) varieties x *Bradyrhizobium*, B) *Bradyrhizobium* x Phosphorus, C) varieties x Phosphorus on shoot dry weight. Vertical lines on bars represent standard error of the statistical means.

4.3.5. Root dry weight

Root dry weight was significantly affected by varieties, *Bradyrhizobium* inoculation, and P application as well as the inoculation x P interaction (Appendix Table 2).

The higher root dry weight (3.40 g plant⁻¹) was recorded from varieties Afgat, while the lower root dry weight (2.98 g plant⁻¹) was recorded from varieties Nova (Table 3). This reflecting inherent genetic differences between the varieties for root dry weight. This result was consistent with the finding of Mahamood *et al.* (2009) and Mugendi *et al.* (2010) who reported significant differences between soybean varieties and observed significant positive responses to P-application.

On the other hand, higher root dry weight (3.33 g plant⁻¹) was recorded from inoculation with *Bradyrhizobium* strain TAL-379 than the root dry weight (3.05 g plant⁻¹) which was recorded from the control (Table 3). Increased root dry weight might be due to that *Bradyrhizobium* inoculation gave N nutrition to soybean crop for root growth through N₂-fixation. In line with this result, Egamberdiyeva *et al.* (2004a) reported that *Rhizobium* inoculation increased soybean shoot and root dry weights by 7 – 23% and 57 – 78%, respectively. Similarly, Erman *et al.* (2011) reported that chickpea inoculation with *Mesorhizobium* strains gave higher root dry weight compared to the control.

Root dry weight of 20 kg P ha⁻¹ supplied soybean was not significantly different from those of 30 kg P ha⁻¹ fertilized soybeans. However, both P rates gave a significantly (P < 0.05) higher root dry weight than 10 kg P ha⁻¹ and the control (Table 3). The result also showed that even 10 kg P ha⁻¹ significantly increased root dry weight plant⁻¹ over the control. Increased root dry weight due to supplied P might be attributed due to promoting effects of root growth and the

formation of lateral fibrous roots when supplied with sufficient P rates. In line with this result, Gentili (2008) reported that sufficient amount of P to soybean enhanced the whole plant fresh and dry mass, nodule dry weight and root dry matter. Tahir *et al.* (2009) also reported that application of P alone significantly increased root length and root dry weight by 33 and 64% over the control.

The interaction effects of *Bradyrhizobium* inoculation x P supply were found to be significant ($P < 0.05$) on root dry weight. As shown in Fig. 6, the interaction result of *Bradyrhizobium* x P supply revealed that greater root dry weight from *Bradyrhizobium* inoculation and the supply of 20 kg P ha⁻¹ followed by *Bradyrhizobium* inoculation with the supply of P at the levels of 30 kg ha⁻¹ and 10 kg P ha⁻¹. Though, both inoculated and un-inoculated soybean seed were statistically at par on root dry weight with the Control (Fig. 6). Similar to these result, Bekere *et al.* (2012) reported that both inoculation and P fertilizer application significantly influenced root dry matter of soybean.

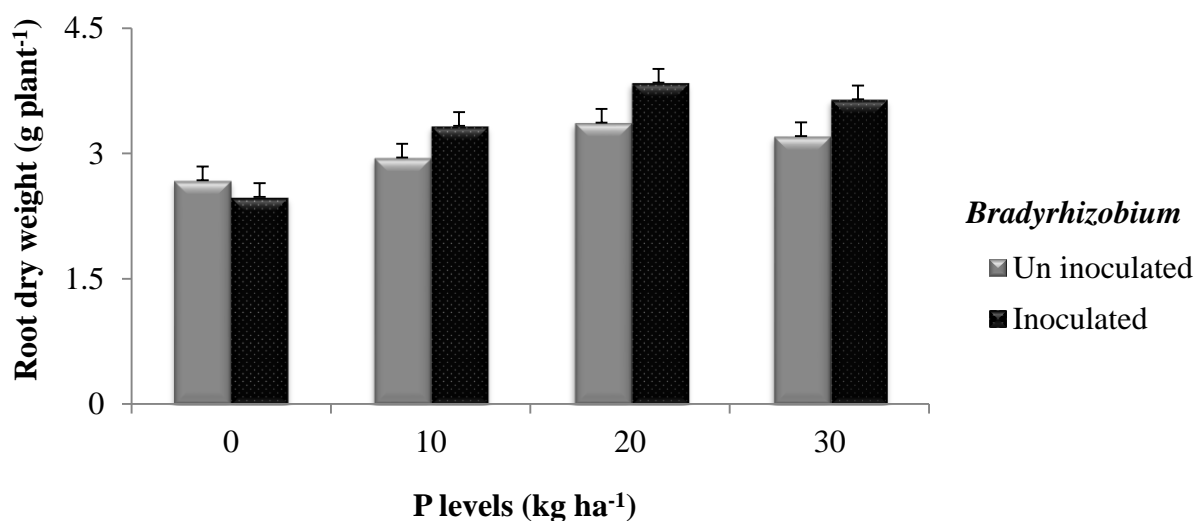


Figure 6: Interaction effects of *Bradyrhizobium* x P rates on root dry weight. Vertical lines on bars represent standard error of the statistical means.

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

4.4.1. Number of primary branches plant⁻¹

Pod bearing branches are considered to be the major contributor to seed yield of legumes. The analysis of variance revealed that soybean varieties and *Bradyrhizobium* strain TAL-379 had significantly ($P < 0.001$) affected the number of primary branches plant⁻¹. However, the main effect of P treatment and all their interaction had no significant effect on the number of primary branches plant⁻¹ (Appendix Table 3).

The maximum primary branches plant⁻¹ (6.78) were recorded from Afgat variety, while the minimum primary branches plant⁻¹ (4.81) were recorded from the Nova variety (Table 4). This observed difference in primary branches production might be attributed due to genetic differences between the varieties. In line with this result, Habtamu *et al.* (2017) stated the differences in number of branches plant⁻¹ among common bean varieties under the field experiment.

Similarly, inoculation with *Bradyrhizobium* strain resulted in marked improvement on number of primary branches plant⁻¹. Moreover, Tairo and Ndakidemi (2013) and Mfilinge *et al.* (2014) reported that *Bradyrhizobium japonicum* inoculation in the field and glasshouse experiments showed significant increase in the number of branches.

4.4.2. Number of pods plant⁻¹

The productive potential of soybean is finally determined by number of pods plant⁻¹ which is a main yield component. Analysis of variance revealed that varieties, *Bradyrhizobium*

inoculation, and application of P had highly significantly ($P < 0.001$) affected the number of pods plant⁻¹. The interaction effects of variety x inoculation, inoculation x P rates as well as variety and P rates were also found to be significant on the number of pods plant⁻¹ (Appendix Table 3).

Of the two varieties the higher number of pods plant⁻¹ (81.09) were recorded from Afgat variety. However, the lower number of pods plant⁻¹ (73.86) were recorded from Nova variety. The observed differences in pod number plant⁻¹ between Afgat and Nova varieties could be attributed due to genotypic differences associated with formation of number of branches and other sink that determines the yield of variety. In line with this result, Muhammad (2002) reported significant variations in the number of pods plant⁻¹ for soybean varieties. Similarly, Tarekegn *et al.* (2017) also reported marked differences among the cowpea varieties on pod numbers plant⁻¹.

