SYMBIOTIC EFFECTIVENESS OF ELITE *Rhizobia* STRAINS ON PRODUCTIVITY OF IMPROVED CHICKPEA (*Cicer arietinum* L.) VARIETIES.

CASE OF DEBRE ZEIT AND WOLAYTA SODO, ETHIOPIA.

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MASTER OF SCIENCE

(Research Methods)

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

2016

Symbiotic Effectiveness of Elite *Rhizobia* Strains on Productivity of Improved Chickpea (*Cicer arietinum* L.) Varieties. Case of Debre Zeit and Wolayta Sodo, Ethiopia.

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Masters of Science in Research Methods at Jomo Kenyatta University of Agriculture and Technology.

2016

DECLARATION

I declare that this project research hereby submitted by me for the Degree of Master of Science (MSc) in Research Methods to the School of Graduate Studies of Jomo Kenyatta University of Agriculture and Technology is my own independent work and has not previously been submitted by me or anybody else at another university. The materials obtained from other sources have been duly acknowledged in the thesis.

Signature _____ Date: _____

Assefa Funga Alemu

This thesis has been submitted for examination with our approval as supervisors

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1. Signature _____ Date: _____

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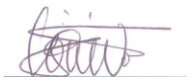
2. Signature  Date: 2016.05.11TH

   Dr. Ojiewo Chris

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   Prof. Githiri Stephen Mwangi

DEDICATION

This research work is dedicated to my beloved wife Mebrat Demissie, my son Bereket Assefa, my daughters Hasset Assefa Imla Assefa also to my parents, brothers and sisters

ACKNOWLEDGEMENT

I take this opportunity to express my gratitude to my supervisors Prof. losenge Turoop (Phytopathologist, JKUAT), Dr. Chris Ojiewo (Legume breeding senior scientist, ICRISAT-Ethiopia) and Prof. Githiri Stephen Mwangi (Plant Geneticist/Breeder, JKUAT) for their constant expert guidance, advice and support through all the stages of the research work. I have special thanks for Dr. Chris Ojiewo not only because of his being my supervisor for the research work but also for his unreserved support and advise starting from the inception of application for the training till the time of my flight to Nairobi. I also wish to extend my appreciation to Dr. Million Eshete (highland pulse crop research team leader, DZARC), Dr. Negash Demissie (biofertilizer production Researcher, DZARC) and Mr. Tesfaye Geleta (Seed Science Technology researcher, DZARC) for their expert consultancy material support.

My gratitude also goes to all technicians in chickpea and lentil research case team; ICRISAT technical staffs at Debre Zeit and laboratory technicians in soil and water laboratory of Debre Zeit Agricultural Research Center for their genuine support provided to me during my research. I also sincerely thank my sister Asnaku Funga, Mr. Mengistu Demissie and Mr. Lijalem Korbu for the keen effort they made in settling issues of my study leave in my absence and uninterrupted support to my every inquiry throughout my study period. My gratitude also goes to RUFOURM for funding my course research work.

Appreciation also goes to my wife, Mebrat Demissie for her support during long period of my study. Special thanks also go to my beloved son Bereket Assefa and daughter Hasset Assefa; they made my work easier and tolerated my absence from home at time when they

needed me most. Above all my special thanks praise with humbled heart go to the Almighty God without His great protection and abundant mercy towards me and my family, this research work would not come to reality. Again and again I love to say to my God Creator of the universe thank you.

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ABBREVIATIONS AND ACRONYMS

AAS: atomic absorption spectrometry

ATP: adenosine triphosphate

BMY: Biomass yield ($kgha^{-1}$)

BNF: Biological nitrogen fixation

CEC: cation exchange capacity

CSA: Central Statistical Agency

DF: Days to 50% flowering

DTM: Days to 90% maturity

DZARC: Debre Zeit Agricultural Research Center

GHI: Grain harvest index

GY: Grain yield ($kgha^{-1}$)

GYCT: Grain yield of control treatment (tons ha^{-1})

HSW: Hundred seed weight (gm)

ICARDA: International Centre for Agricultural Research in Dry Area

ICRISAT: International Crop Research Institute for Semi Arid Tropics

JKUAT: Jomo Kenyatta University of Agriculture Technology

m.a.s.l: meter above sea level

MOA: Ministry of Agriculture

N: nitrogen

NCPP: Nodule count plant per plant

NDW: Nodule dry weight (gm $plant^{-1}$)

NGO: Non Government Organization

NPP: Number of pods per plant

NSP: Number of seeds pod^{-1}

P: Phosphate

PGYI: Percent grain yield increased (%)

PGYI: percentage grain yield increased (%)

PLHT: Plant height (cm)

REML: Residual Maximum Likelihood

SHDW: Shoot dry weight (gm $plant^{-5}$)

SHNC: Shoot nitrogen content (%)

SHNY: Shoot nitrogen yield (gm $plant^{-5}$)

SSA: Sub Saharan Africa

TSP: Triple Super Phosphate

DZ MAXT: Debre Zeit maximum temperature (^{o}C)

DZ MINT: Debre Zeit minimum temperature (^{o}C)

WS MAXT: Wolayta Sodo maximum temperature (^{o}C)

WS MINT: Wolayta Sodo minimum temperature (^{o}C)

ABSTRACT

Chickpea (*Cicer arietinum* L.), is a multi-functional crop with important role in the diet as affordable protein source and in sustaining soil fertility through nitrogen fixation. However, its productivity in Ethiopia of 1.9 t ha^{-1} is lower than its potential of 5.5 t ha^{-1} under well managed conditions, partly due to soil fertility limitations. The study was designed to evaluate effectiveness of elite *rhizobia* strains on productivity of improved chickpea (*cicer arietinum* L.) varieties at Debre Zeit and Wolayta Sodo, Ethiopia. The experiment was conducted at two sites, Debre Zeit (8.73° N, 38.97° E, 1900 m.a.s.l) and Wolayta Sodo (7.04° N, 37.2° E , 1880 m.a.s.l) using three chickpea verities (Natoli, Teketay and ICC-4918) with four levels of rhizobia strains (three indigenous and one commercial inoculants) and one control (non inoculated treatment) arranged in split plot design with three replications. Chickpea varieties as main plot and the rhizobial inoculants as sub plots were assigned to experimental unit randomly. To measure the response of chickpea against the treatments, data were collected on various symbiotic, phenological and yield and yield related traits of chickpea and subjected to statistical analysis using linear mixed model. Inoculated plants produced significantly ($p<0.05$) most of symbiotic, grain yield and yield related traits than non-inoculated treatments. Shoot nitrogen yield was increased in the range of 13.0 – 31.34% by inoculation with strain ICRE-025 over the two test sites. The highest level of N fixation was achieved in genotype ICC-4918 by inoculation with EAL-029 and ICRE-025 which entails that the genotype is of good nitrogen assimilator. Similarly, inoculation enhanced grain yield by 17 - 42%, over control treatment. The highest level of grain yield improvement was achieved by inoculation of chickpea variety with ICRE-05 (42%) and ICRE-03 (36%) at Debre Zeit and Wolayta Sodo respectively. In general, depending on the type of chickpea variety used yield improvement in the context of this finding additional 314 – 1252 $kgha^{-1}$ chickpea grain yields would be

produced due to *rhizobial* inoculation. Investigations at both test sites demonstrated that inoculation of chickpea varieties with native *rhizobial* strains were effective and useful for optimized chickpea production.

CHAPTER ONE

INTRODUCTION

1.1. Background

Chickpea (*Cicer arietinum* L.) is a major grain legume cultivated for its edible seeds. Across the world chickpeas are mainly cultivated in the cool, dry season of the semi-arid tropics on residual moisture. The plant is well adapted to tropical climates with moderate temperatures and is successfully cultivated under irrigation in the cool season of many tropical countries (Bejiga and Van der Maesen, 2006). Well-aerated sandy to sandy loam soils and black cotton soils with a pH ranging from 5 to 7, or even higher, are suitable but salinity and sodicity should be avoided (Damiani at el., 2013; van der Maesen, 1989). Chickpea can grow in places where annual rainfall ranges from 500 to 1800 mm (Bejiga and Van der Maesen, 2006). It is tolerant to drought but does not withstand the humid and hot lowland tropics. Rainstorms during flowering, which may occur during the monsoon season, may harm the crop that is then used mainly for fodder (van der Maesen, 1989). Early summer heat or frost during flowering may also reduce crop yield (Damiani at el., 2013).

Ethiopia being secondary center of origin (Anbessa and Bejiga, 2002; ICARDA, 2009) is one of the largest chickpea producing countries of the world and ranks first in Africa. It is widely grown in the central, northern and eastern highland areas of the country at an altitude range of 1400-2300 m.a.s.l., where annual rainfall ranges between 500 and 2000 mm (Bejiga and Van der Maesen, 2006; Anbessa and Bejiga 2002).

India, Australia, Ethiopia, Iran, Canada, Malawi, Argentina, Algeria, Kazakhstan and Italy are the 10 top producers of chickpea (FAOSTAT 2015). About 14.80 millions of hectare land was devoted for production of 14.24 million of tons of chickpea grain implying that the average productivity of chickpea is 0.96 tons ha^{-1} globally (FAOSTAT, 2015). In Ethiopia chickpea has yield potential of 5.5 tons ha^{-1}on experimental stations (Belay, 2006), though actual productivity is as low as 1.9 tons ha^{-1} (FAOSTAT, 2015). This yield gap between average and potential yield of chickpea could be due to many factors like poor agronomic practices, low soil nutrient, absence of compatible strains, and low population numbers of rhizobia, low infectivity or lack of effectiveness, poor survival rate of rhizobia in the soil or competition amongst strains of rhizobia.

From stand point of environmental impact chickpea is nitrogen fixing legume often used to restore soil fertility before cereal or oilseed crops. It is used as disease cycle breaker and helps to reduce pesticide and herbicide usage (Damiani at el., 2013). Facts from different source indicate that chickpea fixes atmospheric nitrogen in the range of 60 - 140kg N ha^{-1} in one cropping season (Crouch et al., 2004; Shiferaw et al., 2004; Burton, 1984. There is increasing evidence that suggests that more nitrogen can be fixed by existing legume grain crops if they are inoculated more often or with more effective strains of rhizobia (Brockwell et al., 1988). Additionally, Ayaz et al (2010) reported that inoculation of seed with its own specific and suitable *Rhizobium* strain before planting is crucial to fully benefited from grain legume crop in terms of maximum yield and soil improvement. Nevertheless, the process of nitrogen fixation is sensitive to Phosphorus (P) deficiency, which results in reduced nodule mass and low ureide production (Vance, 2001). P plays an important role in N cycling as adenosine triphosphate (ATP) is required in large quantities

for legumes to undergo N fixation (Sinclair and Vadez, 2002). However, study made on some highlands vertisols of Ethiopia including central highlands revealed that there is low availability of P in the soil which can reduce crop productivity in the study area (Mamo and Haque, 1988; Melese et al., 2015).

Previous studies conducted at three regions of Ethiopia (Oromia, Gondar and South Nations, Nationalities and People Region) show some strong responses to inoculation for chickpea (Ali et al, 2004 as cited in Ronner and Giller, 2013). Studies in other countries also indicate that chickpea generally responds well to inoculation, with increases in grain yield from 8 to 40% found in Iran and Canada. There is also an evidence of an 8 to 10% increase in stover yield in Iran and Pakistan. In combination with P-fertilizer (90 kg/ha), grain yields in Pakistan even increased from 1600 to 3100 kg/ha and stover yields from 4350 to 7500 kg/ha (Ali et al, 2004 as cited in Ronner and Giller, 2013). Erman et al (2011) also reported that inoculation with *rhizobium* and *mycorrhiza* improved both grain and stover yields by about 60% in Turkey.

In this regard Ethiopia has greater potential of benefiting from enhanced BNF that may contribute to increased yield and sustained soil fertility. This is because larger area is devoted to grow chickpea as compared to other African countries (FAOSTAT, 2015); there are many rhizobia strains that have been identified and proven to be effective and competitive (Wolde-Meskel, 2012); there are also a number of high yielding released varieties (Hailemariam and Tsige, 2006). However, use of high yielding varieties alone may not result in the required level of productivity without inclusion of compatible and

effective rhizobia strains (Mulongoy, 1995: Shantharam, 1997). A given legume cultivar nodulated by different strains of the same species of rhizobium would fix different amounts of nitrogen. And it is also true that a given strain of *Rhizobium* will nodulate and fix different amounts of N in symbiosis with a range of cultivars of the same plant species (Mulongoy, 1995).

A number of edaphic, climatic, and biotic factors inhibit N fixation. Among these, the absence of specific and effective rhizobia in the soil is the most important (Mulongoy, 1995). Keneni et al (2013) has also suggested that there may be the need to conduct specific tests for specific breeding materials, strain and environments to improve the selection process to gain host genotype compatible to specific rhizobia strain that eventually enhance BNF and subsequently ensure productivity and sustain soil fertility. However, our knowledge on effective rhizobia strains for efficient symbiosis with chickpea varieties is scanty. Besides, the efforts to test and recommend rhizobia inoculums on-farm have been limited. Chickpea farmers therefore traditionally produce the crop without the use of biofertilizers. This study is therefore, evaluated the effectiveness of elite *rhizobia* strains on productivity of improved chickpea (*Cicer arietinum* L.) varieties two locations.

1.2 Statement of Problem

Demand for food production is projected to increase by about 70 percent globally and nearly 100 percent in developing countries by 2050 (Dubois, 2011). This incremental demand for food, together with demand from other competing uses, will place unprecedented pressure on many agricultural production systems across the world. Consequently, land and water resources are often constrained by unsustainable agricultural

practices. Thus particular attention and specific remedial action are required (Dubois, 2011).

Continual mining of soil nutrient caused by growing agricultural crops in developing countries implies depletion of macronutrients and micronutrients which in turn result in a decline of the soil fertility (Hartemink, 2006). Regarding to this, the estimates of soil nutrient losses in Sub Sahara Africa (SSA), Asia and Latin America suggest a net removal of between 20 and 70 kg ha^{-1} of N from agricultural land each year, and these losses are likely to increase. Depletion of soil fertility is the most fundamental cause of low per capita food production in SSA. (Serraj *et al*, 2004; Shiferaw *et al*, 2004).

