ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Genomic diversity, eco-physiological competence and symbio-agronomic characteristics of *Mesorhizobium* species nodulating chickpea (*Cicer arietinum* L.) from Ethiopia

A Thesis Presented to the School of Graduate Studies of Addis Ababa University in Partial Fulfilment of the Requirements for the PhD in Biology (Applied Microbiology Stream)

By

Zehara Mohammed Damtew

Principal Supervisors: **Associate Prof.Fassil Assefa (Addis Ababa University)**

Prof. Douglas Cook (University of California Davis, USA)

Dr. Asnake Fikre (EIAR Ethiopia)

Addis Ababa University **January, 2021**

Dedication

This PhD dissertation is dedicated to my beloved late father Mohammed Damtew, who raised us with great discipline and my mother Ansha Hassen for her consistent and unreserved encouragement throughout my life and carrier.

Declaration

I declare that this PhD thesis is my own independent work that was not submitted elsewhere, and any material obtained from other sources is duly acknowledged in the thesis

Signed on January 5, 2021, the School of Graduate Studies, Faculty of Life Sciences, Addis Ababa University.

PhD Candidate _____

Zehara Mohammed Damtew

Supervisor _____

Dr. Fassil Assefa

Acknowledgements

I would like to thank my major advisor Dr. Fassil Assefa and my co advisor Professor Douglas Cook, from the University of California Davis (UcDavis), USA, USAID-project Director "Feed the Future Innovation Lab for Climate Resilient Chickpea" for their advice insight from the begining of the start up to the end of PhD work. I would also like to thank Professor Douglas Research Group at University of California, Davis and for allowing me to visit and work in their Genomics laboratory. I would also like to thank, Dr. Asnake Fikre, Former Director of Crop Research at Ethiopian Institute of Agricultural Research (EIAR) and Dr. Alex Greenspan, from the University of California, Davis Research Group, for their valuable advice insight and putting long hours of their time in the supervision and guidance of the PhD research.

I am very grateful to Dr. Kassahun Tesfaye for providing me Lab facility and for his cooperation during the collection of the seed materials. I express my gratitude to Dr. Gurja Belay, Dr. Dereja Beyene, Dr. Tesfaye Alemu, Dr. Adey Feleke, Dr Asnake Desalegn, Dr. Yemesrach, Ayele Guchu, Dr. Tassew Siraj and for all staff members of the Department of Microbial, Cellular and Molecular Biology, Addis Ababa University for their support and kindness in various ways throughout the study. My thanks are extended to Dr. Eric von Wettbergg, for his advice during soil characterization process.

My appreciation is due to the Ethiopian Institute of Agricultural Research for granting me study leave to carry out this study programme. Thanks to Debre Zeit Agricultural Research Centre and the entire staff of Crop Research Process for their incredible support during my study. My special thanks go to Nigusie Girma, Tesfaye Gebre, Eresi Megersa, Gezaw Wondimu, Gebrekidan Feleke, Assefa Funga, Dr. Yifru Abera, Dr. Abebe Atilaw, Girma Kassa, Dr. Abebe Atelaw, Dr. Diriba Geleti, Dr. Eshetu Derso, Dr. Solomon Chanyalew, Dr. Mekasha Chichabelu, Worknesh Seid, Dr. Egigayehu Demissie, Dagnachew Bekele, Dr Tulu Degefu, Dr. Asfaw Hailemarium, Dr Sofia Kassa and National soil testing center for their unreserved help techniques in the preparation of inoculant sample material, field experiment vehicle service and analyses.

I also believe that my achievement in this study would not have been possible without the support I received from my family. I am exceedingly grateful to my Sister Zenet Mohammed and my husband Nesro Mohammmed. I acknowledge the support of my dearest friends for visiting and taking, their responsibilities to my family during the whole period of this study, you are a great friend I ever met. My special thanks to my sister Kedja Mohammed and my brother Endris Mohammed for visiting home and support they rendered to my family during my study leave. I find it very difficult to make a complete list of individuals who helped me to complete my thesis work and I prefer to express my deepest thanks for all of them.

Acronyms and abbreviations

ACC	Aminocyclopropane-1-carboxylic acid
ANI	Average nucleotide identity
ATPs	adenosine triphosphates
bp	base pair
CFU	Colony forming unit
CSA	Central Statistics Agency
DAP	Diammonium phosphate
DDH	DNA-DNA hybridization
dH2O	Deionized water
dNTPs	Deoxynucleoside
DAP	Diammonium phosphate
DNA	Deoxyribonucleic acid
EBI	Ethiopian Biodiversity Institute
EC	Exchangeable cation
EIAR	Ethiopian Institute of Agricultural Research
GC	Genome Coverage
GPS	Global Positioning System
ICRISAT	International Crop Research Institute for the Semi-Arid Tropics
Mb	Million base pairs
Milli-Q	Filter sterilized ddH2O
nodA	N-acyl substitution transferred
NodB	chitin-oligosaccharide de-acetylase
nodC	chitin-oligosaccharide synthase
OD	Optical density
ppm	Part per million
RAxML	Randomized Axelerated Maximum Likelihood
rpm	Revolutions per minute
rRNA	Ribosomal RNA
SAS	Statistical Analysis System
TSP	Triple supper phosphate
USDA	United States Department of Agriculture
WGS	Whole Genome Sequence

Table of Contents

List of tables

List of figures

List of appendices

Abstract

Chickpea is among widely cultivated legumes for human consumption and grown as a rotation crop for the enhancement of soil fertility in succeeding cereal crops. The crop has the ability to fix atmospheric nitrogen in association with symbiotic nitrogen fixing bacteria in the genus Mesorhizobium. Thus, in order to fully realize the potential of nitrogen fixation in the crop and improved soil fertility, it is important to understand symbiont diversity, their eco-physiological competence among strains and to identify elite Mesorhizobium strains for their symbio-agronomic effectiveness. To this end, 138 farmer fields throughout major chickpea production regions were sampled for collection of root nodules, soils and recording of their farming practices. Pure rhizobial isolates (80) were subjected to Illumina whole genome sequencing and characterized using average nucleotide identity analyses. 64 strains occur within the Mesorhizobium genus that were assigned to eleven distinct Mesorhizobium genospecies and 3% of the surveyed diversity (genus) previously observed on chickpea, corresponding to genospecies 7A which is M. ciceri. The largest group of 26 strains belonged to M. genospecies 2A, which is conspecific M. spp. ORS3359, corresponding to unnamed symbiont isolated from Acacia seyal in Senegal. Genospecies 2A is related to but distinct from M. plurifarium. Among the diverse clade, 20 representative isolates were tested in-vitro for their eco-physiological competency and symbiotic efficiency in a greenhouse using Natoli and Arerti chickpea varieties. Most isolates were tolerant to high salinity 35%, high temperature 20%, acidity 25%, antibiotics resistance 67% and heavy metal resistance 83% as well as substrate (Carbon, Nitrogen) utilization and inorganic phosphate solubilization. The strains showed difference in nodule number, nodule dry weight and shoot dry weight of which 85% of the Mesorhizobium strains were either effective or highly effective on Natoli and Arerti varieties. Ten of the best performing symbiont strains were tested at three experimental locations for three consecutive years in split plot design using Natoli and Arerti chickpea varieties. Results from field evaluation indicate that the number of nodule and nodule dry weight showed two-fold difference between the low nodulating and high nodulating strains. Similarly, these strains

increased shoot dry weight of Natoli and Arerti varieties (21-40%), above ground biomass accumulation (13-22%) and seed yield (19-31%) compared to uninoculated control irrespective of planting years at three sites. The robust strains comprising of M. loti 45P4S, M. amorphae 80P4S2, M.sp. LSJC280B00 2P3S1-b and M plurifarium 43P2S1 revealed more pronounced response in both nodulation and yield enhancement. These four strains are recommended for future inoculant production after validation with further field trials at different soil conditions. This study demonstrated symbiont diversity, eco-physiological competence and symbio-agronomic potential of selected elite Mesorhizobium strains in soils of chickpea growing regions of Ethiopia.

Keywords: Chickpea, Eco-physiological, Field evaluation, Genetic diversity, *Mesorhizobium*, Phylogeny, symbiotic genes

Manuscripts and articles arising from this thesis

1. Greenlon, A., Chang, P., **Damtew, Z.M**., Muleta, A., Kim, D., Nguyen, H., Sudheer Yadav, S. J Patil, J.S., Udupa, S., Yasin, M., Patil, B., Singh, S., Sarma, B., von Wettberg, E., Kharaman, A., Bukun, B., Assefa, F., Tefsaye, K., Carrasquilla-Garcia, N and Douglas Cook, D. (2019). Global-level population genomics reveals differential effects of geography and phylogeny on horizontal gene transfer in soil bacteria. *Proceeding of the National Academy of Science of the United of America*. 116(30):15200-15209

2. **Zehara Mohammed Damtew**., Douglas R Cook., Greenlon A., Asnake Fikre., von Wettberg E., Marques E., Kassahun Tesfaye., Carrasquilla-Garcia N and Fassil Assefa. (2020). Ecological Competence, Plant Growth Promoting and Symbiotic Characteristics of Different Mesorhizobium Strains Nodulating Chickpea (*Cicer arietinum* L.) from Ethiopia. *Journal of Plant Pathology and Microbiology*. 11(8): 1-11.

3. Zehara Mohammed Damtew., Fassil Assefa., Alex Greenlon., Asnake Fikre von Wettberg E. and Douglas R. Cook. (2021). Survey of genomic diversity of rhizobia (*Mesorhizobium* spp.) nodulating Chickpea (*Cicer arietinum* L.) in Ethiopia (Prepared for submission).

4. Zehara Mohammed Damtew., Douglas R. Cook., Alex Greenlon., Asnake Fikre and Fassil Assefa (2020). Symbiotic performance of elite *Mesorhizobium* species isolated from Ethiopian soils on chickpea (*Cicer arietinum* L.) grown in the field conditions (Prepared for submission).

Chapter 1

1. General Introduction

1.1. Background and Rationale

Nitrogen and phosphorus are the two most limiting elements for crop production. Although nitrogen is abundant in the atmosphere as N_2, it cannot be utilized directly unless it is converted into biologically available form of ammonia (NH_3). The nitrogen availability in the soil is affected by soil type, tillage, N-source, crop rotation, precipitation. The ability of the crops to recover applied Nitrogen chemical fertilizers is usually less than 50% because of their low fertilizer use efficiency (Xuan *et al*.,2017; Di Benedetto *et al*.,2017). Phosphorus is also the next most limiting element for plant growth for 95-99% of the phosphorus in the soil exists in the insoluble, immobilized and precipitated form which is attributed to pH-mediated sorption to the soil (Gupta *et al*.,2015). This is because the readily available phosphate tends to react with calcium and magnesium under high pH; whereas iron or aluminum are fixed under low pH leading to its precipitation making it unavailable for plant uptake (Goswami *et al*.,2016).

Naturally, microorganisms play a very important role in nutrient cycles of nitrogen and phosphorus by mineralizing them from organic matter into inorganic ones through their metabolic processes. Apart from that, inorganic nitrogen (N_2) from the atmosphere is converted into available form of ammonia through Biological nitrogen fixation (BNF) by prokaryotic microrganisms (Bacteria and Archea). It is estimated that prokaryotes annually fix upto 139 to 170 $\times$ 10^6 tons of nitrogen in the terrestrial ecosystem, where more than 70% is fixed by the ensosymbiotic association of the root nodule bacteria with leguminous plants (Khan *et al*.,2019).

Rhizobia effectively protect the plants against different pathogens and confer resistance by enhancing the expression of different genes (Kumar *et al*., 2019). Rhizobacterial strains are considered natural, ecofriendly, and safe besides providing resistance against a broad spectrum of pathogens (Harish *et al*.,2019). Similarly, microorganisms enhance availability of phosphorus by solubilizing complex-structured (insoluble) phosphates viz.

Tricalcium phosphate, rock phosphate, aluminum phosphate through chelating with organic acid production and mineralizing organic phosphorus by liberating enzymes such as phosphatases that ultimately enhance phosphate availability to plants (Vessey,2003; Goswami *et al*.,2016).