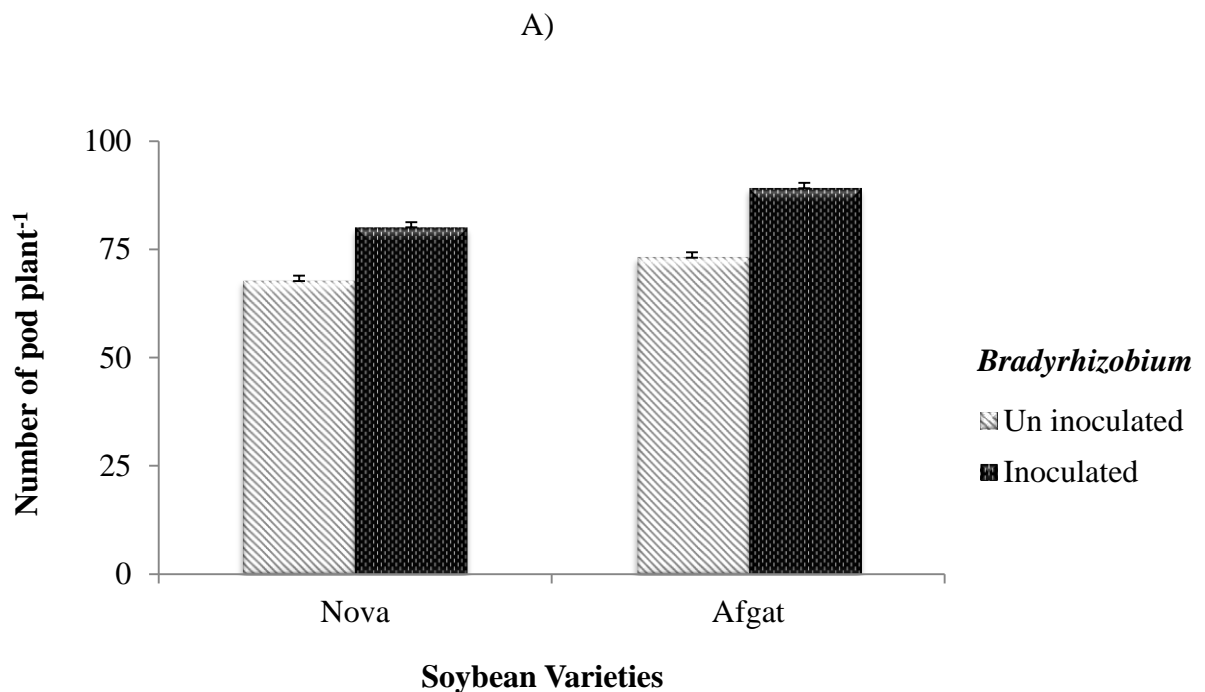
Bradyrhizobium inoculation with strain TAL-379 resulted in marked increase on pod number plant⁻¹ compared to the un-inoculated plants (Table 4). The maximum number of pods plant⁻¹ (84.59) were produced from the inoculated treatment and the minimum pods plant⁻¹ (70.37) were recorded from the un-inoculated treatment. This increased pods plant⁻¹ with *Bradyrhizobium* inoculation could be associated with enhanced growth and higher assimilate accumulation which resulted from better N nourishment due to symbiotic N fixation. This result was in line with the finding of Malik *et al.* (2006) and Dereje (2007) who reported that increased number of pods plant⁻¹ with inoculation in soybean. Similarly, Argaw (2014) also reported similar results in soybean, in that number of pods in soybean increased due to *Bradyrhizobium* inoculation. Positive effect of seed inoculation on number of pods was also

reported by Tahir *et al.* (2009). The authors reported that *Rhizobium* inoculation alone boosted number of pods plant⁻¹ by 85% over un-inoculated treatment. The positive effect of the inoculants might be due to adequate N rendered through N₂-fixation which promoted vegetative growth and plant height and thus improving number of pods plant⁻¹. Contrastingly, Yamur and Engin (2004) reported that inoculation did not affect the number of pod plant⁻¹.

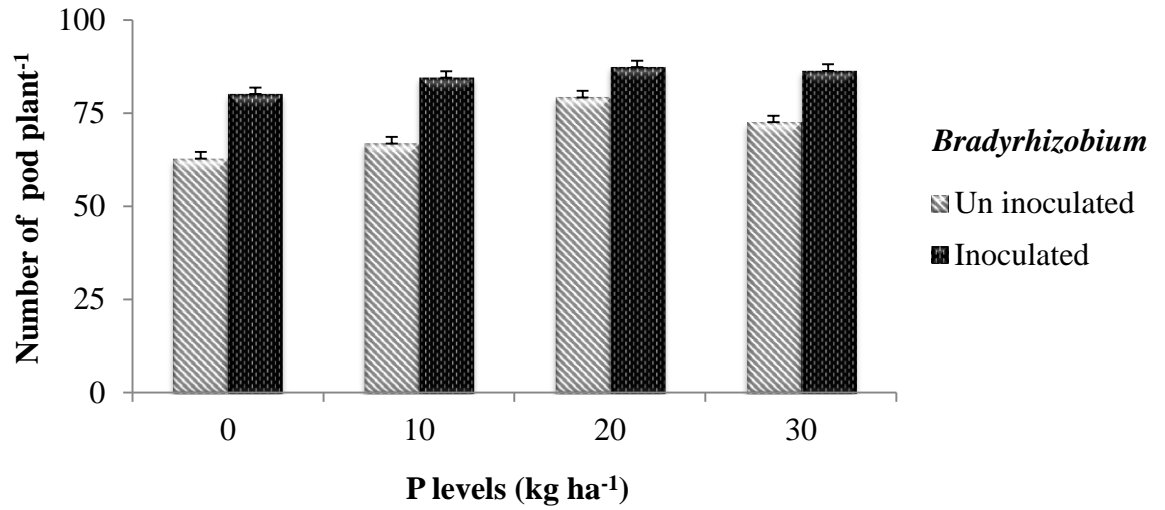
Significantly higher mean pod numbers plant⁻¹ was obtained by the application of 20 kg P ha⁻¹ (83.28) followed by 30 kg P ha⁻¹ (79.47), whereas the lower mean pod number plant⁻¹ (71.46) was obtained from the control (Table 4). The highest number of pods formation from optimum P treated plots might possibly associated with improved reproductive performance of the plants because of enhanced P nutrition. Different authors also reported significant effects of P supplementation on the number of pods plant⁻¹ (Meseret and Amin, 2014; Tessema and Alemayehu 2015; Girma *et al.*, 2017). Furthermore, Abebe and Tolera (2014) and Kiros *et al.* (2015) reported that P application significantly increased number of pods plant⁻¹ on faba bean. In contrast to this result, Gidago *et al.* (2012) reported non-significant effects of supplied P on number of pod plant⁻¹.

Regarding the variety x *Bradyrhizobium* inoculation, the highest number of pods plant⁻¹ was obtained by Afgat variety with *Bradyrhizobium* strain TAL-379 compared to the control, while the lowest number of pods plant⁻¹ was recorded from Nova variety with the control (Fig. 7A). Contrastingly, Solomon *et al.* (2012) reported that, interaction of soybean variety and strain resulted in a non-significant influence on the number of pods plant⁻¹. On the other hand, the interaction of *Bradyrhizobium* x P rates revealed that the greater number of pod plant⁻¹ was obtained from the combination of *Bradyrhizobium* inoculation and supply of 20 kg P ha⁻¹

followed by inoculated seed with 30 kg P ha⁻¹ and 10 kg P ha⁻¹ application compared to zero-P. In the respect of inoculated the supply of 10, 20 and 30 kg P ha⁻¹ was statistically at par (Fig. 7B). In soybean production, P and inoculation with the appropriate *Rhizobium* strains have been reported to have quite prominent effects on nodulation, growth and yield parameters (Shahid *et al.*, 2009; Kumaga and Ofori, 2004). Variety x Phosphorus application interaction was significant on soybean number of pod plant⁻¹. The interaction graph revealed much higher number of pods plant⁻¹ in Afgat variety over Nova variety at 20 kg P ha⁻¹, but decreased number of pods plant⁻¹ at zero-P level for both varieties (Fig. 7C).



B)



C)

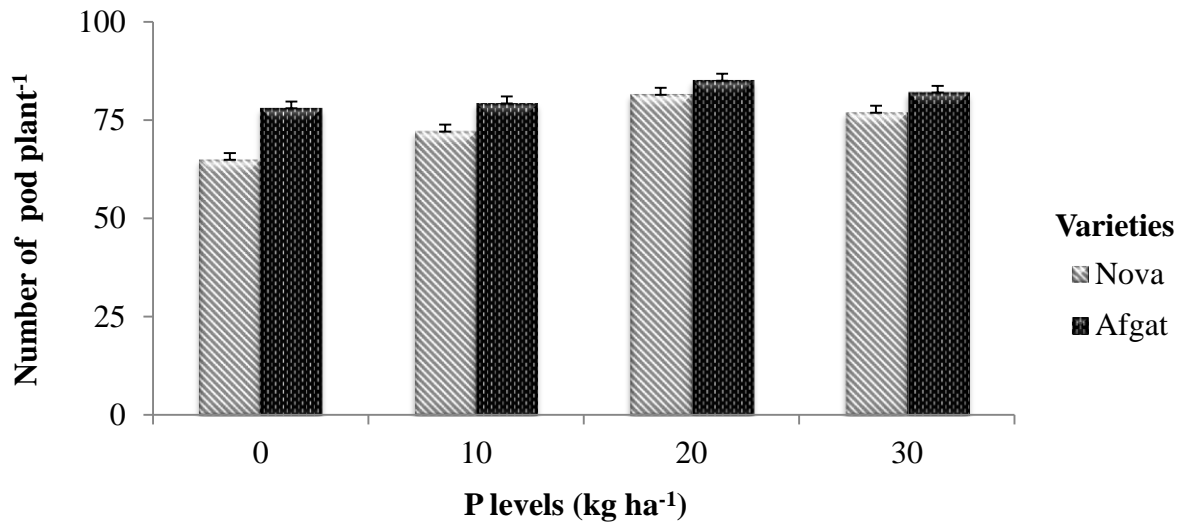


Figure 7: Interaction effects of; A) varieties x *Bradyrhizobium*, B) *Bradyrhizobium* x Phosphorus, C) varieties x Phosphorus on number of pod plant⁻¹. Vertical lines on bars represent standard error of the statistical means.