Ethiopia with a population size of more than 90 million and forecast growth rate of over 2% per annum to 2030 (Economy, 2011) need to produce more to feed its ever increasing population. However, as any developing country, agricultural productivity of the country is low as a whole; in particular chickpea, even though it has yield potential of 5.5 tons ha^{-1}on experimental stations (Belay, 2006), actual productivity is as low as 1.9 tons ha^{-1} (FAOSTAT, 2015) which is below half of the potential productivity of chickpea crop. This yield gap between average and potential yield of chickpea could be due to many factors like poor agronomic practices, low soil nutrient, absence of compatible strains, and low population numbers of rhizobia in the soil, low infectivity or lack of effectiveness, poor survival rate of rhizobia in the soil or competition amongst strains of rhizobia. So there is the need of alleviating such chickpea productivity constraints through identifying specific and compatible elite rhizobia strains proved to be capable of establishing effective symbiosis with chickpea and eventually increase yield and sustained soil fertility.

As matter of fact, Ethiopia has greater potential of benefiting from the output of biotechnology such as biofertilizers than other African countries. This is because larger area is devoted to grow chickpea as compared to other African counties and also there are many indigenous rhizobia strains that has been identified and proven to be effective and competitive (WOLDE-MESKEL, 2012; Hailemariam and Tsige, 2006). However, our knowledge on effective rhizobia strains for efficient symbiosis with chickpea varieties is scanty. Besides, the efforts to test and recommend rhizobia inoculums on-farm have been limited. Chickpea farmers therefore traditionally produce the crop without the use of biofertilizers. This study was conducted to investigate effectiveness of elite *rhizobia* strains on productivity of improved chickpea (*Cicer arietinum* L.) varieties at two locations of Ethiopia.

1.3 Objective of the study

1.3.1 General Objective

The general objective of the study was to investigate symbiotic effectiveness of elite rhizobia strains on productivity of improved chickpea (*Cicer arietinum* L.) varieties in Central highlands of Ethiopia

1.3.2 Specific objectives

1. To evaluate effectiveness of rhizobia strains for symbiotic nitrogen fixation with chickpea varieties in terms of nitrogen uptake.
2. To assess yield increased as a result of inoculating chickpea variety with rhizobia strain.

1.4 Research hypothesis

1. All rhizobium strains are effective for symbiotic nitrogen fixation with all chickpea variety in nitrogen uptake.
2. There would be no yield difference between inoculated and uninoculated chickpea variety in terms of grain yield

1.5 Justification of the Study

This study was conducted to find out ways of enhancing BNF through investigating appropriate inoculant technologies that enable chickpea crops to benefit fully from biologically fixed nitrogen for its growth and subsequently much greater gains in productivity.

The outputs of this study thus contribute significantly to the development of sustainable crop production and improved soil fertility. This in turn reduce yield gaps and soil fertility gaps, without draining soil nutrients while minimizing inputs from external sources. Furthermore, this will help resource poor farmers to overcome the socio-economic limitation regarding access to chemical fertilizer, by providing alternative or complementary sources of nitrogen. Therefore, this study will provide a comprehensive information and understanding on BNF technology, and will be useful to all farming communities, extension workers, students, researchers and scientists involved in BNF enhancement and application of the technology. Further, this study will open chance for future research on related field.

1.6 Scope and Limitation of the Research

This study focuses only on investigation of most appropriate compatible combinations between host plant and rhizobia strain in the effort to ensure sustainable agricultural productivity while maintaining soil fertility though realization of enhanced BNF technology. Therefore, its scope is limited to only improved chickpea varieties developed from germplasm introduced from International Crop Research Institute for the Semi-Arid Tropic (ICRISAT) and International Center of Agricultural Research in the Dry Areas (ICARDA). In terms of area coverage it is also limited to government and non government organizations (NGOs) involved in agricultural activities and farming communities in the mandate area of Debre Zeit Agricultural Research Center (DZARC) and Wolayta Sodo districts from southern region of Ethiopia. However, the result of this study can be used as a reference for other similar areas.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Inclusion of legumes in a cropping system alone does not ensure high BNF. Use of improved crop varieties, improved soil and water management, application of essential nutrients are required to achieve maximum efficiency of BNF. *Rhizobium* inoculation or selection of host genotypes to ensure a higher proportion of nitrogen fixation in the plant is another approach to harness BNF (Rao, 2014).

This study therefore, aimed to find out symbiotic effectiveness of elite rhizobia strains on the productivity of chickpea crop through inoculation to enhance biological fixation of nitrogen. In doing so crop utilizes the fixed nitrogen for its physiological development and that will eventually result in an increased yield. At the same time environmental sustainability will be attained. Thus this review presents discussion focusing on published and unpublished resources that give understanding of enhancing BNF, limiting factors affecting BNF, role of major plant nutrients such as nitrogen and phosphorus in production of legume crops particularly chickpea.

2.2 Chickpea

2.2.1 Origin, Distribution and Global Production Trend

Chickpea, *Cicer arietinum* (L.), one of the most important cool season crops, is believed to have originated in present-day south eastern Turkey and adjoining Syria where several of its natural species are found (Van der Maesen et al, 1987). The crop later spread to India, Europe and subsequently reached Africa, Latin and central American countries. Chickpea

is a self pollinated annual crop that can complete its life cycle in 90 to 180 days depending on the prevailing meteorological conditions. Chickpea can be grown under a wide range of agroclimatic conditions around the world (Singh and Diwakar 1995). Chickpea growing areas can be classified into the following four major geographical regions: Indian subcontinent; West Asia, North Africa, and southern Europe; Ethiopia and East Africa; and the Americas and Australia (Singh and Diwakar 1995).

The crop may grow to a height of 30-70 cm depending on the suitability of the growing environment. It has small feathery leaves on both sides of the stem and sometimes with 2-3 bisexual flowers in a node. The flowers may be white, pink, purple or blue in color. The crop may set 1-3 seeds in a pod and the seed is a good source of protein (23%), carbohydrates (64%), fat (5%) and crude fiber (6%). It also contains 340 mg of phosphorus, 190 mg of calcium, 140 mg of magnesium, 7 mg of iron and 3 mg of zinc in 100 g of seed (Bejiga and van der Maesen, 2006). Chickpea needs a subtropical or tropical climate with well drained fertile soils having pH of 5.5 - 8.6 (Kassie *et al*., 2009; Muehlbauer and Tullu, 1997).

Currently, chickpea is produced worldwide and it is the world's second most important food legume next to dry bean (FAOSTAT, 2015). In 2014, chickpea was grown on about 14.8 million hectares of land across the world with an average productivity of 0.96 ton ha^{-1} globally.

During 1994 – 2005, the annual chickpea production increased by 1.87% and likely to increase (Kassie et al. 2009). Chickpea is among the widely cultivated pulse crops by small

scale farmers of the semi-arid tropics (Anbessa and Bejiga 2002). Generally, Desi and Kabuli types are the two major types of chickpea grown in the world with major differences in seed size, seed colour, surface and thickness of the seed coat. The Desi type is characterized by small seeds with angular appearance, sharp edges and varying colours but usually light brown. On the other hand, the Kabuli type produces large round seeds of white or pale cream or yellow colour.

Nutritionally chickpea provides high quality protein and starch to the predominantly vegetarian population in India and large population sectors in other South Asian and Near-East countries and is considered as health food in developed nations. Chickpea does not contain any specific major anti nutritional factors such as ODAP in grasspea (*Lathyrus sativus L.*), vicin in faba bean (*Vicia faba*), and trypsin inhibitors in soybean (*Glycin max*), although it has oligosaccharides which may cause flatulence (Kumar & Abbo, 2001).

2.2.2. Production and Roles of Chickpea in Ethiopia

In Ethiopia, chickpea is mainly grown in the central, northern and eastern highland areas of the country at an altitude of 1400-2300 m.a.s.l., where annual rainfall ranges between 500 and 2000 mm (Bejiga & van der Maesen, 2006; Anbessa and Bejiga 2002). During the 2014/15 cropping year, Ethiopia produced 458,682.26 tons of chickpea on 239,747.51 ha of land (CSA, 2015). The average productivity of Ethiopian chickpea in the same year was about 1.9 tons ha^{-1} which is below half of the 5.5 tons ha^{-1} that can be produced potentially (Belay, 2006).

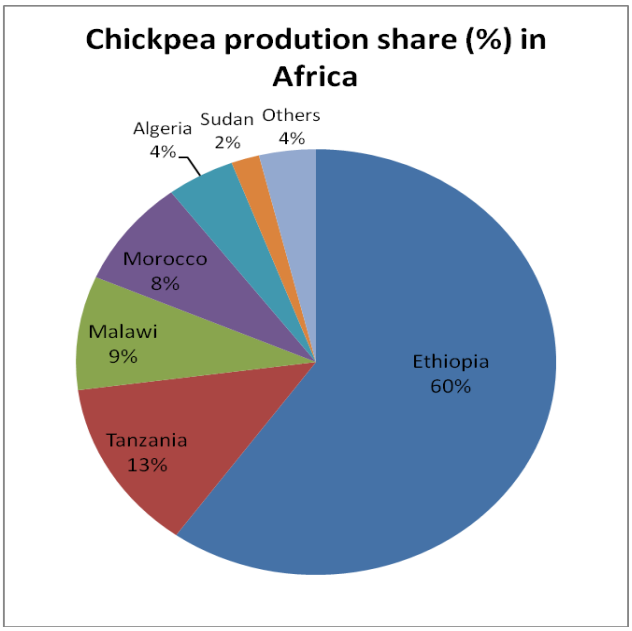

Figure 2.1: Production share of major African chickpea producing countries in 2014

Adapted from FAOSTAT, 2015

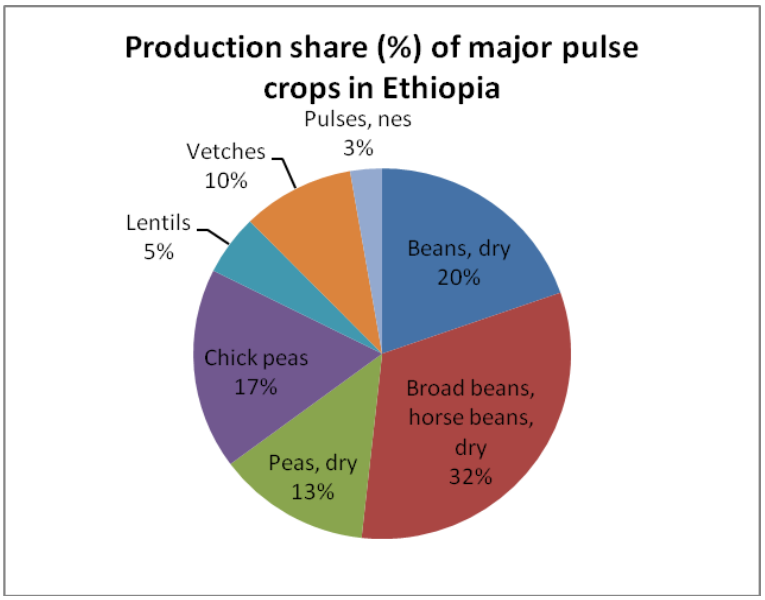

Figure 2.2: Production share of major pulse crops in Ethiopia in 2014

Adapted from FAOSTAT, 2015

The crop can be grown on different soil types as long as good drainage is ensured. However, to achieve optimum growth well drained black soils (usually Vertisols) are

identified as the most suitable soil type (Kassie et al. 2009). In Ethiopia, chickpea is mostly grown on vertisols which have good water holding capacity. Ethiopian farmers essentially plant chickpea on residual moisture after the end of the main rainy season, which is usually in late August -October. Of the two types of chickpea, traditionally the Desi types are more widely cultivated in Ethiopia (Kassie et al. 2009).

Chickpea, a multi-functional crop, has an important role in the diet of the Ethiopian small scale farmers' households and also serves as protein source for the rural poor who cannot afford to buy animal products. The crop also serves as a source of cash income and plays a major role in Ethiopia's foreign exchange earnings through export to Asia and Europe. Its straw is also used for animal feed and due to its capacity of biological nitrogen fixation; chickpea can improve the soil fertility status (Pundir and Mengesha 1995).

Recent research shows that chickpea can fix more nitrogen than other pulse crops, thus enhancing soil fertility for subsequent crops (Machado et al, 2004). The *Rhizobium* bacteria that are compatible for nodule formation on the roots of chickpea are different from those that nodulate peas and lentils. Thus inoculating chickpea seed with the correct inoculant is critical. In spite of all these merits and benefits, the productivity of this crop remains very low in agriculture of Ethiopia.

Even though there exist many improved chickpea cultivars released from Ethiopian Institute of Agricultural Research (EIAR) focused mainly on breeding and selection of improved cultivars with better yield and disease resistance, the mean average productivity is as low as 1.9 tons ha^{-1} (FAOSTAT, 2015) which is much below its potential productivity

of 5.5 tons ha^{-1} (Belay, 2006). In fact variety development can be seen as a component of a package through which crop yield can be improved and it has to be supported by appropriate agronomic management including optimum fertilizer rate, proper weeding, planting at the right time, inoculation, and disease and pest control measures. Therefore, one way of improving yield of leguminous crops is inoculation of the seeds with *Rhizobium* bacteria proven to be effective in other African countries (Woomer 2012).

Facts from different studies indicate that chickpea fixes atmospheric nitrogen in the range of 60kg N ha^{-1} - 140kg N ha^{-1} per growing season (Burton, 1984; Crouch et al., 2004; Shiferaw et al., 2004). There is also increasing evidence that suggests that more nitrogen can be fixed by existing legume grain crops if they are inoculated more often or with more effective strains of rhizobia (Brockwell et al., 1988). Accordingly, if full benefit from grain legume crop is to be achieved in terms of maximum yield and soil improvement, the seed should be inoculated with its own specific and suitable *Rhizobium* strain before planting (Ayaz et al., 2010).

2.3. Nitrogen and Its Role in Crop Production

Nitrogen is known to be an essential nutrient for plant growth and development (Werner and Newton 2005) due to its role in biochemical, physiological and morphological processes of plant production (Novoa and Loomis 1981). Although this critically important element is abundant in the atmosphere, nitrogen is the most limiting element for crop growth worldwide. Healthy plants often contain 3 to 4 percent nitrogen in their above-ground tissues. This is a much higher concentration compared to other nutrients (Caliskan et al, 2008).

Nitrogen is very vital because it is a major component of chlorophyll; it is also a major component of amino acids, the building blocks of proteins. Without proteins, plants wither and die. Some proteins act as structural units in plant cells while others act as enzymes, making possible many of the biochemical reactions on which life is based. Nitrogen is a component of energy-transfer compounds, such as ATP that allows cells to conserve and use the energy released in metabolism. It is also a significant component of nucleic acids such as DNA, the genetic material that allows cells (and eventually whole plants) to grow and reproduce. Therefore, adequate supply of N is necessary to achieve high yield potential in crops. In general, N deficiency causes a reduction in growth rate, general chlorosis, often accompanied by early senescence of older leaves, and reduced yield (Caliskan et al, 2008; Erman et al, 2011). Both yield and quality of crops are highly constrained by low nitrogen availability (Greenland and Nations, 2001). Application of mineral fertilizer, addition of organic material and enhancing biological N fixation through inoculation are the main ways of improving the nitrogen availability to the plants.

