**NODULATION, NITROGEN FIXATION AND YIELD POTENTIAL OF COMMON BEAN *(Phaseolus vulgaris L.)* VARIETIES UNDER SHADE**

**MSc THESIS**


**SELAMAWIT ASSEGID**

**HAWASSA UNIVERSITY**
**COLLEGE OF AGRICULTURE**


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# NODULATION, NITROGEN FIXATION AND YIELD POTENTIAL OF COMMON BEAN *(Phaseolus vulgaris L.)*VARIETIES UNDER SHADE

**SELAMAWIT ASSEGID**

**Major Advisor: Girma Abera (PhD)**

**Co-Advisor: Walelign Worku (Professor)**

**A Thesis Submitted to the School of Plant and Horticultural Sciences, College of Agriculture, Hawassa University**

**In Partial Fulfillment of the Degree of Master of Science in Plant Sciences (Specialization: Agronomy)**

**Hawassa, Ethiopia**

**October, 2021**

## SCHOOL OF GRADUATE STUDIES

## SCHOOL OF PLANT AND HORTICULTURAL SCIENCES, COLLEGE OF AGRICULTURE,

### HAWASSA UNIVERSITY

## APPROVAL SHEET –I

This is to certify that the thesis entitled **"Nodulation, nitrogen fixation and yield potential of common bean *(Phaseolus vulgaris L.)* varieties under shade**" submitted to the Graduate Program of the School of Plant and Horticultural Sciences, College of Agriculture, Hawassa University in partial fulfillment of the requirements for the degree of Masters of Science with specialization in Agronomy. This thesis is a record of original research carried out by Selamawit Assegid, under my supervision, and no part of the thesis has been submitted for any other degree or diploma. The assistance and help received during the course of this investigation have been duly acknowledged. Therefore, we recommend that it to be accepted as fulfilling the thesis requirements.

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|---|---|---|
| GirmaAbera (PhD) | __________________ | ______________ |
| Name of Major Advisor | Signature | Date |
| WalelignWorku (Prof.) | __________________ | _____________ |
| Name of Co-Advisor | Signature | Date |

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## APPROVAL SHEET –II

We, the undersigned members of the boarded of the examiners of the final open defense by Selamawit Assegid have read and evaluated her thesis "N**odulation, nitrogen fixation and yield potential of common bean** *(Phaseolus vulgaris L.)* **varieties under shade",** and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy.

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| ________________ | ________________ | ______________ |
| Name of the Chairman | Signature | Date |
| GirmaAbera (PhD) | ________________ | ______________ |
| Name of Major Advisor | Signature | Date |
| ______________________ | ________________ | ________ |
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Final approval and acceptance of the thesis is contingent upon the submission of the final copy of the thesis to the Council of Graduate Studies (CGS) through the departmental graduate committee (DGC) of the candidate's major department.

Date:-------------------------------------

# DEDICATION

I dedicate this work to my beloved mother Lomi Bekele, my father Assegid Bizuneh my family Biruk Digafe, Bilen Biruk and Yabsira Biruk for their love and support.

# STATEMENT OF THE AUTHOR

I state that this thesis is my work and all sources of materials used for this thesis have been duly acknowledged. I declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate. This thesis has been submitted in partial fulfillment of the requirement for the degree of the Master of Science in Agriculture specialization of Agronomy at Hawassa University College of Agriculture.

Name: Selamawit Assegid Signature:_____________

Date of Submission: ____________________________

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# LIST OF ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of variance |
| BNF | Biological nitrogen fixation |
| CEC | Cation exchange capacity |
| FAO | Food and Agricultural Organization |
| HRC | Hawassa research center |
| MRC | Melkassa research center |
| MT | Metric tone |
| ND | Nitrogen difference |
| NMA | National metrology agency |
| OC | Organic carbon |
| RCBD | Randomized complete block design |
| SAS | Statistical Analysis System |
| t | Tone |
| TN | Total nitrogen |
| TSP | Triple super phosphate |

# Table of Contents

**Contents** **Page No**

# List of Tables

**Table** **Page**

# LIST OF FIGURES

## LIST OF TABLES IN THE APPENDIX

**Page no**

# Nodulation, Nitrogen Fixation and Yield Potential of Common Bean *(Phaseolus vulgaris L.)* Varieties Under Shade

[1]Selamawit Assegid (BSc), [1]Girma Abera (PhD) and [1]Walelign Worku (Prof.)

[1]School of Plant and Horticulture Science, College of Agriculture, Hawassa University,

Hawassa, Ethiopia

## ABSTRACT

*Common bean (Phaseolus vulgaris L.) is a herbaceous annual crop which in a symbiotic relationship with specific soil bacteria, 'fixes' atmospheric nitrogen ($N_2$) into amino form that can be used for plant growth.A field experiment was conducted to determine the effect of shade and Rhizobium inoculation on nitrogen fixation, nodulation, yield and yield components of three common bean varieties at Hawassa University Research Farm, Hawassa Southern Ethiopia. The experimental treatments consisted of a factorial combination of 2 levels of shade (25% shading and non-shading), 2 levels of inoculation (inoculated and non-inoculated) and three common bean varieties (HawassaDume, Nasir and Ibbado). The experiment was laid out as a randomized complete block design with four replications. The result showed that inoculation of common bean with Rhizobium strain HB-429 significantly affected all parameters compared to the non-inoculated plants in the field trial. The interactions of variety,shade and inoculation affects leaf area, leaf area index, specific leaf area,grain yield and 100 seed weight of common bean. The highest nodule number was recorded from rhizobium inoculated plots, while the lowest nodule number was recorded from the 25% shade treatments. Similarly the highest grain yield was recorded from inoculated Hawassa dume variety of open treatments while the lowest grain yield was recorded from non-inoculated Ibbado variety at 25% shade treatments.Also this study indicated inoculated Hawassa dume variety from the open treatments derived the greatest percentage of N from $N_2$ fixation and the lowest percentage of N was derived from non-inoculated Ibbado variety from the 25% shade treatments N uptake of common bean grown on full light had significantly greater N content than wheat this increase in N uptake of common bean is presumably derived from N2-fixation capacity of the legumes. Therefore, variety Hawassa dume inoculated with rhizobium strain HB-429 in open generated the highest seed yield. It would be worthwhile to do similar experiments on the area with the inclusion of more shade levels to refine the findings*

***Keywords**: Common bean, nitrogen fixation, inoculation and shade*

# 1. INTRODUCTION

Common bean (*Phaseolus Vulagris* L) is an annual crop which belongs to the family Fabaceae. It grows best in warm climate at temperature of 18 to 24$^{o}$C Teshale *et al.,(* 2005). It is highly polymorphic, warm-season, herbaceous annual crop and one of the most important cash crops and source of protein in many lowlands and mid land areas. It is high in starch, dietary fiber content and is an excellent source of potassium, selenium, molybdenum, thiamine, vitamin B6, and folic acid Maiti and Singh,( 2007). It is used as food in different form; the green unripe pods are cooked or conserved as vegetable and the ripe seeds cooked for "nifro" MOARD, (2009).

Among the pulse crops, common bean is the second in area of production in the Ethiopia CSA, (2015). As High in nutrients and commercial potential, common bean holds great promise for fighting hunger, increasing income and improving soil fertility in Sub Saharan Africa. The national average yield of common beans is low ranging from 1.4 tone ha$^{-1}$, which is far below the corresponding yield recorded at research sites (2.5-3 tones ha$^{-1}$) using improved varieties CSA,(2015). Common bean is highly preferred by Ethiopian farmers because of its early maturing characteristics (short season crop) that enable households to get cash income required to purchase food when other crops have not yet matured Legese *et al.,(* 2006).

Like other legumes, common bean associates with Rhizobium bacteria in the soil, which form root nodules. Through nitrogenase activity, the bacteria within the nodules fix atmospheric nitrogen to form ammonia, which the bean plant uses as a nitrogen source, reducing the need for externally applied fertilizers Ramos, (2003). However, the nitrogen-fixing capacity of common bean varies by variety and is generally less than that of other agronomically important legumes, such as soybeans, which tend to have larger root nodules with higher nitrogenase activity Isoi and

Yoshida, 1991; Hardarson,( 1993). Several adverse environmental factors present in regions where beans are commonly grown, such as drought, flooding, either high or low temperatures and shade effect from various cropping systems may impact nitrogen fixation. Rhizobium populations, nodulation, ammonium assimilation and nitrogenase activity are all reduced under these conditions Devi, 2012; Beebe, 2014; Hungria and Kaschuk, (2014). Planting beans in soil where they have not been grown before can also result in poor nitrogen fixation, due to insufficient Rhizobium in the soil to initiate nodulation Wortmann, (2006).

Biological nitrogen fixation is one of the most important processes for ecosystem to access available N for all living organisms. Although $N_2$ consists 78% of atmosphere, but the triple bond between two N atoms is very stable, and only a few group of prokaryotes can fix $N_2$ to ammonia by the enzyme nitrogenase. Annual rate of natural nitrogen fixation is estimated about 232 x $10^6$ t, and the 97% depends on biological nitrogen fixation Bloom, (2011). This exceeds the rate of chemical nitrogen fertilizer uses about 100 x $10^6$ t in 2009. Common bean can use $N_2$, though symbiosis with nitrogen fixing soil bacteria, rhizobia, to make root nodules for harboring them.

Nitrogen is among the most abundant element on the Earth, it is the critical limiting element for growth of most plants due to its unavailability Vance, (2001). Plant acquire N from two principal sources the first is from the soil, through commercial fertilizer, manure, and/or mineralization of organic matter; and the second is from the atmosphere through symbiotic $N_2$ fixation. Nitrogen fixation by micro-organisms is the major source of soil nitrogen which contributes about 75% of total nitrogen content SubbaRao,( 2001). Worldwide, legumes are grown on approximately 250 million ha and fix about 90 Tg (or $9 \times 10^7$ t) of dinitrogen ($N_2$) per year. Rhizobium spp. and Bradyrhizobium spp. are major contributors to overall $N_2$ fixation through the legume- Rhizobium symbiosis and have been used for over a hundred years as beneficial microorganisms (Xavier *et al*., 2004).

Shade regardless of its source reduces the irradiance predominantly in the photosyntetically active region of the spectrum (400 to 700 nm) and thereby affect the photosyetnsis rates with common bean. The level of irradiance is a major ecological factor that influences plant growth. Plants respond to different level of irradiance through both genetic adaptation and phenotypic acclimation Lambers *et al*., 1998; Bell *et al*., (2000). When plants are shaded by neighbors two type of reaction can occur; shade-acclimation response maximize light harvesting in shade condition through increase in specific leaf area and reduced chlorophyll a/b ratio Evans and Poorter,(2001) whereas shade avoiding responses maximizes light capturing by positioning the leaves out of the shade Vandenbussche *et al*., (2005).