4.4.3. Number of seed pod⁻¹

The number of seeds pod⁻¹ is perceived as a significant constituent that directly imparts in exploiting potential yield recovery in leguminous crops (Devi *et al.*, 2012). Both varieties and P had shown significant effects ($P < 0.05$) on the number of seed pod⁻¹, while *Bradyrhizobium* inoculation and their interactions were non-significant effect on the number of seed pod⁻¹ (Appendix Table 3).

The higher number of seeds pod⁻¹ was recorded from the Afgat variety (2.92 seeds pod⁻¹); whereas it was lower in the Nova variety (2.79 seeds pod⁻¹) (Table 4). This might be due to inherent genetic difference between the soybean varieties for seed production pod⁻¹. Consistent with this result, Tesfaye *et al.* (2018) observed significant variations in number of seeds pod⁻¹ among soybean genotypes. The report is also in agreement with the findings of Dugje *et al.* (2009) and Singh *et al.* (2011), who reported that the differences among the cowpea varieties due to their genetic makeup.

Significantly highest number of seeds pod⁻¹ was obtained from the application of 20 kg P ha⁻¹ (2.98) followed by 30 kg P ha⁻¹ (2.91), whereas the lowest seeds pod⁻¹ (2.76) was obtained by application of 10 kg P ha⁻¹. However, plants fertilized with 0, 10 and 30 kg P ha⁻¹ were statistically at parity (Table 4). The increase of seeds pod⁻¹ with increasing P fertilizer application up to optimum level might be due to the promoting effects of P fertilizer for nodule formation, protein synthesis, fruiting and seed formation. This result is in line with that of Shahid *et al.* (2009) who reported that application of adequate amount of P resulted in significantly increase yield of soybean plants.

4.4.4. Hundred seed weight

Hundred seeds weight is also an important yield component that reflects the magnitude of seed development which ultimately determines the final yield of a crop. The result of analysis revealed that, there was significant effect on hundred seed weight because of varieties, *Bradyrhizobium* inoculation, P rates and their interaction (Appendix Table 3).

Concerning the main effect of varieties, the heavier seed weight was recorded from variety Afgat (20.78 g) than variety Nova (12.41 g). Increase hundred seed weight in variety Afgat might be due to its inherent larger seed size. Moreover, the observed differences in hundred seed weight between the varieties might be due to the difference in translocation and partitioning efficiency of assimilates from source to sink (El Naim and Jabereldar, 2010). Similar to this result, Solomon *et al.* (2012) and Tessema and Alemayehu (2015) reported a significant differences in hundred seed weight by the main effect of soybean and common bean varieties, respectively.

Bradyrhizobium inoculation with strain TAL-379 significantly increased hundred seed weight (17.52 g) compared to the control (15.67 g) (Table 4). Increased hundred seed weight as a result of *Rhizobium* inoculation could be attributed due to significant contribution of N₂-fixation which supplied extra N for the crop as it is a major constituent of amino acids and many biological compounds that play major roles in photosynthesis which eventually increased seeds weight. Similar findings were reported by Tairo and Ndakidemisi (2013) who stated that *Rhizobium* inoculation significantly increased hundred seeds weight of soybean by 91%. Likewise, Roy *et al.* (1995) and Kazemi *et al.* (2005) also indicated that inoculation of soybean seeds increased hundred seeds weight.

Although increasing P levels from 0 to 20 kg P ha⁻¹ showed unpredictable increase in hundred seeds weight, the highest hundred seeds weight (17.05 g) and the lowest hundred seeds weight (15.83 g) were obtained from 20 and 0 kg P ha⁻¹ application, respectively. However, application of 30 kg P ha⁻¹ was statistically at par with unfertilized control and other P rates. The heavier seed weight due to adequate P fertilization could be increase translocation and partitioning of assimilates from source to grain. These results are in agreement with Badini *et al.* (2015) who reported that application of adequate amount of P resulted in markedly increase seed weight of legumes. In line with this result, Tairo and Ndakidemisi (2013) found a significant increase of hundred seeds weight by 5 – 18% due to P application from 20 to 80 kg P ha⁻¹ over the control treatment. In contrast to the results of this study, Negash *et al.* (2015) reported that the different levels of P rates (46, 69, and 92 kg P₂O₅ ha⁻¹) did not result in a significant difference in hundred seed weight of common bean.

The variety x phosphorus x *Bradyrhizobium* strain TAL-379 interaction was found to be highly significant (P < 0.001) on hundred seed weight of soybean. This implies that soybean varieties responded differently to the combinations of supplied P levels and *Bradyrhizobium* treatments. The greater hundred seed weight was detected in Afgat variety with inoculation and 10 kg P ha⁻¹ levels over Nova varieties (Fig. 8). The seeds inoculated with *Bradyrhizobium* TAL-379 alone show greater hundred seed weight than the control, 10, 20 and 30 kg P ha⁻¹ supplements alone in both varieties (Fig 8). The results also showed the heavier seed weight from both varieties with TAL-379 inoculation alone than the supply of 30, 20 and 10 kg P ha⁻¹ alone (Fig. 8). Hundred seed weight generally increased with bacterial inoculation at all P levels compared to un-inoculated plants (Fig. 8). The increase in seed weight might be due to availability of more resources. Similarly, inoculation x P effect on

thousand-seed weight was reported by Shahid *et al.* (2009). In conformity with this result, Gobeze *et al.* (2015) found that varieties and their interactions with P fertilizer had significant effect on bean thousand seed weight. Tarekegn *et al.* (2017) was also reported similar results, number of nodules plant⁻¹, number of seeds pod⁻¹ and hundred seed weight were significantly affected by the interaction effect of *Bradyrhizobium* inoculation and variety.

The correlation analysis indicated that hundred grain weight was found to be positively and very highly significant correlated with days to flowering ($r = 0.74^{***}$), days to maturity ($r = 0.91^{***}$), number of nodule plant⁻¹ ($r = 0.57^{***}$), nodule dry weight ($r = 0.51^{***}$), shoot dry weight ($r = 0.48^{***}$), root dry weight ($r = 0.46^{***}$), number of primary branches plant⁻¹ ($r = 0.75^{***}$), number of pod plant⁻¹ ($r = 0.52^{***}$), grain yield ($r = 0.52^{***}$), above ground biomass yield ($r = 0.44^{**}$) and also significant and positively correlated with number of seed pod⁻¹ ($r = 0.30^*$) and harvest index ($r = 0.35^*$) (Appendix Table 4).

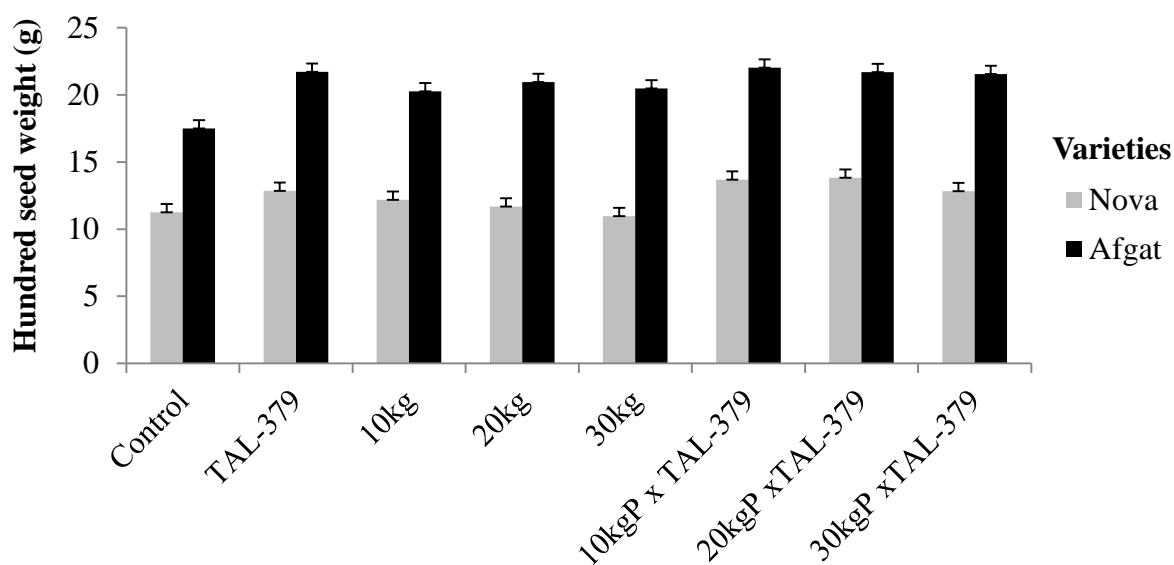


Figure 8: Interaction effects of; Variety x Phosphorus x *Bradyrhizobium* TAL-379 inoculation on hundred seed weight. Vertical lines on bars represent standard error of the statistical means.