Seen from stand view of environmental sustainability, nitrogen fixation by crop legumes reduces the need for fertilizer nitrogen (N) and emissions of nitrous oxide to atmosphere (Ryder et al., 2014). However, application of nitrogen fertilizer is reported (Namvar et al., 2011) to have positive effects on morphological traits and yield of crops in general and chickpea in particular. The usage of 100 kg urea ha^{-1}, for example, resulted in the highest biomass production and grain yield of chickpea as compared to the control and lower rates of fertilizer on a silty loam soil in Iran. Compared to the control, addition of 100 kg urea ha^{-1} improved grain yield of chickpea by 36% (Namvar et al, 2011).

2.3.1 Biological Nitrogen Fixation

Biological nitrogen fixation (BNF) is a process by which N in the atmosphere is reduced into a biologically useful, combined form of N-ammonia by living organisms (Herridge et al., 2008; Giller, 2001). The greatest proportion of N found on the earth is located in the atmosphere, as nitrogen molecule. Nevertheless, the majority of organisms cannot utilize this free and abundant, but highly stable source of N because they can only use N which is combined with other atoms into plant usable forms, such as ammonium, nitrates and ammonia (Giller 2001).

The process of making N available constitutes a specialized and intricately evolved interaction of soil microbes and higher plants via the formation of nodules (Sessitsch et al. 2002). Nodules are formed on roots or, in some cases, stem (Tamimi and Timko 2003). Though estimate of biologically fixed nitrogen varies among different literatures, recent review article has witnessed that estimate of global BNF in natural terrestrial ecosystems contributes about 107 million tons of nitrogen while marine N fixation contributes 121 million tons of nitrogen each year. Cultivation-induced BNF in agricultural crops and fields adds 33 million tons per year out of which a symbiotic BNF by *Rhizobium* associated with seed legumes accounts 10 million tons (range 8-12 million tons), leguminous cover crops (forages and green manures) 12 million tons $year^{-1}$, non *Rhizobium* N fixing species 4 million tons $year^{-1}$, cyanobacteria in wet rice fields 4-6 million tons and entophytic N fixing organisms in sugarcane 1-3 million tons $year^{-1}$ (Rao, 2014). The most important N fixing agents in agricultural systems are the symbiotic associations between legumes and the microsymbiont *rhizobia* bacteria (Giller 2001).

Regionally, nitrogen fixation with annual legumes may contribute 15–120 kg N $ha^{-1}y^{-1}$ in tropical Africa (Dakora and Keya, 1997) and 20–120kg N $ha^{-1}y^{-1}$ in temperate regions (Pearson et al, 1995). Depending on the availability and effectiveness of the native rhizobia, one way of improving N fixation in grain legumes is inoculation of the crop seeds with effective strains of *rhizobia*. Despite being mentioned by some as a promiscuous host (Rivas et al. 2007), there is consensus that both nodulation and growth of chickpea can be improved by inoculation (Giller 2001).The growth and yield of chickpea can be improved by inoculating seeds with competitive strains of rhizobia and this can be an economically feasible way of increasing productivity of chickpea (Ben Romdhane et al. 2008).

Across 16 on-station trials inoculation of chickpea with *Rhizobium* increased grain yield by an average 342 kg ha^{-1} (Wani et al., 1995). Likewise, depending on cultivar, effectiveness of bacterial strain and environmental factors the association of chickpea and *Mesorhizobium cicer* produced up to 176 kg N ha^{-1} annually in India (Rupela et al, 1987a). Another study conducted at three regions of Ethiopia (Oromia, Gondar and South Nations Nationalities and People Region) show some strong responses to inoculation (source of strains unknown) for chickpea (Ali et al, 2004 as cited in Ronner and Giller, 2012). Similarly studies in other countries also indicate that chickpea generally responds well to inoculation, with increases in grain yield from 8 to 40% found in Iran and Canada.

There is an evidence of an 8 to 10% increase in stover yield in Iran and Pakistan. In combination with P-fertilizer (90 kg/ha), grain yields in Pakistan even increased from 1600 to 3100 kg/ha and stover yields from 4350 to 7500 kg/ha (Ali et al, 2004 as cited in Ronner and Giller, 2013). Erman et al. (2011) also reported that inoculation with *rhizobium* and

mycorrhiza improved both grain and stover yields by about 60% in Turkey. Results of these aforementioned studies reveal that productivity of chickpea has significantly increased. Moreover, increasing and extending the role of biofertilizers such as *Rhizobium* can reduce the need for chemical fertilizers and decrease adverse environmental effects. Therefore, in the development and implementation of sustainable agriculture techniques, biofertilization has great importance in alleviating environmental pollution and deterioration of nature (Vessey and Chemining'wa, 2006; Erman et al., 2011)

2.4 Specificity and Effectiveness of Rhizobium Strain

There are roughly 1,300 leguminous plant species in the world. Of these, nearly 10% have been examined for nodulation, 87% of which were nodulated. Thus not all legumes are infected by rhizobia. A *Rhizobium* that nodulates cowpea may not nodulate *Leucaena* and vice versa. Leguminous species mutually susceptible to nodulation by a particular group of bacteria constitute a cross-inoculation group (Mulongoy, 1995).

Not all symbioses fix N_2 with equal effectiveness. This means that a given legume cultivar nodulated by different strains of the same species of *Rhizobium* would fix different amounts of nitrogen. Selection of elite strains of *Rhizobium* is based on this observation. Similarly, a given strain of *Rhizobium* will nodulate and fix different amounts of N_2 in symbiosis with a range of cultivars of the same plant species (Mulongoy, 1995). In like manner, Rhizobium species producing nodules in chickpea are specific only to this crop and inoculation with effective strains is advised in soils with no or weak bacterial presence (Rupela et al. 1987a). These factors may influence the growth of microorganisms in the free-living state, the process of plant infection or nodule development, and the fixation of N after the symbiosis has been established (Giller 2001). A number of edaphic, climatic,

and biotic factors inhibit N_2 fixation. Among these, the absence of specific and effective rhizobia in the soil is the most important factor (Mulongoy, 1995).

2.5. Factors Affecting Biological Nitrogen Fixation

Interactions between the microsymbiont and the plant are complicated by edaphic, climatic, and management factors. A legume-r*hizobium* symbiosis might perform well in a loamy soil but not in a sandy soil, in the sub humid region but not in the Sahel, or under tillage but not in no-tillage plots. These factors affect the microsymbiont, the host-plant, or both (Mulongoy 1995). Giller (2001) also reported that ability of symbiotic nitrogen fixing agents to fix N is strongly influenced by the prevailing environmental conditions that can mainly be categorized as physical factors, chemical factors, and nutrient deficiencies.

2.5.1. Edaphic Factors

Soil moisture status, drought, soil acidity, P deficiency, excess mineral N, and deficiency of Calcium, Molybdenum, Cobalt and Boron are the main edaphic factors limiting biological nitrogen fixation (Mulongoy, 1995; Giller 2001; Slattery et al. 2001).

Excessive moisture and water-logging prevent the development of root hair and sites of nodulation, and interfere with a normal diffusion of O_2 in the root system of plants except those legumes like *Sesbania rostrata* and *Aeschynomene* species that can actively fix N_2 under these conditions because they are located on the plant stems, rather than on the roots (Mulongoy, 1995).

On the other hand, drought can be considered to be among the most harmful abiotic constraints to BNF, mainly due to its effect on soil physical and biological characteristics (Kantar et al. 2010). Drought has a pronounced effect both on the number of rhizobia and

N fixation rates. N fixation is more sensitive to reductions in soil water content than other physiological processes (Giller 2001). The effect of drought on the soil microbial community can be in two ways: either reducing the number of water filled pores and the thickness of water films around soil particles or increasing the salt concentration in the soil solution.

***2.5.1.1 Drought*:** reduces the number and diversity of rhizobia in soils, and inhibits nodulation and N_2 fixation. Prolonged drought will promote nodule decay. Deep-rooted legumes exploiting moisture in lower soil layers can continue fixing N_2 when the soil is drying (Mulongoy, 1995).

2.5.1.2 Soil acidity and related problems: Calcium deficiency and aluminum and manganese toxicity adversely affect nodulation, N_2 fixation and plant growth. (Mulongoy, 1995; Giller, 2001; Slattery et al, 2001). In like manner, the soil pH greatly influences rhizobia content of soils and their ability to nodulate pulse crops (Slattery et al, 2001). Soil acidity reduces nitrogen fixation in legumes, particularly affecting *Rhizobium* survival in soil and reducing nodulation. Nodulation of soya bean and haricot bean was drastically reduced at pH of 4.5, whereas a pH of 5.2 resulted in good nodulation as well as satisfactory N fixation (Hungria and Vargas 2000). Moreover, production of grain legumes is severely reduced in salt affected soils mainly due to the impairing effect of both salinity and sodicity on the plant ability to form and maintain nitrogen fixing nodules (Rao et al. 2002). For chickpea, very small nodule dry mass was recorded for all the genotypes tested under highly saline soils (Rao et al. 2002). With increasing salinity, a sharp decrease in both nodule number and nodule biomass was observed for all chickpea genotypes tested.

***2.5.1.3 Phosphorus deficiency*:** is commonplace in tropical Africa and reduces nodulation, N_2 fixation and plant growth. Moreover, being a building block of a plant energy source, P is important in N cycling because adenosine triphosphate is required in large quantities by legumes to develop nodules and undergo the fixation process (Sessitsch et al. 2002). As N fixation is an energy demanding process (Giller 2001), larger P quantities are needed by N fixing plants than by mineral N supplied plants.

In this regard, poor nodulation and poor plant vigour have been observed in beans grown in soils low in extractable P (Amijee and Giller 1998) while acute deficiency of phosphorus can even prevent nodulation by legumes (Giller 2001), showing the sensitivity of the process of N fixation to P deficiency. The role of mycorrhizal fungi in increasing plant P uptake with beneficial effects on N_2 fixation has been reported. Dual inoculation with effective rhizobia and mycorrhizal fungi shows synergistic effects on nodulation and N_2 fixation in low P soils. The use of local rock phosphate has been recommended, particularly in acid soils, as an inexpensive source of P.

The addition of P-solubilizing microorganisms, particularly of the general *Psemdamaias, Bacillus, Penicillium,* and *Aspergillus* can solubilize rock phosphate and organically bound soil P (which constitutes 95 - 99% of the total phosphate in soils). However, the use of these microorganisms is not widespread. Some reports show nodulation response to K under field conditions. However, other investigators consider the K effect to be indirect, acting through the physiology of the plant (Mulongoy, 1995; Giller 2001; Slattery et al. 2001).

2.5.1.4 Excess Soil Mineral Nitrogen: inhibits the *Rhizobium* infection process and also inhibits N_2 fixation. The former problem probably results from impairment of the recognition mechanisms by nitrates, while the latter is probably due to diversion of photosynthesis toward assimilation of nitrates. Application of large quantities of fertilizer N inhibits N_2 fixation, but low doses not more than 30 kg N ha^{-1} of fertilizer N can stimulate early growth of legumes and increase their overall N_2 fixation. The amount of this starter N must be defined in relation to available soil N (Mulongoy, 1995; Namvar et al. 2011).

***2.5.1.5 Various microelements*:** like Copper, Molybdenum, Cobalt, and Boron are necessary for N_2 fixation. Some of these are components of nitrogenase for example Molybdenum (Mulongoy, 1995).

2.5.2 Climatic Factors

The two important climatic determinants affecting BNF are temperature and light.

***2.5.2.1 Extreme temperatures*:** affect N_2 fixation adversely. This is easy to understand because N_2 fixation is an enzymatic process. However, there are differences between symbiotic systems in their ability to tolerate high (>35°C) and low (<25°C) temperatures (Kantar et al. 2010; Mulongoy, 1995). However, some chickpea rhizobia have shown their maximum growth at 20°C (Rodrigues et al. 2006).

***2.5.2.2 The availability of light*:** regulates photosynthesis, upon which biological nitrogen fixation depends. This is demonstrated by diurnal variations in nitrogenase activity. A very few plants can grow and fix N_2 under shade (e.g., *Flemingia congesta* under plantain canopy). In alley farming if hedgerows are not weeded, or if trees are planted with food

crops like cassava, their nitrogen fixation and growth will be reduced due to shading. Early growth of legume trees is slow and they cannot compete successfully for light (Mulongoy, 1995).

2.5.3. Competition between Rhizobia

Indigenous rhizobia in soils can vary in population density and infectivity from place to place ranging from < 10 to 107 cells g^{-1} of soil. Competition in case of rhizobia is mostly used to refer to the competition for nodule occupancy (Giller 2001), which is a complex and controversial area in the study of the legume-rhizobium symbiosis (Thies et al. 1992). However, competition between inoculated and indigenous rhizobia is most strongly influenced by the size of indigenous rhizobial populations whereas environmental factors did not play a major role other than affecting the size of the native rhizobial population. Decreasing nodule occupancy by inoculant strains was observed with increasing number of indigenous rhizobia for lima bean and cowpea (Thies et al. 1992).

2.5.4. Agronomic Management

Establishment of effective symbiosis between rhizobia and the host plant primarily requires optimal conditions that are necessary for growth of the host plants. In this regard, agronomic practices have a profound influence on both the soil and the crop under consideration. For example, the organic matter content of the soil is influenced by the agronomic management and has several positive influences on soil fertility, moisture holding capacity and microbial activity. Agronomic factors that influence BNF by affecting both the crop and the microbial activity in the rhizosphere include tillage practices, selection of effective or responsive crops, appropriate cropping systems, method of sowing, time of sowing, use of agrochemicals, use of *Rhizobium* cultures and its

frequency, the way of handling the inoculants and the method of inoculation (Kantar et al. 2010).

2.6. Phosphorus and Its Importance in Crop Production

Next to nitrogen, phosphorus is the most important element for adequate grain production. The evolution of science, particularly in the past century, has clearly demonstrated the significance of phosphorus for all animal and plant life on the earth (Ryan et al. 2012). Rock phosphate is the global source of raw material for P fertilizer and it is wise to efficiently use this finite resource. Substantial amount of P is found in different parts of plants. Large quantities of P are found in seed and fruit, and it is considered essential for seed formation (Gidago et al. 2012). Especially in the early stages of plant development, adequate supply of P is required for development of the reproductive parts and P has a positive effect on root growth, early maturity, and reduced disease incidence.