Intercropping is practiced by the majority of farmers in the tropics and subtropics as a yield maximization and risk minimization strategy. Nearly 80% of all beans produced are grown with maize at some time during the annual crop cycle Francis, (1977). It is mainly intercropped with late-maturing cultivars of maize and sorghum. The amount of light under the taller cereal canopy changes during the various phenological phases though the variation during the latter phases is smaller. For instance, in late cultivars of maize, the maize crop starts to have a closed canopy starting from six weeks from emergence and continues up to the beginning of physiological maturity.

The poorer performance of legumes when intercropped may be partly because the quantity of light reaching their canopy is reduced by the taller companion crop Stirling *et al.*, (1990). Dry matter production by a canopy of a given size varies in proportion to the amount of intercepted energy, at a rate governed by its radiation use efficiency Squire, (1990). The review by Laing *et al*.,( 1984) indicates that competition for light among components is the most important factor determining productivity of the intercropping system when other factors are non-limiting.

The effects of light availability on $N_2$-fixation are less certain. Studies have suggested that competition for light can decrease $N_2$-fixation by decreasing growth. Carlsson *et al,(*.2009) found that competition for light from grasses may have decreased plant growth and consequently limited $N_2$-fixation at the plant level. Jensen, (1996) reported that competition has a negative effect on $N_2$-fixation of intercropped pea, also presumably because of increased competition for light. As annual intercrops are increasingly found in intercropping systems, the effects of light dynamics on biogeochemical processes are emerging as an important interaction.

Shading may influence the quality of legumes Wekesa *et al*., (2015). However, the information about their effect on nodulation and, nitrogen fixation were studied little. Therefor my objective was to examine effect of inoculation and shade on common bean varieties in relationship to growth, grain yield, nodulation and nitrogen fixation.

## 1.2. Objectives

### 1.2.1. General Objective

- To assess the effects of shade condition and Rhizobial strains HB-429 on the nodulation, nitrogen fixation and yield potential of common bean varieties.

### 1.2.2. Specific Objective

- To investigate the differences in nodulation and $N_2$ fixing capacity of inoculated and non-inoculated common bean varieties under shade conditions.
- To evaluate the effect of Rhizobial strains on yield and yield components of common bean varieties under shade conditions.

# 2.LITERATURE REVIEW

## 2.1. Origin and Distribution of Common Bean

Wild common bean occurs from northern Mexico Acosta-Diaz *et al.*, (2015) to northwestern Argentina and distinct differences in both morphological characteristics and molecular markers have been identified in the northernmost and southernmost populations Singh, (1991); Freyre, (1996). The climate where common bean originated is sub-tropical to temperate, with defined wet and dry seasons, and bean prefers regions with moderate rainfall, rather than dry regions or areas with excessive rain Beebe, (2014). Excessive temperatures cause flowers to abscise, and low temperatures delay pod production and can result in empty pods Liebenberg, (2009). Common bean prefers well-drained, sandy clay or sandy loam soils, with balanced fertility and moderate acidity pH 5.8-6.5 Liebenberg, (2009).

Common bean originated from the New World two centers of origin were identified Andean and Mesoamerican Hornakova *et al.*, (2003); Logozzo *et al*., (2007). The domestication occurred independently in South America and Central America/Mexico leading to two different domesticated gene pools, the Andean and Mesoamerican, respectively Papa and Gepts,( 2003); Petry *et al*., (2015). This crop is native to Mexico and Guatemala where the greater part of the diversity of varieties is found Arenas *et al*.,( 2013).

Common bean is the most widely distributed of the related species and has the broadest range of genetic resources Gomez, (2004) and is frequently used as food crop throughout the world, especially in Latin America and Africa. Common bean was introduced to Europe probably from Cuba immediately after Columbus's voyage/ since the first half of the sixteenth century. It was distributed widely in all parts of Europe and the Mediterranean area where many landraces and varieties evolved that were grown to provide dry seed or fresh pods Logozzo *et al*., (2007).

The species was perhaps introduced to the eastern part of Africa by Portuguese traders in the sixteenth century Wortmann *et al*., (2004). Ethiopia is the third largest producer of common beans in Eastern and Southern Africa and the leading exporter in Africa. The country exported 40 percent of its common beans out of the total production in 2010 FAOSTAT, (2015).

### 2.2. Economic Importance of Common Bean in Ethiopia

Common bean is one of the most important cash crops and source of protein for farmers in many lowlands and mid-altitude areas of Ethiopia. Of the country's export earnings estimated for pulses over 85% comes from common bean, exceeding that of other pulses such as lentils, horse (faba) bean and chickpea Negash, (2007). According to FAOSTAT,( 2010) report overall, common bean ranks third as an export commodity in Ethiopia, contributing about 9.5% of total export value from agriculture. Another report showed that common bean is also highly preferred by Ethiopian farmers because of its fast maturing characteristics that enables households to get cash income required to purchase food and other household needs when other crops have not yet matured Gabre-Madhin, (2012).

Ethiopia has a geographic comparative advantage over other competitive countries. It takes nine weeks for sea shipments of beans from China to reach EU markets, whereas it only takes three weeks from Ethiopia Ferris and Kaganzi, (2008). ERCA report shows that common bean exports increased in total value from 19 million $ in 2005 to 134 million $ in 2014, quantity of 43 thousand MT in 2005 to 171 thousand MT in 2014, exhibiting a growth of more than threefold ERCA, (2014).

## 2.3. Production and Management of Common bean

Almost all haricot beans were produced by smallholder farmers CSA, (2015). The average farm size for smallholder farmers is between 0.25-0.5 hectares. There is a wide range of common bean types grown in Ethiopia including mottled, red, white and black varieties Frehiwot, (2010). The most commercial varieties are pure red and pure white colored beans and these are becoming the most commonly grown types with increasing market demand Ferris and Kaganzi, (2008). To support both the growth in domestic and export bean markets, Ethiopian Institute of Agricultural Research EIAR, (2014) has developed a range of high yielding, multi disease resistant bean varieties.

The focus of this genetic improvement program has been on the pure red and white beans to support the commercial sector Ali *et al*., (2003). Within the red bean types, the most favored and most commercially accepted varieties include Red Melka, a mottled medium sized red; Red Wolayita, a medium sized pure light red; and Nasser, a small pure dark red variety Ferris and Kaganzi ,(2008). Among the country pulse crops common bean is the second both cultivated area and in volume of production accounting 21% and 19% respectively CSA,( 2015).

Common beans productivity depends on good weather condition and use of appropriate technologies (fertilizer, improved seed, and herbicide) with the recommended rate and time. The national agricultural research system has generated a number of improved agricultural technologies and recommendations such as crop variety, agronomic practices, crop protection measures as well as other technical advices and practices. The technologies promoted include improved varieties, recommended fertilizer rates and types, improved agronomic and weed control practices. The average common bean productivity was about 1.6 tons $ha^{-1}$ CSA, (2015). However, the experience from experimental plots indicates that 2.5-3.0 tons $ha^{-1}$ can be obtained EIAR, (2014).

The seed yield of bean is the result of many plant growth processes which ultimately influence the yield components such as pods/plant, seeds/pod and unit weight of seed. The highest seed yields were obtained when all the above got maximized Tsubo *et al*., (2004). The spatial distribution of plants in a crop community is an important determinant of yield and many experiments have been conducted to determine the spacing between rows and between plants that maximizes yield Egli, (2008). Two general concepts are frequently used to explain the relationship between spacing, plant density, and yield. First, maximum yield could be only if the plant community produced enough leaf area to provide maximum light interception during reproductive growth Tsubo *et al*., (2004). Secondly, equidistant spacing between plants affected interplant competition Pendleton and Hartwing, (2000). Hence, it will be very important to adjust the spatial distribution of the recommended population in order to have maximum yield. The spacing for haricot bean is 40 cm between rows and 10 cm between plants.

Even though haricot bean has the ability to fix nitrogen it is required to apply additional chemical fertilizers. Depending on the fertility status of the soil, applications of diammonium phosphate (DAP) up to the rate of 100 kg ha-1 and urea up to 50 kg ha-1 are required to enhance productivity. Both fertilizer doses can be side dressed at the side of the planting furrow just prior to sowing. Plants require nitrogen more than any other mineral nutrient because of its critical role in growth and development. Moreover, this element is deficient in most farmers' fields due to losses in leaching and volatilization. Thus, it is essential to use bio fertilizers for haricot bean production in addition to chemical fertilizers. Use of bio fertilizers is one of the natural fertility maintenance methods, which is accomplished by microscopic living organisms that live in association with pulse crops. These microorganisms are bacteria called Rhizobia.

The bacteria live in association with the plants in special structures called nodules. The nodules are found attached to the roots of pulse crops. These bacteria change gaseous nitrogen in to mineral nitrogen, which is a form that can be utilized by the plants.

The field should be weeded twice during the period between two weeks after sowing and flower initiation. Hand weeding is more appropriate for haricot bean though it is possible to use chemicals alternatively. First weeding needs to be done 2-3 weeks after planting. The second weeding should be done before flowering at about 4 to 5 weeks after planting, depending on the varieties. Harvesting should be started when most of the pods (80 to 90% of pods) begin to dry. Walelign,(2015)

### 2.4. Biological Nitrogen Fixation in Legumes

Biological nitrogen fixation (BNF) or symbiotic nitrogen fixation is the process whereby legume crops and specific Rhizobium bacteria (rhizobia) work together to make nitrogen from the soil air surrounding the roots available for use by the plant. The symbioses between rhizobia and legume plants are mainly a mutualistic interaction. Soon after the crop germinates, rhizobia enter the root hairs Long, (1996). Once inside, the bacteria penetrate further into the root through an infection thread. The rhizobia rapidly multiply within the root and the plant responds by forming specialized structures called nodules, in which the rhizobia are contained. The process of root infection and nodule formation is referred to as "nodulation." It may take three to four weeks after seed germination before nodulation is evident on the plant roots. Air held in small pores in the soil contains approximately 78 percent nitrogen in a gaseous form ($N_2$). In this form, the nitrogen is not available for plant use. However, the Rhizobium bacteria in the root nodules can "fix" this form of nitrogen gas by binding it with hydrogen and forming ammonia ($NH_3$), which is available for use by plants. The nitrogen fixation process requires a considerable amount of energy which is provided by the plant. The plant also provides nutrients and water to the rhizobia in the nodules and, in return, the rhizobia provide fixed nitrogen to the plant Vance,( 2002). The amount of nitrogen fixed varies with the type of crop, crop health, the supply of nitrogen already available in the soil, and other environmental conditions. Under ideal conditions, pulse crops can fix as much as 50-80 percent of their total nitrogen requirement, with the remaining nitrogen coming from soil or fertilizer sources. Pulse crops can be ranked according to their estimated ability to fix nitrogen: faba bean > soybean > pea >chickling vetch > chickpea > lentil >lupin> dry bean Vance, (2002). The range of nitrogen fixing varying from 20 to 180 kg N $ha^{-1}$ annually Clarke and Russel, (1977) for example lentil fix 37-45 kg N $ha^{-1}$ and published estimates of chickpea N balance vary enormously, from –47 to +135 kg N $ha^{-1}$ similarly on average, 36% of common bean N can be derived from BNF (%Ndfa) under farmers' field conditions Herridge *et al*., (2008)

The rhizobia can have two lifestyles: as endo-symbiont inside nodules or as free-living saprophytes in the soil or rhizosphere. As endo-symbiont, the rhizobia promote growth of the host plant by supplying fixed nitrogen and in turn rhizobia get carbohydrates and energy from the host legumes.