Table 4: Yield and yield components of soybean varieties in response to *Bradyrhizobium* inoculation and P application at Alage, during 2020 belg cropping season

Treatments	Number of branch plant ⁻¹	Number of pod plant ⁻¹	Number of seed pod ⁻¹	Hundred seed weight (g)	Grain yield (t ha ⁻¹)	Above ground biological yield (t ha ⁻¹)	Harvest index (%)
Varieties							
Nova	4.81 ^b	73.86 ^b	2.79 ^b	12.41 ^b	2.13 ^b	4.99 ^b	42.04 ^b
Afgat	6.78 ^a	81.09 ^a	2.92 ^a	20.78 ^a	2.53 ^a	5.56 ^a	45.98 ^a
LSD0.05	0.50	2.43	0.12	0.53	0.10	0.36	3.82
Inoculation							
Un-inoculated	5.17 ^b	70.37 ^b	2.84	15.67 ^b	1.79 ^b	4.36 ^b	41.39 ^b
TAL-379	6.12 ^a	84.59 ^a	2.88	17.52 ^a	2.86 ^a	6.19 ^a	46.62 ^a
LSD0.05	0.50	2.43	ns	0.53	0.10	0.36	3.82
P levels (kg ha⁻¹)							
0	5.42	71.46 ^d	2.78 ^b	15.83 ^b	1.94 ^c	4.59 ^c	41.44
10	5.67	75.70 ^c	2.76 ^b	17.04 ^a	2.31 ^b	5.18 ^b	44.13
20	5.78	83.28 ^a	2.98 ^a	17.05 ^a	2.64 ^a	5.93 ^a	45.89
30	5.72	79.47 ^b	2.91 ^{ab}	16.46 ^{ab}	2.41 ^b	5.40 ^b	44.57
LSD0.05	ns	3.42	0.17	0.75	0.14	0.51	ns
CV (%)	15.09	5.38	7.25	5.44	7.37	11.64	14.88

Mean values followed by dissimilar letters in a column are significantly different at * : $P \leq 0.05$;

** : $P \leq 0.01$; *** : $P \leq 0.001$; NS: Non-significant; LSD: Least significant difference and CV:

Coefficient of variation.

4.4.5. Grain yield

Analysis of variance revealed that varieties, *Bradyrhizobium* inoculation and application of P highly significantly ($P < 0.001$) affected the grain yield. The interaction effect of inoculation x variety as well as P x variety were also found to be significant on the grain yield (Appendix Table 3).

The maximum (2.53 t ha^{-1}) and the minimum grain yields (2.13 t ha^{-1}) were obtained from Afgat and Nova varieties, respectively (Table 4). The significant variation in grain yield between the varieties is largely due to differences in inherent yielding potential of the varieties. 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 to genetic makeup of the varieties examined. The current study also in agreement with the work of Girma *et al.* (2017), who reported that a significant difference between common bean varieties on grain yield.

The results of analysis of variance revealed that grain yield was significantly ($P < 0.001$) affected by *Bradyrhizobium* inoculation. The greater grain yield (2.86 t ha^{-1}) was obtained from plants inoculated with strain TAL-379, while the lower grain yield (1.79 t ha^{-1}) was recorded from the control (Table 4). The significant increase in grain yield in response to inoculation with stain TAL-379 might be attributed to the increased availability of N in the soil for uptake by plant roots, through fixed N (Nyoki and Ndakidemi, 2013). Ulzen *et al.* (2016) also observed a significant increase in grain yield of cowpea after inoculation with *Bradyrhizobium* inoculant. This result was in line with the findings of Shahid *et al.* (2009) who reported that grain production can increase by 70 – 75% when the proper rhizobia isolates

are inoculated. Similar, several researchers had also reported the improvement legume yields due to rhizobial inoculation (Abbasi *et al.*, 2010; Ibrahim *et al.* 2011; Lamptey *et al.*, 2014).

Phosphorus rates significantly ($P < 0.001$) influenced grain yield of soybean (Table 4). The highest grain yield (2.64 t ha^{-1}) was recorded from 20 kg P ha^{-1} fertilized soybeans and closely followed by 30 kg P ha^{-1} (2.41 t ha^{-1}) supplied ones. Fertilization of 20 kg P ha^{-1} significantly improved grain yield of soybean over the other levels (0, 10 and 30 kg P ha^{-1}). The grain yields of the crop obtained under applications of 10 and 30 kg P ha^{-1} were not significantly different. However, the grain yield of 10, 20 and 30 kg P ha^{-1} supplied soybeans was significantly greater than grain yield of the unfertilized crop. The decrease in seed yield at the lowest and highest P application was most likely due to the fact that the growth and development of soybean was influenced by nutrient deficiency or nutrition surplus (Xiang *et al.*, 2012). The result is in agreement with those of Pauline *et al.* (2010), and Aise *et al.* (2011) who reported similar findings on seed yield of soybean under the condition of the proper P application. 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; Kamara *et al.*, 2010; Tarekegn *et al.*, 2017). Likewise, Sabir *et al.* (2001) reported similar findings and concluded that the number of pods plant^{-1} , seeds pod^{-1} , hundred grain weight and seed yield were significantly increased by different P rates. However, the result is contrasting with the finding of Meseret (2006) who reported the non-significant yield response of mung bean at Hawassa area as a result of high P sorption.

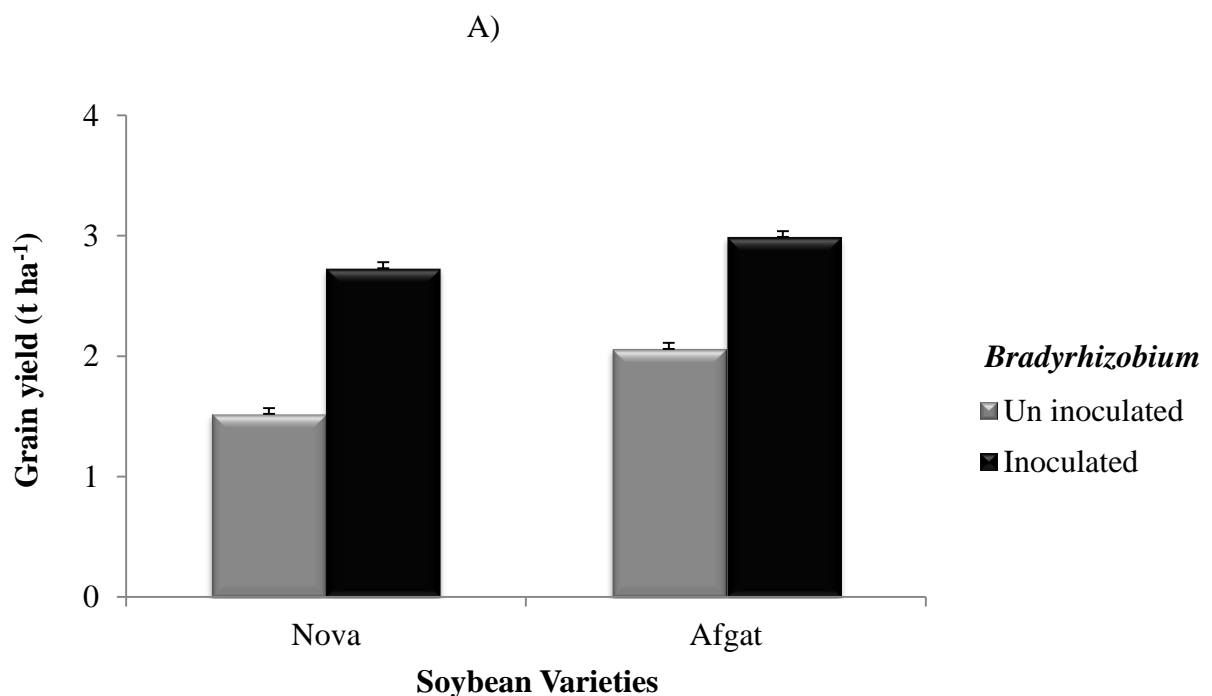
Varieties x inoculation interaction revealed that the highest grain yield (2.99 t ha^{-1}) from the variety Afgat with strain TAL-379. However, the lowest grain yield (1.52 t ha^{-1}) was obtained

from the variety Nova without inoculation (Fig. 9A). This indicates that Afgat variety produces more nodulation than Nova varieties through symbiosis with *Bradyrhizobium japonicum* inoculation, which resulted in more N₂-fixation that leads to increased yield of the variety. In line with this result, Habtamu *et al.* (2019) reported that common bean variety with *Rhizobium* strain HB-429 produced greater grain yield than the control. The current study was in contrary with the finding of Solomon *et al.* (2012) who reported that soybean variety with strain had no interaction effect on the seed yield. Also, the result was contradicts with the finding of Tarekegn *et al.* (2017) who reported that the interaction effects of cowpea varieties and *Bradyrhizobium* strain did not significant difference on seed yield ha⁻¹.