Despite the presence of a large P pool, Africa is suffering from shortage of available P, which remains a yield limiting factor. Also in the highlands of Ethiopia, available P is often a limiting element in crop production (Mamo et al 1988), with 70 to 75% of the agricultural soils deficient in P.

Application of small amounts of P fertilizer (26 kg P ha^{-1}) dramatically increased nodulation, N accumulation and seed yields of haricot bean grown on farmers' fields in northern Tanzania (Giller et al. 1998).

Data obtained from multi location experiments, consistent yield response of faba bean to P fertilization was observed (Ghizaw et al. 1999). Likewise, application of 10 kg P ha^{-1} significantly improved grain yield and biological yield of haricot bean (Gidago et al. 2012). The same study revealed that application of P enhanced physiological maturity and

yield components, though not in a statistically significant way. Similarly, application of P fertilizer enhanced mid-season dry matter accumulation and tissue P accumulation of both Desi and Kabuli chickpea, but grain yield was increased only modestly for Desi chickpea, while yield of Kabuli chickpea was not affected by application of P (Walley et al. 2005)

CHAPTER THREE

MATERIAL AND METHODS

3.1 The Test Environment

The experiment was conducted under field conditions at Debre Zeit (representing the central highlands) and Wolayta Sodo (representing southern region) of Ethiopia during the main cropping season of 2015/2016 (September–January). Soil samples from both locations were collected from the rhizosphere at the depth 0 to 30 cm for physico-chemical characterization (table 3.1).

Table 3.1 Description of test sites and physico-chemical properties of soil.

Parameters	Debre Zeit	Wolayta Sodo
Latitude	8.73° N	7.04°N
Longitude	38.97° E	37.2°E
Altitude (m,a.s)	1900	1880
pH (1:1.25 H2O)	6.61 (Slightly acidic)	6.00 (Slightly acidic)
Organic Carbon (%)[a]	1.2(Low)	1.67(medium)
Organic Matter (%)	2.1(Low)	2.87(Low)
Total Nitrogen (%)[b]	0.16(medium)	0.19(medium)
Available phosphorus (ppm)[c]	73.25 (excess)	9.17 (Low)
Exch K (cmol)/kg[d]	0.40(High)	0.23(High)
CEC (cmol)/kg[e]	39.89(High)	19.70(Medium)
Moisture content (%)	4.54	1.49
Soil texture [f]		
Clay (%)	61.60	64.6
Silt (%)	31.40	23.4
Sand (%)	7.0	12.0

Method: a=Walklay and Black; b= Kjeldahl; c= Olsen; d &e =Ammonium acetate; f =Hydrometer; ppm= parts per million

3.2. Experimental Design and Layout

The experiment was conducted using three chickpea varieties (Natoli, Teketay and ICC-4918) with four levels of *rhizobia* strains (EAL-029, ICRE-025, ICRE-03 and ICRE-05) and a control (non-inoculated) were used in split plot design with three replications. Chickpea varieties as main plot and the four *rhizobium* inoculants and a control as sub plots were assigned to experimental unit randomly. Each of the main plots was divided into five sub plots having plot size of 4m by 1.2m ($4.8m^2$). The spacing of 30 cm and 10 cm was used between and intra row respectively. Accordingly, each sub plots consisted of four rows out of which the outer two rows were used as border rows and in each rows 40 – 45 seeds were planted. Phosphorus fertilizer in the form of TSP was applied to all plots at the rate of 100 kg ha^{-1}. All other crop management and protection practices were applied uniformly to all experimental units as required.

3.3 Inoculants Preparation and Method Used

Three elite *rhizobia* strains (ICRE-025, ICRE-03 and ICRE-05) proven to be effective at greenhouse and laboratory condition were obtained from the soil microbiology laboratory of the School of Plant and Horticultural Sciences, Hawassa University, Ethiopia and the commercial biofertilizer, known as EAL-029 was obtained from National Soil Laboratory of Ethiopia. The inoculum was received at the concentration of 10^9 cells g^{-1} of peat carrier.

All the necessary aseptic measures were given due attention when preparing inoculums and inoculation. Accordingly, using peat based inoculation method at the recommended rate of 10g per kilogram of seed. 200g of chickpea seed was soaked by 30 ml water of 5% sugar solution. The contents were stirred well. Sugar solution improves the adhesion of inoculant to the seed. Then 2g of inoculant was added on the wetted seed and mixed in a plastic bag

until all the seeds were uniformly coated. The whole inoculation procedure was completed in shaded area as sunlight affects the bacteria. Seed was sown immediately after inoculation. All varieties were evaluated for parameters such as nodulation, crop phenological data yield and yield component traits and total nitrogen uptake traits.

3.4 Soil Characteristics of the Study Sites

The soil textural class of the experimental fields was found to be heavy clay with average proportions of 61.6% clay, 31.4% silt and 7% sand for Bishoftu (Debre Zeit) and 64.6% clay, 23.4% silt and 12% sand at Wolayta Sodo. The pH value of the soil ranged from 6.0 to 6.6 which were classified under slightly acidic for both Wolayta Sodo and Debre Zeit. Thus the values were within the optimum range of pH for crop production (6.0 and 8.2) (Horneck at el, 2011). It was also found to be favorable for chickpea infective strains of rhizobia (Rodrigues et al. 2006).

The total nitrogen content (TN) of the soil was 0.16% and 0.19% for Debre Zeit and Wolayta Sodo respectively and can be rated as medium amount (Havlin et al. 1999). The available phosphorus content of the soil was analyzed using the Olsen et al. (1954) method which classify the values of available P as low (< 10 ppm), medium (10 – 25 ppm), high (25 – 50 ppm) and excess (>50). Accordingly, the available phosphorus content of soil sampled from Debre Zeit experimental field ranged over 38.06 ppm to 108.44 ppm having the average value of 73.25 ppm. Thus the soil at experimental field of Debre Zeit was classified as soil with excess available P content. This could be due to continual application of phosphorus fertilizer to cereal crops like Tef and wheat. Usually at Debre Zeit Agricultural Research Center Tef and wheat crops are grown on the same field successively in a rotation scheme.

On contrary, the available phosphorus content of soil sampled from Wolayta Sod was found to be 9.17 ppm and hence classified as soil with low content of available P. The value of soil organic carbon (OC) ranged from 1.2 to 1.67%, which is within the range of low organic carbon content (Landon 2014). The cation exchange capacity (CEC) value obtained from soil sample test was 39.89 cmol kg^{-1} for Debre Zeit and 19.70 cmol kg^{-1} for Wolayta Sodo. According to Landon (2014) CEC rating scheme for top soils CEC values less than 5 cmol kg^{-1} are considered to be very low, values between 5 and 15 cmol kg^{-1} low, value from 15-25 cmol kg^{-1} medium, values from or 25-40 cmol kg^{-1} high and greater than 40 cmol kg^{-1} very high. Based on this rating the soil of Debre Zeit experimental field had high CEC value implying high capacity of the soil to retain and release elements such as K, Ca, Mg, and Na. Soil of Wolayta Sodo lies in the rate of medium class.

Generally soils at the two experimental sites showed moderate differences in their initial content of major nutrients and characteristics. However, Debre Zeit soil was a bit more fertile than Wolayta Sodo soil.

The amount of rainfall received by the two test sites was nearly the same within the growing season of chickpea. Debre Zeit received 228 mm while Wolayta Sodo received 187 mm rainfall from August to December 2015(figure 3.1). However, it was much lower than the minimum requirement (500 mm) reported by Bejiga and van der Maesen (2006). During the study period the weather variables recorded deviated much from the long-term trends at both locations, causing irregular distribution and low amount of rainfall with low mean minimum temperature (figure 3.2). The growing season was generally characterized

by erratic form of rainfall and hence growth of chickpea and activity of micro organisms in the soil to some extent might be adversely affected.

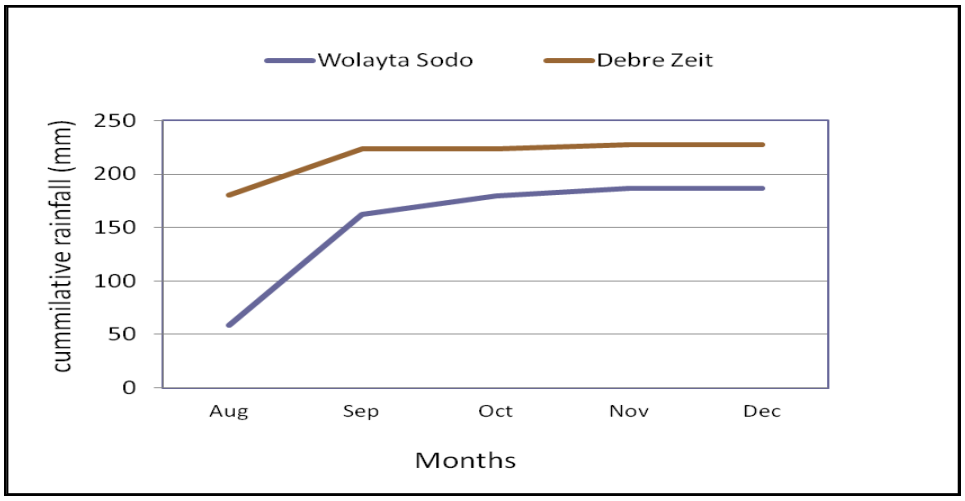

Figure 3.1 Monthly rainfalls (mm) during 2015 chickpea growing season at Debre Zeit and ayta Sodo

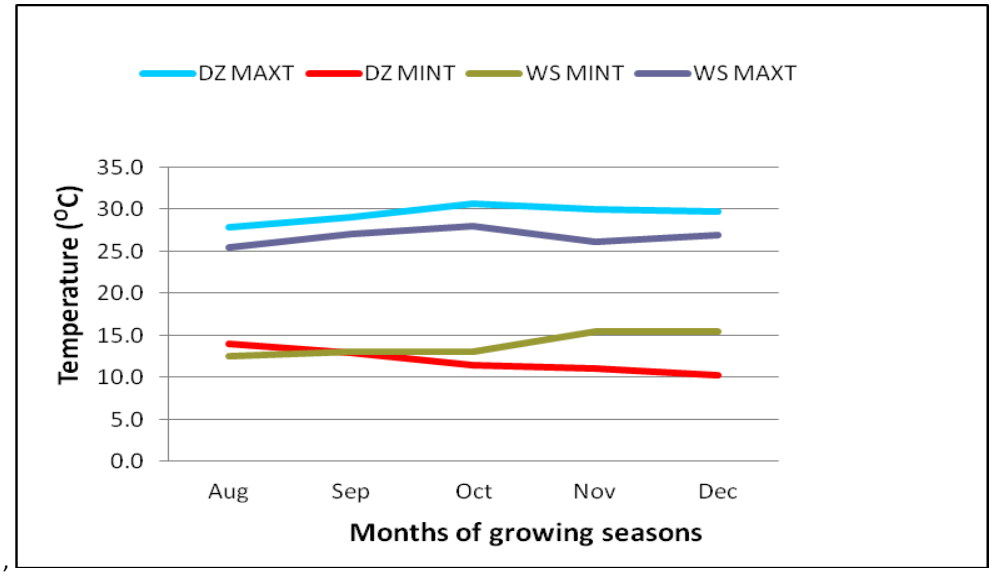

Figure 3.2 Maximum and minimum temperature (^{0}C) during 2015 chickpea growing season at Debre Zeit and Wolayta Sodo

3.5. Observations and Data Collection

3.5.1. Nodulation and Symbiotic Related Data

Nodulation assessment was conducted at flowering stage (Days to 50% flowering). Accordingly, Nodules count per plant (NCPP) was done by counting number of nodule per each randomly selected 5 plants and the average of 5 random plants taken. Nodule dry weight per plant (NDW) after recording NCPP from five randomly selected plants, then nodules were severed from the roots, oven dried at 70°C for 48 hours and their dry weight were recorded to give nodule dry weight per plant.

Plant tissue analysis for nitrogen content: Five plants from each plot were sampled at the time of harvest and separated into grain and straw. The straws were oven dried at 70°C for 48 hours, milled with mechanical miller and passed through a 1mm sieve. Subsequently, the straw was analyzed for total N. The straw total N uptake was calculated as a product of their respective straw yield obtained from five plants and N content obtained from the lab analysis i.e., N uptake (shoot dry weight x N concentration) plant^{-5}. Because of the absence of non nodulating check nitrogen fixed was determined using the procedure formulated by Yaman and Cinsoy, (1996) as follow:

N fixed (N uptake in an inoculated variety – N uptake in uninoculated variety) were calculated. Thus, the following data were recorded as: shoot nitrogen content (SHNC), shoot nitrogen uptake in gm plant^{-5} (SHNY). Percent change (increase or decrease) in data of nodulation traits, grain yield and nitrogen uptake for inoculated treatments was obtained by using the formula:

$$\%\text{Change } (\Delta) = \left[\frac{Mi-Mu}{Mu}\right] x\ 100$$

Where, Mi = Mean of inoculated variety, and Mu = Mean of uninoculated variety

3.5.2. Crop Phenology

Days from sowing to the stages when 50% of the plants have started flowering was recorded from each plot as Days to 50% flowering (DF). Similarly, Days from sowing to the stages when 90% of the pods mature was recorded from each plot as Days to 90% maturity (DTM) and measurement of plant height (PLHT) was taken from five randomly selected plants from the ground to the tip using a ruler at maturity.

3.5.3. Grain Yield, Yield Component Traits and Biomass Production

Average of actual count of number of pods per each selected five plants was counted as Number of pods plant^{-1}(NPP). The ratio of total number of seeds to number of pods per plant was calculated as Number of seeds pod^{-1}(NSP). Hundred seed weight (HSW) of randomly selected hundred seeds weighed on a sensitive balance in gram was taken. Biomass yield (BMY) weight of all above ground plant part per plot was taken and then converted to kgha^{-1}. Weight of seeds harvested from central two rows per plot in gram was taken and then converted to kgha^{-1} as Grain yield (GY). Grain Harvest index (GHI) was also calculated as the ratio of grain yield to biological yield.

3.6. Data Analysis

Statistical analysis of data collected from the field and laboratory was analyzed by SAS version 9.3 statistical software using mixed linear model procedure after checking the compliance of the data with the assumptions of the statistical test. Mean separation test was done using LSD at p-value 0.05.