As common to most pulse crops, common beans have the ability to form a symbiotic relationship with soil bacteria capable of trapping nitrogen gas ($N_2$) from the atmosphere and converting it into ammonia, which can be used by the plant for growth, development and seed production.

Atmospheric nitrogen is converted to ammonia by the nitrogenase enzyme in a process known as biological nitrogen fixation (BNF) Bhatia *et al*.,( 2001). The capacity of legumes to fix atmospheric nitrogen gives them an advantage over non-leguminous crops when grown on soils low in nitrogen (N) content. However, the nitrogen-fixing capacity of common bean varies by variety and is generally less than that of other agronomically important legumes, such as soybeans, which tend to have larger root nodules with higher nitrogenase activity Hardarson *et al*.,( 1993). Common bean roots are colonized by a wide range of native *Rhizobium* species and strains, some of which have little or no nitrogenase activity Ribeiro *et al,*. (2013), and this may be one of the reasons for reduced nitrogen-fixing capacity.

In many regions of the world where common beans are grown, nitrogen fixation is limited by unfavorable soil conditions such as acidity, drought (water stress), floods and high temperature. *Rhizobium* populations, nodulation, ammonium assimilation and nitrogenase activity are all reduced under these conditions Devi *et al*., (2012); Beebe *et al*., (2014); Hungria and Kaschuk, (2014). Low soil phosphorus and manganese levels as well as low soil pH are also associated with sub-optimal nitrogen-fixing capacity Graham and Vance, (2003); Ramos, (2003); Wortmann, (2006). *Rhizobium*-mediated nitrogen fixation can be enhanced by increasing planting density Graham, (1981), Temperature has been reported to affect nodulation, survival and persistence of rhizobial strains in soil. Depending on their natural habitat, tolerance of rhizobia to temperature varies across various strains Mohammadi *et al*., (2012). Additionally, temperatures greater than 40 ºC inhibit infection of root hairs by rhizobia Hungaria and Franco, (1993) and nitrogen fixation Long, (2001). Moreover, nitrogenase activity was lower when common beans formed nodules at

35 ºC Piha and Munns, (1987). There is also evidence that intercropping may inhibit nitrogen fixation by increasing competition for water and soil nutrients Graham, (1981).

In their study, Michiels *et al.,(*1994) attributed ineffectiveness of rhizobium symbiosis at temperatures greater than 40ºC to deletion of the symbiotic plasmids. Low temperatures also limit nitrogen assimilation Gibson, (1965), photosynthate assimilation, malate production, and nitrogenase activity Bordeleau and Prevost, (1994). Moisture stress has greater impact on nodulation and nodule activity than on plant root and shoot metabolism Albrecht *et al*., (1984). Rhizobial strains vary in their adaptability to water stress.

### 2.5. Factors Affecting Biological Nitrogen Fixation

Rhizobium sp. survival in soil is influenced by a combination of factors including acidity, salinity, alkalinity, soil temperature, moisture, fertility - nutrient deficiencies and soil structure Slattery *et al*., (2001). In addition, rhizobia must compete with other soil organisms, including other bacteria, fungi and protozoa.

Soil acidity is the major significant problem for agricultural production in many legumes growing and production areas of the world. Most leguminous plants require a neutral or slightly acidic soil for growth, especially when they depend on symbiotic $N_2$ fixation. Soil pH significantly influences rhizobia content of soils and their ability to nodulate legumes Slattery *et al.*, (2004). Soil acidity reduces nitrogen fixation in legumes, particularly affecting Rhizobium survival in soil and reducing nodulation. The optimum pH for rhizobial growth is considered to be between 6.0 and 7.0, and relatively few rhizobia grow well at pH less than 5.0.

The fast growing strains of rhizobia have generally been considered less tolerant to low pH than have slowly growing strains of Bradyrhizobium Graham *et al*., (1994). Vassilava *et al*.,(1997) reported that, nodulation of legumes is reduced in acidic soil, mainly because of sensitivity of early nodulation events, such as attachment, root hair curling and initiation of infection thread formation.

High temperatures strongly affect bacterial infection and $N_2$ fixation in several legume species. Critical temperatures for $N_2$ fixation are 30°C for clover and pea and range between 35 and 40°C for soybean, guar, peanut, and cowpea Zaharan, (1999). Rhizobium strains differ in their ability to grow, nodulate their host plants and expression of nitrogenase activity at extreme temperatures. Nodulation and symbiotic nitrogen fixation depend on the nodulating strain in addition to the plant cultivar.

Temperature affects root hair infection, bacteroid differentiation, nodule structure, and the functioning of the legume root nodule. High (not extreme) soil temperatures will delay nodulation or restrict it to the subsurface region. For most rhizobia, the optimum temperature range for growth in culture is 28 to 31°C, and many are not capable to grow at 37°C. Rhizobia have a poor growth at temperature below 10 $^{o}$C Graham, (1992). Nodulation in sativum is affected to greater extent by low temperature. In extreme temperature in addition of nodulation and physiological disorders, rhizobial plasmid also loss and genomic rearrangements can be affected.

On the other hand moisture content of the soil can have a significant effect on growth, survival of soil rhizobia and production of crop. Symbiotic $N_2$ fixation of legumes is also highly sensitive to soil water deficiency. Soil moisture deficiency has a pronounced effect on $N_2$ fixation because nodule initiation, growth, and activity are all more sensitive to water stress than are general root and shoot metabolism Giller,( 2001). The response of nodulation and $N_2$ fixation to water stress depends on the growth stage of the plants. It was found that water stress imposed during vegetative growth was more detrimental to nodulation and nitrogen fixation than that imposed during the reproduction stage.

There was little chance for recovery from water stress in the reproductive stage. The wide range of moisture levels characteristic of ecosystems where legumes have been shown to fix nitrogen suggests that rhizobial strains with different sensitivity to soil moisture can be selected. Different Strains of rhizobia and different legume cultivars display differences in tolerance of low water activity Zahran, (1999). Optimization of soil moisture for growth of the host plant is generally more sensitive to moisture stress than bacteria, results in maximal development of fixed-nitrogen inputs into the soil system by the Rhizobium-legume symbiosis. Drought also affects both the number of rhizobia and $N_2$ fixation rates. It is the most harmful abiotic constraints to BNF, mainly due to its effect on soil physical and biological characteristics Zahran,( 1999).

Naturally occurring Rhizobium often exist at populations of 1.0 × 104 to 1.0 × 107 cells $g^{-1}$ of soil Brockwell *et al*.,( 1995). Where naturalized rhizobia are few or absent the introduction of new strains by seed or soil inoculation is normally successful, provided due care is exercised Brockwell *et al*., (1995). On the other hand, where large populations of naturally occurring rhizobia exist, inoculation is invariably futile. At intermediate levels of naturalized rhizobia ($10^{-1}$, 000 $g^{-1}$ of soil) competition between naturalized and introduced rhizobia for nodule formation is of practical concern Brockwell *et al*., (1995). To alleviate this, Brockwell *et al*., (1995) proposed certain desirable characteristics when selecting rhizobial strains suitable for use as inoculants is the ability of rhizobial strains to colonies with the soil and tolerate environmental stresses, ability to compete for nodule formation with background population of rhizobia and ability to form effective nodules that fix N and to have no deleterious effects on non-target hosts.

Strains of rhizobia differ widely in their ability to survive, nodulate and fix N in the soil environment, thus strain selection plays a major part in its survival and infective ability (Brockwell *et al*., 1995).Additionally, population density, effectiveness and competitive ability are primary characteristics of indigenous rhizobial populations that affect inoculation responses (Thies *et al.*, 1991).

**2.6. Relation of Light Intensity with Growth and Photosynthesis**

Light is not only a major source of energy but it also is one of the environmental determinants for plant growth. The light intensity and quality are essential for the plant development, morphology and various physiological responses. Changes in light spectrum have strongly influenced on the leaf anatomy, morphology and physiology Macedo *et al.,(* 2011). In order to adjust to different light regimes of the environment, plants have developed many mechanisms including morphological and physiological changes at various levels Zhang *et al.,(* 2003); Fan *et al.,(* 2013). Low radiation intensity can lead to increase specific leaf area (SLA) and plant height. These adaptations aimed to maximize available light absorption for photosynthesis Steinge *et al., (*2003). Intercropping of legumes with tall cereals may result in shading of the shorter legumes. This will reduce the amount of photo synthetically active radiation that reaches to legumes.

Meanwhile, high radiation intensity is associated with many physiological and morphological characteristics that are appropriate to environmental conditions, such as reduced SLA to protect plants from high radiation exposure; increase leaf thickness by increasing the number of cell layers or increasing the development of palisade and spongy tissue. This modification helps to prevent or mitigate the damage caused by excessive illumination by light energy, ensuring good photosynthesis Matos *et al.,(* 2009). In plant tissues such as stems and leaves, the synthesis of secondary metabolites may change due to physiological, biochemical, and genetic factors in which light is one of the photoreceptors Lefsrud *et al., (*2008).

On the other hand, according to Terashima *et al., (*2009) the light in the red and blue regions of the spectrum are mainly absorbed by photosynthetic pigments. About 90% absorption by plant leaves are blue or red light Terashima *et al., (*2009). Thus, photosynthetic rate, physiology and plant growth, development are significantly influenced by blue or red light Chen *et al.,(* 2014). In most crop plants, even a slight increase or decrease in light intensity leads to considerable changes in photosynthetic characteristics Wu *et al*., (2017). Light intensity affects the central processes of crops such as physiology, biochemistry and cell division Kong *et al*., (2016); Yang *et al*., (2018b); Wu *et al*., (2018). The plant processes impair with decreasing light intensity which bring dramatic developmental and physiological changes, leading to a rapid decrease of these processes Yang *et al*., (2015); Wu *et al*., (2016).. Rates of physiological processes rise while the photosynthetic yield reduces Yang *et al*., (2018a). The photosynthetic rate is the major driver of crop plant carbon balance, optimum and continuous availability of light should also be considered into account to study the plant responses to shade stress.