The interaction effect of variety x phosphorus revealed the variation on soybean grain yield. The highest grain yield was recorded from the variety Afgat with the 20 kg P ha⁻¹, followed by the same variety with the P rates of 30 and 10 kg ha⁻¹ over zero-P level (Fig. 9B). However, there was no marked difference between both Afgat and Nova variety on grain yield at zero-P level. This implies that, the cultivars which produced higher grain yield might have either better ability to absorb the applied P from the soil solution or translocate and use the absorbed P for grain formation than the low yielding. Similarly, the study on soybean, Farani (1988) indicated that seed yield was increased with seed inoculation and varying P rates up to 26 kg P ha⁻¹. The result was in line with the finding of Alemu (2018) who reported that soybean variety with P rates had interaction effect on the seed yield.

As it is indicated in Appendix Table 4, the correlation analysis revealed that grain yield was highly significant and positively correlated with days to maturity ($r = 0.43^{**}$), and also very highly significant and positively correlated with number of nodule plant⁻¹ ($r = 0.95^{***}$), nodule

dry weight ($r = 0.88^{***}$), shoot dry weight ($r = 0.92^{***}$), root dry weight ($r = 0.60^{***}$), number of primary branches plant^{-1} ($r = 0.55^{***}$), number of pod plant^{-1} ($r = 0.86^{***}$), hundred seed weight ($r = 0.52^{***}$), above ground biomass yield ($r = 0.85^{***}$) and harvest index ($r = 0.58^{***}$). This indicates that the development of effective and promising nodules of the crop due to P supply and *Bradyrhizobium japonicum* inoculation could promote N uptake through the process of BNF which ultimately improves the final grain yield and yield attributes of the crop. The yield of plant is a dependent variable, depends upon all other growth and yield contributing components. Therefore, it is generally correlated with all other components. Direct relationships of biomass yield, nodulation, shoot and root dry matter yields with grain yield are strongly in support of Bekere *et al.* (2012).



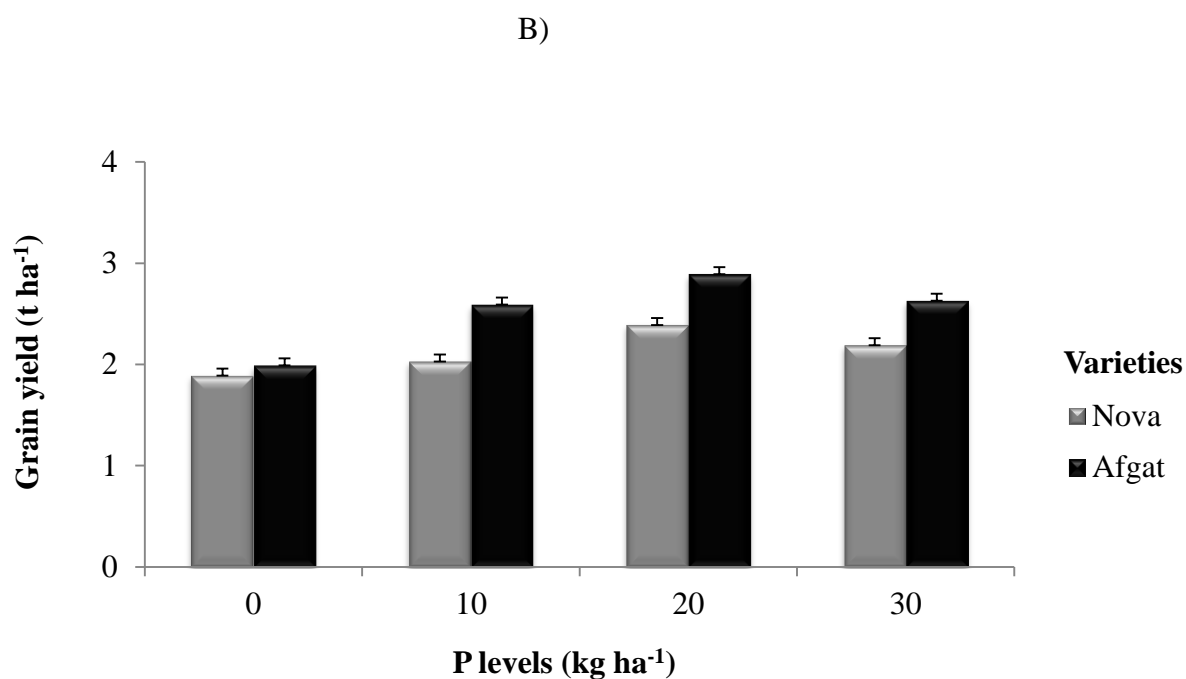


Figure 9: Interaction effects of; A) varieties x *Bradyrhizobium*, B) varieties x Phosphorus on grain yield. Vertical lines on bars represent standard error of the statistical means.

4.4.6. Above ground biological yield

Above ground biological yield was significantly affected by varieties, *Bradyrhizobium* inoculation, P application and variety x inoculation interaction (Appendix Table 3).

The higher above ground biological yield was recorded (5.56 t ha⁻¹) from Afgat variety, while the lower value was recorded (4.99 t ha⁻¹) from Nova variety (Table 4). The observed difference in above ground biological yield between soybean varieties might be due to inherent genetic difference. Moreover, the higher above ground biological yield could be attributed to the better plant shoot growth and grain formation ability of Afgat variety. The finding was in line with the work of Tesfaye *et al.* (2018) on soybean. The current study is

also in agreement with the finding of Dereje *et al.* (2015) who reported marked difference in above ground biomass yield among the varieties of common bean.

Bradyrhizobium inoculation with strain TAL-379 resulted in a highly significant increase in above ground biological yield compared to the control treatment. The highest above ground biological yield (6.19 t ha⁻¹) was recorded from plants inoculated with strain TAL-379 and the lowest above ground biological yield (4.36 t ha⁻¹) was recorded from the control (Table 4). A similar result was detected by Abbasi *et al.* (2010) who stated that above ground biomass yield of soybean was quadratic-ally increased up to 75% by the inoculation of different strains of rhizobia. Similarly, Alam *et al.* (2015) also reported a significant increase in soybean shoot biomass when the plants were inoculated with *Bradyrhizobium*. Since, N is a key factor in many biological compounds that plays a major role in photosynthetic activity and chlorophyll synthesis which ultimately resulted in vigorous vegetative growth and more biomass accumulation.

Likewise, the highest above ground biological yield was achieved from P application at the rate of 20 kg P ha⁻¹ (5.93 t ha⁻¹) followed by 30 kg P ha⁻¹ (5.40 t ha⁻¹) and 10 kg P ha⁻¹ (5.18 t ha⁻¹). Whereas, the lowest above ground biological yield was achieved from P application at the rate of 0 kg P ha⁻¹ (4.59 t ha⁻¹) (Table 4). This increase in dry matter yield with the application of P might be due to the adequate supply of P could be attributed to an increase in the number of branches plant⁻¹, and leaf area. This in turn increased photosynthetic area and number of pods plant⁻¹ as the result increased above ground biological yield. Similar to these results, Lamptey *et al.* (2014) reported an increase in above ground biomass yield of soybean as a result of increased levels of P fertilizer. However, this result contradicted the finding of

Gobeze and Legese (2015) who found that the main effect of P supply did not affect significantly biomass yield at Amaro.

Variety x *Bradyrhizobium* inoculation interaction also revealed significantly ($P < 0.05$) greater above ground biological yield with *Bradyrhizobium* inoculation strain TAL-379 for both variety Afgat and Nova. While, the lower above ground biological yield was obtained for both variety Afgat and Nova with un-inoculated treatment. However, there was no marked difference between both varieties with *Bradyrhizobium* inoculation (Fig. 10). Generally, above ground biological yield was increased with *Bradyrhizobium* inoculation strain TAL-379 for both varieties compared to the control. The results also indicated that N₂-fixation by *Bradyrhizobium japonicum* inoculation in both varieties enhanced the vegetative growth of soybean, which resulted in substantial increase in its biomass yield. Tahir *et al.* (2009) reported an increase in plant biomass due to inoculation of *Bradyrhizobium japonicum* alone by 62.8% over the control.