CHAPTER FOUR

RESULTS

4.1. Effect of Inoculation on Nodulation and Symbiotic Nitrogen Fixation of Chickpea

Nodulation exhibited no consistent pattern with variety, rhizobia strain type. Nodules were mostly observed along the middle of main root in most of varieties forming crown (whorls) around the root and very few nodules were observed on lateral /secondary roots (Plate 4.1 c and d). Generally mean nodule number observed from the two test sites (Debre Zeit and Wolayta Sodo) rated under fair (few) to good category ranging over 5.2 to 25.2 regardless of variety and type of strains used. However, the number of nodule alone does not indicate effectiveness of rhizobia isolates. Similar findings were found by (Abd El-Maksoud and Keyser, 2010), who reported that a great number of nodules can be formed by a strain fixing little or no nitrogen, even in the presence of effective strains.

(a) (b) (c) (d)

Plate 4.1: Effective nodule color (a and b) and most commonly observed nodule position (c and b) in chickpea root

The nodule color was rated as slightly dark red/ dark pink (plate 4.1 a and b). Inoculation of chickpea varieties by *rhizobial* strains affected significantly ($p < 0.05$) nodule count per plant (NCPP), nodule dry weight (NDW) and shoot nitrogen content (SHNC) at Wolayta Sodo. Shoot dry weight (SHDW), and shoot nitrogen yield (SHNY) were also affected

significantly ($p<=0.05$) at Debre Zeit. Inculcation did not affect the nodulation status and nodule dry weight of chickpea at Debre Zeit. However, inoculated varieties produced higher number of nodules over control treatments.

In the experiment conducted at Wolayta Sodo, varieties inoculated with strains ICRE-03 produced the highest nodule dry weight (0.29 gm plant^{-1}), followed by those treated with strains EAL-029 (0.28gm plant^{-1}) and ICER-05 (0.28 gm plant^{-1}) (table 4.1 and 4.2).

Shoot nitrogen content (SHNC) of the chickpea varieties was not affected by inoculation at Debre Zeit. However, inoculated varieties had higher nitrogen content over control treatment. Similarly, in an experiment conducted at Wolayta Sodo the maximum shoot nitrogen content was recorded in varieties inoculated with strain ICRE-025 (0.62%) followed by ICRE-03(0.61%).

Rhizobium inoculation affected shoot dry weigh of chickpea varieties at Debre Zeit and increased by 5.7 -16.0% over control. The highest increment was achieved by inoculation with strain ICRE-03. At Wolayta Sodo inoculation did not affect shoot dry weight but varieties inoculated with *rhizobia* strains were all increased numerically resulting in an increment by 9.4 - 21.6% over control. The highest increment achieved by inoculation with ICRE-05 (Table 4.2 and figure 4.1).

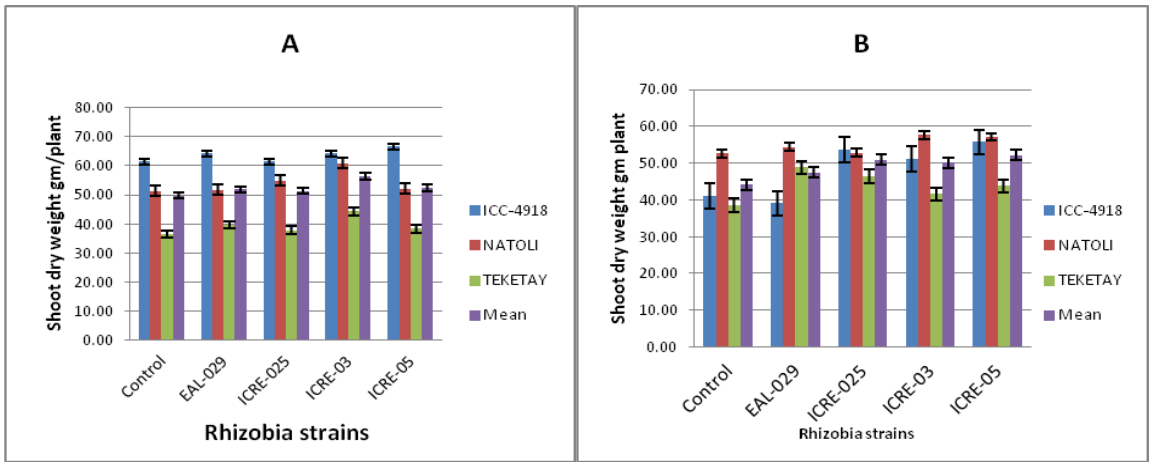

Figure 4.1: Response of chickpea shoot dry weight at Debre Zeit (A) and Wolayta Sodo (B) (gm plant^{-1}) to inoculation

Shoot nitrogen yield (SHNY) was also affected by *rhizobial* inoculation at both test sites. In an experiment conducted at Debre Zeit the highest shoot nitrogen yield (23.7 gm plant^{-5}) was recorded from varieties inoculated with *rhizobia* ICRE-03, followed by strain EAL-029 (23.24 gm plant^{-5}). Similarly, at Wolayta Sodo the highest shoot nitrogen yield (32.1 gm plant^{-5}) was recorded from varieties inoculated with *rhizobia* ICRE-025, followed by strain ICRE-03 (30.8 gm plant^{-5}) (table 4.1 and 4.2 and figure 4.2).

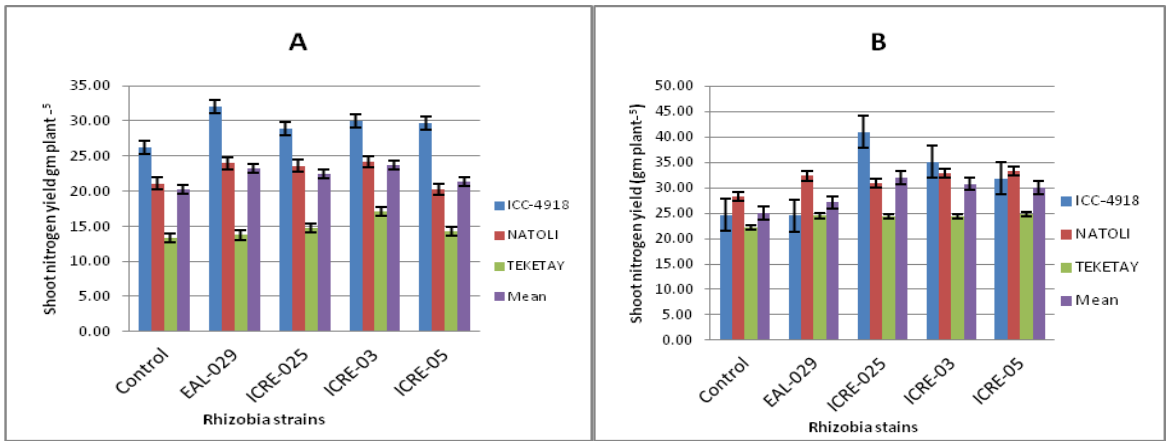

Figure 4.2: Response of chickpea shoot nitrogen yield at Debre Zeit (A) and Wolayta Sodo (B) (gm plant^{-5}) to inoculation.

Table 4.1 Effect of Rhizobia inoculation on nodulation and symbiotic nitrogen fixation of chickpea varieties against rhizobia strains tested at Debre Zeit, Ethiopia.

Variety	Rhizobia strain	NCPP	NDW	SHDW	SHNC	SHNY	N fixed	RE (%)
ICC-4918	Control	10.3	0.19	61.5	0.41	26.3	-	-
	EAL-029	10.4	0.22	64.2	0.50	32.0	5.8	33.2
	ICRE-025	13.3	0.33	61.4	0.47	28.9	2.7	19.1
	ICRE-03	13.9	0.26	64.2	0.46	29.9	3.7	20.8
	ICRE-05	14.3	0.29	66.6	0.44	29.6	3.4	18.0
NATOLI	Control	14.4	0.16	51.4	0.41	21.1	-	-
	EAL-029	15.9	0.32	51.7	0.47	24.0	2.9	25.8
	ICRE-025	11.5	0.23	54.8	0.42	23.6	2.5	16.4
	ICRE-03	14.3	0.32	60.9	0.39	24.1	3.1	18.9
	ICRE-05	13.9	0.30	52.1	0.39	20.2	-0.8	8.0
TEKETAY	Control	12.1	0.14	36.5	0.37	13.4	-	-
	EAL-029	13.4	0.32	39.7	0.35	13.7	0.4	12.6
	ICRE-025	13.4	0.23	37.8	0.38	14.7	1.3	3.6
	ICRE-03	12.1	0.19	44.3	0.39	17.0	3.7	24.2
	ICRE-05	14.5	0.22	38.3	0.37	14.3	0.9	19.5
	----------**Variety** ----------							
	ICC-4918	12.4	0.26	63.6a	0.46	29.4a	3.9	22.8
	NATOLI	14.0	0.27	54.2ab	0.42	22.6ab	1.9	17.3
	TEKETAY	13.1	0.22	39.3b	0.37	14.6b	1.6	15.0
	---------- Rhizobia strains ----------							
	Control	12.27	0.16b	49.79	0.397	20.24	0.00	0.00
	EAL-029	13.24	0.29a	51.85	0.439	23.24	3.00	23.88
	ICRE-025	12.71	0.26a	51.35	0.423	22.42	2.18	13.03
	ICRE-03	13.47	0.26a	56.46	0.417	23.70	3.46	21.29
	ICRE-05	14.24	0.27a	52.35	0.397	21.38	1.14	15.18
	Mean	**13.19**	**0.25**	**52.36**	**0.41**	**22.20**	**2.45**	**18.35**
P-value (< 0.05)								
	Variety	ns	Ns	*	ns	*	ns	ns
	Rhizobia	ns	*	ns	ns	ns	ns	ns
	R x V	ns	Ns	ns	ns	ns	ns	ns

NCPP = nodule count plant-1, NDW =nodule dry weight plant-1, SHDW = shoot dry weight gm plant-1, SHNC = shoot nitrogen content (%), SHNY = shoot nitrogen yield gm plant-5, N fixed = nitrogen fixed gm plant-5 and RE(%) = Relative effectiveness (%) ** = Significant (P <= 0.01), ** = significant (P <= 0.05) and ns=non-significant (P> 0.05), V= Variety, R= Rhizobia strain and V*R= the interaction of variety and Rhizobia strain. Means with the same letter are not significantly different

Table 4.2 Effect of Rhizobia inoculation on nodulation and symbiotic nitrogen fixation of Chickpea varieties tested at Wolayta Soda, Ethiopia.

Variety	Rhizobia strain	NCPP	NDW	SHDW	SHNC	SHNY	N fixed	RE (%)
ICC-4918	Control	10.7	0.11	41.0	0.60	24.6	-	-
	EAL-029	13.4	0.29	36.6	0.63	24.5	2.0	2.1
	ICRE-025	11.9	0.24	53.6	0.76	41.0	16.4	69.7
	ICRE-03	15.2	0.33	51.2	0.68	35.0	10.5	47.5
	ICRE-05	11.3	0.27	55.8	0.56	31.9	7.3	32.4
NATOLI	Control	15.8	0.24	52.7	0.53	28.2	-	-
	EAL-029	20.4	0.37	54.4	0.60	32.3	4.1	16.7
	ICRE-025	19.1	0.36	52.8	0.58	30.9	2.6	15.4
	ICRE-03	16.7	0.34	57.7	0.56	32.8	4.6	20.4
	ICRE-05	16.4	0.41	57.1	0.58	33.3	5.1	23.5
TEKETAY	Control	6.7	0.07	38.6	0.58	22.3		-
	EAL-029	8.6	0.18	48.9	0.50	24.5	2.2	9.6
	ICRE-025	6.9	0.16	46.5	0.52	24.3	2.1	8.9
	ICRE-03	10.1	0.21	41.7	0.60	24.4	2.1	9.6
	ICRE-05	7.1	0.17	43.8	0.56	24.7	2.5	12.0
	---------- Variety ----------							
	ICC-4918	12.5b	0.25ab	48.4	0.65	31.4	9.0	3.9
	NATOLI	17.7a	0.34a	54.9	0.57	31.5	4.1	19.0
	TEKETAY	07.9c	0.16b	43.9	0.55	24.0	2.2	10.0
	---------- Rhizobia strains ----------							
	Control	11.04b	0.14b	44.11	0.569	25.03c	-	-
	EAL-029	14.13a	0.28a	47.45	0.576	27.11bc	2.77	15.15
	ICRE-025	12.64ab	0.25a	50.98	0.621	32.06a	7.02	31.34
	ICRE-03	13.98a	0.29a	50.18	0.612	30.76ab	5.73	25.84
	ICRE-05	11.60b	0.28a	52.24	0.569	29.97ab	4.94	22.65
	Mean	**12.68**	**0.25**	**48.99**	**0.59**	**29.46**	**5.12**	**23.75**
P-value (< 0.05)								
	Variety	**	*	ns	ns	ns	ns	ns
	Rhizobia	*	**	ns	ns	*	ns	ns
	R x V	ns	ns	ns	*	ns	ns	ns

NCPP = nodule count plant-1, NDW =nodule dry weight plant-1, SHDW = shoot dry weight gm plant-1, SHNC = shoot nitrogen content (%), SHNY = shoot nitrogen yield gm plant-5, N fixed = nitrogen fixed gm plant-5 and RE(%) = Relative effectiveness (%) ** = Significant (P <= 0.01), ** = significant (P <= 0.05) and ns=non-significant (P> 0.05), V= Variety, R= Rhizobia strain and V*R= the interaction of variety and Rhizobia strain. Means with the same letter are not significantly different

4.2. Effects of Inoculation on Phenological Traits of Chickpea Varieties.

Rhizobial inoculation affected significantly ($p <= 0.05$) days to 50% flowering (DF) and days to 90% maturity (DTM) at Debre Zeit and only DF at Wolayta Sodo. No significant difference for plant height at both test sites. DTM also found to be non significant at Wolayta Sodo. The earliest DF was recorded form varieties inoculated with *rhizobia* strain EAL-029 (42 days) at Debre Zeit and ICRE-025(44 days) at Wolayta Sodo. Late DF was observed from varieties inoculated with strain ICRE-03 at both test sites, 43 days at Debre Zeit and 45 days at Wolayta Sodo being par with strain EAL-029 (45 days).

Inoculation significantly affected Days to 90% maturity (DTM) at Debre Zeit the difference between the earliest and late maturing days were recorded form varieties inoculated with ICRE-025 (91.8 days) and ICRE-03 (93 days). Significant difference for DTM at Wolayta Sodo was not detected. No significant variation in plant height (PLHT) was detected at both test sites. However, inoculated varieties had taller PLHT (table 4.3).