The main important changes by shade stress in crop growth are ascribed to its huge reduction of net photosynthesis Yang *et al*., (2018b). Reductions in photosynthesis could occur by two main principle mechanisms Yang *et al*.,( 2017) the first one is through decrease of $CO_2$ diffusion into leaves, since the decrease internal and stomata conductance and the second is by metabolic potential inhibition for photosynthesis by inhibiting the leaf growth and enlargement by controlling the cell proliferation Wu *et al*.,( 2017, 2018).

## 2.7. Response of Common Bean to Light

Intercropping of common bean is very common with maize or other cereals in smallholder farming systems of southern Ethiopia. The intercropping effect on component crops are complex, it might be due to competition for sun light (shading), moisture and nutrients. However, evaluation of some of the effects is not easy. Therefore, simulation studies via controlled shades could pinpoint the effects of intercropping systems on the component crops. Intercropping is practiced by the majority of farmers in the tropics and subtropics as a yield maximization and risk minimization strategy. Nearly 80% of all beans produced are grown with maize at some time during the annual crop cycle Francis, (1977). It is mainly intercropped with late-maturing cultivars of maize and sorghum.

The amount of light under the taller cereal canopy changes during the various phenological phases though the variation during the latter phases is smaller. For instance, in late cultivars of maize, the maize crop starts to have a closed canopy starting from six weeks from emergence and continues up to the beginning of physiological maturity. The poorer performance of legumes when intercropped may be partly because the quantity of light reaching their canopy is reduced by the taller companion crop Stirling *et al*., (1990). Dry matter production by a canopy of a given size varies in proportion to the amount of intercepted energy, at a rate governed by its radiation use efficiency Squire, (1990). The review by Laing *et al*.,(1984) indicates that competition for light among components is the most important factor determining productivity of the intercropping system when other factors are non-limiting. As an understory component, the amount of light that reaches the legume crop is largely reduced.

In a maize-bean intercropping, Gardiner and Craker, (1981) indicated that at low (18 000 plants $ha^{-1}$) and high (55 000 plants $ha^{-1}$) maize densities, the amount of solar radiation available for the bean was 50% and 20% of the incident light for the low and high densities, respectively. The highest density was accompanied by 70% yield reduction as compared with the sole crop yield. Chickoye *et al*,.(1996) in their simulation study of competition for photosynthetically active radiation showed that shading of the bean canopy by common ragweed, *Ambrosia artemisiifolia*, accounted for 50–70% of the yield loss when both emerged together.

**2.8. Effect of inoculation on growth, N-fixation and yield of common bean**

Rhizobia inoculation is the process of introducing the appropriate Rhizobium bacteria to the soil in numbers sufficient to ensure successful nodulation Brockwell *et al*., (1995). This is done by coating the seed with a liquid or peat-based powder inoculant, or by treating the soil with a granular or liquid inoculant. Commercial inoculants, when properly applied, ensure that every plant is exposed to a sufficient number of bacteria to initiate nodulation and start the nitrogen fixation process. Rhizobium bacteria are not very mobile in the soil, and thus, the inoculant must come in contact with the developing seedling for infection of root hairs to occur. Specific pulse crops require specific Rhizobium species for nodulation Brkić *et al.*, (2004). Rhizobium species capable of nodulation in lentil and pea crops is not capable of nodulation in common bean. If the wrong Rhizobium species is used, inoculation will have no beneficial effect. Soils commonly lack sufficient numbers of the correct Rhizobium bacteria to optimize the nitrogen fixation process Brockwell *et al*.,( 1995).

$N_2$-fixing legumes are a cheaper and more sustainable alternative to chemical N fertilizers for small-scale farmers. Nodulated legumes are known to contribute substantial amounts of symbiotic N to cropping systems Mohale *et al*,.(2014). The inclusion of nodulated grain legumes like common bean in cropping systems can improve crop yields and thus replenish soil N Maina *et al*,. (2011).$N_2$ fixation in legumes is the second most important biological process after photosynthesis Hayat *et al*,.*(* 2008); Unkovich *et al*,.*(*2008), and contributes N to meet the legume's N demand, as well as for succeeding crops Peoples *et al*,. (2009). Rhizobial strains including Rhizobium leguminosarumbiovarphaseoli are therefore widely used as inoculants to improve growth, symbiotic performance and grain yield of common bean under both glasshouse and field conditions Kellman *et al*,.*(* 2005). However, $N_2$ fixation by the common bean symbiosis with soil rhizobia is hardly adequate to meet plant growth and grain yield Kabahuma,( 2013); Sánchez *et al*,. (2014). This is due, in part, to susceptibility of the crop to nutritional and environmental constraints, its short maturity period, and the ineffectiveness of indigenous soil rhizobia Kabahuma, (2013). Pre-inoculation of seeds with elite rhizobial strains was shown to increase symbiotic performance and grain yield of common bean planted in farmers' fields Akter *et al*,.( 2014); Tabaro,(2014).

# 3. Materials and Methods

## 3.1. Description of the Study Area

The experiment was conducted at Research Farm of Hawassa University, which is located at 273 km south west of Addis Ababa in Sidama Regional State. The site is located 7° 4’N latitude and 38° 31’E longitude and at an altitude of (1692m). The average rainfall of the area is 800 to 1100 mm annually. The average annual maximum, minimum and mean temperature of the area is 27, 12, and 20°C, respectively. The main rainy season extends from April to September and it is interrupted by some dry, sun shine and sometimes from May to July NMA,( 2015). The most common food crop productions in the study area include maize, enset and common bean.

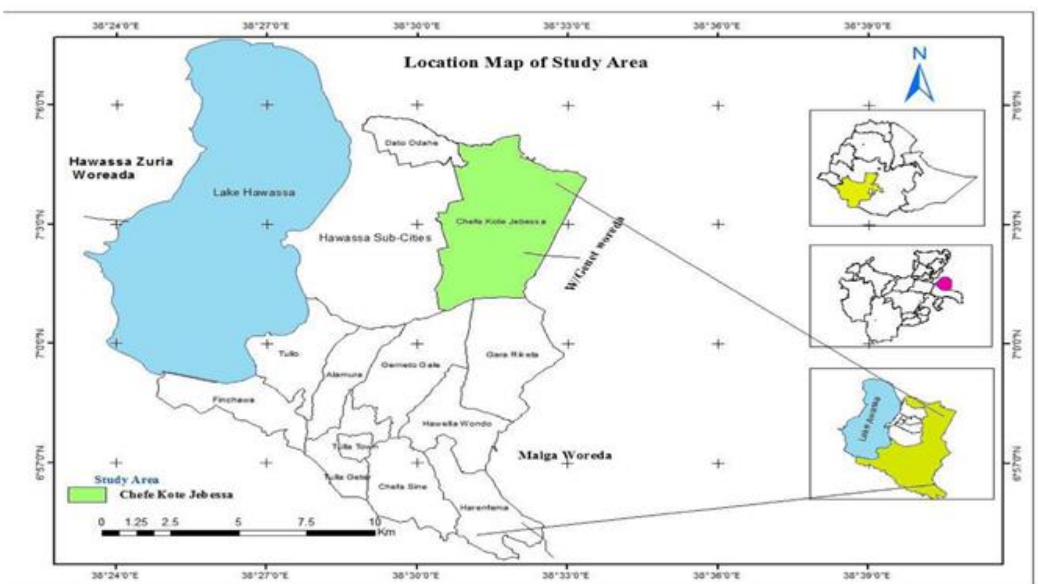


Figure 1: Geographical location of study area Source; Ethio GIS 2015

### 3.2. Description of Experimental Materials

Rhizobium inoculant, HB-429 was obtained from Menagesha Biotech PLC. The inoculant was brought with standard carrier and kept in the laboratory until treated to seed during planting. Triple super phosphate (TSP) was used as a source of $P_2O_5$ fertilizer. The common bean varieties used for the study were Hawassa dume, Nasir and Ibbado.

**Table 1.**Common bean varieties used for the experiment

| Variety | Year of release | Growth duration | Growth habit | Seed color | Released institute |
|---|---|---|---|---|---|
| Hawassa dume | 2008 | 85-110 | Indeterminate semi-bush | Dark red | HRC |
| Ibbado | 2003 | 90-110 | Determinate bush | Speckled-red | HRC |
| Nasir | 2003 | 85-95 | Indeterminate semi-climbing | Dark red | MRC |

HRC-Hawassa research center   MRC-Melkasa research center

### 3.3. Treatments and Experimental Design

The treatments consisted of three common bean varieties (Hawassa dume, Nasir and Ibbado) two levels of shade (open and 25% shade) and two level of bacterial strain (inoculated and non-inoculated). The treatments were arranged in a factorial combination using randomized complete block design (RCBD) with four replications. The recommended P (20 kg $ha^{-1}$) and starter N (23 kg $ha^{-1}$) to facilitate N fixation of common bean were applied at planting Tarekegn *et al*., (2018). The source of P was Triple Superphosphate (TSP; 46% $P_2O_5$), whereas Urea was a source for N. The gross plot size was (1 m × 2.4 m =2.4m$^2$) and the plot had six rows and 20 seeds were sown per row.

The common bean cultivars were sown at 40 cm spacing between rows and 10 cm spacing between plants. The net plot size was (4 rows × 0.4 m = 1.6 $m^2$).

The spacing between blocks and plots was 1.5 and 1m, respectively. This Rhizobium phaseoli strain HB-429 was widely used previously to enhance the symbiotic performance and yield of common bean under field condition, and it is considered as an elite strain for common bean cultivation in Ethiopia Tarekegn, (2012).

### 3.4. Experimental Procedures

The experimental plots were prepared before planting. Urea was used as the source of N and 23kg $ha^{-1}$ was used. At the time of planting, *Rhizobium* inoculant (strainHB-429) was mixed with a sugar solution in which the seed is soaked. The sugar solution ensures the strain adheres to the coating of the seed and that the *Rhizobia* quickly colonize the interior of the plant after germination. The seed inoculation was done just before planting under shade to maintain the viability of cells at the rate of 10 g/kg of seed. Seeds were allowed to air dry for a few minutes before planting to avoid fungal growth. The uninoculated seed was planted first to avoid bacterial contamination with inoculated seeds.

All plots were isolated with ridges to minimize the movement of bacteria from one plot to another. A total of 12 treatment combinations were used in the experiment. The experiments were designed as three-factor experiments in a randomized complete block design (RCBD). There were four replications of each treatment. All standard local cultural practices were accomplished throughout the growth period. Manual weeding was done whenever required. Shading was created by nets which reduced the incoming solar radiation by 25%. The shade level was compared with full sunlight (0% shade).