Other microorganisms in the rhizosphere are involved in several direct and indirect activities that enhance plant growth and development. The rhizosphere is the zone of soil surrounding a plant root where the biology and chemistry of the soil are influenced by the root (Jha and Saraf, 2015). Bacteria that colonize rhizosphere and plant roots that exert beneficial effects on plant growth are referred to as plant growth promoting rhizobacteria (PGPR) (Mohite,2013). PGPR include both free living microorganisms, endophytes colonizing plant tissues and bacteria that are able to establish symbiotic relationships with plants (Wdowiak-Wrobel *et al*.,2017).

Mechanisms by which PGPR stimulate plant growth are broadly categorized as direct or indirect and some traits are considered as direct and at the same time as an indirect mechanism (Glick, 2012). The direct plant growth enhancement mechanisms include; nutrient acquisition (via N-fixation, phosphate solubilization and siderophore production) and modulating phytohormones. The indirect growth promoting mechanisms of PGPR include their ability to act as biocontrol against phytopathogens through various forms of antagonism like competition, production of antibiotics, siderophores and lytic enzymes, production of hydrogen cyanide, producing bacteriocin (Glick, 1995; Gupta *et al*., 2000), detoxifying virulent factors (Compant *et al*., 2005), pathogenic signal interference (Lugtenberg and Kamilova, 2009) and triggering induced systemic resistance (Glick, 1995; Glick, 2012).

Chickpea (*Cicer arietinum* L.) is second most important pulse crop after common bean grown in many countries worldwide (Diapari *et al*.,2014). It is originated 10,000 years ago in the Mesopotamian region of South Eastern Turkey and spread to five recognized centers of diversity; the Mediterranean bassin, and Central and West Asia (Redden and Berger,2007). It moved to India ~6,000 years ago and arrived in Ethiopia as early as 2,000-3,000 years ago (von Wettberg *et al*., 2018).

Ethiopia is one of the secondary centers of diversity of the crop (Geletu Bejiga and van der Maesen, 2006) and the major chickpea growing country contributing to more than 40% of production in Africa (Redden and Berger, 2007). In spite of its widespread cultivation in the country, the productivity of chickpea is low with an average of 2 t/ha compared to its potential 4-5 t/ha (CSA, 2018). In conventionally farmed fields chickpea (both Desi and Kabuli types) yields are below 2 t/ha, while farmers adopting improved technologies can harvest up to 2-4 t/ha (Sultan *et al*.,2018).

The yield gap is attributed to many factors the most important ones are; low soil fertility, severe drought or erratic rainfall patterns, soil salinity/acidity, occurrence of diseases, pests and weeds (Sultan *et al*.,2018). Its deficiency is also attributed to low biological nitrogen fixation (BNF) (Dar *et al*.,2016). It is one of the major protein sources of the majority of the population in the country due to its high protein content (17-22%) (Jukanti *et al*., 2012). It is grown as a rotation crop with other cereal crops to improve soil fertility and enhance yield for it derives 70% of its N through converting atmospheric dinitrogen (N_2) to ammonia (NH_3) through biological nitrogen fixation (Khaitov and Abdiev, 2018).

That might improve the N content of soil which is available for the subsequent crop. The production of high content and quality proteins by chickpea is attributed to biological nitrogen fixation (Zhang *et al*, 2018). Benefits of symbiotic nitrogen fixation (SNF) can be maximized by optimizing the amount of N_2 fixed by rhizobial bioinoculants for legume symbioses. The use of rhizobial inoculants in agricultural production is aimed at ensuring that the most effective microsymbiont occupies the largest proportion of nodules formed on the target host legume in the field (Thies *et al*.,2001). Although chickpea is capable of fixing inorganic nitrogen, its production is constrained by poor soil fertility mainly nitrogen and phosphorus. This is due to variation in effectiveness of the crop in biological nitrogen fixation.

Effectiveness in symbiotic nitrogen fixation (SNF) in legumes is influenced by the existence of native rhizobia in the soils, genetic variation in bacterial strains, the number of infective cells applied and symbiotic response of the host cultivar (Ben Romdhane *et al*., 2007b; Nasr Esfahania *et al*.,2014; Dwivedi *et al*., 2016; Sinclair and Nogueira, 2018). For the several years now, there has been a lot of interest to study on phenotypic and symbiotic properties of different groups of chickpea rhizobia from different parts of Ethiopia (Mulissa Jida and Fassil Assefa, 2012; Tulu Degefu *et al*.,2013; Daniel Muleta and Fassil Assefa, 2015; Wondwosen Tena *et al*, 2016; Wubayehu Gebremedhin *et al*., 2018; Tassew Sirage and Fassil Assefa, 2018) and plant growth promoting properties of the rhizobia and rhizobacteria from the rhizosphere of chickpea (Mulissa Jida and Fassil Assefa, 2012).

Although few studies were undertaken on genetic diversity of the rhizobia and a symbiotic effectiveness trial of few isolates in Southern Ethiopia (Wondwosen Tena *et al.*, 2016; Endalkachew Wolde-Meskel *et al*., 2018), most of them were limited to studies of collecting large samples of cognate isolates of rhizobia from different chickpea growing regions and screening for their symbiotic properties in greenhouse trials. Moreover, chickpea rhizobial genetic diversity and its symbiosis has not been extensively explored. Similarly, the hitherto studies lacked to explore into untouched chickpea rhizobial harbor in terms of geographic distribution and relationship within strains diversity and their symbiotic performance in different soil and chickpea varieties has not been extensively tested. Thus, the purpose of this study is to explore the diversity of indigenous *Mesorhizobium* in Ethiopia, identifying effective and competitive strains being used to suggesting the potential strains for formulation of *Mesorhizobium* inoculants for chickpea.

1.2. Objective of the study

1.2.1. General objective

- Develop genomic diversity of rhizobia strains nodulating chickpea for enhancing chickpea production in Ethiopia.

1.2.2. Specific objective

- To determine the phylogenetic diversity of chickpea nodulating strains from Ethiopian soils

- To establish phylogenetically representative strains for ecologically competent qualities, plant growth promoting capability (*in vitro*) and symbiotic effectiveness on two chickpea varieties in greenhouse condition

- To evaluate selected elite *Mesorhizobium* strains for their symbio-agronomic characteristics on two chickpea varieties under field conditions that can be used for future inoculant production.

Chapter 2

2. Literature Review

2.1. The significance of chickpea production

Chickpea is originated in ~10,000 years ago in the Mesopotamian region of Southeastern Turkey*;* thereafter spread to India ~6,000 years ago and arrived in Ethiopia as early as 2,000-3,000 years ago (van der Maesen et al., 2007; Redden and Berger, 2007).Currently, chickpea is produced in more than 60 countries worldwide and the world's second most important grain legume after common bean with particular importance in the semi-arid tropics of sub-Saharan Africa and South Asia (Diapari *et al*.,2014) and (Zafar *et al*., 2017) estimated that the crop contributes to more than 20% of world pulse production covering 15% of the total land area.

It is grown on in South Asia and Sub-Saharan Africa, which accounts for more than 75% of the world chickpea area. Thus, the major chickpea producing countries are; Australia (629,400 tonnes), Myanmar (562,163 tonnes), Ethiopia (458,682 tonnes), Turkey (450,000 tonnes), Pakistan (399,030 tonnes) of the chickpea cultivation area. The crop has two types; the desi type characterized by brown to dark colored and rough surfaced seeds that account for up to 85% of production; whereas the cream to yellow colored seeded colour kabuli type controbutes to 15% of the production (Imran *et al*.,2015; Thudi *et al* 2017; Zafar *et al*., 2017).

Chickpea is a vital source of carbohydrates, protein, minerals (calcium, phosphorus, magnesium, zinc, iron), vitamins (riboflavine, niacin, thiamin), unsaturated fatty acids (linoleic, oleic acids), dietary fibre and lipids; of which, carbohydrates account to about 60%, protein content (17-22 %), dietary fibre 17.4%, total Sugar (10.4%) and 6% fat (Jukanti *et al*.,2012). It is also the most important commodity crop in the agricultural economy in advanced developing economies (Turkey) and developed countries (USA, Australia and Canada).

Chickpea is sown with low water inputs, largely reliant on stored residual moisture following wet seasons and can grow well even in the marginal soil and soils of varying textures (Imran *et al*.,2015). Consequently, it is a relatively drought tolerant grain legume grown in semi-arid regions may act as an insurance crop in poor seasons when the main crop fails due to drought (Ogola, 2015).

Chickpea obtains its nitrogen from conversion of atmospheric nitrogen into ammonia, by a process known as biological nitrogen fixation through a symbiotic relationship with *Mesorhizobium* (Nour *et al*., 1994). Nitrogen fixation increases the nutritional protein content of the seeds and enhance soil fertility and benefits both chickpea host and its following crops there by reducing the use of fertilizer (Khaitov and Abdiev, 2018), When chickpea is grown in a rotation, it can reduce rate of weeds and diseases and pests (Erdemic *et al*., 2017). As reported by (Greenlon *et al*., 2018) estimated that up to 181 kg N per hectare is obtained when chickpea pods are harvested and crop residues are tilled into the soil. Studies conducted in Turkey indicated that inoculation with *Mesorhizobium ciceri* increased the average shoot dry weight by 12% and nitrogen % by 7.9% (Elkoca *et al*., 2015).

2.2. The process of biological nitrogen fixation (BNF)

The biological nitrogen fixation is a process converting inert atmospheric dinitrogen (N_2) to biologically available form ammonia (NH_3) by prokaryotic micro-organisms. Prokaryotic organisms (bacteria and the archaea) (diazotrophs) have the enzymatic nitrogenase, encoded by the *nif* gene; to break the strong triple bond between the two N atoms and make them reactive with hydrogen atoms to form ammonia (Kumar *et al*.,2019; Valentine *et al*.,2011). BNF is categorized into two categories; nonsymbiotic nitrogen fixing group that are either free living, associative or endophytic and endo-symbiotic nitrogen fixation. A symbiotic nitrogen fixation is mediated by bacteria such as *Cyanobacteria*, *Azotobacter*, *Azospirillum*, *Acetobacter diazotrophicus*, *Azoarcus* (Ipek *et al*. 2019). The second category includes; nodule forming bacteria (*Rhizobiaceae* family*)* with Leguminous plants and the actinomycete *Frankia with* non-leguminous plants. It is estimated that Biological nitrogen fixation (BNF) contributes ~139 to 170 × 10^6 tons of

nitrogen per year to the terrestrial ecosystem compared to the ~65 $\times 10^6$ tons of nitrogen added in the form of synthetic fertilizer per year (Khan *et al*.,2019).

2.3. Rhizobia legume Symbiosis

Rhizobium-legume symbiosis is a host specific association and the need to identify specific strains and the diversity of rhizobia associated with legumes is vital for better exploitation of the benefits of the rhizobia as biofertilizers (Koskey *et al*.,2018). The efficiency of nitrogen fixation varied between strain either due to genomic background of the rhizobia and/ or the combinations between strains, plant varieties and soil factors (Wang *et al*., 2019). It is, therefore, of paramount importance to understand the major phases of the general symbiotic process involved such as plant infection, nodulation and nodule maturation, senescence, release of rhizobia and persistence of rhizobial populations in soil (Musarrat *et al*.,2010). The developmental stages start with the attachment of the bacteria within root hairs followed by root hair curling, epidermal invasion and crack entry for the formation of root nodules that provide an environment suitable for nitrogen fixation by rhizobia (Oldroyd *et al*.,2011). The success of the symbiosis depends on the recognition of rhizobia by the legume host of to activate the expression of a group of bacterial nodulation(*nod*) genes leading to initiation of bacterial infection (Gibson *et al*.,2008).

2.4. Rhizobial infection and nodulation genes

Rhizobium-legume symbioses are species-specific and each host plant may be nodulated by one or a few microsymbiont species (Wielbo *et al*., 2015). For this reason, the nodulation process begins with an intricate signal exchange and recognition by the symbionts, the control of the plant defense responses, nodule organogenesis, and efficient N_2 fixation and ammonium assimilation (Olivares *et al*., 2013). The plant initiates by releasing signal molecules such as flavonoids (secondary plant metabolites), phenolics, sugars, dicarboxylic acids and amino acids. The rhizobia detect the compounds and respond by aggregating and attaching them around the root hair of the host (Fauvart and Michiels,2008).

The signal molecules in the rhizosphere define specificity, competitiveness, infectivity and effectiveness of the legume rhizobium symbiosis because different legumes produce different types and mixes of compounds (Unay and Perret, 2019). Flavonoid signal molecules activate nodulation protein D (NodD) in rhizobia by stimulating the binding of the transcriptional regulator NodD of the nodulation genes promoter (Mus *et al*.,2016).