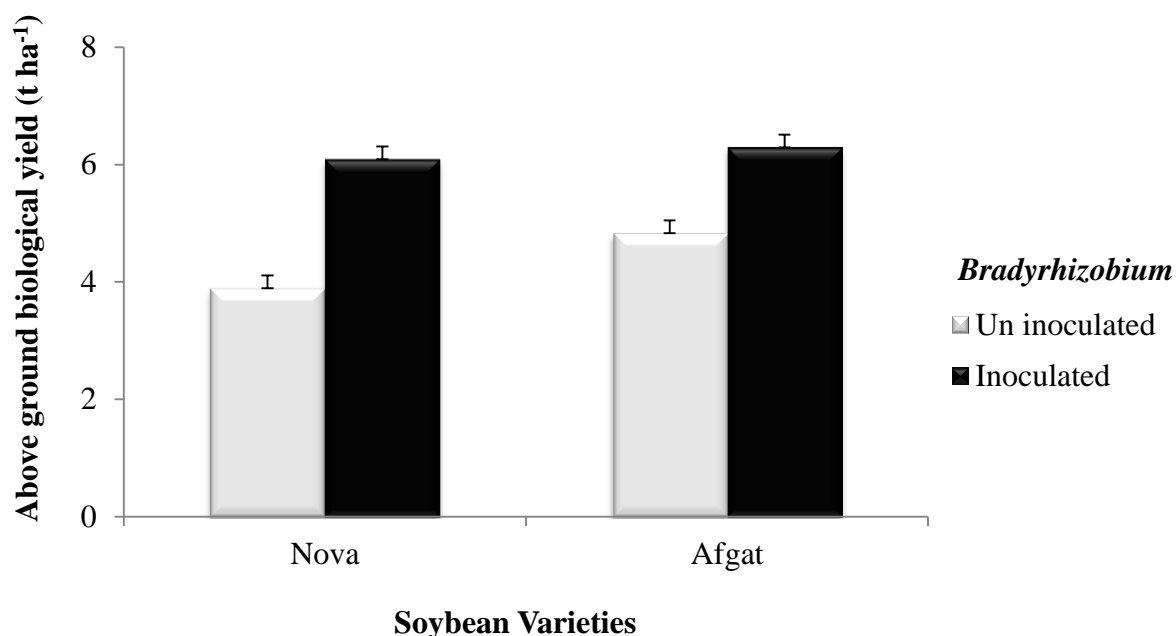


Figure 10: Interaction effects of varieties x *Bradyrhizobium* on above ground biological yield. Vertical lines on bars represent standard error of the statistical means.

4.4.7. Harvest index

Harvest index is very useful in measuring nutrient partitioning in crop plants, which provides an indication of how efficiently the plant utilized acquired nutrients for grain production. The analysis of variance on harvest index showed that there was a significant difference in the effect of varieties and *Bradyrhizobium* inoculation, but there was no significant difference on P application and their interactions (Appendix Table 3).

Of the two varieties, Afgat recorded significantly higher harvest indices of (45.98%). While, variety Nova recorded the lower harvest index (42.04%) (Table 4). The observed difference was related to genotypic difference and is directly linked to productivity or partitioning capacity of the crop. In agreement with this result, Ano (2005) reported that the differences in

harvest index might be due to the inherent varietal characteristics, environmental factors and other cultural practices.

Bradyrhizobium inoculation with strain TAL-379 resulted in a significant increase of harvest index compared to the control. The greater value (46.62%) was recorded from plants inoculated with strain TAL-379 and the lower harvest index was obtained from the control (Table 4). The results indicated that adequate supply of N through biological N₂-fixation enhanced dry matter partitioning in favor of grain showing a greater harvest index. A similar result was reported by Roy *et al.* (1995) who observed that soybean seeds inoculation increased harvest index.

4.5. Effect of *Bradyrhizobium* Inoculation and P on Soil Properties after Harvest

There were no significant differences in the main effect of soybean varieties on soil chemical properties. But, soil pH and total N was significantly affected by *Bradyrhizobium* inoculation as well as soil pH was significantly affected by the application of P (Table 5).

Bradyrhizobium inoculation with strain TAL-379 resulted in a marked improvement in the soil pH compared to the control. The highest soil pH (8.01) was recorded from plots inoculated with strain TAL-379, while the lowest soil pH (7.83) was recorded from un-inoculated treatment (Table 5). In contrast to this result, Geletu *et al.* (2017) reported that *Rhizobium* inoculation did not influence soil pH after harvest. Total N content was also significantly ($P < 0.01$) affected by *Bradyrhizobium* inoculation with strain TAL-379. The higher total N (0.22%) was recorded from plots inoculated with strain TAL-379 compared to the control (Table 5). This might be due to the fact that *Bradyrhizobium* bacteria contributed for better N_2 -fixation that sufficiently used for it and further N to the soil. Similarly, to this result several authors Semira *et al.* (2008); Tesfaye *et al.* (2018) also reported that inoculation of legumes with *Rhizobium* strains significantly increased soil N after harvest.

Application of P fertilizer at the levels of 30 kg ha^{-1} showed significant increased soil pH (8.01) as compared to the control treatment (Table 5). P is the most important nutrient element required for growth and development of plants. Generally, the result showed that the available P in soil decreased during the growing period in all treatments (Table 5). The highest reduction of available P from the original value (11.48 mg kg^{-1}) was 1.56 mg kg^{-1} in the control, whereas the lowest reduction of 1.24 mg kg^{-1} was gained from the application of 20 kg P ha^{-1} . Possible reasons for reduction might be crop use, fixation and leaching of P. On the

other hands, N₂-fixation by itself requires high amount of P and after growing of legume crops the soil available P will decreased (Silva *et al.*, 2014).

Table 5: Soil chemical properties of the study area after harvesting

Treatments	Soil pH	Total nitrogen (%)	Available phosphorus (mg kg ⁻¹)
Varieties			
Nova	7.91	0.19	9.50
Afgat	7.94	0.20	9.39
LSD0.05	ns	ns	ns
Inoculation			
Un-inoculated	7.83 ^b	0.18 ^b	9.40
TAL-379	8.01 ^a	0.22 ^a	9.49
LSD0.05	0.07	0.01	ns
P levels (kg ha⁻¹)			
0	7.84 ^b	0.19	9.92
10	7.86 ^b	0.20	10.11
20	7.97 ^{ab}	0.20	10.24
30	8.01 ^a	0.21	10.17
LSD0.05	0.09	ns	ns
CV (%)	1.42	10.71	3.18

Mean values followed by dissimilar letters in a column are significantly different at * : $P \leq 0.05$; ** : $P \leq 0.01$; *** : $P \leq 0.001$; NS: Non-significant; LSD: Least significance difference and CV: Coefficient of variation.

4.6. Partial Budget (Economic) Analysis of Treatment Effects

Partial budget is a method of organizing experimental data and information about the costs and benefits of various alternative treatments. It is a way of calculating the total costs that vary and the net benefits of each treatment (CIMMYT, 1988). From the result of this study, the mean yield of all 16 treatments tested was obtained. According to CIMMYT (1988), the average yield was adjusted downward by 10%. This is the reason that, the researcher have assumed that using the same treatments the yield from experimental plots and farmers field is vary, thus average yield obtained from the treatment tested should be adjusted downward. According to the economic analysis data (Table 6) all marginal rates were above 100% that is in a rage of acceptance (CIMMYT, 1988). On this study, net benefit were calculated by current fertilizer (TSP) cost of 18.50 ETB kg⁻¹, *Bradyrhizobium* strain TAL-379 400.00 ETB kg⁻¹, daily laborer expense due to treatment effects was 40.00 ETB and field price of 1 kg of soybean that farmers receive for the crop when they sale it as adjusted yield was 25.00 ETB.