A destructive sample was taken from the t two rows for estimation of nodulation parameter. While at physiological maturity, plants from the central two rows of a net plot size of (0.4m x 2=0.8m$^2$) were harvested and used for determining yield and yield components, while reference crop wheat newly released variety Fantale was planted in the same area (1x2.4m) plots with 0.2m between rows and 0.05m between plants and consists of a total of 12 rows Tamiru, (2019)

### 3.5. Soil sampling and analysis

Twelve pre-planting soil samples from the experimental site was collected in a zigzag pattern from the depth of 0-20 cm using an auger and the samples were mixed thoroughly to produce one representative composite sample of 1 kg. The soil sample was air-dried and ground to pass 2 mm sieve and analyzed for total N, available P, pH, organic carbon (OC), exchangeable cations and physical properties at Horticoop Ethiopia soil and water analysis laboratory. The soil pH was measured in the supernatant suspension of a 1: 2.5 soil to water ratio using a standard glass electrode pH meter Rhoades, (1982). Organic carbon was determined by Walkley and Black (1934) method. Total N was determined using Kjledahl method Bremner and Mulvaney, (1982). Available P was determined by Olsen *et al.*, (1954) method using ascorbic acid as the reducing agent. The cation exchange capacity (CEC) in cmol (+) kg$^{-1}$ was measured using 1 M-neutral ammonium acetate method Jackson, (1967). The soil-particle size distribution was determined using the Bouyoucos hydrometer method Bouyoucos, (1962).

## 3.6. Data collection

### 3.6.1. Crop Phenology

**Days to flowering (DTF):** was determined by taking the number of days from emergency to the date of 50% of the plants in each plot start flowering.

**Days to maturity (DTM)**: was determined by counting the number of day from sowing to harvesting. Harvesting time was determined when pod color is turned yellow and the seed is matured.

### 3.6.2. Growth parameters

**Plant height (cm):** was measured at physiological maturity from the ground level to the apex of each plant from five randomly selected plants in each plot.

**Shoot dry weight (g):** it was recorded by measuring the above ground biomass at the mid flowering stage from plants that were sampled for nodulation. The samples were placed in labeled perforated paper bags and oven-dried for 48 hours at $70^{o}C$ to constant weight. The averages were recorded as shoot dry weight $plant^{-1}$.

**Leaf area ($cm^2$):** was measured from plants harvested for shoot weight determination from each plot at mid flowering stage. The average of the five plants leaf area was taken as the leaf area of the plant. It was measured by using leaf area meter (Model-LI-3100Area meter, Li-Cor, Lincoln, USA)

**Leaf area index**: was calculated as the ratio of total leaf area to the respective ground area occupied by the crop (40 cm x 10 cm). Then the Specific leaf area (SLA) is the ratio of leaf area per unit of leaf dry weight.

### 3.6.3 .Nodulation parameter

**Number of Nodules**: the total number of nodules was determined from five plants randomly taken from each plot at flowering by carefully digging and exposing with the bulk of root mass and nodules. The roots were washed and the nodules were separated from the soil and roots and the total number of nodules was determined by counting.

**Nodule dry weight**: the nodules dry weight was measured by drying nodules at 70 ºC for 48 hr. then the dry weight was reported as g plant$^{-1}$.

### 3.6.4. Yield and yield components

**Number of pods per plant:** was determined by counting the number of pods per plant of five randomly taken plants from each plot at harvest and then the number of seeds per pod was recorded from five randomly taken pods from each plot at harvest.

**Hundred seed weight (g):** was determined by taking the weight of 100 randomly sampled seeds from the total harvest from each plot and the weight was adjusted to 10% moisture level.

**Total above ground dry biomass**: was obtained from oven dried five sample plants per plot for 48 h at 85 ºC and the weight was converted to t ha$^{-1}$.

**Grain yield (kg ha$^{-1}$):** was recorded from the two central rows after drying and threshing and then the seed yield were adjusted to moisture level of 10%. Finally, yield per plot was converted to t ha$^{-1.}$

**Harvest index (HI):** was computed as the ratio of grain yield (t ha$^{-1}$) to total above ground dry biomass.

### 3.6.5. $N_2$-Fixation Measurements

The N-difference method was used to detect fixed N in common bean and wheat plants. Seed samples of common bean and non $N_2$-fixing reference plants (wheat) were dried and finely ground (<0.1 mm), for analysis of total N (Kjeldahl analysis). Nitrogen fixed by the ND method was calculated as reported by Unkovich *et al.,(* 2008).

Quantity of $N_2$ fixed = (N yield fixing species/legumes) – (N yield non-fixing species/reference)....... (1)

### 3.7. Statistical Analysis

The collected data were subjected to the analysis of variance (ANOVA) using SAS software version 9.0 (SAS Institute, 2002). Mean separation was carried out using least significance difference (LSD) test as described in Gomez and Gomez,( 1984). Moreover, correlation analysis was also carried out to study the nature and degree of relationship between yield and yield components of common bean varieties, shade and inoculation. Correlation coefficient values (r) were calculated and test of significance was analyzed using Pearson correlation procedure.

# 4. RESULTS AND DISCUSSION

## 4.1 Soil properties

The soil textural class of the experimental site was clay loam with average proportions of 31% clay, 28% silt and 41%. The pH value of the study soil was slightly alkaline (pH 7.4). According to Landon, (1991) soils having pH value in the range 5.5 to 7.5 are considered suitable for most agricultural crops. Total nitrogen content (TN) of the study soil was 0.12%, thus classified as medium (0.15-0.25%). As far as soil organic carbon (OC) is concerned, the value was 1.40%, which was within the range of medium organic carbon content Landon, (1991), and the cation exchange capacity (CEC) of the study soil was 18 Cmolc (+) $kg^{-1}$ which is in the medium (15-25 cmol (+) $kg^{-1}$) category.

Table 2. Soil physico-chemical properties of the experimental Site

| Soil characteristics | Values | Status | Reference |
|---|---|---|---|
| pH | 7.4 | Acidic | Tekelign (1991) |
| Total N (%) | 0.12 | Medium | Tekelign (1991 |
| CEC cmol(+) $kg^{-1}$ soil | 18 | Medium | Landon(1991) |
| OC (%) | 1.40 | Medium | Landon(1991) |
| Soil texture | | | |
| Clay (%) | 31 | | |
| Sand (%) | 41 | | |
| Silt (%) | 28 | | |
| Soil texture Class | Clay Loam | Clay loam | Tekelign(1991) |

### 4.2. Phenological and Growth Parameters

#### 4.2.1. Days to flowering

Days to 50% flowering of common bean was significantly ($P<0.001$) influenced by the main effects of shade and rhizobium inoculation (Table 3). However, days to flowering were not significantly affected by the variety.Similarly both the two-way interaction and the three-way interactions did not show significance difference (Appendix Table 1). Inoculated plants relatively early flowered than non-inoculated plants. Similar result was reported that state chickpea early flowers when inoculated Zamans *et al*., (2011).

The results also revealed that none-shaded plants relatively reached 50% flowering early than shaded plants. Increasing shade percentage delayed flowering this is attributed to compensating growth of yield components, when distribution of some yield limiting resources is affected by genetics and environmental factors Van schoonhoven and Voysest,( 1991). Similarly there was a delay in days to flowering under intercropping this might be due to effect of shading from the maize canopy, which thereby slowed down the physiological processes, and higher competition for available light, nutrients, and moisture among the companion crops. Similar results were reported by Ijoyah and Dzer,(2012) in maize/okra intercropping.

#### 4.2.2 Plant height

Plant height was significantly influenced by the main effects of Rhizobium inoculation and shade, however, plant height was not significantly affected by the main effect of variety, the two-way interaction and the three-way interactions (Appendix Table 3). The longest plant heights (43.18cm) were recorded from the plots inoculated with HB- 429 (Table 3). The reasons for increase in plant height under inoculation could be due to the increased vegetative growth with nitrogen fixation. This result is in line with the report of Kubota *et al.,(* 2008) who stated that plant height of soybean was increase with N in the presence of Rhizobium inoculants. Other authors also reported similar results from researches conducted on chickpea and faba bean El-Wakeil and El-Sabai, (2007) indicating that Rhizobium inoculation significantly increased plant height.

Shade had significant ($P \leq 0.05$) effect on plant height (Table 3). The shortest plant height (40.22 cm) was recorded from the open treatment which was significantly lower than the 25%shade treatment. This could be due to low light conditions negatively affected the soybean morphology by increasing plant height and reducing stem diameter which in turn caused soybean lodging especially under intercropping conditions Liu *et al*., (2016)

Table 3.Main effect of variety, shade and rhizobia inoculation on days to flowering (DF) and plant height(PH).

| Treatments | Days to Flowering | Plant Height |
|---|---|---|
| Variety | | |
| Hawassa dume | 46.25 | 42.2 |
| Nasir | 45.87 | 41.19 |
| Ibbado | 45.68 | 40.55 |
| Shade | | |
| Open | 43b | 40.22b |
| 25% | 46.62a | 42.40a |
| Rhizobium | | |
| Inoculated | 45.66b | 43.18a |
| Uninoculated | 46.20a | 39.45b |
| CV(%) | 1.67 | 4.84 |
| LSD | 1.54 | 6.36 |

*CV = Coefficient of variation,ns; non-significant. Means in the same column followed by the same letter(s) are not significantly different at 0.05 % probability level*

### 4.2.3 Days to physiological maturity

Days to physiological maturity was significantly ($P$<0.05) affected by the interaction of rhizobium inoculation and shade (Table 4). However, Days to physiological maturity was not significantly affected by the main effect of variety (Appendix Table 1). Open treatment and inoculation of common bean with rhizobium strains (HB- 429) decreased days to physiological maturity as compared to the shade and non-inoculated treatments.The results are supported by earlier research findings inoculation significantly reduced days to physiological maturity as compared to the non-inoculated in haricot bean Gidago *et al*.,( 2011)

There was slight difference in days to physiological maturity between open and shade treatments. Open treatments had shortest days (86.5) to reach physiological maturity while shade treatment had the longest day (92.83) to reach physiological maturity. Increasing shade percentage delayed physiological maturity this is attributed to compensating growth of yield components, when distribution of some yield limiting resources is affected by genetics and environmental factors.Van schoonhoven and Voysest,(1991).