Implicating bacterial NodD protein triggers the transcription of a range of genes within the rhizobia (Larrainzar *et al*.,2015). The nodulation genes are important for the synthesis and secretion of nod factors (lipochitin-oligosaccharides) that are receptors for the plant flavonoid signal to induce structural and functional alterations within the plant root (Velazquez *et al*.,2010). Apart from the Nod factors, various bacterial cell structures such as the lipopolysaccharides, β-glucans, exopolysaccharides, capsular proteins and K antigen are also recognized by the plants to determine the host specificity (Kour *et al*.,2019).

More than 30 different *nod*, *nol* and *noe* genes are involved in the synthesis and secretion of Nod factors (Wang *et al*.,2019). The common nodulation genes (*nodABC, nodD, nodIJ*) exist in all symbiotic rhizobia except some *Bradyrhizobium* strains (Bekuma, 2017). Among these genes *nodABC* encode the enzymes required for the synthesis of the core Nod factor structure of an *N*-acetyl glucosamine oligosaccharide backbone with a fatty acyl chain at the non-reducing end (Andrews *et al*.,2018). Nod factors also trigger curling of the root hair towards the attached bacterium and generating a shepherd hook structure that entraps the bacterial microcolony attached to the tip of the root hair (Clua *et al*.,2018). At this point, the root hair plasma membrane invaginates and start formation of intracellular infection thread (ITs) in which the bacterium enters into the plant root interior (Murray,2011). In this way, cell division along the infection thread into the root cortex initiate the formation of the nodule primordium or the infected cells (Fauvart and Michiels,2008).

The bacteria released from tip of the infection thread into root cortical cells via endocytosis. Subsequently, the bacteria continue to divide and fully internalized by the host cell to become an intracellular organelle surrounded by a host-derived symbiotic membrane (Stonoha-Arther and Wang, 2018). The enveloping membrane (symbiosomes)

controls provision of energy, sequestration of free oxygen molecules and nutrient exchange between the symbionts to create suitable environment for nodule development (Mus *et al*.,2016). The type of nodules formed by legumes is classified into determinate and indeterminate Nodules. Nodules can be determinate (which grows up to maturity, stops the development and starts N_2 fixation), such as in soybean, common beans and indeterminate nodules (which holds a meristematic zone that guarantees a continuous growth of the organ concomitantly with N_2 fixation), such as in *Medicago truncatula*, alfalfa and pea (Valentine *et al*.,2011).

Within the symbiosome (nitrogen-fixing unit of the nodule) rhizobia undergo cell division and differentiation to form the nitrogen fixing entity known as the bacteroids. Bacteroid is a metabolic switch that converts free-living rhizobia into N_2-fixing organelles, that express the nif and fix genes clusters required for nitrogenase enzyme complex assembly and functioning (Unay and Perret, 2019). They are also sites for transport (exchange) of reduced carbon compounds from the plant to the nodule and of fixed nitrogen from the bacteroids to the host plant cytoplasm (Coba de la Pena and Pueyo,2012).

The Nitrogenase enzyme requires a FeS-cluster and other metal-dependent cofactors for electron transduction; thus, nitrogenase complex consisted of two metallo protein componets; an iron-protein coded by dinitrogenase reductase *(nifH)* structural gene and iron-molybdenum encoded by α and β subunits dinitrogenase (*nifD, nifK)* (Dos Santos *et al*.,2012). Both the Fe and MoFe components of nitrogenase are O_2 labile. Bacteria fix nitrogen inside root nodules in which low oxygen and abundant carbon sources are provided (Olivares *et al*., 2013). Commonly, the nitrogenase reduces atmospheric nitrogen through a series of energy-demanding metabolic steps. The rhizobia require reduced carbon essential for bacterial physiology as well as to produce (16 ATPs) required to fuel symbiotic N_2 fixation (Desbrosses and Stougaard, 2011). The nitrogenase catalyzes the following reaction;

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$$

Nitrogen fixation requires the construction of specialized symbiotic nodules structures and protection against excess oxygen. For this reason, the internal layer of the nodules

acquires thick mucilaginous layer and a high concentration of hemoglobin that is essential to control oxygen homeostasis and protect the rhizobial nitrogenase enzyme complex from oxidation (Sinclair and Nogueira, 2018). In general, the rhizobium legume symbiosis contains a set of nod, nif and fix genes which is important to entice the formation of proficient nodules on roots of legume crops for the continual function of nitrogenase or symbiotic nitrogen fixation (Unay and Perret, 2019).

2.5. The genome and phylogeny of symbiotic rhizobia

Rhizobial genomes are considered to have two main components of core and accessory genes. Core genome is mostly chromosomal, essential for cellular function, shared across all members of species contain higher GC. The accessory genome is the one located on chromosomal islands including symbiosis genes, increase adaptive potential of host through provision of phenotypic advantageous for various niches and contain lower GC (Laranjo *et al*.,2014; Bekuma, 2017; Wang *et al*.,2019). A common feature of the rhizobial genomes is that genes involved in nodulation and N_2-fixation are clustered on symbiotic plasmid (pSym) or incorporated into the chromosome as symbiotic islands (Skorupska *et al*., 2010). The symbiosis genes in rhizobia refer to nodulation genes (nod, noe and nol) which are responsible for nodulation and nitrogen fixation genes (nif and fix) that are involved in atmospheric nitrogen fixation (Laranjo *et al*.,2008).

Rhizobia typically have large and complex multipartite genomes compared to most bacteria, ranging in size from ~5-10 Mb that enable them to adapt to different habitats (Bellon,2018). Rhizobia have one chromosome and several plasmids and/or megaplasmids that may represent 50% of the genome (Alexandre *et al*.,2006), that contribute to an evolutionarily dynamic genome through the process of horizontal gene transfer (Gibson *et al*.,2008). In many cases, the phylogenetic positions of the symbiosis genes, especially the nodulation genes, are different from those of the chromosomal (housekeeping) genes (Wang *et al*.,2019).

Rhizobial symbiosis genes are often carried on symbiotic islands or plasmids (pSym) that can be transferred (horizontal transfer) between different bacterial species within and across genera (Andrews *et al*.,2018). Integrative and conjugative elements (ICEs) are

generally regarded as regions of contiguous DNA integrated within a bacterial genome that are capable of excision and horizontal transfer via conjugation (Haskett *et al*., 2017). As ICEs are universal mobile genetic elements present as "genomic islands" within bacterial chromosomes; symbiosis islands are the largest documented ICEs and their transfer converts nonsymbiotic *Mesorhizobia* into nitrogen N_2- fixing symbionts of leguminous plants (Haskett *et al*., 2016).

2.6. Taxonomy and diversity of nitrogen fixing rhizobia

The geographic distribution, diversity and phylogenetic relatedness of rhizobia could highlight their evolutionary origin as well as their unique characteristics which can be utilized for manipulation of symbiotic nitrogen fixation in legumes (Koskey *et al*.,2018). This suggests that representative strains from the large genetic diversity of rhizobial species can be selected for developing elite rhizobial bioinoculants for precision agriculture (diCenzo *et al*.,2018). Wang *et al*., (2019) suggested that rhizobial diversity depends on four factors; long evolutionary history, environmental selection for their survival (chromosome genes), host selection for nodulation (symbiosis genes) and lateral transfer of symbiosis gene (creating novel combinations of chromosome and symbiosis genes). Strain competition and cohabitation in the vicinity enables the horizontal transfer of genetic material between bacteria which may enrich the genetic pool of individual strains and increase intrinsic population diversity (Wielbo *et al*., 2015).

Traditionally, taxonomy of rhizobia was based on the host specificity of the rhizobial strains, or cross inoculation group concept. However, this criterion was not useful to classify species of rhizobia due to the possibility of natural transfer of symbiotic plasmids among bacterial strains in the soil and occurrence of some rhizobial strains which are capable of nodulating a wide range of legumes. The location of symbiotic genes was also used as a genotypic tool to differentiate between the fast and slow growing legume nodulating bacteria, they are typically chromosome located for the slow growing *Bradyrhizobium* and on plasmids for fast growing *rhizobium* strains (Shamseldin *et al*.,2017).

Later on, phenotypic features (morphological, physiological, biochemical) of bacteria have been used for the description of new bacterial species in numerical taxonomy at 80% phenotypic similarity. Phenotypic analysis is used for preliminary classification of bacteria into the genus as well as selection of metabolic, physiological, ecological and plant growth promoting characteristics of rhizobial strains that may beneficially influence plant growth and development and for providing information about ecological competence of the rhizobia in the organism's habitat (Ltaief *et al*.,2007; Wdowiak-Wrobel *et al*.,2017).

The advent of molecular analyses, together with the isolation and characterization of more root nodule from many legumes has revolutionaized rhizobial taxonomy. These include; DNA base composition (G + C content), DNA-DNA hybridization, sequences of the 16S ribosomal RNA genes and Fatty Acid Methyl Ester (FAME) profiles (O'hara *et al*., 2016). DNA-DNA hybridisation (DDH) technique has been applied as the gold standard method and strains classified in the same species should have 70% DDH relatedness among each other (Aserse *et al*.,2017). However, DDH results vary between different laboratories and this incurs inconsistent classification of the same species.

Apart from the 16S rRNA gene, PCR-based RFLP analysis of 16S-23S rRNA gene intergeneric spacer have been widely used at a faster rate than the 16S rRNA, thus adding valuable information to the analysis. Despite, the 16S rRNA gene is highly conserved to allow separation of closely related species; the genomes of rhizobia may lose or gain plasmids or genomic islands (HGT) and genetic recombination in 16S rRNA genes leading to insufficient rhizobial taxonomy (Azcarate *et al*., 2011). Later, Multilocus Sequence Analysis (MLSA) of housekeeping protein coding genes including 16S rRNA, *atpD* (ATP synthase F1, beta subunit), *recA* (recombinase A) and *rpoB* (RNA polymerase, beta subunit) was established to discriminate between closely related species at 96-97% similarity (Azcarate *et al*., 2011; O'hara *et al*.,2016; Mousavi *et al*., 2017). These genes have a faster rate of evolution than the 16S rRNA gene but are conserved enough to retain genetic information useful for taxonomic purposes (O'hara *et al*.,2016).

Several genomic approaches have been employed to define and demonstrate the involvement of *rhizobial* genomes in the symbiotic events. Recently genomic data analysis tools based on whole genome sequences such as Average Nucleotide Identity (ANI) have

provided scientifically valid taxonomic standards for classifications; that accommodate the rapidly expanding field of genomics to classify microbial diversity (Mahato *et al*., 2017). The standard ANI value of 95-96% has been applied for species threshold, while 75 and 70% could be the thresholds for genus and family, respectively (Wang *et al*.,2019).

Generally, whole genome sequencing offers the ability to explore rhizobial diversity using culture independent methods (Greenlon *et al*., 2019) and to use genomic data to assess species relationships using methods such average nucleotide identity (ANI) and pan-genome enumeration (Goris *et al*., 2007; Richter *et al*., 2009). Whole genome tools also provide opportunities to discriminate more nuanced strain-level diversity, which may facilitate prediction and selection of rhizobial diversity better suited for use as legume biofertilizers (as postulated by Greenlon *et al*., 2019).

The use of polyphasic approach (combination of phenotypic, genotypic and symbiotic characters together with the exploration of more legumes for their endosymbionts) contributes to the advancement of rhizobial classification for several decades. Thus, the root nodule bacteria that are classified under the family *Rhizobiaceae* have been classified into two classes, *Alphaproteobacteria* and *betaproteobacteria*, 13 genera and more than 98 species (Vargas *et al*.,2010; Velazquez *et al*.,2019). The well-known members of the Alphaproteobacteria are the genera *Rhizobium*, *Mesorhizobium*, *Ensifer* (former *Sinorhizobium*), *Azorhizobium*, *Bradyrhizobium*, *Methylobacterium*, *Devosia*, *Ochrobactrum*, *Phyllobacterium*, *Shinella*, *Microvirga.* The two genera belonging to the class *betaproteobacteria* are the genera *Burkholderia* and *Cupriavidus* (Wdowiak-Wrobel *et al*.,2017).