Table 4.3 Effect of inoculation on crop phenology of chickpea varieties tested at Debre Zeit and Wolayta Sodo, Ethiopia.

Description		Debre Zeit			Wolayta Sodo		
Variety	**Rhizobia**	**DF**	**DTM**	**PLHT**	**DF**	**DTM**	**PLHT**
ICC-4918	Control	40.67	96.67	33.33	40.67	116.3	34.57
	EAL-029	40.67	95.67	32.47	40.5	117.7	35.5
	ICRE-025	40.33	97.33	34.3	41.33	117.3	34.07
	ICRE-03	44.00	97.33	36.5	42	118.3	34.97
	ICRE-05	41.33	98.33	34.47	40.67	117.0	36.6
NATOLI	Control	45.67	90.33	33.1	56	110.3	40.17
	EAL-029	45.67	89.67	33.8	57.33	111.3	39.17
	ICRE-025	45.33	90.00	34.3	55.67	110.3	40.03
	ICRE-03	45.0	91.67	33.6	56.67	111.7	39.83
	ICRE-05	46.0	91.33	34.4	56.00	110.0	39.23
TEKETAY	Control	41.0	89.67	35.97	36.33	110.0	37.1
	EAL-029	40.33	90.33	35.77	36.67	109.0	36.73
	ICRE-025	41.67	88.00	35.13	35.00	110.0	36.4
	ICRE-03	40.67	90.00	35.93	36.67	109.7	34.2
	ICRE-05	40.67	88.33	35.93	36.67	110.7	37.77
	----------Variety ----------						
	ICC-4918	41.4b	97.07a	34.21	41.07b	117.3	35.11
	NATOLI	45.53a	90.6b	33.84	56.33a	110.7	39.69
	TEKETAY	40.87b	89.27b	35.75	36.27c	109.9	36.44
	----------Rhizobia strains ----------						
	Control	42.44	92.22	34.13	44.33	112.2	37.28
	EAL 029	42.22	91.89	34.01	45.11	112.7	36.54
	ICRE-025	42.44	91.78	34.58	44	112.6	36.83
	ICRE-03	43.22	93.00	35.34	45.11	113.2	36.33
	ICRE-05	42.67	92.67	34.93	44.44	112.6	37.87
P-value							
	Variety	**	**	ns	**	ns	ns
	Rhizobia	ns	Ns	ns	ns	ns	ns
	V x R	*	Ns	ns	ns	ns	ns

** = Significant (P <= 0.01), * = significant (P <= 0.05) and ns=non-significant (P> 0.05), V= Variety, R= Rhizobia strain and V*R= the interaction of variety and Rhizobia strain. DF = days to 50% flower, DTM = days to 90% maturity, PLHT = plant height in cm. Means with the same letter are not significantly different

4.3. Effects of Rhizobium Inoculation on Yield and Yield Related Traits of Chickpea Varieties.

The effect of *Rhizobium* inoculation on number of pods plant-^{1} (NPP), number of seeds pod-^{1} (NSP), hundred seed weight (HSW), biomass yield (BMY), grain yield (GY), grain harvest index (GHI) and percentage grain yield increased (PGYI) are presented in table 4.4 for experiment conducted at Debre Zeit and table 4.5 for experiment conducted at Wolayta Sodo. Inoculation treatments significantly ($p <= 0.05$) affected HSW, GY and GHI at two test sites. No significant differences were detected for NPP, NSP and BMY under experiments conducted at Debre Zeit. However, inoculated treatments showed numerically higher performance for these traits than the control at both test sites.

Under experiments conducted at Debre Zeit the highest HSW (27.53 gm) obtained from varieties inoculated with *rhizobia* strain ICRE-05, followed by ICRE-025 (26.71 gm) and the lowest (25.58 gm) value for HSW was recorded by varieties in the control group. Similarly, at Wolayta Sodo the highest HSW (27.81 gm) and the lowest (27.20 gm) values for HSW were observed from varieties inoculated with EAL-029 and varieties in control group respectively (table 4.5 and figure 4.3).

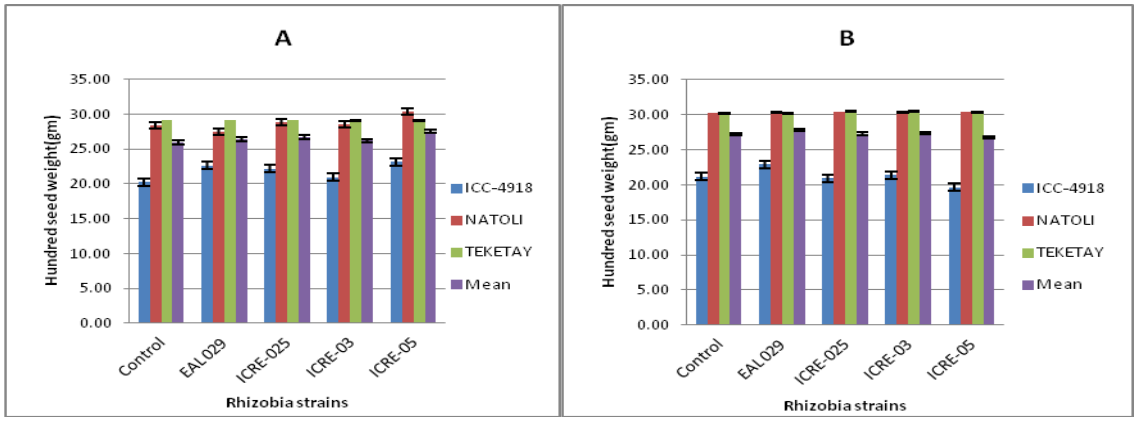

Figure 4.3: Response of chickpea hundred seed weight at Debre Zeit (A) and Wolayta Sodo (B) (gm) to inoculation

BMY was significantly (p <=0.05) affected by inoculation at Wolayta Sodo and enhanced in the range of 15.4 – 21.6% over control. The highest increment was achieved by varieties inoculated with ICRE-05(Table 4.5 and figure 4.4).

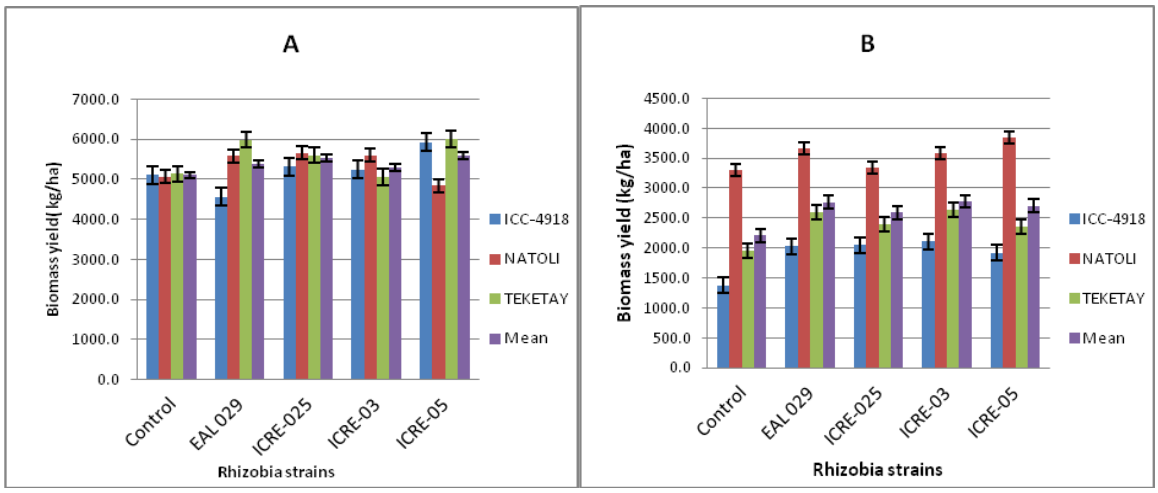

Figure 4.4: Response of chickpea biomass yield at Debre Zeit (A) and Wolayta Sodo (B) ($kgha^{-1}$)

GY of chickpea varieties were significantly ($p<0.05$) affected by inoculation at both test sites. The highest GY was observed from varieties inoculated with strain ICRE-05 (3609 $kgha^{-1}$), followed by ICER-03 (3433 $kgha^{-1}$) and the lowest GY was obtained from varieties in control treatment (2628 $kgha^{-1}$) at Debre Zeit (table 4.4). Similarly, under experiment conducted at Wolayta Sodo the highest GY was recorded from varieties inoculated with strain ICRE-03 (2527 $kgha^{-1}$) followed by ICRE-03 (2460 $kgha^{-1}$) and the lowest GY was registered by varieties in the control treatment (2009 $kgha^{-1}$) (Table 4.5 and figure 4.5).

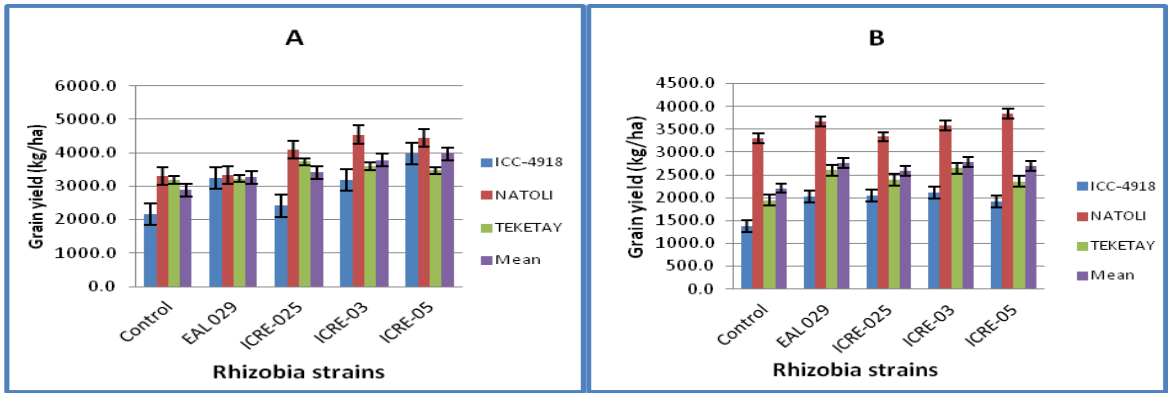

Figure 4.5 Response chickpea grain yield at Debre Zeit (A) and Wolayta Sodo (B) (kgha^{-1}) to inoculation.1

GHI which is the ratio of grain yield to biomass weight was affected by inoculation significantly ($p <= 0.05$) at both test sites. Chickpea varieties inoculated with strain ICRE-05 (0.60) had the highest value of GHI, followed by strain ICRE-025 (0.57) and the lowest value of GHI (0.54) was recorded from varieties in control treatment at Debre Zeit (table 4.4). Similarly, in an experiment conducted at Wolayta Sodo, higher GHI was recorded from varieties inoculated with strain EAL-029 (0.53), followed by varieties inoculated with strain ICRE-03 and the lowest value of GHI (0.48) was obtained from non inoculated varieties (Table 4.5 and figure 4.6).

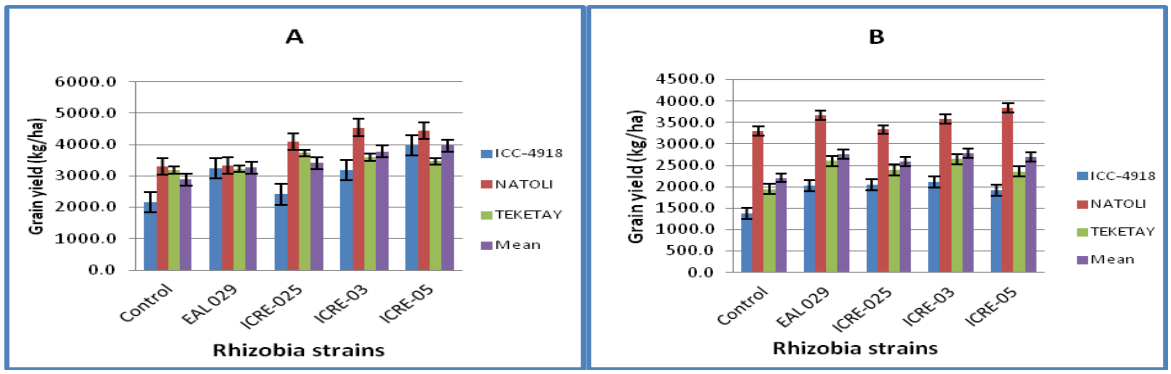

Figure 4.6: Response of chickpea grain harvest index at Debre Zeit (A) and Wolayta Sodo (B) to inoculation

Table 4.4 Effect of Rhizobia inoculation on agronomic attributs in ckickpea varieties tested at Debre Zeit, Ethiopia.

Variety	Rhizobia strain	NPP	NSP	HSW	BMY	GY	GHI	PGYI
ICC-4918	Control	62.00	1.13	20.27	4710	1973	0.42	-
	EAL-029	53.67	1.11	22.59	5997	2949	0.49	49.07
	ICRE-025	40.00	1.14	22.18	5106	2195	0.43	11.31
	ICRE-03	59.67	1.06	20.97	5938	2892	0.49	46.22
	ICRE-05	61.00	1.15	23.14	7266	3628	0.49	81.73
NATOLI	Control	43.67	1.09	28.33	5458	3013	0.55	-
	EAL-029	37.33	1.12	27.51	4974	3036	0.61	1.46
	ICRE-025	48.33	1.06	28.83	5870	3729	0.66	26.2
	ICRE-03	53.67	1.18	28.49	6780	4135	0.61	37.91
	ICRE-05	46.67	1.1	30.39	5994	4041	0.68	34.6
TEKETAY	Control	41.00	1.09	29.13	4642	2899	0.63	-
	EAL-029	42.67	1.13	29.15	4827	2936	0.6	0.24
	ICRE-025	47.33	1.15	29.11	5347	3400	0.64	18.85
	ICRE-03	47.33	1.09	29.06	5387	3272	0.61	14.99
	ICRE-05	45.67	1.12	29.06	4987	3157	0.63	8.34
	----------Variety ----------							
	ICC-4918	55.27	1.12	21.83b	5803	2727b	0.46b	37.67
	NATOLI	45.93	1.11	28.71a	5815	3591a	0.62a	20.04
	TEKETAY	44.80	1.12	29.1a	5038	3133ab	0.62a	08.48
	----------Rhizobia strains ----------							
	Control	48.89	1.1	25.0b	4937	2628b	0.535	-
	EAL-029	44.56	1.12	26.42ab	5266	2973ab	0.566	16.9
	ICRE-025	45.22	1.12	26.71ab	5441	3108ab	0.575	18.8
	ICRE-03	53.56	1.11	26.17ab	6035	3433a	0.57	33.0
	ICRE-05	51.11	1.12	27.53a	6082	3609a	0.60	41.6
	Mean	**48.61**	**1.12**	**26.71**	**5706**	**3280.75**	**0.58**	**27.58**
P-value (0.05)								
	Variety	ns	ns	**	ns	*	**	*
	Rhizobia	ns	ns	Ns	ns	ns	ns	ns
	V x R	ns	ns	Ns	ns	ns	ns	ns

** = Significant (P <= 0.01), * = significant (P <= 0.05) and ns = non-significant (P> 0.05), V= Variety, R= Rhizobia strain and V*R= the interaction of variety and Rhizobia strain. NPP = number of pods plant-1, NSP = number of seeds plant-1, HSW = hundred seed weight (gm), BMY = biomass yield (kgha-1), GY = grain yield (kgha-1), GHI = grain harvest index and PGYI = Percent grain yield increased (%), Means with the same letter are not significantly different.