Table 4. Interaction effect of rhizobium inoculation and shade level on days to maturity

| | Shade | |
|---|---|---|
| Inoculation | Open | 25% |
| Inoculated | 86.5c | 87.66bc |
| Uninoculated | 89.16b | 92.83a |
| CV(%) | 2.13 | |
| LSD | 6.98 | |

*CV = Coefficient of variation,. Means in the same column followed by the same letter(s) are not significantly different at 5 % probability level*

### 4.2.4. Leaf area

The analysis of data revealed significant ($P \leq 0.05$) difference due to interaction effects of variety, shade and rhizobium inoculation on leaf area (LA) and specific leaf area (SLA). The largest LA (1115.5cm$^2$) is obtained from inoculated Hawassa dume variety with shade treatment while the lowest LA (455.6cm$^2$) is obtained from non-inoculated Ibbado variety from open treatment (Table 5). This could be due to that plants that grow in a shady environment invest relatively more of the products of photosynthesis and other resources in leaf area to increase light harvesting and photosynthetic surface Lambers *et al.*, (1998) Similarly the largest SLA (337.14 cm$^2$/g) is obtained from inoculated Hawassa dume variety from the shade treatment while the lowest SLA (112.9 cm$^2$/g) is obtained from non-inoculated Ibbado variety with open treatment. This is due to increasing shade increases SLA. Worku *et al*., (2004) found that SLA increased as an acclimation characteristic to reduce irradiance in common bean.

### 4.2.5. Leaf area index

The analysis of data revealed significant (P ≤ 0.05)difference due to interaction effects of Variety, shade and Rhizobium inoculation on leaf area index (LAI).The largest LAI (2.78cm$^2$) is obtained from inoculated Hawassa dume variety from the shade treatment while the lowest LAI (1.13cm$^2$) is obtained from non-inoculated Ibbado variety from open treatment (Table 5). Shade increase led to higher LAI this is due to plants that grow in a shady environment invest relatively more of the products of photosynthesis and other resources in leaf area to increase light harvesting and photosynthetic surface Lambers *et al.*, (1998).

Table 5. Interaction effect of variety, rhizobium and shade level on leaf area , specific leaf area and leaf area index

| Variety | Shade level | Inoculation | Leaf area($cm^2$) | Specific leaf area($cm^2/g$) | Leaf area index |
|---|---|---|---|---|---|
| Hawassa Dume | open | Inoculated | 657.6cde | 165.32cd | 1.64cde |
| | | Uninoculated | 611.0de | 152.46cde | 1.52de |
| | 25% | Inoculated | 1115.5a | 337.14a | 2.78a |
| | | Uinoculated | 751.9cd | 219.02b | 1.87bcd |
| Nasir | open | Inoculated | 656.2cde | 164.86cd | 1.64cde |
| | | Uninoculated | 573.5ef | 133.77de | 1.43ef |
| | 25% | Inoculated | 844.1b | 228.11b | 2.11b |
| | | Uninoculated | 717.9bcde | 193.59bc | 1.79bcde |
| Ibbado | Open | Inoculated | 616.8de | 159.75cde | 1.54de |
| | | Uninoculated | 455.6f | 112.90e | 1.13f |
| | 25% | Inoculated | 784.5bc | 220.65b | 1.96bc |
| | | Uninoculated | 665.2cde | 190.83bc | 1.66cde |
| CV(%) | | | 14.4 | 17.62 | 14.42 |
| LSD | | | 338.3 | 102.33 | 0.84 |

*CV = Coefficient of variation, Means in the same column followed by the same letter(s) are not significantly different at 5 % probability level.*

## 4.3. Yield and Yield Components of common bean

### 4.3.1. Number of pod per plant (NPPP)

The number of pods/plant was significantly ($P<0.001$) affected by the main effect of Rhizobium inoculation and shade (Table 6). However, both the two-way and three way interaction were not showed significant ($P>0.05$) difference to number of pod per plant (Appendix Table 2). Similarly number of pod per plant was not significantly affected by the main effect of variety. The lowest number of pods/plant (10.95) was recorded from the non-inoculated and the highest number of pod/plant (14.02) was recorded from the Rhizobium strain HB-429 inoculated treatment. The increased pod number with applied inoculants could be associated with enhanced growth and higher assimilate accumulation which resulted from better N nourishment due to symbiotic $N_2$-fixation. The result is in agreement with the work of Malik *et al*.,( 2006) and Dereje, (2007) who concluded that increased number of pods per plant with *Brady rhizobium japonicum* inoculation in soybean. The number of pods/plant was significantly ($P<0.05$) affected by shade (Table 6). The lowest number of pods/plant (11.44) was recorded from 25% shade treatments and the highest number of pod/plant (13.53) was recorded from the open treatments this means that shading had significant negative effect on seed yield and yield related parameters and pod number . This is might be due to the lower photosynthetic rate and biomass accumulation (Khalid *et al*., 2019)

### 4.3.2. Number of seeds per pod (NSPP)

The analysis of variance revealed that number of seeds/pod was significantly ($P<0.001$) affected by Rhizobium inoculation and shade. However, both the two-way and three way interaction were not showed significant ($P>0.05$) difference to number of seed per pod (Appendix Table 2). Similarly number of seed per pod was not significantly affected by the main effect of variety.

The lowest number of seeds/pod (4.12) was recorded from the non-inoculated treatment and the highest (4.89) was recorded from the inoculation of strain HB-429 (Table 6). The result shows that Rhizobium inoculation played an important role in common bean generative growth and therefore made a marked increase in the number of seeds/pod. The significant difference on number of seeds/pod among inoculation and the non-inoculated was in line with the finding of Muhammad,( 2002) who found that, seed inoculation increased the number of seeds/pod in addition to grain yield.

The analysis of variance revealed that number of seeds/pod was significantly $P<0.05$) affected by shade (Table 6). The lowest number of seeds/pod (4.28) was recorded from the 25% shaded treatment and the highest (4.89) was recorded from the open treatments. This means that shading had significant negative effect on seed yield and on number of seed per pod. This is might be due to the lower photosynthetic rate and biomass accumulation. Khalid *et al*., (2019)

### 4.3.3. Shoot dry weight

The analysis of variance revealed that shoot dry weight was significantly affected due to main factors effects of Rhizobium inoculation ($P<0.05$) and shade ($P<0.001$) (Table 6). However, both the two-way and three way interaction was not showed significant ($P>0.05$) difference to shoot dry weight (Appendix Table 1). Similarly shoot dry weight was not significantly affected by the main effect of variety .Inoculation with strain HB-429 gave relatively higher shoot dry weight (22.95g) plant$^{-1}$ that was greater than the non-inoculated. The observed benefits on bean by Rhizobium inoculation seem to be the supply of N to the crop through symbiotic $N_2$-fixation Togay *et al*., (2008). Similar to this result, the research outcomes of Bhuiyan *et al*., (2008) showed that the highest dry matter accumulation on mung bean was obtained from inoculation with Rhizobium. Sharma *et al*.,(2000) reported the significant effect of seed inoculation on dry weight biomass compared to the non-inoculated treatments.

Shading had significant ($P<0.001$) effect on shoot dry matter accumulation of common bean compared to open (Table 6). Thus, 25% shading gave relatively higher shoot dry weight (24.06 g) plant$^{-1}$ that was greater than the open treatment. Increase in shoot dry weight with increasing shade levels could be due to an increase in vegetative growth duration and decreased root/shoot ratio under shade. Sidique *et al*., (1990: Urbas and Zobel, (2000), which allocated more assimilates for shoot rather than root growth.

Table 6. Main effect of variety, shade and rhizobia inoculation on number of pod plant $^{-1}$ (NPP), on number of seed per pod$^{-1}$ (NSP) and on shoot dry weight (SDW) plant$^{-1}$.

| Treatments | NPP | NSP | SDW(g) |
|---|---|---|---|
| Variety | | | |
| V1 | 13.07a | 4.60a | 22.29a |
| V2 | 12.43a | 4.55a | 21.89a |
| V3 | 11.9b | 4.36b | 21.10b |
| Shade | | | |
| S1 | 13.53a | 4.73a | 19.46b |
| S2 | 11.44b | 4.28b | 24.06a |
| Inoculation | | | |
| R1 | 14.02a | 4.89a | 22.95a |
| R2 | 10.95b | 4.12b | 20.58b |
| CV(%) | 10.65 | 8.16 | 13.52 |
| LSD | 5.23 | 1.31 | 7.12 |

*CV = Coefficient of variation, ns; non-significant. Means in the same column followed by the same letter(s) are not significantly different at 5% probability level.*

### 4.3.4. Grain yield

The analysis of variance revealed significant ($P \leq 0.05$) difference due to interaction effects of variety, shade and rhizobium inoculation on grain yield. However, the two way interaction was not showed significant ($P>0.05$) difference to grain yield (Appendix Table 4). The largest grain yield (4.03t $ha^{-1}$) was obtained from inoculated Hawassa dume variety from the open treatment while the lowest grain yield (1.68 t $ha^{-1}$) was obtained from non-inoculated Ibbado variety from shaded treatment (Table 7).The significant increase in grain yield in response to Rhizobium stain HB-429 inoculation may be attributed to the increased availability of N in the soil for uptake by plant roots, through fixed $N_2$. The results coincide with the findings of Sajid *et al*., (2011), who concluded that the treatments with Rhizobium inoculation gave higher grain yield than those of non-inoculated. It may also be due to more number of pods and seeds productions due to Rhizobium inoculation. A similar increasing effect of Rhizobium inoculation on grain yield of soybean has also been reported by Abbasi *et al*.,( 2008). It has been found that shading conditions significantly decreased the soybean yield and yield components Wu *et al*., 2016; Iqbal *et al*., (2018b) similar to our results others has reported that seed yield of soybean plants significantly decreased in shading conditions as compared to normal conditions Yang *et al*.,( 2017).

### 4.3.5. 100 seed Weight

Seed weight was significantly ($P<0.05$) affected by interaction effect of variety, Rhizobium inoculation and shade (Table 7). However, the interaction of shade with inoculation were not showed significant ($P>0.05$) difference to 100 seed weight (Appendix Table 1). Inoculation of common bean by HB-429 increased hundred seed weight as compared to other treatment. The results suggest that Rhizobium inoculation could contribute to increase the efficiency of major nutrient use and thus led to the higher grain weight and yield. Morad *et al*., (2013) also observed that seed inoculation with proper rhizobium at early growth stage stimulated the root nodulation and improved grain weight. In conformity with this result, Tairo and Ndakidemi, (2013) found 91% increase in hundred seed weight by inoculation. The other most important reason for variation in hundred seed weight might be due to genetic characteristics of the varieties for this trait.