2.7. Chickpea rhizobia

Chickpea establish symbiosis with root nodule bacteria belonging to the specific genus *Mesorhizobium* with an intermediate growth rate between the genera *Rhizobium* and *Bradyrhizobium* (Nour *et al.,* 1994). Although chickpea rhizobia are considered for long as very host specific to *M ciceri* and *M mediterraneum* (Nour *et al*., 1995). Later studies have shown that chickpea is able to establish symbioses with several species of

Mesorhizobium, such as *Mesorhizobium amorphae*, *Mesorhizobium loti*, *Mesorhizobium tianshanense*, *Mesorhizobium muleiense*, *Mesorhizobium abyssinicae* and *Mesorhizobium shonense* (Alexandre *et al*., 2009; Zhang *et al*., 2018).

Recently, (Greenlon *et al*., 2019) reported that the *Mesorhizobium* genus encompasses 36 distinct species; of which eight *Mesorhizobium* species were cosmopolitant that nodulate chickpea globally; and 20 chickpea symbionts had not been previously recognized. The symbiosis genes of *Bradyrhizobium* and *Mesorhizobium* species are located in chromosomal symbiosis islands, the exceptions described to date being *Mesorhizobium amorphae* (Wang *et al*., 1999) and *Mesorhizobium huakuii* (Laranjo *et al*.,2008). The phylogenetic analysis of symbiosis genes *(nifH* and *nodC)* has been used for determining the symbiovar of rhizobial strains; all chickpea-nodulating rhizobia should be assigned to the symbiovar *ciceri* that is *Mesorhizobium* spp. sv. *ciceri* (Wang *et al*., 2019). Symbiovar represents a group of bacterial strains distinguishable from other strains of the same species on the basis of physiological or biochemical characters, which can be shared by different species due to lateral gene transfer (Dwivedi *et al*.,2016).

2.8. Plant growth promoting rhizobacteria (PGPR)

Rhizosphere is the zone of soil surrounding a plant root where the biology and chemistry of the soil are influenced by the root. Bacteria that colonize rhizosphere and plant roots that exert beneficial effects on plant growth are referred to as plant growth promoting rhizobacteria (PGPR) (Mohite,2013). PGPR include both free living microorganisms, endophytes colonizing plant tissues, and bacteria that are able to establish symbiotic relationships with plants (Wdowiak-Wrobel *et al*.,2017). Based on the degree of association with root cells, PGPR are also grouped into extracellular plant growth promoting rhizobacteria (ePGPRs) and internal plant growth promoting rhizobacteria (iPGPRs) (Sharma *et al*., 2013).

The extracellular plant growth promoting rhizobacteria (ePGPRs) exist in the rhizosphere, on the rhizoplane or in the spaces between the cells of root cortex; that include the bacterial genera such as *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus* and *Pseudomonas* (Gupta *et al*.,2015). The intracellular plant growth promoting rhizobacteria(iPGPR) are located inside the specialized nodular structures of root cells belongs to the family of Rhizobiaceae;

Rhizobiaceae, includes *Allorhizobium, Bradyrhizobium, Mesorhizobium* and *Rhizobium* endophytes and Frankia species, both of which can symbiotically fix atmospheric nitrogen with some groups of higher plants (Nagargade *et al*.,2018). PGPRs benefit not only the growth of plants but the entire community, including bacteria, plants and soil fauna; such benefits include an increase in growth and N- and P-uptake by plants, increased photosynthesis and decreased carbon costs, through the inoculation of elite strains (Perez-Fernandez and Alexander, 2017). The application of PGPR in diverse crops has been reported to approximately increase the yield by 20-40% (Kumar *et al*.,2019). They promote plant growth through direct and indirect mechanisms.

2.8.1. Plant growth promoting mechanisms

PGPR play great role in facilitate nutrient uptake or increase nutrient availability and regulating plant hormone levels. PGPR directly improved plant growth by providing the plants with bacterial synthesized organic molecules such as (amino acids, carbohydrates, enzymes, inorganic substances), promoting plant growth through production of plant growth regulators (Auxins, abscisic acid, cytokinins, gibberellic acid) and produce the siderophore for solubilize and sequestering of iron (Fe) from the soil and supply it to the plant cells and synthesis of hydrogen cyanide (Siyar *et al*.,2019; Ipek *et al*.,2019). They produce several types of organic molecules that include; amino acids, carbohydrates, and enzymes and inorganic substances (Siyar *et al*.,2019).

This mechanism includes biofertilizer activity through nutrient fixation (biological nitrogen fixation, nutrient solubilization (phosphate solubilization, potassium solubilization), biostimulators (production of phytohormones) and siderophores to

enhance growth and Fe uptake by plants, respectively (Velazquez *et al*.,2019). PGPR can promote plant growth through indirect mechanisms through production of antagonistic substances and promotion of host Induced Systemic Resistance (ISR), against pathogens (Di Benedetto *et al*.,2017). They produce HCN (Perez-Fernandez and Alexander, 2017), Antibiotics, Bactreiocin, HCN, NH_3, Hydrolytic enzymes, ExoPolysaccharides, Growth substance, polysaccharides and produces protecting enzymes 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity (Ahmed *et al*.,2019) that indirectly benefit the host plants.

2.8.1.1.PGPR as biofertilizer (provision of nitrogen, phosphorus and iron)

Biofertilizer is a substance containing living microorganisms (organic compounds), when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Di Benedetto *et al*.,2017). Mode of action of PGPR as biofertilizers to enhance the nutrient status of host plants included; biological N_2 fixation by free living, associative and symbiotic nitrogen fixing bacteria, increasing the availability of nutrients (P, Fe, etc) in the rhizosphere, inducing increases in root surface area, enhancing other beneficial symbioses of the host and combination of modes of action (Vessey,2003). PGPR are involved in both solubilization of inorganic (insoluble) phosphates and mineralizing organic phosphorus by liberating enzymes such as phosphatases to make phosphorus available for plants (Kour *et al*., 2019).

Microorganisms mineralize organic phosphorus in soil by solubilizing complex-structured phosphates viz. Tricalcium phosphate, rock phosphate, aluminum phosphate; that turns organic phosphorous to inorganic form ultimately aiding the phosphate availability to plants (Goswami *et al*.,2016). Phosphorus is taken up in soluble forms (H2PO4- and HPO4 2-); this bioavailable fraction constitutes only the 0.1% of the total phosphorus content in soils and phosphorus deficiency imposes an important constraint on chickpea production (Riva *et al*.,2019). Phosphorus is required for many plant functions including vital nucleic acid and adenosine triphosphate (ATP) production; a decrease in phosphorus availability to the plant causes a decrease in nodule formation and nitrogen fixation due to lack of ATP metabolic actions in the associated plant cell (Adeleke *et al*.,2019).

Reductions in the concentration of ATP and energy charge in inorganic phosphate deficient nodules results in significant declines in nitrogenase activity, symbiotic nitrogen fixation (SNF) capacity and the growth and productivity of legume crops (Nasr Esfahania *et al*.,2014). PGPR are also capable of solubilizing iron by the production of iron binding molecules like siderophores which can form Fe-siderophore complex, readily available to plants under iron deficiency conditions.

Many bacteria produce organic compounds called siderophores that chelate Fe^{3+} and increase its availability for plant uptake after the reduction of Fe^{3+} to Fe^{2+} or directly as siderophore-Fe^{3+}complex (Vessey, 2003; Riva *et al*.,2019).

2.8.1.2.PGPR as plant growth regulators

PGPR also produce multitudes of phytohormones such as auxins (Indole acetic acid; IAA), gibberellins, cytokinins, abscisic acid and ethylene ammonia (Kour *et al*.,2019). Indole acetic acid (IAA) is a common product of L- tryptophan metabolism produced by PGPRs helps in the production of longer roots with increased number of root hairs and root laterals which are involved in nutrient uptake (Mohite,2013). It been estimated that more than 80% of the soil bacteria are able to produce auxins, especially IAA, indolebutyric acid or similar compounds derived from tryptophan metabolism (Vargas *et al*.,2010). The naturally occurring auxin indole-3-acetic acid (IAA) promote root elongation lateral root development and is involved in the early steps of nodule organogenesis (Coba de la Pena and Pueyo,2012). Cytokinins mediate the rhizobial infection in legumes and increase chlorophyll content.

Abscisic acid (ABA) is an important plant hormone related to plant response to drought; Abscisic acid could inhibit nodulation, rhizobial infection and gene expression of several nodule associated genes (Valentine *et al*.,2011). The production of gibberellins stimulates the root system and few bacterial species such as *Bacillus pumilus* and *Bacillus licheniformis* are capable of producing the hormone (Jha and Saraf, 2015). In rhizobia, cytokinin leads to nodule development by regulating the different Nod factors pathway and initiating the cortical cell division (Kumar *et al*., 2019).

2.8.1.3. PGPR as biocontrol agents (Antagonism)

PGPR protect plants from the attack of plant pathogens by different direct and indirect mechanisms that create conducive environment for normal plant growth (Kour *et al*.,2019). Siderophores bind the soluble form of iron from soil to make it available to plants; thus, siderophore-Fe complex is up taken by plant roots making the soil environment Fe-deficient for pathogenic fungi (Ahmed *et al*.,2019).

Thus, siderophore producing rhizobacteria improve plant health by improving iron nutrition and inhibiting the growth of other microorganisms with the release of their antibiotic molecule and hinder the growth of pathogens. Iron deficiency causes growth inhibition, decrease in nucleic acid synthesis inhibition of sporulation, and causes changes in cell morphology of the pathogen (Jha and Saraf, 2015). The ability of PGPR to synthesize antibiotics and other extracellular metabolite such as subtilin, sublancin, chlorotetain, rhizoctinins, surfactins hinders the growth of phytopathogens even at low concentration (Adeleke *et al*.,2019).

PGPR also induce systemic resistance to plants against different pathogens by the activation of plant defense mechanisms and directly suppress broad spectrum of pathogens pathogesn by competition, antagonism and producing antipathogenic compounds (Harish *et al*.,2019; Kumar *et al*., 2019; Siyar *et al*.,2019). PGPR also modulate a wide range of environmental stresses like high temperature, cold, drought, salinity, alkalinity UV, and pathogen infection; abiotic stress is the primary cause of crop loss world wide by more than 30% (Goswami *et al*.,2016). Under acidity, salinity, drought, plants produce ethylene to overcome the stressful conditions. The PGPR possess the ability to produce an enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme to decrease the ethylene synthesis by competing with plant ACC oxidase (Vargas *et al*.,2010).

Chapter 3

3. Survey of genomic diversity of rhizobia (*Mesorhizobium* spp.) nodulating Chickpea (*Cicer arietinum* L.) in Ethiopian

Zehara Mohammed Damtew[1,2*]. Fassil Assefa[1], Alex Greenlon[4]., Asnake Fikre[2,] Noelia Carrasquilla-Garcia[3] Eric J. B. von Wettbergg[5] and Douglas R. Cook[3]

[1]Department of Microbial Cellular and Molecular Biology, College of Natural Sciences, Addis Ababa University, Addis Ababa, 32853 Ethiopia; [2]Debre Zeit Agricultural Research Center, Ethiopian Institute for Agricultural Research, Bishoftu; [3]Department of Plant Pathology, University of California, Davis, CA 95616;[4]University of California, Berkeley, Environmental Science, Policy and Management;[5]Department of Plant and Soil Science, University of Vermont, Burlington, VT 05405;

Abstract

Chickpea nodulated by a very specific group of root nodule Mesorhizobia. The two specific well known species are Mesorhizobium ciceri and Mesorhizobium mediterreanum, that were mainly studied from the Mediterranean Area. However, recent studies from different chickpea grown areas revealed the presence of more diverse groups that nodulate chickpea. This necessitates the screening of more isolates from different centers of origin of the crop to fully reveal the phylogenetic diversity of the endosymbionts from the presumably large genetic diversity of rhizobial species in order to select and develop elite rhizobial bio-inoculants for demanding agriculture. In this study, chickpea nodules (80) were surveyed from 138 farmer fields from which 64 isolates were obtained and characterized based upon amplicon sequencing. All 64 strains fell into the genus Mesorhizobium grouped into eleven distinct species, of which two strains genospecies7A (3% of the surveyed diversity) were aligned to the previously observed M. ciceri. The largest group is M. genospecies 2A containing 26 strains, was conspecific to M. spp. ORS3359, an unnamed symbiont isolated from Acacia seyal in Senegal. The Genospecies

2A is also related to but distinct from M. plurifarium. A single additional strain is conspecific with M. spp. WSM3876, isolated from Astragalus pelecinus in Eritrea. Fifty three percent of the strains were presumed to represent eight novel species, encountered here for the first time. Mapping of strain diversity to geographic origin revealed wide distribution of most clades, with minimal or no geographic structuring.