Table 4.5 Effect of Rhizobia inoculation on agronomic attributs in ckickpea varieties tested at Wolayta Sodo, Ethiopia

Variety	Rhizobia strain	NPP	NSP	HSW	BMY	GY	GHI	PGYI
ICC-4918	Control	29.33	1.33	21.16	3362	1251	0.37	-
	EAL-029	38.00	1.19	21.51	3698	1705	0.46	44.38
	ICRE-025	35.67	1.52	20.89	4404	1862	0.42	53.62
	ICRE-03	43.67	1.19	21.37	4346	1919	0.44	61.53
	ICRE-05	40.67	1.18	19.66	4385	1743	0.39	35.53
NATOLI	Control	40.67	1.10	30.22	5254	3004	0.57	-
	EAL-029	34.00	1.56	30.32	5549	3341	0.63	12.50
	ICRE-025	42.33	1.09	30.38	4953	3035	0.62	5.34
	ICRE-03	48.33	1.15	30.32	5618	3257	0.58	11.58
	ICRE-05	50.33	1.15	30.39	5956	3493	0.59	18.44
TEKETAY	Control	29.00	1.06	30.21	3607	1774	0.49	-
	EAL-029	35.00	1.13	30.10	4686	2365	0.50	35.00
	ICRE-025	33.67	1.10	30.52	4384	2176	0.49	22.90
	ICRE-03	34.00	1.21	30.45	4386	2405	0.55	36.07
	ICRE-05	36.33	1.13	30.30	4225	2144	0.51	21.36
	----------Variety ----------							
	ICC-4918	37.43	1.29	20.88b	4039b	1696b	0.42b	39.01
	NATOLI	43.13	1.21	30.33a	5466a	3226a	0.60a	09.57
	TEKETAY	33.60	1.13	30.32a	4257b	2173b	0.51ab	23.07
	----------Rhizobia strains ----------							
	Control	33.00b	1.16	17.08	4074b	2009b	0.48	-
	EAL-029	34.89b	1.27	18.08	4645a	2471a	0.53	30.63a
	ICRE-025	37.22b	1.24	19.23	4580b	2358a	0.51	27.29b
	ICRE-03	42.00a	1.18	21.59	4783a	2527a	0.52	36.39a
	ICRE-05	42.44a	1.15	21.80	4855a	2460a	0.49	25.11b
	Mean	**39.14**	**1.21**	**20.18**	**4716**	**2454**	**0.51**	**29.86**
P-value (0.05)								
	Variety	ns	Ns	**	*	**	*	ns
	Rhizobia	**	Ns	ns	*	*	ns	*
	V x R	ns	**	ns	ns	ns	ns	ns

** = Significant (P <= 0.01), * = significant (P <= 0.05) and ns = non-significant (P> 0.05), V= Variety, R= Rhizobia strain and V*R= the interaction of variety and Rhizobia strain. NPP = number of pods plant-1, NSP = number of seeds plant-1, HSW = hundred seed weight (gm), BMY = biomass yield (kgha-1), GY = grain yield (kgha-1), GHI = grain harvest index and PGYI = Percent grain yield increased (%), Means with the same letter are not significantly different

CHAPTER FIVE

DISCUSSION

5.1 Soil Characteristics of the Study Sites

The amount of rainfall received by the two test sites was more or less the same within the growing season of chickpea. Debre Zeit received 228 mm while Wolayta Sodo received 187 mm rainfall from August to December 2015. It was much lower than the minimum requirement (500 mm) by which the crop fully grow physiologically to give good harvest as reported by Bejiga & van der Maesen (2006).

During the study period, the weather variables recorded deviated much from the long-term trends at both locations, causing irregular distribution and low amount of rainfall. The growing season was generally not favorable for most of the crops grown in the country. Therefore the trial should be repeated at the same locations during a more favorable year due to the unusual weather conditions experienced during the experimental period.

The physico-chemical properties of the soils from the two test locations, Debre Zeit and Wolayta Sodo, showed medium levels of nitrogen contents (0.16 - 0.19%) but high levels of K. The levels of CEC(39.89 cmol kg^{-1}) was high at Debre Zeit and medium(19.70 cmol kg^{-1}) at Wolayta Sodo with pH values slightly acidic ranging from 6.0 to 6.61 which is within the optimum range pH for crop production (6.0 - 8.2) (Horneck et al, 2011). The conditions were favorable for chickpea infecting rhizobia strains (Rodrigues et al. 2006). The level of soil phosphorus was excessively high at Debre Zeit (73.25 ppm) and low at Wolayta Sodo (9.17 ppm) (Table 3.1). Generally soils at the two experimental sites

showed minor differences in their initial content of major nutrients and characteristics, with Debre Zeit soil being somewhat more fertile than Wolayta Sodo soil.

5.2. Effect of Inoculation on Nodulation and Symbiotic Performance of Chickpea

In the present study all chickpea varieties showed nodulation in uninoculated treatments. This confirms the presence of native rhizobia in the soil. Significant variation as a result of inoculation was observed among the genotypes for most of symbiotic nitrogen fixation and a number of associated characters (Table 4.1 and 4.2) at both test sites.

Inoculated varieties produced higher nodule count, nodule dry weight, shoot dry weight, shoot nitrogen content and shoot nitrogen yield at the two test sites. However, the response to inoculation was higher at Wolayta Sodo for most of measurements of symbiotic performances. For instance mean performance of shoot nitrogen content (0.59 %) was higher than that of SHNC (0.42 %) at Debre Zeit. SHNY (28.99 gm plant^{-5}), amount of nitrogen fixed (5.11 gm plant^{-5}) and relative effectiveness in terms of percentage of fixed nitrogen increased over non inoculated treatment (23.75%) were also higher than that of Debre Zeit (18.35%) (Table 4.1 and 4.2) The reason could be due to the presence of lower fertility soil (table 3.1) and low presence of native population of rhizobia at Wolayta Sodo (Abdula, 2013). Imran, (2015) also reported that the inoculation response was more significant in soil having poor indigenous rhizobial population and fertility.

On the other hand Debre Zeit was less responsive to inoculation for symbiotic characters of chickpea. The possible reason for the difference was soil fertility status (table 3.1). In fact, Debre Zeit is the center for chickpea breeding and production for many years and hence there might be high rate of native rhizobia population in the soil capable of

hindering the development of the new test rhizobia strains used in the represent study. There have been several reports by many authors such as Jones and Hardarson (1979) Alexander (1982), Dughri and Bottomley (1983), Dudeja and Khurana (1988) and Sheoran et al (1997) that the presence of native rhizobia is a major hindrance for inoculant performance under field conditions. Rhizobium inoculation would be beneficial only when the inoculant strain competes with the native rhizobia and forms nodules.

Depending on variety and rhizobia strain used, the percentage of fixed nitrogen increased over control (uninoculated treatment) of the two test sites ranged from 13.01- 31.34% (Table 4.1 and 4.2) in chickpea shoot dry weight. The minimum percentage of increased fixed nitrogen was obtained from the varieties inoculated with the commercial strain EAL-029 while the maximum percentage increase of fixed nitrogen was from the varieties inoculated with ICRE-025 at Wolayta Sodo (Table 4.1 and 4.2) The potential of fixation by the genotypes may be generally limited because of a shortage of soil moisture as chickpea crop normally grown on residual moisture (Keneni, 2013). Moreover, the climate of the growing season deviated from the long term trend due to *El Niño* effect that caused short and erratic form of rainfall resulting in longer dry occasions that induced drought effect to the development of crop and soil micro organisms.

Concerning effect of low soil moisture, Beck and Rupela (1996) reported that fixation in winter-sown chickpea reached over 80–81%, where as spring-sown chickpea, where moisture was a limiting factor, fixed only 8–27%. Additionally, Bergersen (1970) reported that the nature of soil rhizobial populations may affect the nitrogen fixation potential of legumes. For successful inoculation with an effective isolate that would result in an enhanced nitrogen fixation, one or both the following conditions should be in place. First,

the number of available invasive rhizobia may be insufficient to nodulate the host adequately. Second, the average effectiveness of the population in the soil has to be inadequate to support the host's fixed nitrogen requirements. Difference in method of determination of the amount of nitrogen may also underestimate the amount of nitrogen fixed (Dibabe et al, 2001).

Rhizobial inoculation also affected other associated characters of symbiotic nitrogen fixation. Chickpea varieties inoculated with strain EAL-029 produced higher nodule number, nodule dry weight and shoot nitrogen content followed by strain ICRE-03 at par for the two test sites. Similarly varieties inoculated with ICRE-03 also produced higher value of shoot dry weight and shoot nitrogen yield. High fixed nitrogen in the shoot part of chickpea and high percentage increase of fixed nitrogen over the control treatment was also obtained from varieties inoculated with ICRE-03 followed by ICRE-025 (Table 4.1 and 4.2) at the two test sites with variable magnitude implying that the elite rhizobia strains were performing as good as the standard check (EAL-029). Generally chickpea varieties treated with rhizobial inoculant produced higher mean value of symbiotic traits. The current results are similar to previous reports (Tena et al, 2016; Beshir et al, 2015; Abdula, 2013; Argaw, 2012) conducted on chickpea and other grain legumes in which inoculation increased parameters of symbiotic and agronomic traits.

Ali et al, (2006 as cited in Ronner and Giller, 2013) also reported that response of chickpea to inoculation was strong in the study conducted at three regions of Ethiopia (Oromia, Gondar and South Nations, Nationalities and People Region). There was also an evidence of an 8 to 10% increase in stover yield in Iran and Pakistan. In combination with P-

fertilizer (90 $kgha^{-1}$), grain yields in Pakistan even increased from 1600 to 3100 $kgha^{-1}$ and stover yields from 4350 to 7500 $kgha^{-1}$ (Ali et al, 2006 as cited in Ronner and Giller, 2013). Erman et al. (2011) also reported that inoculation with *Rhizobium* and *mycorrhiza* improved both grain and stover yields by about 60% in Turkey.

Though, significant variation was not detected among interaction of variety with rhizobia strain for symbiotic traits, comparing interaction mean performance of those parameters revealed significant performance over the control treatment. For instance inoculation of genotype ICC-4918 with EAL-029 gave the highest mean value for measurements of SHNC (0.50%), SHNY (32.05 gm $plant^{-1}$) fixed nitrogen (5.79 gm $plant^{-5}$) and percentage of fixed nitrogen increased (33.25%) at Debre Zeit. However, the trend of specificity of variety ICC-4918 to only one strain at Wolayta Sodo was not observed. Rather it oscillated among ICRE-025 and ICRE-03 and ICRE-05 (Table 4.1 and 4.2).

Variety Teketay also constantly performed best when inoculated with ICRE-03 for symbiotic traits such as SHDW(44.34 gm $plant^{-1}$), SHNC (0.39%), SHNY (17.03 gm $plant^{-5}$) amount of fixed nitrogen (3.66 gm $plant^{-5}$) and percentage of fixed nitrogen increased (24.20%) at Debre Zeit. As it was observed in the interaction of genotype ICC-4918 with rhizobia strain similar trend of being specific for variety Teketay with rhizobia ICRE-03 also was not remain constant, rather it performed best first with ICRE-05, second with ICRE-03 and then with EAL-029 (Table 4.1 and 4.2). The implication is specific compatibility of rhizobia strain to certain chickpea varieties or chickpea variety to specific rhizobial inoculant was not observed in the current study because the strain that best performed with genotype ICC-4918 at Debre Zeit was also performed best with another

variety Teketay and the interaction (variety x rhizobia strain) effect was also statistically non significant for almost all parameters measured.

There was a strong genotypic/varietal effect on nodulation and nitrogen fixation in this study. Genotype ICC-4918 was included to the experiment as non nodulating check but was found to be superior for measurements of symbiotic traits exceeding Natoli and Teketay varieties. Regarding non nodulating genotypes of chickpea, there are recently published reports of findings which are contradicting. Gul et al (2014) reported that the four genotypes (ICC 4993, ICC 19183, ICC 4918 and ICC19181) purchased from ICRISAT did not produce any nodule in control as well as inoculated treatment while other treatments produced nodule even without inoculation. On another finding Gul et al (2011) also point out that ICC-4918 was late maturing and low yielding genotype being used as non nodulating check. In contrast to this Keneni et al (2013) reported that a non nodulating genotype ICC-19180 was found to be nodulating with best performance for grain nitrogen yield and assimilation efficiency of fixed nitrogen and suggested that whether a change in environment alone can induce nodulation of a genotype that is naturally non-nodulating in another environment needs to be investigated in the future.

In the current study the nodules were observed and collected not only from inoculated seed of the genotype ICC-4918 but also was from the uninoculated plots where ICC-4918 was planted at both test sites. Additionally, the highest shoot dry weight (63.39 gm plant^{-1} at Debre Zeit) and the highest nitrogen concentration (0.46% at Debre Zeit, 0.65% at Wolayta Sodo) were also observed from straw sample of genotype ICC-4918. The possible explanation for high shoot dry weight and high nitrogen concentration in genotype ICC-

4918 was its late maturity. In fact DTM for ICC-4918 was 97 days and 117 days at Debre Zeit and Wolayta Sodo respectively (table 4.3).

According to Bidlack et al (2007) findings, late maturing varieties accumulated more biomass in vegetative shoot components (leaf and stem) while early maturing varieties partitioned more photosynthate into reproductive structures (flower, pods, seeds). The findings from this study supports the suggestion given by Keneni et al (2013) but postulates that the reason for ICC-4918 failing to nodulate in earlier studies in India was basically due to lack of compatible *Rhizobia* strains. It is plausible to conclude here that the efficiency of nodulation and nitrogen fixation depends on the compatibility between the chickpea genotype and the *Rhizobia* strain and the environmental (mostly soil factors) conditions.