Table 7. Interaction effect of variety, shade and Rhizobium inoculation on grain yield and100 seed weight of common bean

| Variety | Shade level | Inoculation | Grain yield(t/ha$^{-1}$) | 100seed weight(g) |
|---|---|---|---|---|
| Hawassa dume | Open | inoculated | 4.03a | 29.12cd |
| | | uninoculated | 2.15g | 27.5cde |
| | 25% | inoculated | 2.95d | 28.25cd |
| | | uninoculated | 1.92h | 25def |
| Nasir | Open | inoculated | 3.55b | 24ef |
| | | uninoculated | 1.98h | 21.75f |
| | 25% | inoculated | 2.67e | 22.25ef |
| | | uninoculated | 1.81i | 20.5f |
| Ibbado | Open | inoculated | 3.16c | 46.5a |
| | | uninoculated | 1.96h | 36.25b |
| | 25% | inoculated | 2.36f | 40.75b |
| | | uninoculated | 1.68j | 30.5c |
| CV(%) | | | 2.71 | 12.79 |
| LSD | | | 0.96 | 9.77 |

*CV = Coefficient of variation,. Means in the same column followed by the same letter(s) are not significantly different at 5 % probability level*

### 4.3.6. Above ground biomass

The analysis of variance revealed significant ($p<0.001$) difference due to interaction effects of variety, shade and rhizobium inoculation on above ground biomass. The heights (6.07 t $ha^{-1}$) above ground biomass was obtained from inoculated Ibbado variety in open treatment while the lowest (2.85t $ha^{-1}$) above ground biomass was obtained from non-inoculated Hawassa dume variety from the shaded treatment (Table 8). Malik *et al*,*(*2006) reported that nitrogen fixation by rhizobium hastened the vegetative growth that improved the yield components which are the possible reason for substantial increase for biological yield . On the other hand, the increments of biomass yield could be due to sufficient nitrogen supply mainly from biological nitrogen fixation. In line with this result (Abbasid *et al*., 2010) also pointed above ground biomass yield of soybean were increased ranging from 39 to 75% by the inoculation of different strains of rhizobia. Similarly, previous researchers have found that biomass accumulation is directly associated with the availability of light intensity and reductions in light decreased the biomass production ((Kiniry *et al*., 2004; Maddonni and Otegui, 2004).

### 4.3.7 Harvest index

The analysis of variance for harvest index showed significant ($P \leq 0.001$) difference among the interaction effect of variety, shade and rhizobium inoculation. However, the two factors was not showed significant ($P>0.001$) difference to harvest index. (Appendix Table 4) The highest harvest index (42%) was recorded from the variety Hawassa dume inoculated under open (non-shaded) condition treatment while the lowest harvest index (21%) was recorded from the variety Ibbado non-inoculated in shade treatment (Table 8). This could be due to the increase of vegetative growth duration and decreased root/shoot ratio under shade which allocated more assimilates for shoot rather than root growth (Sidique *et al*., 1990; Urbas and Zobel, 2000). The highest harvest index (HI) was obtained due to inoculation of HB-429 strain. This may be due to higher partitioning of dry matter into grain. Similar results reported by (Kamara *et al.,* 2007).

Table 8. Interaction effect of variety, shade and Rhizobium inoculation on above ground biomass and harvest index of common bean

| Variety | Shade level | Inoculation | Above ground bio mass (t/ha$^{-1}$) | Harvest index |
|---|---|---|---|---|
| Hawassa dume | Open | inoculated | 4.88c | 0.42a |
| | | uninoculated | 4.17e | 0.28d |
| | 25% | inoculated | 3.66f | 0.34b |
| | | uninoculated | 2.85h | 0.25e |
| Nasir | Open | inoculated | 5.40b | 0.42a |
| | | uninoculated | 4.37d | 0.27de |
| | 25% | inoculated | 4.12e | 0.33b |
| | | uninoculated | 3.36g | 0.23f |
| Ibbado | Open | inoculated | 6.07a | 0.35b |
| | | uninoculated | 4.79c | 0.26de |
| | 25% | Inoculated | 4.13e | 0.29c |
| | | uninoculated | 3.38g | 0.21g |
| CV(%) | | | 1.92 | 3.32 |
| LSD | | | 1.34 | 0.13 |

*CV = Coefficient of variation,. Means in the same column followed by the same letter(s) are not significantly different at 5 % probability level.*

## 4.4. Nodulation parameter

### 4.4.1 Nodule number per plant

Nodules number/plant was significantly (P<0.001) affected by rhizobium inoculation and shade. However, both the two-way and three way interaction was not showed significant (P>0.001) difference to nodule number per plant (Appendix Table 2). Similarly nodule number per plant was not significantly affected by the main effect of variety. The highest nodules number (43.17) was recorded from the strain HB-429 while minimum nodules number per plant (35.98) was recorded with non-inoculated treatment. The mean nodule number per plant was (39.57) (Table 9). The increased nodules number with rhizobium inoculation could be associated with the efficiency of introduced rhizobia to compete with indigenous bacteria dwelling in the soil. These results are in line with the findings of Sajid *et al*.,( 2011) who revealed that the rhizobium inoculation significantly enhanced nodules number.

Shade significantly reduced the number of nodules non-shaded plants possessed more nodules (40.92) than those at 25% shade (38.23) (Table 9). Differences in nodule number per plant due to shading treatments can be attributed to the lower production of roots and hence nodules. Nodule mass of plants grown in highest light was greater than those under shade. Reduction in nodule mass was due both to reduced nodule number and to reduction in the average weight per nodule. Kry and Joel (1980).

### 4.4.2. Nodule dry weight

The analysis of variance revealed that nodule dry weight was significantly ($P<0.001$).affected by rhizobium inoculation and shade. However, both the two-way and three way interaction was not showed significant ($P>0.05$) difference to nodule dry weight (Appendix Table 2). Similarly nodule dry weight was not significantly affected by the main effect of variety Inoculated treatment had the highest nodule dry weight (0.51g) palnt$^{-1}$ while the non-inoculated treatments had the lower nodule dry weight (0.37g) plant$^{-1}$ (Table 9). Similar effects of seed inoculation on nodule dry weight have also been reported by Dereje, (2007) and Bhuiyan *et al*., (2008) on soybean who stated that inoculation significantly increase nodule dry weight of legumes under field condition. According to Fatima *et al*., (2007) high nodule dry weight can be generally a prerequisite for increasing $N_2$-fixation in legumes rather than number of nodules.

Plants that were grown in full light possessed more nodules than those at 25% shade. Differences in nodule number per plant due to shading treatments can be attributed to the lower production of roots and hence nodules. Nodule mass of plants grown in highest light was greater than those under shade. Reduction in nodule mass was due both to reduced nodule number and to reduction in the average weight per nodule. Kry and Joel( 1980).

Table 9. Main effect of variety, shade and rhizobium inoculation on nodule number(NN) and nodule dry weight(NDW).

| Treatments | NN | NDW(g) |
|---|---|---|
| Variety | | |
| Hawassa dume | 42a | 0.47a |
| Nasir | 40a | 0.45ab |
| Ibbado | 36b | 0.41b |
| Shade | | |
| Open | 41a | 0.48a |
| 25% | 38b | 0.40b |
| Inoculation | | |
| Inoculated | 43a | 0.51a |
| Uninoculated | 31b | 0.37b |
| CV(%) | 10.35 | 16.76 |
| LSD | 12.76 | 0.23 |

*CV = Coefficient of variation, ns; non-significant. Means in the same column followed by the same letter(s) are not significantly different at 5 % probability level.*

## 4.5. Percentage of N derived from Biological nitrogen fixation

The results showed greater N content in Hawassa Dume(0.06t) when inoculated with strain HB-429 under open condition, in contrast to the much lower n content in Ibbado(0.02t) (Table 10) in non-inoculated conditions grown under 25% shade. Hawassa Dume was symbiotically more effective than Ibbado, similarly it contributed much more symbiotic N and therefore, produced significantly greater gain yield when compared to Ibbado variety. The increased plant growth as a result of Rhizobium inoculation also resulted in greater grain yield. This was evidenced in this study by the significantly positive correlation between percent N derived from fixation and bean grain yield, as well as significantly marked correlation between amount of N-fixed and grain yield .Rhizobium-inoculated common bean plants accumulated significant amounts of symbiotic N when compared to non-inoculated.

This improved N nutrition from enhanced N supply via $N_2$ fixation by the introduced strains resulted in greater photosynthate production for higher grain yield. These results are consistent with the findings of Kawaka *et al*., (2014), which showed that bean inoculation with an effective strain increased N nutrition and grain yield. These findings are also in agreement with those of Elkocaetal,(2010) and Zafar-Allahetal,(2007),who reported a stimulatory effect of Rhizobium inoculation on growth and symbiotic performances of common bean.On the other hand similar results have been previously reported by lentil and pea Abi-Ghanem *et al*., (2011). This implies that improving nodulation is important traits to enhance the total N in plant tissue. Variation in plant N accumulation among varieties could be due to the presence of variability in BNF among common bean varieties Hardarson *et al*., 1993; Nleya *et al*., (2002).

Similarly previous studies found that plant N concentration in different pulse crops is influenced by the host plant cultivar as well as by Rhizobium strain Hungria and Neves,(1987); Neves*et al*.,(1987); Hardarson*et al*., (1993 ). On the other hand some studies have found that light limitation can decrease the $N_2$-fixation capabilities of legumes Jensen, (1996); Swift *et al*., (2004.). For instance, shading has been shown to decrease $N_2$-fixation rates in white clover Høgh-Jensen *et al*., (2002).

Table 10. Result of N content (%) of treatments

| Variety | Shade level | Inoculation | %N fixed | N conten(t) |
|---|---|---|---|---|
| Hawassa dume | Open | inoculated | 1.67 | 0.06 |
| | | uninoculated | 1.48 | 0.03 |
| | 25% | Inoculated | 1.42 | 0.04 |
| | | Uninoculated | 1.32 | 0.02 |
| Nasir | Open | inoculated | 1.57 | 0.05 |
| | | uninoculated | 1.47 | 0.03 |
| | 25% | inoculated | 1.38 | 0.04 |
| | | uninoculated | 1.26 | 0.02 |
| Ibbado | Open | Inoculated | 1.56 | 0.05 |
| | | uninoculated | 1.45 | 0.03 |
| | 25% | inoculated | 1.34 | 0.03 |
| | | Uninoculated | 1.1 | 0.02 |

The %N fixed was calculated from seed.

## 4.6. Associations between agronomic traits

Grain yield of the experiment was positively and significantly correlated with number of pod per plant (r= 0.84**), number of seed per pod (r=0.75 **), nodule number (r= 0.72**), and nodule dry weight (r= 0.69**) (Table 11). Grain yield had negative significant correlation with date of flowering (r= -0.44**). Leaf area was positively and significantly correlated with shoot dry weight (r= 0.68**), leaf area index (r=0.68**), and specific leaf area (r=0.81** ) (Table 11).