Key words: Nodule, *Mesorhizobium*, nucleotide, Rhizobia, whole genome

3.1. Introduction

Chickpea (*Cicer arietinum* L.) is the second most important food legume in the world after common bean (*Phaseolus vulgaris* L.) (FAOSTAT, 2015). It is widely grown in the semi-arid regions across the Indian sub-continent, the Middle East, Mediterranean regions, Australia, Mexico and Ethiopia. Ethiopia is one of the second centers of diversity of the crop (Geletu Bejiga and van der Maesen, 2006). Traditionally, chickpea was thought to be nodulated by specific group of moderate to slow growing rhizobia and later were identified into the genus *Mesorhizobium* and two species *M. ciceri* (Nour *et al*., 1994) and *M mediterraneum* (Nour *et al*., 1995). Later on, other *Mesorhizobium* species; *M. loti* isolated from lotus species, *M. amorphae* (Amorpha fruticosa) and *M. tianshanense* from Astragalus
(Rivas *et al*., 2007), *M. huakuii* a symbiont of Astragalus sinicus (Alexandre *et al*., 2009), *M. opportunistum* from Biserrula pelecinus (Laranjo *et al*., 2012) and *M. muleiense* isolated from Cicer arietinum in china (Zhang *et al*., 2012b) and unnamed novel *Mesorhizobium* genospecies (Elias and Herridge, 2015) were also identified from different parts of the world.

Several studies showed that local *Mesorhizobium* species acquired chickpea-nodulating ability from the typical *M. ciceri* or *M. mediterraneum* (introduced to an area together with the crop) through lateral transfer of symbiotic genes (Zhang *et al*, 2017). The diversity of chickpea rhizobia also varies with geographical locations and other environmental conditions like soil pH, salinity (Alexandre *et al*., 2009). Greenlon *et al*., (2019), recently showed symbiosis with chickpea involves an unexpectedly wide diversity of *Mesorhizobium* species varying according to geographic patterns and land use practices.

The hitherto studies in Ethiopia showed that chickpea is nodulated by diverse groups of rhizobia (Mulisa Jida and Fassil Assefa, 2012; Daniel Muleta and Fassil Assefa, 2015; Wubayehu Gebremedhin *et al*., 2018). However, most of this diversity were limited to phenotypic characteristics and with little support from molecular analysis. Recently, Wondwosen Tena *et al*., (2017) identified four different genospecies of which three of them were highly related to *Mesorhizobium* species not previously known to nodulate chickpea.

In Ethiopia (Daniel Muleta and Fassil Assefa, 2015) found a total of 70 chickpea root nodule bacteria from Central and Northern Ethiopia of which only 52% were rhizobia, the remaining were endophytes and nodulating rhizobia based up on presumptive and authentication tests. Wubayehu Gebremedhin *et al*., (2018) isolated 39 root nodule bacteria from chickpea growing areas of the Eastern, Southeastern and Southern parts of the country, of which 23 isolates (59%) were identified as chickpea root nodule bacteria with the same identification methods. Recently (Tassew Siraj and Fassil Assefa, 2018) screened 24 genetically diverse indigenous *Mesorhizobium* spp for their potential to ecological adaptations at laboratory and their symbiotic effectiveness on two chickpea varieties under greenhouse conditions.

However, effective *Mesorhizobium* strains compatible to multiple varieties of the crop inoculation have not been intensively characterized from representative regions of Ethiopia and we lack an understanding of diverse *Mesorhizobium* strain in relation to their eco-physiological competence. This shows introduction of competitive recipient *Mesorhizobium* strains which displayed a large spectrum persistence adaptive mechanism with different types of chickpea varieties to addressed developing inoculum towards promising impact on chickpea production is stil a current challenge. Ethiopia is one of the centers of diversity of chickpea varieties/landraces and the crop is grown in diverse agro-ecological conditions (Geletu Bejiga and Ketema Daba, 2006; Gemechu Keneni *et al*., 2012).

This presupposes the existence of more diverse groups of chickpea rhizobia in the Ethiopian soil that mirrors the postulated secondary diversification of endemic crops postulated by (Harlan,1969). Since chickpea is commonly cultivated on marginal and

stress conditions, studying its *Mesorhizobial* diversity is highly important to select efficient strains to enhance the productivity of the crop. It can be hypothesized that collection and genomic analysis of a large number of symbiotic bacteria from Ethiopia's chickpea growing areas is expected to reveal genomic diversity and in so doing provides a rational basis to prioritize a manageable set of strains for functional testing. The long-term goal is to identify strains that can maximize nitrogen fixation efficiency in Ethiopia's chickpea production system.

3.2. Materials and Methods

3.2.1. Regional coverage and sampling of farmer fields

Chickpea fields were surveyed at 138 locations in the two major producing regional states of Amhara and Oromia (Figure 3.1) and each *Mesorhizobium* strains administrative zone and geographical origin presented (appendix 1) during the 2014/2015 cropping season. Hierarchical N2-fixing root nodules sampling scheme from chickpea across soil types, climates, growing seasons, agricultural methodologies, histories of cultivation and multiple geographic scales. Farmers were interviewed using a standardized questionnaire to obtain information about the history of cultivation (Table 2.2). At each field, the roots of five plants were excavated and healthy looking nodules were removed from the root and preserved in vials filled with silica gel beads at 4°C until further processing. Isolation, characterization and molecular analyses were conducted at the University of California, Davis, California, USA.

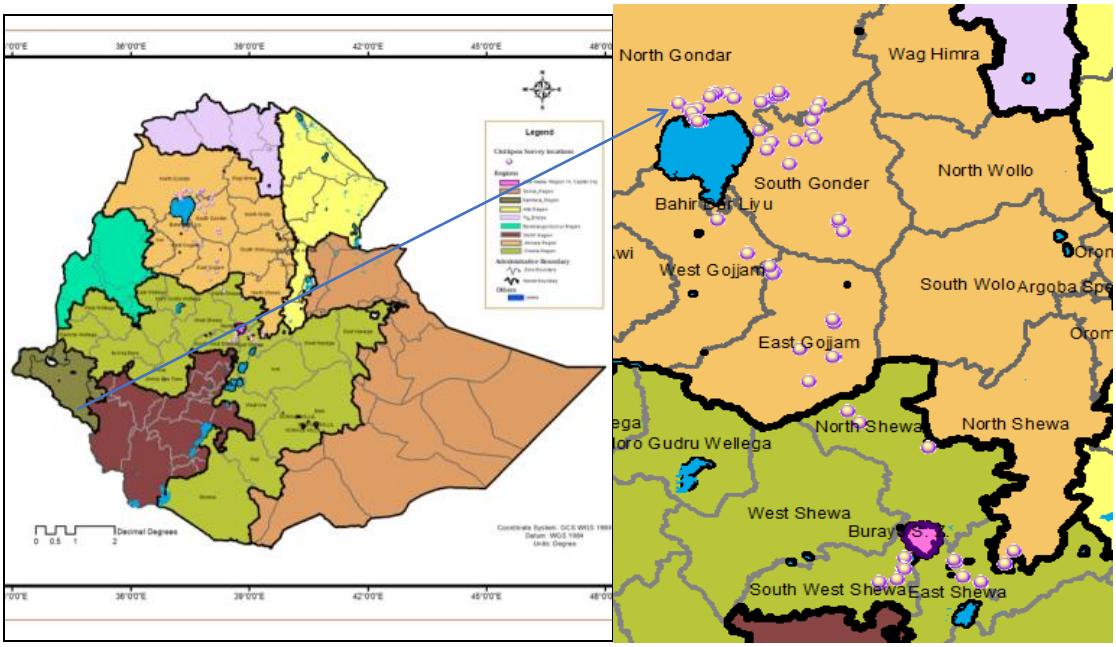


Figure 3.1. Distribution of Sample Collection sites

Bacteria were isolated and purified as described by (Somasegaran *et al*., 1994). Nodules were rehydrated by immersing them in sterile water overnight and were briefly surface-sterilized with 70% (v/v) ethanol 2% (v/v) sodium hypochlorite solution for 2 minutes, rinsed with five change of sterile distilled water. Individual nodules were crushed and loopful of the suspension was streaked on sterilized yeast extract mannitol agar plates (YEMA) medium containg (mannitol 10g/l, K2HPO4 0.5g/., MgSO4.7H2O 0.2g/l, NaCl 0.1g/l, yeast extract 0.5g/l and agar 15g/l). The plates were incubated at 28°C for 5-7 days. Single colonies were picked and purified the sub-cultured by serial re-streaking on the same medium plates. A total of 106 purified colonies were preserved in 20% (v/v) glycerol stocks at -80^{o}C for further tests.

3.2.2. Genomic DNA and Whole genome analysis

Individual isolates from glycerol stocks were grown in YM broth until they became turbid and pelleted in a microfuge for total genomic DNA extraction. Thus, DNA of 106 isolates was extracted using the Qiagen's Blood and Tissue DNeasy kit (Qiagen Ltd., CA, USA) following the corresponding protocol for Gram-negative bacteria. DNA purity was

assessed using a Nanodrop Ó 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, USA) based on the ratio of absorbance at 260 nm/230 nm and 260 nm/280 nm. DNA concentration was determined using Picogreen fluorescent dye (Invitrogen) on Tecan fluorescence plate reader, with a subset of samples confirmed using Qubit ® 2.0 fluorometry (Invitrogen, CA, USA) with the accompanying DNA quantity standards. The samples were resolved on an agarose gel to assess DNA integrity. DNA quantity data was used to normalize DNA concentration for subsequent genome libraries.

Finally, based on these DNA integrity assessment 80 pure isolates were selected for Illumina whole genome sequencing libraries. Genome libraries were prepared for sequencing using the Illumina Nextera XT kit Handbook (Qiagen, CA, USA), following the manufacturer protocols (Illumina, 2012). Libraries were normalized to a uniform concentration using Qubit ® 2.0 fluorometry (Invitrogen, CA, USA) and pooled them together. Nextera XT libraries were sequenced using the Illumina MiSeq platform (Illumina, San Diego, CA, USA) at University of California Davis, Genome Center DNA Core Facility USA

Illumina libraries were demultiplexed using Cassava (Illumina Inc, version 1.7). Quality assessment and filtering of sequenced data were accomplished using FastQC (

http://www.bioinformatics.babraham.ac.uk/projects/download.html - fastqc) at a threshold of 0.001 (Phred score of 30). Raw Illumina reads were trimmed and filtered using

Trimmomatic version 0.36 (Bolger *et al*., 2014) to remove adaptors, low-quality sequence reads and overlapping sequences not supported by as paired ends. Trimmed reads were assembled into contigs using SPAdes version 3.10.1 (Bankevich *et al.,* 2012). Resulting contigs were aligned using BLAST version 2.2.30+ (Camacho *et al.*, 2009) to reference genomes and verified by CheckM version 1.0.7 (Parks *et al*.,2015) and Phylosift (Darling *et al*.,2014) to check the completeness and contamination. Reads that comprised *Mesorhizobium*-mapped contigs were then assembled using SPAdes version 3.10.1 (Bankevich *et al.,* 2012). Genomes with greater than 10% contamination reported by CheckM were excluded from further analysis (e.g., ANI). Genes were predicted from

contigs and annotated *de novo* in draft Illumina assemblies using the Prokka pipeline version 1.13 (Seemann, 2014).

Phylogenetic placement of Ethiopian chickpea isolates was performed using a multilocus protein sequencing phylogeny. The ortholog genes from a set of assemblies, along with available related assembled genome retrieved from NCBI in the bacterial family *phyllobacteriaceae* were inferred. Alignment and concatenation of 400 ubiquitous phylogenetically informative set of protein sequences were performed in PhyloPhlAn program version 0.99 (Segata *et al*., 2013). Thus, a phylogenetic tree was constructed using maximum likelihood (ML) methodology on the basis of protein-coding sequences (CDS) of 400 conserved single-copy, universal marker genes, present in all 64 strains. The confidence levels of the phylogenetic trees are evaluated as bootstrap values based on 1,000 replications. The phylogenetic analysis of symbiotic genes was inferred based on alignments of each symbiotic gene *nodC* and *nifH* with RaxML version 7.2.7(computed under the GTRCAT model of nucleotide substitution with 100 bootstrap repetitions) (Stamatakis, 2014).