The results displayed that Afgat varieties recorded the highest marginal rate return (1400.0%) in percent among all the treatments. The highest net benefit recorded from inoculation of *Bradyrhizobium* strain TAL-379 and 20 kg P ha⁻¹ was due to highest yield was produced from these treatments. Therefore, from the budget summary of economic analysis, the highest net return (54525 Birr ha⁻¹) was recorded from Afgat varieties with acceptable marginal rate of return (1400.0%). Concerning the *Bradyrhizobium* inoculation strain TAL-379, the highest net return (63750 Birr ha⁻¹) was obtained from *Bradyrhizobium* inoculation, while the lowest net economic return (40275 Birr ha⁻¹) was recorded in the un-inoculated treatment. Among the applied P fertilizer levels, the highest net return (57150 Birr ha⁻¹) was recorded from 20 kg P

ha⁻¹. The lowest net economic return (43650 Birr ha⁻¹) was recorded the control treatment (Table 6). Therefore, from this study, to obtain optimum economic return from the production of soybean at the study area, the use of Afgat varieties, *Bradyrhizobium* inoculation with strain TAL-379 and 20 kg P ha⁻¹ application could be recommended for profit production of soybean at Alage soil Central Rift Valley of Ethiopia.

Table 6: Cost benefits analysis of *Bradyrhizobium* inoculation and P application for soybean production at Alage soil during 2020 belg cropping season

Treatments	Average Yield (t ha⁻¹)	Adjusted Yield (t ha⁻¹)	P cost (ETB ha⁻¹)	STC (ETB ha⁻¹)	Seed Price (ETB ha⁻¹)	LC (ETB ha⁻¹)	TVC (ETB ha⁻¹)	GFB (ETB ha⁻¹)	Net benefit	Dominance	MRR (%)
Varieties											
Nova	2.13	1.917	0	0	1800	0	1800	47925	46125	-	
Afgat	2.53	2.277	0	0	2400	0	2400	56925	54525	UD	1400.0
Inoculation											
Un inoculated	1.79	1.611	0	0	0	0	0	40275	40275	D	
TAL-379	2.86	2.574	0	200	0	400	600	64350	63750	UD	1137.5
P levels (kg ha⁻¹)											
0	1.94	1.746	0	0	0	0	0	43650	43650	D	
10	2.31	2.079	925	0	0	400	1325	51975	50650	D	
20	2.64	2.376	1850	0	0	400	2250	59400	57150	UD	600.0
30	2.41	2.169	2775	0	0	400	3175	54225	51050	D	

Where, GB = Gross benefit, STC = Strain cost, LC = Labor cost, TVC = Total variable cost, MRR = Marginal rate of return, D Dominated, UD = Undominated, Nova variety, Afgat variety, Inoculation and Phosphorus fertilizer

5. SUMMARY AND CONCLUSION

Soybean is one of oil-bearing crops that popularly and most widely grown in different parts of the world and produced in some parts of our country. However, soybean yield is low compared to other legume crops due to many factors affecting its production which include suitable varieties, poor agronomic practices such as fertility management including *Bradyrhizobium* inoculation, appropriate fertilizer rate, and time of application, untimely and inappropriate field operations. Therefore, dependable information on agronomic management practices such as crop varietal selection, *Bradyrhizobium* inoculation as N sources and P fertilizer rates and crop response to this *Bradyrhizobium* inoculation and P fertilizer and their interaction is relatively very important to arise crop production and productivity. To achieve this, field research was undertaken to investigate the growth, symbiotic, and yield response of soybean varieties to *Bradyrhizobium* inoculation and P fertilizer application at Alage Agricultural Technical and Vocational Education Training College, during the 2020 belg cropping season. The experiment consisted of two soybean varieties (Afgat and Nova), two inoculation levels (un-inoculated and inoculated with strain TAL-379) and four levels of P fertilizer (0, 10, 20 and 30 kg P ha⁻¹) in the form of TSP and in randomized complete block design with a factorial arrangement using three replications.

In general, there were highly significant ($P \leq 0.05$) varietal responses for all of the parameters measured. Among the varieties, Afgat took the longest days to emergence, days to flowering and days to maturity than Nova variety. Moreover, the Afgat variety produced higher nodule number, nodule dry weight, shoot dry weight, root dry weight, number of branches, number of

Pods, number of seeds, hundred seed weight, grain yield, above ground biomass and harvest index as compared to Nova varieties (Table 2, 3 and 4).

On the other hand, *Bradyrhizobium* inoculation with strain TAL-379 showed highly significant differences on days to maturity, nodule number, nodule dry weight, shoot dry weight, root dry weight, number of branches, number of pods, hundred seed weight, grain yield, above ground biomass yields and harvest index (Table 2, 3 and 4). In contrast, days to emergence, days to flowering, plant height and number of seed pod⁻¹ were not statistically affected by inoculation of strain TAL-379.

The application of P fertilizer significantly reduced all phenological parameters like days to emergence, days to flowering and days to maturity. However, the supply of P at the levels of 20 kg P ha⁻¹ significantly increased nodule number, nodule dry weight, plant height, shoot dry weight, root dry weight, number of pods, number of seeds, hundred seed weight, grain yield and above ground biomass as compared to control and other P rates (Table 2, 3 and 4). On the other hand in some parameters such as the number of branches and harvest index were not statistically affected by supply of P levels.

The interaction effect of varieties x inoculation, inoculation x P rates and varieties x P rates caused significant variation on days to maturity, numbers of nodules, nodule dry weight, shoots dry weight, root dry weight, number of pods plant⁻¹, hundred seed weight, above ground biomass yield and grain yield. Thus, the highest grain yield (2.99 t ha⁻¹) was recorded from the use of Afgat variety with *Bradyrhizobium* TAL-379 inoculation followed by the interaction of Afgat variety and the supply of P at the level of 20 kg ha⁻¹ (2.89 t ha⁻¹) (Figure

9). It could be deduced that use of Afgat variety with *Bradyrhizobium* TAL-379 inoculation markedly increases the productivity of soybean at Alage, Central Rift Valley of Ethiopia.

Based on the partial budget analysis, the highest net benefit (54525 Birr ha⁻¹) was recorded from Afgat varieties with acceptable marginal rate of return (1400.0%). Concerning the *Bradyrhizobium* inoculation strain TAL-379, the highest net benefit (63750 Birr ha⁻¹) was obtained from *Bradyrhizobium* inoculation, while the lowest net return (40275 Birr ha⁻¹) was recorded in the un-inoculated treatment. Among the applied P fertilizer levels, the highest net benefit (57150 Birr ha⁻¹) was obtained from 20 kg P ha⁻¹ supplied. However, the lowest net return (43650 Birr ha⁻¹) was recorded from the control (Table 6). Therefore, the use of Afgat variety, *Bradyrhizobium* strain TAL-379 inoculation and phosphorus fertilizer application at the level of 20 kg ha⁻¹ could be recommended to soybean producers in Alage area to achieve superior yield and better economic return. However, verification of this result on different areas and seasons could be required in order to put the recommendation in a firm ground.

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7. APPENDICES

Appendix Table 1: Mean squares of ANOVA phenological parameters of soybean varieties in response to *Bradyrhizobium* inoculation and P application at Alage, during 2020 belg cropping season

Mean square of phenological parameters				
Source of variation	DF	Days to emergence	Days to flowering	Days to maturity
Replication	2	0.33333	6.521	2.46
Variety (V)	1	6.02083 ^{**}	507.000 ^{***}	5954.11 ^{***}
Inoculation (I)	1	1.02083 ^{ns}	10.083 ^{ns}	709.94 ^{***}
Phosphorus (P)	3	7.90972 ^{***}	38.167 ^{***}	108.74 ^{***}
V x I	1	1.02083 ^{ns}	0.750 ^{ns}	269.80 ^{***}
V x P	3	0.07639 ^{ns}	6.500 ^{ns}	13.95 ^{ns}
I x P	3	0.07639 ^{ns}	7.583 ^{ns}	12.46 ^{ns}
V x P x I	3	0.07639 ^{ns}	5.139 ^{ns}	5.61 ^{ns}
Error	30	0.64444	2.854	5.10
CV (%)		10.52	4.10	3.55

Where, DF = degree of freedom, CV = coefficient of variation, ^{***} = Significant at 0.001, ^{**} = Significant at 0.01, ^{*} = Significant at 0.05 and ns = non-significant at 0.05 level of probability.