Harvest index exhibited a significant positive association among number of pod per plant (r= 0.49**), number of seed per pod (r= 0.44**), nodule number (r=0.46**), grain yield (r= 0.68**), leaf area (r=0.60**), specific leaf area (r=0.57**) and nodule dry weight (r=0.41**) (Table 11).

**Table 11.Correlation Coefficients of common bean**

| | DF | NPPP | NSPP | NN | SDW | LA | LAI | SLA | SW | NDW | BIOM | GY | HI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DF** | 1.00 | | | | | | | | | | | | |
| **NPPP** | -0.390* | 1.00 | | | | | | | | | | | |
| **NSPP** | -0.44** | 0.67** | 1.00 | | | | | | | | | | |
| **NN** | -0.32* | 0.57** | 0.59** | 1.00 | | | | | | | | | |
| **SDW** | 0.36* | -0.08ns | -0.03ns | -0.03ns | 1.00 | | | | | | | | |
| **LA** | 0.27ns | 0.04ns | 0.05ns | 0.17ns | 0.68** | 1.00 | | | | | | | |
| **LAI** | 0.27ns | 0.04ns | 0.05ns | 0.17ns | 0.68** | 1.00** | 1.00 | | | | | | |
| **SLA** | 0.43** | -0.05ns | -0.01ns | 0.12ns | 0.58** | 0.81** | 0.81** | 1.00 | | | | | |
| **SW** | -0.18ns | -0.01ns | -0.11ns | -0.05ns | -0.23ns | -0.29* | 0.29 | -0.20ns | 1.00 | | | | |
| **NDW** | -0.26ns | 0.59** | 0.69** | 0.53** | 0.04ns | 0.07ns | 0.07 | 0.01ns | -0.004ns | 1.00 | | | |
| **BIOM** | -0.70*** | 0.63** | 0.57** | 0.48** | -0.36* | -0.36* | 0.36 | -0.42** | 0.37* | 0.52** | 1.00 | | |
| **GY** | -0.44** | 0.84** | 0.75** | 0.72** | 0.02ns | 0.16ns | 0.16 | 0.08ns | 0.10* | 0.69** | 0.64** | 1.00 | |
| **HI** | 0.12ns | 0.49** | 0.44** | 0.46** | 0.38** | 0.60** | 0.60 | 0.57** | 0.28* | 0.41* | 0.10* | 0.68** | 1.00 |

DF= Days to flowering NPPP= Number of pod per plant NSPP= Number of seed per pod NN=Nodule number SDW=Shoot dry weight LA=Leaf area LAI=Leaf area index SLA=Specific leaf area SW=1000 Seed weight NDW=Nodule dry weight BIOM= Biomass GY=grain yield HI= Harvest index *ns*, *, ** *and* *** = *non-significant, significantly different at 0.05, 0.01, 0.001 probability levels respectively.*

# 5. SUMMARY AND CONCLUSION

Common bean is particularly suitable for food security due to its short growing cycle and adaptability to different cropping systems. However; the impacts of shade on nodulation, nitrogen fixation of common bean were studied little. The study was conducted at Hawassa University experimental site under field condition during the 2019/20 main cropping season. A field experiment was conducted to evaluate the nodulation, nitrogen fixation and yield potential of common bean variety under shade. The treatments in the experiment were two levels of shade (open and 25 %shade), three common bean variety(Hawassadume, Nasir and Ibbado)and two level of rhizobium inoculation(inoculated and non-inoculated). The experiment was laid out in Randomized complete black design with factorial arrangement with four replication and a total of 12 treatments.

The result of the study revealed that days to 50 % flowering and plant height was significantly ($p<0.001$) affected by shade and rhizobium inoculation but variety as well as interaction effects did not show significance difference. Days to physiological maturity was significantly ($P<0.05$) affected by the interaction of rhizobium inoculation and shade. Number of pods per plant, number of seed per pod, shoots dry weight, nodule number and nodule dry weight was significantly affected by the main effect of Rhizobium inoculation and shade but variety as well as interaction effects did not show significance difference.

The analysis of variance showed significant ($P \leq 0.001$) difference among the interaction effect of variety, shade and rhizobium inoculation on grain yield, 100 seed weight, above ground biomass and harvest index.

The mean value of nodule numbers varied between 38.23 to 40.92for shade and 35.98 and 43.17 for rhizobium inoculation. The highest nodule number was recorded from rhizobium inoculation. The mean value of grain yield varied between 4.03ton $ha^{-1}$ to 1.68ton $ha^{-1}$. The highest grain yield was recorded from Inoculated Hawassa dume variety of open field treatments while the lowest grain yield was recorded from Non-inoculated Ibbado variety of 25 % shade treatments.

There for the result of this experiment indicated that inoculation of rhizobial strain HB-429 combined with variety Hawassa dume in open improves number of nodule, nodule dry weight, shoot dry weight, number of pod per plant, number of seed per pod, grain yield, N content and nitrogen fixation as compared to25 %shade and non-inoculated treatments. This implies that variety Hawassa dume inoculated with rhizobium strain HB-429 in open generated the highest seed yield.

It would be worth while to do similar experiments on sites of the area with the inclusion of more shade levels to refine the findings.

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# 7. APPENDICES

**Appendix 1.**Mean square values of days to flowering, days to maturity, shoot dry weight and 100 seed weight.

| SV | Df | DF | DM | SDW | 100SW |
|---|---|---|---|---|---|
| Rep | 3 | 0.409 | 1.13 | 8.40 | 22.27 |
| Variety(V) | 2 | 1.312ns | 7.64ns | 5.91ns | 1115.69*** |
| Shade(S) | 1 | 22.68*** | 70.08** | 253.64*** | 13.55ns |
| Rhizobium | 1 | 3.52* | 184.08*** | 67.45** | 287.63*** |
| V*S | 2 | 0.56ns | 0.77ns | 2.39ns | 3.42ns |
| V*R | 2 | 0.77ns | 0.02ns | 0.76ns | 86.19** |
| S*R | 1 | 0.02ns | 18.75* | 30.88ns | 35.88ns |
| V*S*R | 2 | 0.27ns | 0.06ns | 0.04ns | 49.63* |
| Error | 33 | 0.59 | 3.59 | 8.65 | 14.09 |
| CV | | | 2.13 | 13.52 | 12.79 |

**, ** &***, ns indicate significant, highly significant and non-significant variations respectively, SV: sources of variations, Df=degree of freedom, Rep=replications, CV= coefficient of variation*

**Appendix 2.**Mean square values of number of nodule, number of pod per plant, number of seed per pod and nodule dry weight.

| SV | Df | NN | NPPP | NSPP | NDW |
|---|---|---|---|---|---|
| Rep | 3 | 23.99 | 0.42 | 0.07 | 0.01 |
| Variety | 2 | 18.94ns | 5.09ns | 0.24ns | 0.01ns |
| Shade | 1 | 86.67* | 52.5*** | 2.36** | 0.08** |
| Rhizobium | 1 | 619.2*** | 113.46*** | 7.13*** | 0.21*** |
| V*S | 2 | 1.52ns | 0.10ns | 0.001ns | 0.0008ns |
| V*R | 2 | 0.40ns | 0.002ns | 0.02ns | 0.0008ns |
| S*R | 1 | 3.96ns | 2.90ns | 0.01ns | 0.0008ns |
| V*S*R | 2 | 6.13ns | 1.35ns | 0.27ns | 0.0008ns |
| Error | 33 | 16.78 | 1.76 | 0.13 | 0.005 |
| CV | | 10.35 | 10.65 | 8.16 | 16.76 |

**, ** &***, ns indicate significant, highly significant and non-significant variations respectively, SV: sources of variations, Df=degree of freedom, Rep=replications, CV= coefficient of variation*

**Appendix 3.**Mean square values of plant height, leaf area, leaf area index and specific leaf area.

| SV | Df | PH | LA | LAI | SLA |
|---|---|---|---|---|---|
| Rep | 3 | 1.09 | 5775 | 0.03 | 1367.5 |
| Variety | 2 | 11.05ns | 94699** | 0.59** | 10155.8** |
| Shade | 1 | 57.13** | 570571*** | 3.56*** | 83429.2*** |
| Rhizobium | 1 | 167.29*** | 269790*** | 1.68*** | 24888.1*** |
| V*S | 2 | 1.88ns | 20360ns | 0.12ns | 3909.4* |
| V*R | 2 | 0.26ns | 10410ns | 0.06ns | 1223.9ns |
| S*R | 1 | 8.40ns | 33869ns | 0.21ns | 2801.6ns |
| V*S*R | 2 | 0.50ns | 35159* | 0.21* | 4290.5* |
| Error | 33 | 3.99 | 10307 | 0.06 | 1118.8 |
| CV | | | 14.42 | 14.42 | 17.62 |

*, ** &***, ns indicate significant, highly significant and non-significant variations respectively, SV: sources of variations, Df=degree of freedom, Rep=replications, CV= coefficient of variation

**Appendix 4.** Mean square values of grain yield, above ground biomass and harvest index

| SV | Df | GY | AGB | HI |
|---|---|---|---|---|
| Rep | 3 | 13649 | 1574 | 0.046*** |
| Variety | 2 | 61909*** | 319506*** | 0.011*** |
| Shade | 1 | 271472*** | 3569752*** | 0.079*** |
| Rhizobium | 1 | 648*** | 1522969*** | 0.0003ns |
| V*S | 2 | 8532** | 49190*** | 0.0045*** |
| V*R | 2 | 22613*** | 10769** | 0.0086*** |
| S*R | 1 | 1043*** | 26602*** | 0.0014*** |
| V*S*R | 2 | 2630** | 15502*** | 0.0012** |
| Error | 33 | 396146 | 1035 | 0.0001*** |
| CV | | 2.71 | 1.88 | |

*, ** &***, ns indicate significant, highly significant and non-significant variations respectively, SV: sources of variations, Df=degree of freedom, Rep=replications, CV= coefficient of variation

# SKETCH OF BIOGRAPHY

The author was born in 1980 at Negele borana, in the South Zone of Oromia, Ethiopia. She attended elementary education (grade 1-8) at Ogoba primary school at Negelle borana. After completion of elementary education, she joined Ambo secondary school at ambo and attended grade 9-12. In 2001 she was one among the students who have joined Hawassa University College of Agriculture to follow first degree and she graduated with the Bachelor of Science in Plant science in July 3, 2011. Selamawit has served as junior researcher for five years from 09/02/2015 till now in Ethiopia Biodiversity Institute. Then, by the sponsorship of Ethiopia Biodiversity Institute she joined Hawassa University in October 2018 to pursue her Degree of Masters in Plant Sciences (Specialization Agronomy) under school of postgraduates of Hawassa University, Ethiopia.