The phylogenetic tree was then plotted using Interactive Tree of Life (iTOL) version 4 (Letunic and Bork, 2019). For the sub-species demarcation patterns, pairwise average nucleotide distance on genes from the phylophlan marker set calculated using the JGI's gANI calculator version 1(Varghese et al., 2015). Clustered genomes into groups using mothur version 1.39.5 (Schloss et al., 2009). To further determine genetic relationships among the strains, the principal coordinate analysis (PCoA) was performed with GenAlEx 6.5 (Peakall and Smouse, 2006) software. The details of whole genome and taxonomic characterization were compiled with global level population genomic as part of the thesis work in **Paper I** (Greenlon *et al*., 2019). The Genome sequence assembly for the whole genome sequence projects has been deposited at EMBL under an umbrella BioProject PRJNA453501 with accession numbers PRJNA453501.

3.2.3. Soil characterization of sampling sites

For each sampled field, 500 gm of soil was collected and characterized for macro and micronutrients following standard protocols (von Wettberg *et al*., 2018) at Western Laboratories (Modesto, CA, USA). The elements measured included; total Cu (mg/kg), total Zn (mg/kg), total Fe (%), total Ca (g/kg), total Mn (g/kg), total Na (g/kg), total K (g/kg), total Mg (g/kg), nitrogen (all forms, as a percentage), total organic carbon, total inorganic carbon, total sulfur (ppm), Lime-$CaCO_3$ (%), pH, EC (µS/cm), and Potassium (P_2O_5, in mg/kg). Soil pH, plant available phosphorus, potassium, magnesium, calcium, Copper, Iron, Zinc, Sulfur, manganese, Boron were interpreted according to (Benton, 2003), Organic Matter rating (Tekalign Tadese, 1991) and ratings of cation exchange capacity (Hazelton and Murphy, 2007) were performed.

3.2.4. Authentication of root nodule formation at greenhouse

The ability of each strain to elicit nodule formation on the host was carried out under greenhouse conditions at the University of California Davis. Seeds of domesticated chickpea genotype ICCV 96029 were surface sterilized using surface sterilized with 4% sodium hypochlorite for 3 min, then rinsed with five changes of sterile distilled water and allowed to germinate on 1% (w/v) water agar until radical emergence.

Germinated chickpea seedlings were transferred into deep pot containers (D40H) and seedlings were inoculated with 1 ml of cells from fresh, fully turbid culture containing ~10^8 viable cells (colony forming units) for each respective strain. After 30 days, the plants were harvested to check nodule formation.

3.3. Results and Discussion

3.3.1. Diversity of bacterial symbionts of chickpea

A total of one hundred thirty eight smallholder farms were surveyed covering top eight chickpea producing zones, representing forty-six special districts in two regional states. Sampling elevations spanned 1,130 meters above sea level. Farmers interviews revealed that 66% of fields were sown with chickpea landraces, often preferred for local consumption, with the remaining 34% planted to one of five improved cultivars (Table

3.1) as *genospecies* level and presented in (appendix 1) for each strain. Almost 84% of farmers reported no use of fertilizer inputs as summarized (Table 3.1) and presented in (appendix 2) for each field. Subsequently, 14% used diammonium phosphate (DAP) and a single as compost as fertilizer with no history of rhizobial inoculation input.

Table 3.1. Sampling sites biophysical factors associated *Mesorhizobium* genospecies clustering

Cultivation history	***M. Genospecies***											
	2A	**8A**	**1B**	**1E**	**4A**	**4B**	**3A**	**1A**	**1D**	**7A**	**9A**	**Total (%)**
Number of fields	26	6	3	5	6	8	2	2	3	15	2	**46**
Chickpea type (%)												
Landrace	19	2	1	5	5	6	0	2	2	0	1	**66**
Improved cultivars	7	4	2	0	1	2	2	0	1	2	0	**34**
Rotation pattern (%)												
Tef-Chickpea	14	0	1	1	2	2	1	1	0	0	1	**36**
Wheat-Chickpea	1	0	0	1	1	2	0	0	0	0	0	**8**
Barely-Chickpea	2	6	1	1	3	3	0	0	1	2	0	**28**
Sorghum-chickpea	7	0	1	2	0	1	1	1	2	0	0	**22**
Faba bean-chickpea	0	0	0	0	0	0	0	0	0	0	0	**1**
Chickpea -chickpea	1	0	0	0	0	0	0	0	0	0	0	**1**
Gibeto-Chickpea	1	0	0	0	0	0	0	0	0	0	0	**1**
Fertilizer Used												

DAP	2	0	1	2	1	2	1	0	0	0	0	**14**
Not used	24	6	2	3	5	6	0	2	3	2	1	**84**
Compost	0	0	0	0	0	0	1	0	0	0	0	**1**
Desired traits												
Consumption	20	4	3	4	5	5	2	2	2	2	1	**78**
Market	6	2	0	1	1	3	0	0	1	0	0	**22**
Diseases symptom												
Medium	0	2	0	0	0	0	0	0	0	0	0	**3**
Low	26	4	3	5	6	8	2	2	3	2	1	**97**

3.3.2. Whole genome sequencing and average nucleotide identity analysis distinction

Sequence reads of 80 bacterial cultures genomes were evaluated for completeness and genomes suitability for further analyses. Excluding sixteen strains with contamination in excess of 10% or genomes estimated to be less than 85% complete. The genome statistics such as genome coverage, GC content, *N*50, lengths of contigs and scaffolds for each strain presented in (Appendix 3). Thus, 64 genome assemblies resulted in the generation of 2564 average contigs and 2569 average scaffolds. The average genome size among the set was calculated to be 6,960382 base pairs long with average N50 and GC content of 7443bp and 63.3%, respectively. Genomes annotated in draft whole genome assemblies predicted 64 strains belonged to nitrogen-fixing bacterial symbionts of chickpea share highest affinity with genus *Mesorhizobium*.

Subsequently, based on the criterion that strains with average nucleotide identity >95% (ANI_{95}) are members of the same species (Goris *et al*., 2007), the set of strains further resolved into eleven distinct species. We refer to these as "genospecies 1A", "genospecies 1B", etc (Table 3.2, Figure 3.2 and Appendix 3) or as ANI_{95} groups. These independent Ethiopian strain genospecies categories were derived from the associated study of (Greenlon *et al*. 2019) who showed that 95% ANI as the lower boundary of phylophlan marker genes for confirming six countries chickpea rhizobia with their global close relatives and with each other.

It can be argued that the assembly genomes size in this study is comparable with other previously sequenced *Mesorhizobium* species from gene bank (Haskett *et al.,* 2016; Wang *et al*., 2018). Based on this for some species such as *M. huakuii* 7653R (6.88Mb), *M. loti*

R7A (6.52Mb), *M. opportunistum* WSM2075(6.88Mb), *M. ciceri* CC1192(6.99Mb), *M. amorphae* CCNWGS0123 (7.34Mb) and *M. australicum* WSM2073 (6.20Mb) were reviewed by (Atsede Muleta, 2020). This might be the case that high quality draft assemblies from genomic libraries were contribute for analysis of genome content and revealed their quality for further phylogenetic analyses and species assignment.

Table 3.2.Genome sequencing characteristics for each *Mesorhizobium* genospecies at averaged based

No	Sample strains	ANI95 group	N50	Scaffolds (No)	Genome size	Longest Scafold	GC %	Completeness
1	*M.genospecies* 2A	2A	7731	3037	7306586	51709	63.6	91.8
2	*M.genospecies* 8A	8A	9548	1495	6795981	69012	62.5	97.7
3	M.genospecies 7A	7A	17281	1496	6447211	87575	62.5	99.5
4	*M.genospecies* 9A	9A	6418	710	7144145	11576	62.5	96.2
5	*M.genospecies* 1B	1B	3486	4218	8140628	43994	63.1	93.9
6	*M.genospecies* 1E	1E	7009	1975	7109814	68530	63.1	96.6
7	*M.genospecies* 4A	4A	7873	1956	6661177	57476	63.6	95.3
8	*M.genospecies* 4B	4B	9674	1834	6639088	64528	63.8	96.1
9	*M.genospecies* 3A	3A	6940	2206	6476389	54387	63.2	98.0
10	*M.genospecies* 1A	1A	10220	1103	7804200	27193	62.7	97.3
11	*M.genospecies* 1D	1D	3615	3246	6660960	26445	63.5	96.3

3.3.3. Advent of phylogenetic analysis of protein coding gene in native root nodulating bacteria

Phylogenetic sequence analysis based on 400 phylogenetically conserved bacterial protein-coding genes predicted from whole-genome alignments, that permitted accurate placement of these 64 strains within the broader context of known *Mesorhizobium*, as well within the bacterial family *Phyllobacteriaceae* (Figure 3.2). This genome based phylogenetic analysis confirmed and added detail to the assignment of strains to *Mesorhizobium* genospecies (Figure 3.2). The phylogenetic analysis based on these concatenated of 400 conserved single-copy, universal marker genes revealed numerous well supported relationships within and among distinct clades and well-supported together with the type strains of their closest *Mesorhizobium* species. The majority of which contain numerous strains within individual genospecies. Ethiopian strains were clustered into six phylogenetic groups together with the type strains of their closest *Mesorhizobium*

species and their phylogenetic breadth concentrated in seven broad clades relative to bacterial strains that nodulate chickpea globally occur throughout the full diversity of the genus *Mesorhizobium* (Figure 3.2).

Only two of the 64 strains correspond to species previously observed on chickpea, both of which are in genospecies 7A *M. ciceri*. The largest species group (26 strains in total) is *M. genospecies* 2A, which is conspecific with *M. spp*. ORS3359(NZ-CCNC01000214.1), an unnamed symbiont isolated from Acacia in Senegal. *Genospecies* 2A is related to but distinct from *M. plurifarium* STM8773, which is exemplary of genospecies 2C (Greenlon *et al*., 2019). The specific species *Mesorhizobium plurifarium* that previously described to form nodules on tree and shrub legumes throughout the Old and New World tropics (Diouf *et al*.,2015). Two strains, 80P4S2 and 10P4S2 are conspecific with *M. spp*. WSM3876(NZ-NSGA01000056.1), isolated from *Astragalus pelecinus* in Eritrea and belonging to *genospecies* 3A. The fact that the genome-based species assignments are most often to symbionts previously reported from distant legume relatives is consistent with the conclusion that host range of the bacterium is governed by a highly mobile integrated conjugative element (ICE) that is sufficient to convert a wide range of *Mesorhizobium* species into chickpea symbionts (Greenlon *et al*., 2019).

The remaining 34 strains represent eight new genospecies, not previously reported in public genome databases. These include genospecies 1A, B, D, E, 4A, B, 8A and 9A. While several of these novel genospecies have phylogenetic proximity to named species, none of the corresponding named species are calculated to be conspecific with this newly described diversity. For example, six strains are assigned to *genospecies* 8A, for which the closest named strain is *M. australicum* WSM2073. While bootstrap values reveal 100% association of *genospecies* 8A with WSM2073, they are nevertheless not the same species. *Mesorhizobium australicum* strain WSM2073 was isolated from root nodules on the pasture legume *Biserrula pelecinus* growing in Australia in 2000(Nandasena *et al*., 2009). Later on, strains belonged to this species also described as symbionts of chickpea (Greenlon *et al*.,2019) and inoculation of chickpea cultivars with native Ethiopian *Mesorhizobium* strains relative to *Mesorhizobium australicum* WSM2073 increased in

nodule number, nodule dry weight and shoot dry matter at greenhouse conditions in Ethiopia (Zehara *et al*.,2020).