Appendix Table 2: Mean squares of ANOVA nodulation and growth of soybean varieties in response to *Bradyrhizobium* inoculation and P application at Alage, during 2020 belg cropping season

Mean square of Nodulation and Growth parameters						
Source of variation	DF	Nodule number plant ⁻¹	Nodule dry weight (g plant ⁻¹)	Plant height(cm)	Shoot dry weight (g plant ⁻¹)	Root dry weight (g plant ⁻¹)
Replication	2	0.439	0.00019	5.350	0.434	0.00886
Variety (V)	1	111.935 ^{***}	0.03203 ^{***}	194.408 ^{***}	27.241 ^{***}	2.07917 ^{***}
Inoculation (I)	1	558.285 ^{***}	0.14963 ^{***}	4.941 ^{ns}	142.003 ^{***}	0.90475 ^{**}
Phosphorus (P)	3	26.487 ^{***}	0.01569 ^{***}	63.054 ^{***}	28.454 ^{***}	2.42589 ^{***}
V x I	1	6.235 ^{***}	0.00008 ^{ns}	0.003 ^{ns}	11.761 ^{***}	0.04142 ^{ns}
V x P	3	0.336 ^{ns}	0.00109 ^{ns}	0.473 ^{ns}	1.873 ^{**}	0.08645 ^{ns}
I x P	3	0.662 [*]	0.00289 [*]	4.075 ^{ns}	1.312 [*]	0.30459 [*]
V x P x I	3	0.167 ^{ns}	0.00036 ^{ns}	3.025 ^{ns}	0.202 ^{ns}	0.00447 ^{ns}
Error	30	0.184	0.00081	4.682	0.381	0.08146
CV (%)		4.98	21.65	2.38	7.92	9.49

Where, DF = degree of freedom, CV = coefficient of variation, ^{***} = Significant at 0.001, ^{**} = Significant at 0.01, ^{*} = Significant at 0.05 and ns = non-significant at 0.05 level of probability.

Appendix Table 3: Mean squares of ANOVA yield and yield components of soybean varieties in response to *Bradyrhizobium* inoculation and P application at Alage, during 2020 belg cropping season

Mean square of Yield and Yield components								
Source of variation	DF	Number of primary branch plant ⁻¹	Number of pod plant ⁻¹	Number of seed pod ⁻¹	Hundred seed weight (g)	Grain yield (t ha ⁻¹)	Above ground biological yield (t ha ⁻¹)	Harvest index (%)
Replication	2	0.5781	2.60	0.02601	0.042	0.0072	0.3505	62.764
Variety (V)	1	33.1669 ^{***}	628.58 ^{***}	0.21467 [*]	839.344 ^{***}	1.9080 ^{***}	3.8760 ^{**}	186.875 [*]
Inoculation (I)	1	10.9252 ^{***}	2426.79 ^{***}	0.02125 ^{ns}	41.329 ^{***}	13.7924 ^{***}	40.2967 ^{***}	327.973 ^{**}
Phosphorus (P)	3	0.2985 ^{ns}	308.22 ^{***}	0.12724 [*]	3.999 ^{**}	1.0192 ^{***}	3.7001 ^{***}	41.925 ^{ns}
V x I	1	0.0469 ^{ns}	40.52 [*]	0.01802 ^{ns}	0.090 ^{ns}	0.2338 ^{***}	1.6133 [*]	0.819 ^{ns}
V x P	3	1.1758 ^{ns}	52.22 ^{**}	0.03139 ^{ns}	1.286 ^{ns}	0.1268 ^{***}	0.5111 ^{ns}	86.012 ^{ns}
I x P	3	0.3785 ^{ns}	58.72 ^{**}	0.01302 ^{ns}	1.466 ^{ns}	0.0310 ^{ns}	0.5401 ^{ns}	54.731 ^{ns}
V x P x I	3	1.0624 ^{ns}	14.75 ^{ns}	0.01387 ^{ns}	2.331 [*]	0.0114 ^{ns}	0.5728 ^{ns}	24.056 ^{ns}
Error	30	0.7039	9.22	0.05072	0.577	0.0145	0.2864	40.655
CV (%)		15.09	5.38	7.25	5.44	7.37	11.64	14.88

Where, DF = degree of freedom, CV = coefficient of variation, ^{***} = Significant at 0.001, ^{**} = Significant at 0.01, ^{*} = Significant at 0.05 and ns = non-significant at 0.05 level of probability.

Appendix Table 4: Correlation results among parameters

	DE	DF	DM	NN	NDW	PH	SDW	RDW	NPB	NPPP	NSPP	HSW	AGBY	GY	HI
DE	1														
DF	0.48***	1													
DM	0.42**	0.85***	1												
NN	-0.14 ^{ns}	0.30*	0.56***	1											
NDW	-0.23 ^{ns}	0.24 ^{ns}	0.46***	0.91***	1										
PH	-0.43**	-0.62***	-0.61***	0.03 ^{ns}	0.09 ^{ns}	1									
SDW	-0.27 ^{ns}	0.15 ^{ns}	0.34*	0.88***	0.89***	0.22 ^{ns}	1								
RDW	-0.37*	0.13 ^{ns}	0.27 ^{ns}	0.61***	0.69***	0.19 ^{ns}	0.72***	1							
NPB	0.17 ^{ns}	0.49***	0.68***	0.62***	0.52***	-0.34*	0.46***	0.36*	1						
NPPP	-0.18 ^{ns}	0.25 ^{ns}	0.49***	0.89***	0.82***	0.17 ^{ns}	0.81***	0.62***	0.57***	1					
NSPP	-0.08 ^{ns}	0.11 ^{ns}	0.22 ^{ns}	0.32*	0.42**	0.10 ^{ns}	0.39**	0.41**	0.24 ^{ns}	0.41**	1				
HSW	0.28 ^{ns}	0.74***	0.91***	0.57***	0.51***	-0.50***	0.48***	0.46***	0.75***	0.52***	0.30*	1			
AGBY	-0.26 ^{ns}	0.19 ^{ns}	0.37*	0.85***	0.83***	0.14 ^{ns}	0.83***	0.58***	0.48***	0.78***	0.31*	0.44**	1		
GY	-0.20 ^{ns}	0.20 ^{ns}	0.43**	0.95***	0.88***	0.11 ^{ns}	0.92***	0.60***	0.55***	0.86***	0.23 ^{ns}	0.52***	0.85***	1	
HI	-0.05 ^{ns}	0.13 ^{ns}	0.27 ^{ns}	0.49***	0.38**	0.01 ^{ns}	0.50***	0.33*	0.32*	0.45**	-0.02 ^{ns}	0.35*	0.10 ^{ns}	0.58***	1

Where, DE = days to emergence, DF = days to flowering, DM = days to maturity, NN = number of nodule, NDW = nodule dry weight, PH = plant height, SDW = shoot dry weight, RDW = root dry weight, NPB = number of primary branch, NPPP = number of pod per plant, NSPP = number of seed per pod, HSW = hundred seed weight, AGBY= above ground biological yield, GY= grain yield, HI= harvest index, *** = Significant at 0.001, ** = Significant at 0.01, * = Significant at 0.05 and ns = non-significant at 0.05 level of probability.

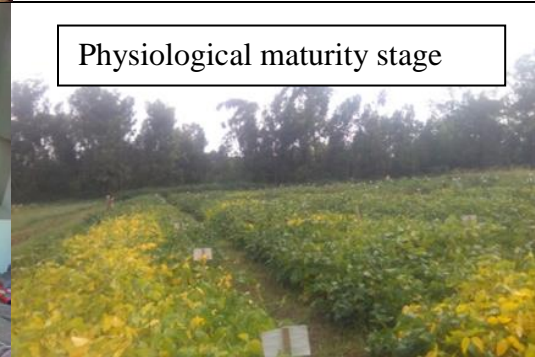
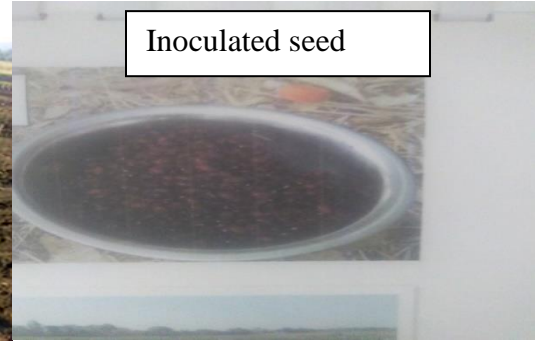


Figure 11: Some pictures during experimentation time

BIOGRAPHICAL SKETCH

The Author, **Ayana Yadeta**, was born on February 10, 1993, in Oromia Regional State, from his father Mr. Yadeta Gameda and his mother Mrs. Yeshe Jiru at Tibe hara district, Gobu-Sayo Woreda, East Wollega Zone. When he reached school age he moved to neighboring Tibe hara kebele to attend Goda Hara elementary primary school from 2000-2007. He then joined Anno Senior Secondary and Preparatory School to attend his secondary and preparatory education from 2008-2011. He then joined Mizan-Tepi University in 2012 and graduated with a degree of Bachelor of Science in Plant Science in 2014. Upon graduation, he was employed by the Ministry of Agriculture as a College instructor in Alage Agricultural Technical and Vocational Educational Training College on October 08, 2015, where he is working until now. In June 2018, he joined Hawassa University to pursue a postgraduate study leading to the Degree of **Master of Science** in Plant Science with specialization in **Agronomy**.