Regarding compatibility and effectiveness of elite indigenous rhizobia in the current study, strains ICRE-03, IRCE-025 and ICRE-05 were performing superior in most symbiotic parameter measurements to the strandard check (EAL-029) otherwise perform equally. For instance comparison of rhizobia strains performance in measurements of shoot dry weight and relative effectiveness (Percentage of nitrogen increased), chickpea varieties inoculated with strain ICRE-03 had the highest shoot dry weight (56.46 gm plant^{-1}) followed by strain ICRE-05(52.35 gm plant^{-1}) at Debre Zeit. Similarly in the experiment conducted at Wolayta Sodo varieties inoculated with strains ICRE-05 had the highest shoot dry weight (52.24 gm plant^{-1}), followed by strain ICRE-025 (50.98 gm plant^{-1}).

Other comparison criteria for effectiveness of rhizobia in symbiotic nitrogen fixation is a parameter known as relative effectiveness (RE) as reported by Imran et al (2015) and

Maâtallah et al (2002). Thus, chickpea varieties inoculated with strain EAL-029 had the highest (23.88%), relative effectiveness value followed by strain ICRE-03 (21.29%) at Debre Zeit. Similarly, under experiment conducted at Wolayta Sodo varieties inoculated with strain ICRE-025 gave the highest relative effectiveness (31.34%) followed by strain ICRE-03 having relative effectiveness value of 25.84% implying that all the elite rhizobia strains under the study were compatible and effective in symbiotic nitrogen fixation as long as biotic and abiotic factors kept constant.

5.3. Effect of Inoculation on Agronomic Performance of Chickpea Varieties

Rhizobium inoculation or selection of host genotypes to ensure a higher proportion of nitrogen fixation in the plant that eventually enhance crop productivity is one of the approaches to harness biological nitrogen fixation in a cropping system (Kennedy and Tchan 1992). Improving biological nitrogen fixation (BNF) in food crops may increase plant-based protein for human consumption and increase growth of subsequent crops with lesser chemical inputs (Imran et al, 2015).

The seed inoculation with rhizobium significantly increased most of the agronomic traits measured in the current study. Biomass and grain yield suggest that rhizobial inoculation increased the BMY and GY of the chickpea varieties in both test sites (Tables 4.4 and 4.5). At Debre Zeit inoculation enhanced BMY by 9%, 11%, 21% and 25% over the control treatments, by inoculation with strains EAL-029, ICRE-025, ICRE-03 and ICRE-05 respectively. Similarly, at Wolayta Sodo BMY was increased by 15.4%, 16.9% 21.1% and 21.7% over the control treatments by inoculation with EAL-029, ICRE-025, ICRE-03 and ICRE-05 respectively. Grain yields at Debre Zeit test site were increased by 16.9%, 18.8%, 33.0% and 41.6% over the control treatments, when inoculated with strains EAL-029,

ICRE-025, ICRE-03 and ICRE-05 respectively. Similarly, grain yields were increased at Wolayta Sodo by 30.6%, 27.3%, 36.4% and 25.1% over the control treatments, by inoculation with EAL-029, ICRE-025, ICRE-03 and ICRE-05, respectively. The current results are similar to previous reports of Bhuiyan et al. (1998) that reported Rhizobium inoculation increased nodulation and seed yields up to 35%. Gupta and Namdeo (1996b) also reported that seed inoculation with rhizobium increased chickpea seed yields by 9.6-27.9%.

Unlike performance for symbiotic traits, Debre Zeit was found to be superior in agronomic performance of chickpea variety when comparing the amount of biomass yield $kgha^{-1}$ and grain yield $kgha^{-1}$ produced (table 4.3 and 4.4).The highest biomass and grain yield produced when inoculated with rhizobia strain ICRE-05 were 6082 $kgha^{-1}$ and 3609 $kgha^{-1}$ while that of Wolayta Sodo were 4855 $kgha^{-1}$ and 2547 $kgha^{-1}$ when inoculated with ICRE-05 and ICRE-03, respectively.

The reason of higher productivity of chickpea at Debre Zeit could be soil fertility status. Result of soil analysis revealed that soil at Debre Zeit was more fertile than Wolayta Sodo (table 3.1). There was medium (0.16%) content of nitrogen, excess (73.25 ppm) available phosphorus and high potassium (0.40 cmol kg^{-1}) in the soil at Debre Zeit. Actually Debre Zeit is a center for chickpea breeding and production for many years due to the existence of favorable environmental conditions. However, at Debre Zeit response to inoculation for symbiotic traits was lower compared to Wolayta Sodo due to possible existence of high rate of native rhizobia population in the soil capable of hindering the development of the new test rhizobia strains used in the represent study as stated earlier.

Significant effect of inoculation was also detected for parameters such as HSW, NPP, and GHI by which higher mean values of traits measured were observed in an inoculated treatment group compared to control at two test sites. Though, it was reported by Appunu *et al* (2008) that the efficiency of *Rhizobium* legume symbiosis adversely affected due to various climatic and edaphic factors, when grown under field conditions, effectiveness of strains on percent improvement in grain yield of the current study has demonstrated a potential of increasing the productivity of chickpea. Generally, the study revealed that the highest grain yield increased by the rate of 42% when chickpea varieties were inoculated with strain ICRE-05 and low grain yield increment was found (17%) when varieties were inoculated with strain EAL-029. The average grain yield increment of inoculated treatment group was 28%. By implication, depending on the type of variety used in context of the current data an additional yield gain of 314 – 1252 $kgha^{-1}$ of chickpea grain yield would be produced though inoculation (Table 4.4 and 4.5).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6. 1 Conclusions

In this study the indigenous elite rhizobia strains was evaluated for effectiveness and productivity on chickpea under field condition at Debre Zeit and Wolayta Sodo. The results of the current study indicated that chickpea yield can be improved through proper *rhizobium* inoculation by different rhizobial strains had a pronounced effect on grain yield, yield components, nodulation, total N uptake, amount of nitrogen fixed in the shoot part of the plant as compared to non-inoculated treatments. Indigenous *rhizobium* strain ICRE-05 and ICRE-03 was found to have a more significant effect on most of the studied parameters, followed by ICRE-025. These results indicated that the indigenous chickpea rhizobial strains used in this study are better adapted to the soil environment and survived in adequate numbers as compared to the commercial inoculants EAL-029. ICRE-03, ICRE-05 and ICRE-025 were competitive influenced the chickpea yield in the same even in higher magnitude to the commercial strain EAL-029 treatment. These three strains not only increased the chickpea yields but also enhanced the shoot nitrogen content which in turn could increase protein content of the seed. This is particularly important in that these strains could be used for inoculation to increase the number of inoculants of chickpea in the study area of the country hence could be further studied on a wider range of soils to evaluate the likelihood of its successful incorporation into the existing cropping system.

6.2 Recommendations

The results of this study suggest that there is a potential of increasing chickpea productivity under field condition that the use of *Rhizobium* inoculants will increase

chickpea production. However, the experiment was done only at two test sites representing the central highlands and southern mid altitude of Ethiopia for one season. In fact, there is high variability among agro ecological zones of Ethiopia for rainfall, soil type and climatic conditions. Consequently, though encouraging and attractive yield growth improvement were observed with the strains chickpea varieties used, I would suggest that the experiments would have to be replicated over locations in order to ascertain the finding of the current study.

In this study chickpea variety responded to inculcation uniquely. Genotype ICC-4918 was included to the experiment as non nodulating check but was found to be nodulating superior for measurements of symbiotic traits such as higher shoot dry weigh shoot nitrogen content hence higher amount nitrogen fixation was observed over control treatment exceeding varieties Natoli Teketay. Thus further investigation need to be conducted to identify whether stimulation of nodulation is from activity of indigenous rhizobia strains or host genotypes adaptation to the new environment.

As the success of inoculation depends on the potential to improve the competitiveness of rhizobia under field conditions, it is advisable to know background history of native rhizobia harboring in the soil prior to application of inoculation.

Since there are limited numbers of inoculant producing private companies and governmental organizations in the country attempts should be made to further strengthen the capacity of inoculant producing companies so that availability of biofertilizer on the market would be guaranteed.

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Appendix

SAS code for separate analysis

The most relevant SAS program statements for mixed linear procedure are presented below, with classification variables for location, replication, genotypes rhizobia labeled as LOC, Rep, Gen Rhz, respectively. Note that Loc 1 = Debre Zeit, Loc 2= Wolayta Sodo

```
proc sort data=SASUSER.HH;
by Loc;
run;

Proc mixed data=SASUSER.HH;
Class Rep Gen Rhz;
Model GY = Gen Rhz Rhz*Gen/ddfm=satterth;
     Random Rep Rep*Gen;
     LsMeans Gen Rhz;
     Lsmeans Rhz* Gen;
     by Loc;
     title 'Mixed linear model anova for the RCBD with a
     Split Plot Arrangement';
     run;
```

SAS OUTPUTS

```
---------------------------- Loc=1 -----------------------------------
                           Dependent Variable           NCPP

                                Type 3 Tests of Fixed Effects

                 Effect    Num      Den      F      Pr > F
                           DF       DF     Value

                   Gen       2       4      0.15   0.8673

                   Rhz       4      24      0.24   0.9120

                 Gen*Rhz     8      24      0.41   0.9042

---------------------------------- Loc=2 -----------------------------
                        Dependent Variable           NCPP

                                Type 3 Tests of Fixed Effects
                                     Num     Den
                          Effect      DF      DF    F Value    Pr > F
```

Gen	2	4	19.56	0.0086
Rhz	4	24	3.65	0.0184
Gen*Rhz	8	24	1.14	0.3718

---------- Loc=1 ----------

Dependent Variable NDW

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	6	0.28	0.7683
Rhz	4	24	4.01	0.0125
Gen*Rhz	8	24	1.47	0.2215

---------- Loc=2 ----------

Dependent Variable NDW

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	4	14.17	0.0153
Rhz	4	24	5.90	0.0019
Gen*Rhz	8	24	0.44	0.8832

---------- Loc=1 ----------

Dependent Variable SHDW

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	6	6.93	0.0275
Rhz	4	24	0.54	0.7052
Gen*Rhz	8	24	0.16	0.9937

---------- Loc=2 ----------

Dependent Variable SHDW

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	6	3.05	0.1218
Rhz	4	24	2.19	0.1002
Gen*Rhz	8	24	1.66	0.1603

---------- Loc=1 ----------

Dependent Variable SHNC

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	4	3.32	0.1413
Rhz	4	24	1.66	0.1911
Gen*Rhz	8	24	1.25	0.3153

---------- Loc=2 ----------

Dependent Variable SHNC

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	6	2.42	0.1698
Rhz	4	24	1.56	0.2182
Gen*Rhz	8	24	2.43	0.0438

---------- Loc=1 ----------

Dependent Variable SHN

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	6	4.84	0.05
Rhz	4	24	0.88	0.4922
Gen*Rhz	8	24	0.28	0.9664

---------- Loc=2 ----------

Dependent Variable SHNY

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	6	1.67	0.2652
Rhz	4	24	3.11	0.0339
Gen*Rhz	8	24	1.93	0.1010

---------- Loc=1 ----------

Dependent Variable BMY

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	30	2.05	0.1470
Rhz	4	30	1.53	0.2197
Gen*Rhz	8	30	0.81	0.6005

---------- Loc=2 ----------

Dependent Variable BMY

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	4	7.20	0.0473
Rhz	4	24	2.94	0.0415
Gen*Rhz	8	24	1.31	0.2845

---------- Loc=1 ----------

Dependent Variable GY

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	30	4.81	0.0154
Rhz	4	30	2.30	0.0815
Gen*Rhz	8	30	0.84	0.5788

---------- Loc=2 ----------

Dependent Variable GY

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	4	23.37	0.0062
Rhz	4	24	3.09	0.0347
Gen*Rhz	8	24	0.54	0.8134

---------- Loc=1 ----------

Dependent Variable GHI

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	4	18.86	0.0092
Rhz	4	24	1.47	0.2422
Gen*Rhz	8	24	0.98	0.4730

---------- Loc=2 ----------

Dependent Variable GHI

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F

Gen	2	6	7.54	0.0230
Rhz	4	24	0.96	0.4456
Gen*Rhz	8	24	0.40	0.9122

---------- Loc=1 ----------

Dependent Variable DF

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	28	65.87	<.0001
Rhz	4	28	0.88	0.4871
Gen*Rhz	8	28	2.23	0.0559

---------- Loc=2 ----------

Dependent Variable DF

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	28	1063.01	<.0001
Rhz	4	28	1.43	0.2513
Gen*Rhz	8	28	0.53	0.8243

---------- Loc=1 ----------

Dependent Variable DTM

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	6	25.66	0.0011
Rhz	4	24	1.29	0.3026
Gen*Rhz	8	24	1.59	0.1800

---------- Loc=2 ----------

Dependent Variable DTM

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	4	4.07	0.1084
Rhz	4	24	0.45	0.7696
Gen*Rhz	8	24	0.60	0.7698

---------- Loc=1 ----------

Dependent Variable NPP

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	6	1.51	0.2934
Rhz	4	24	0.72	0.5895
Gen*Rhz	8	24	0.68	0.7040

---------- Loc=2 ----------

Dependent Variable NPP

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	4	2.88	0.1678
Rhz	4	24	6.88	0.0008
Gen*Rhz	8	24	1.73	0.1432

---------- Loc=1 ----------

Dependent Variable NSP

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	6	0.03	0.9723
Rhz	4	24	0.15	0.9623
Gen*Rhz	8	24	1.34	0.2708

---------- Loc=2 ----------

Dependent Variable NSP

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	4	2.24	0.2227
Rhz	4	24	1.03	0.4142
Gen*Rhz	8	24	3.65	0.0065

---------- Loc=1 ----------

Dependent Variable PLHT

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F

Gen	2	6	1.26	0.3501
Rhz	4	24	1.20	0.3360
Gen*Rhz	8	24	1.15	0.3684

---------- Loc=2 ----------

Dependent Variable PLHT

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	4	3.15	0.1509
Rhz	4	24	1.41	0.2604
Gen*Rhz	8	24	1.35	0.2675

---------- Loc=1 ----------

Dependent Variable HSW

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	28	84.51	<.0001
Rhz	4	28	1.18	0.3412
Gen*Rhz	8	28	0.68	0.7037

---------- Loc=2 ----------

Dependent Variable HSW

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Gen	2	6	41.26	0.0003
Rhz	4	24	0.28	0.8882
Gen*Rhz	8	24	0.37	0.9248