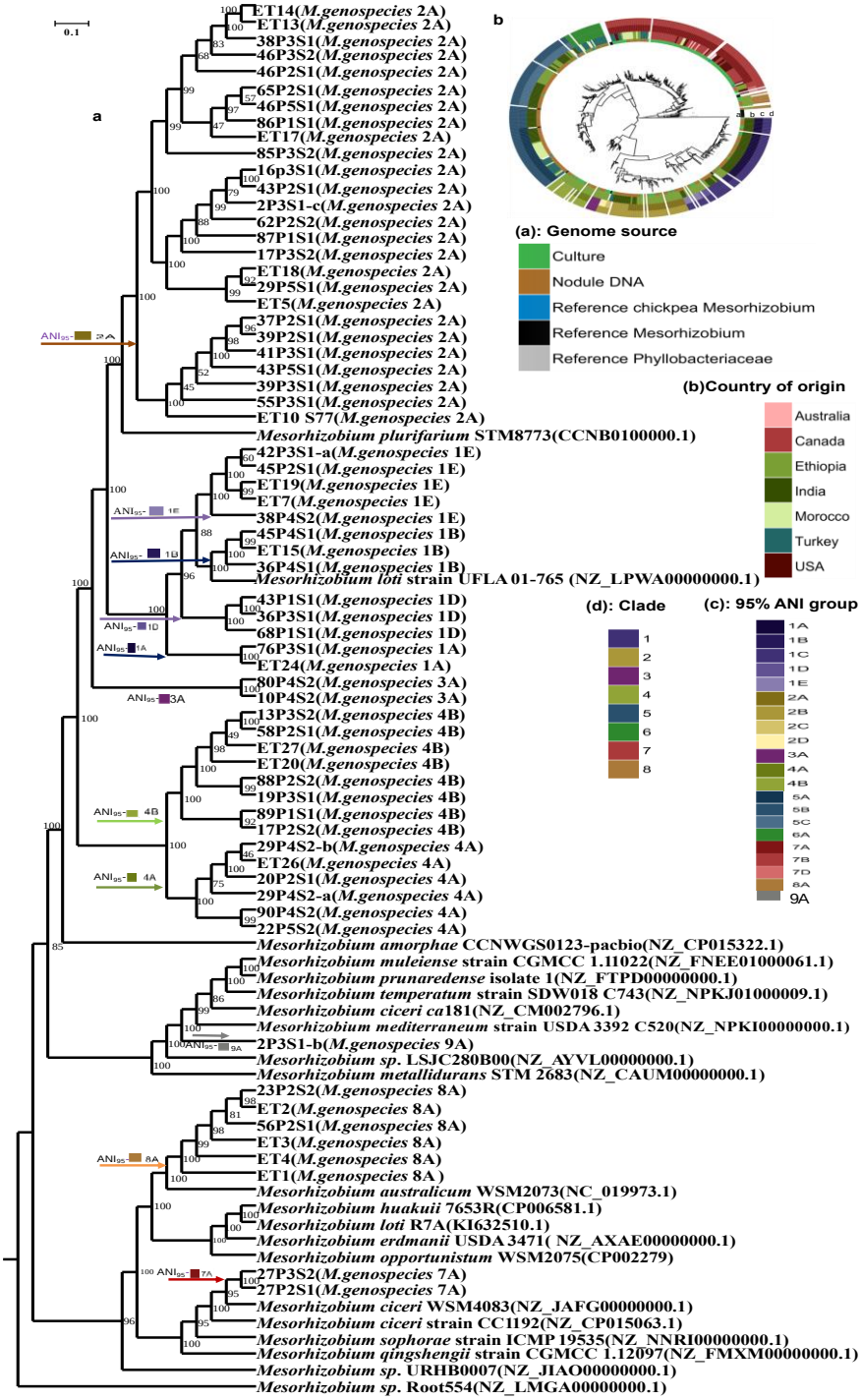


Figure 3.2. (a) Phylogenetic relationships and species assignments of Ethiopian chickpea nodulating bacterial strains based on 400 phylogenetically conserved bacterial protein-coding genes predicted from whole-genome sequence assemblies and bootstrap values based on 1000 replications at each node, b) Global collection of chickpea's *Mesorhizobium* symbiont; concentric rings represent from inner to outer a) Genome source, b) country of origin, c) 95% Average Nucleotide Identity group, d) major clades of bacterial strains that nodulate chickpea globally occur throughout the full diversity of the genus *Mesorhizobium*.

A second group of 14 strains forms two sister clades with 100% bootstrap support (*genospecies* 4A and 4B), but for which there is no related genome and therefore no assignable species name. Instead, 4A and 4B are sister to a large and diverse group of strains including genospecies 1A, B, D, E and 2A. Thus, Ethiopian *Mesorhizobium* strains on chickpea are a highly diverse and span much of the known diversity of *Mesorhizobium* (Greenlon *et al*., 2019). Moreover, the observation that many strains are conspecific with strains from diverse legume species, including members of the genera *Acacia* and *Astragalus*, indicates that the genome backgrounds of chickpea symbionts are also ecologically diverse.

Generally, strains such as 1A, 1D, 4A and 4B corresponding to relative species *M. amorphae*, which was originally described as symbionts of the leguminous shrub *Amorpha fruticosa;* later this species being a common chickpea nodulating symbionts in Spain (Rivas *et al*., 2007). Similarly, strain 1B and E affinity to *M. loti*, that previously reported as chickpea symbionts in Morocco (Maatallah *et al*., 2002). Therefore, the present study revealed significant genomic diversity of chickpea nitrogen-fixing bacterial symbionts in the genus *Mesorhizobium* throughout chickpea growing regions of Ethiopia. This outcome might be due to methodical sampling of the root nodules and the use of massively parallel whole genome sequencing.

3.3.4. Phylogenetic analysis of symbiotic *nodC* and *nifH* genes

The sequence analysis of nodC genes of the Ethiopian strains confirmed existence of diversity of *Mesorhizobium* strains to nodulating Chickpea in Ethiopia (Figure 3.3). The phylogenetic trees of *nod* C clustered the strains into four clades, together with the type strains of their closest *Mesorhizobium* species (Figure 3.3). According to the nodC

gene phylogeny 46 strains (29%) showed close resemblance to the reference type strain *Mesorhizobium ciceri* strain CC1192, isolated from *Cicer arietinum* in Israel (Corbin *et al*.,1977). In parallel, 7 strains (5%) were grouped together in a separate clade along with reference strain *Mesorhizobium ciceri ca181*, isolated from Cicer arietinum in India (Yadav, 2006).

The other, 5 Strains (3%) scattered along with *Mesorhizobium muleiense strain CGMCC 1.11022*, isolated from Cicer arietinum in China (Zhang *et al*., 2017). The remaining, 3 strain (2%) clade together with *Mesorhizobium mediterraneum strain USDA 3392 C520*, from Cicer arietinum in France.

As indicated above, *Mesorhizobium* genes involved in legume symbiosis are both diverse and highly mobile (Greenlon *et al*, 2019). Thus, while the phylogenetic patterns of such genes reflect their individual histories, there is often limited consensus between such genes and with the background genome (Gaby and Buckley, 2014). nodC is a chitin synthase, widely distributed in and specific to rhizobia where it is involved in the synthesis of the chito oligosaccharide backbone of nod factor. *nifH* encodes the dinitrogenase reductase subunit of nitrogenase that is common to all nitrogen fixing organisms.

The *nodC* phylogenetic tree confirmed the high diversity of *Mesorhizobium* strains nodulating chickpea (Figure 3.3). As expected, the phylogenetic placement of the *nodC* orthologs revealed widespread disagreement with the genospecies concept, as exemplified by strain nomenclature and by comparison to the phylogenetic analysis of 400 core genes in (Figure 3.2). These stark differences highlight the relatively stable nature of the core genome and the highly transferrable nature of components of the symbiosis machinery.

To further highlight the incongruity of species relationships and *nodC* phylogenetic placement in (Figure 3.3), we point to the placement of strains from genospecies 7A, which is *M. ciceri* the native symbiont of *C. echinospermum* (Greenlon *et al*., 2019). None of the four *M. ciceri* strains shown in (Figure 3.3) have similar phylogenetic placement based on nodC, despite their occurrence in a conserved core ANI_{95} group and a single well supported clade based on 400 Phylophlan orthologs (Figure 3.3). Thus, these *M. ciceri*

nodC orthologs have very different evolutionary histories. This discordance between symbiotic genes and species assignment is even more evident considering the 26 strains of genospecies 2A, which are distributed throughout every major branch of the *nodC* phylogeny (Figure 3.3).

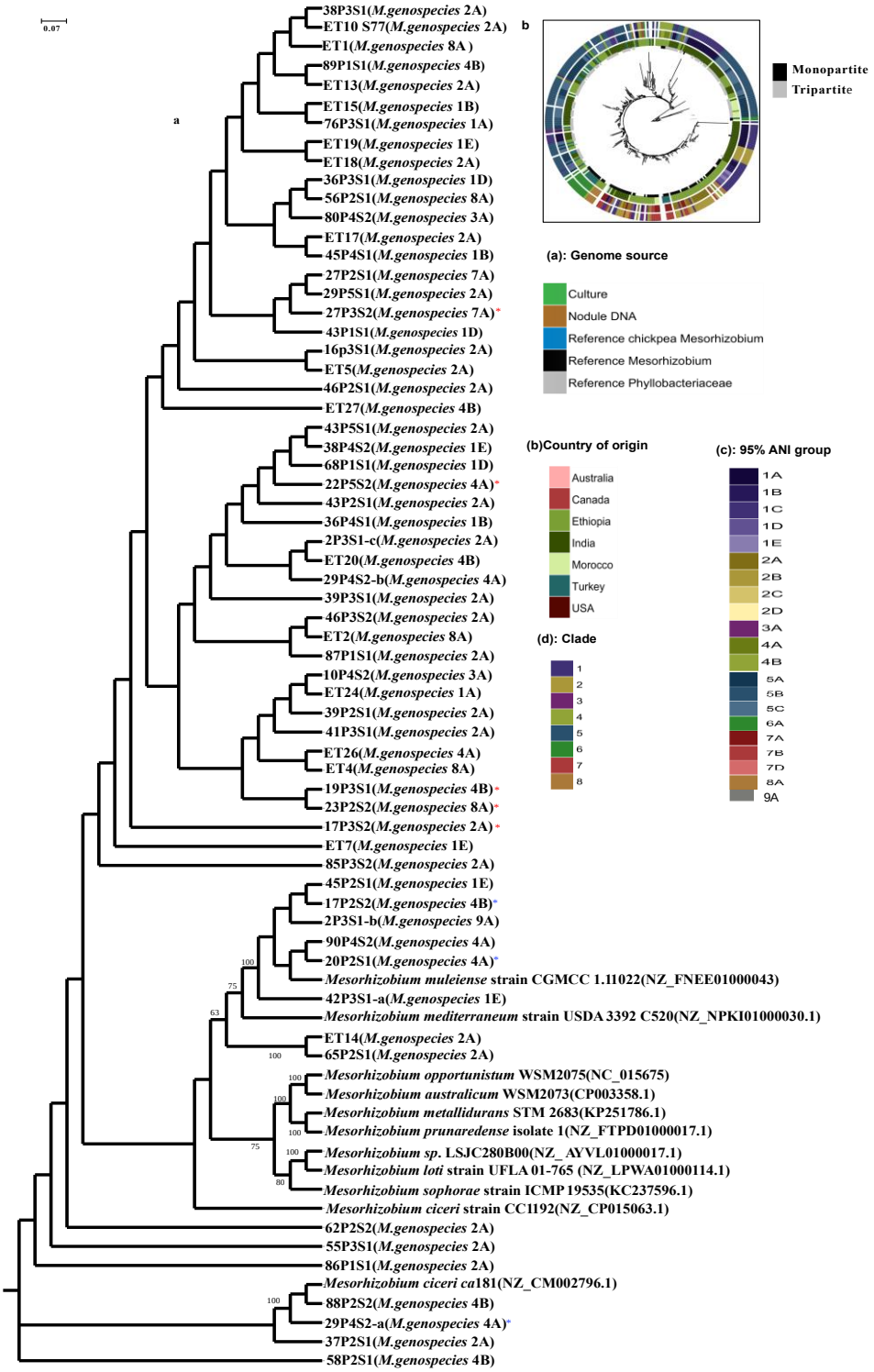


Figure 3.3. (a) Phylogenetic relationships of Ethiopian strains based on sequences of symbiosis-related genes *nodC* genes predicted from whole-genome assemblies; (b) Global collection of chickpea's *Mesorhizobium* symbiont; concentric rings represent from inner to outer a) Genome source, b) country of origin, c) 95% Average Nucleotide Identity group, d) major clades of bacterial strains that nodulate chickpea globally occur throughout the full diversity of the genus *Mesorhizobium*. Bootstrap values over 60% (based on 1000 replications) are shown at each node

Similarly, the symbiotic nitrogen fixation genes *nifH* phylogenetic tree were confirmed many *M. genospecies* strains which showed a closed relative with four *Mesorhizobium* species described previously to nodulating Chickpea (Figure 3.4). The symbiotic position of the strains based on *nifH* gene assigned into four closed relative group, the largest main clades correspondent to *Mesorhizobium ciceri* strain CC1192 containg 40 strains (26%).

The other strains formed minor clades with *Mesorhizobium mediterraneum* strain USDA 3392 C520 included 9 strain (5%), followed by *Mesorhizobium muleiense* strain CGMCC 1.11022 related group included 8 Strains (5%). Subsequently, 4 strains (3%) being differentiated congruent with *Mesorhizobium ciceri* ca181 and one strains belonging with *Mesorhizobium loti R7A;* which reported to nodulate chickpea (Laranjo *et al*., 2008). The sequence analysis of *nod* C and *nifH* clustering related to available closest *Mesorhizobium* species; showed that the chickpea nodulating strains shared related *nod* C and *nifH* gene sequences regardless of their different chromosomal backgrounds and site of origin. That might reflecting the host specificity of these symbiotic genes for chickpea (Rivas *et al*., 2007; Laranjo *et al*., 2008; Zhang *et al*., 2017).

The symbiotic nitrogen fixation gene *nifH* reveals a similarly complicated phylogenetic history (Figure 3.4), with no resemblance to the well-supported species assignments (based on ANI and as in Figure 3.2). Given the known promiscuity of *Mesorhizobium's* symbiotic ICE, one might expect the phylogenetic signal among symbiosis genes to be coherent, however comparison of the placement of sets of strains between the nodC and *nifH* phylogenetic trees (Figures 3.3 and 3.4, respectively) reveals an almost complete lack of correlation. The chaotic history of *nodC* and *nifH* orthologs relative to each other and to species' evolution almost certainly reflects a history of frequent and successive gene transfer events among strains, at the level of individual genes or gene neighborhoods between integrative conjugative elements (ICEs).

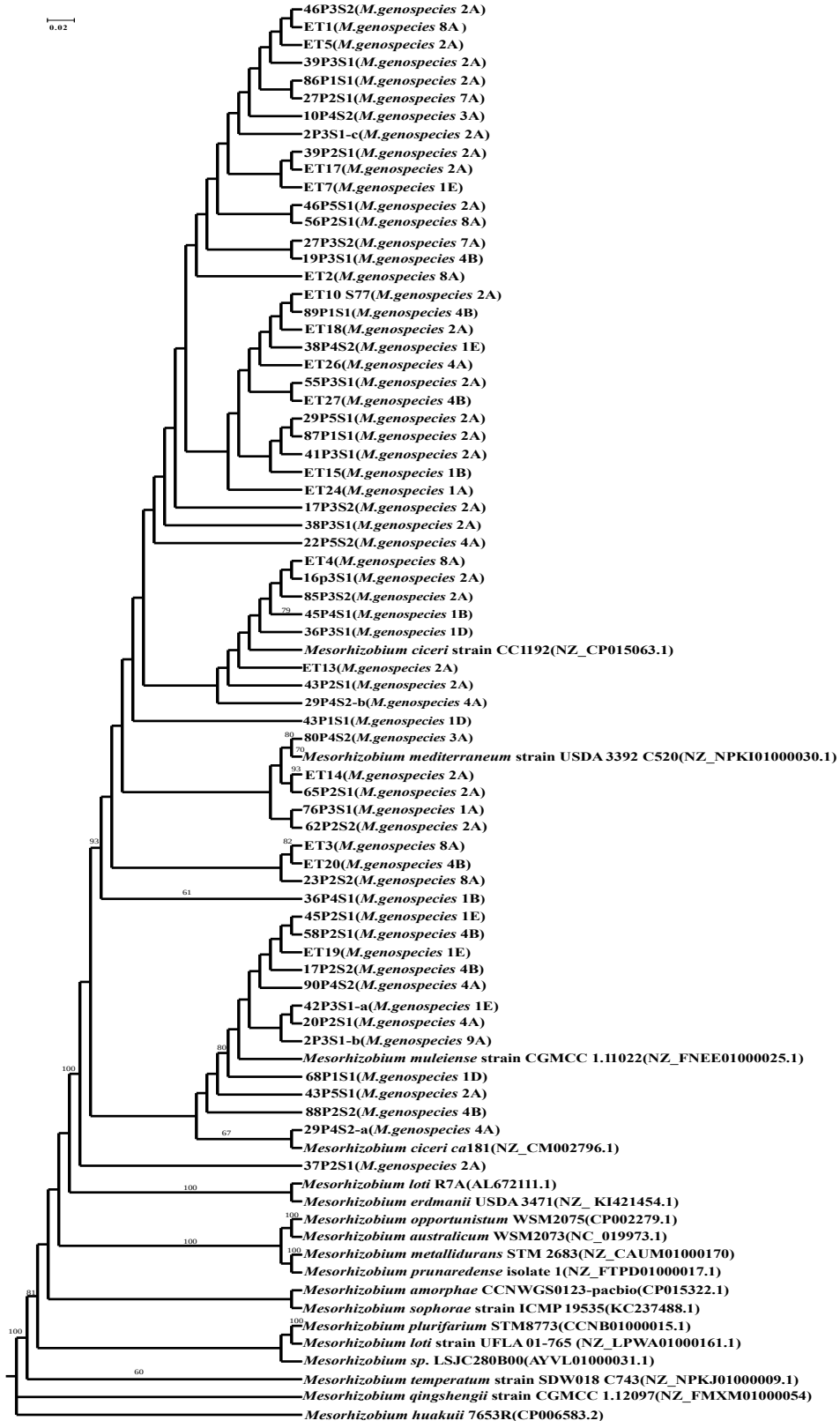

0.02
46P3S2(M.genospecies 2A)
ET1(M.genospecies 8A)
ET5(M.genospecies 2A)
39P3S1(M.genospecies 2A)
86P1S1(M.genospecies 2A)
27P2S1(M.genospecies 7A)
10P4S2(M.genospecies 3A)
2P3S1-c(M.genospecies 2A)
39P2S1(M.genospecies 2A)
ET17(M.genospecies 2A)
ET7(M.genospecies 1E)
46P5S1(M.genospecies 2A)
56P2S1(M.genospecies 8A)
27P3S2(M.genospecies 7A)
19P3S1(M.genospecies 4B)
ET2(M.genospecies 8A)
ET10 S77(M.genospecies 2A)
89P1S1(M.genospecies 4B)
ET18(M.genospecies 2A)
38P4S2(M.genospecies 1E)
ET26(M.genospecies 4A)
55P3S1(M.genospecies 2A)
ET27(M.genospecies 4B)
29P5S1(M.genospecies 2A)
87P1S1(M.genospecies 2A)
41P3S1(M.genospecies 2A)
ET15(M.genospecies 1B)
ET24(M.genospecies 1A)
17P3S2(M.genospecies 2A)
38P3S1(M.genospecies 2A)
22P5S2(M.genospecies 4A)
ET4(M.genospecies 8A)
16p3S1(M.genospecies 2A)
85P3S2(M.genospecies 2A)
45P4S1(M.genospecies 1B)
36P3S1(M.genospecies 1D)
Mesorhizobium ciceri strain CC1192(NZ_CP015063.1)
ET13(M.genospecies 2A)
43P2S1(M.genospecies 2A)
29P4S2-b(M.genospecies 4A)
43P1S1(M.genospecies 1D)
80P4S2(M.genospecies 3A)
Mesorhizobium mediterraneum strain USDA 3392 C520(NZ_NPKI01000030.1)
ET14(M.genospecies 2A)
65P2S1(M.genospecies 2A)
76P3S1(M.genospecies 1A)
62P2S2(M.genospecies 2A)
ET3(M.genospecies 8A)
ET20(M.genospecies 4B)
23P2S2(M.genospecies 8A)
36P4S1(M.genospecies 1B)
45P2S1(M.genospecies 1E)
58P2S1(M.genospecies 4B)
ET19(M.genospecies 1E)
17P2S2(M.genospecies 4B)
90P4S2(M.genospecies 4A)
42P3S1-a(M.genospecies 1E)
20P2S1(M.genospecies 4A)
2P3S1-b(M.genospecies 9A)
Mesorhizobium muleiense strain CGMCC 1.11022(NZ_FNEE01000025.1)
68P1S1(M.genospecies 1D)
43P5S1(M.genospecies 2A)
88P2S2(M.genospecies 4B)
29P4S2-a(M.genospecies 4A)
Mesorhizobium ciceri ca181(NZ_CM002796.1)
37P2S1(M.genospecies 2A)
Mesorhizobium loti R7A(AL672111.1)
Mesorhizobium erdmanii USDA 3471(NZ_ KI421454.1)
Mesorhizobium opportunistum WSM2075(CP002279.1)
Mesorhizobium australicum WSM2073(NC_019973.1)
Mesorhizobium metallidurans STM 2683(NZ_CAUM01000170)
Mesorhizobium prunaredense isolate 1(NZ_FTPD01000017.1)
Mesorhizobium amorphae CCNWGS0123-pacbio(CP015322.1)
Mesorhizobium sophorae strain ICMP 19535(KC237488.1)
Mesorhizobium plurifarium STM8773(CCNB01000015.1)
Mesorhizobium loti strain UFLA 01-765 (NZ_LPWA01000161.1)
Mesorhizobium sp. LSJC280B00(AYVL01000031.1)
Mesorhizobium temperatum strain SDW018 C743(NZ_NPKJ01000009.1)
Mesorhizobium qingshengii strain CGMCC 1.12097(NZ_FMXM01000054)
Mesorhizobium huakuii 7653R(CP006583.2)

Figure 3.4. Phylogenetic relationships of Ethiopian strains based on sequences of symbiosis related genes *nifH* genes predicted from whole-genome assemblies and annotations are the same as *nodC* in fig 3.3). Bootstrap values over 60% (based on 1000 replications) are shown at each node

Greenlon *et al*., (2019) reached a similar conclusion with a quantitative analysis of global phylogenetic coherence among symbiosis genes. The current analysis complements the results from (Greenlon *et al*.,2019) by analyzing the detailed phylogenetic history of two key symbiosis genes among a larger set of strains from Ethiopia, which despite their more limited geographic distribution are equally non-coherent with each other and with their background genomes. ICE elements can be divided into two categories based on the structural features, so called monopartite and tripartite ICE elements (Haskett *et al*., 2017) and the majority of Ethiopian symbiotic gene belonged to monopartite (Figure 3.5).

3.3.5. Distribution of chickpea *Mesorhizobium* in the sampling sites

Each sample collection regional zones contain a unique species or similar phylogenetic group of strains; 75% of strains are from five genospecies that occur in both Amhara and Oromia as summarized (Table 3.3) and presented in (appendix 1) for each strain. With this pattern *M. genospecies* (2A) occurs in seven of the eight sampled regional zones. *M. genospecies* (2A) representing 43% of collection wide strains predominantly at Amhara and Oromia occupies (35%).

Table 3.3. Distribution of *Mesorhizobium* genospecies group in the sampling zones

No	Sample strains	WSH	ESH	NSH	WG	EG	NG	SG	SW	Score (%)
1	*M.genospecies* 2A	6	1	0	2	1	13	3	0	43.3
2	*M.genospecies* 8A	0	2	1	0	0	0	3	0	9.4
3	M.genospecies 7A	0	0	0	0	2	0	0	0	3.1
4	*M.genospecies* 9A	1	0	0	0	0	0	0	0	1.6
5	*M.genospecies* 1B	0	0	0	1	0	2	0	0	4.7
6	*M.genospecies* 1E	0	0	0	0	0	3	2	0	7.8
7	*M.genospecies* 4A	2	0	0	0	3	1	0	0	9.4
8	*M.genospecies* 4B	6	0	0	0	0	1	1	0	12.5
9	*M.genospecies* 3A	0	0	1	0	0	0	0	1	3.1
10	*M.genospecies* 1A	0	0	0	0	0	2	0	0	3.1
11	*M.genospecies* 1D	0	0	0	1	1	1	0	0	4.7
	Total strains	15	3	2	4	7	23	9	1	64

Table legend: WSH=West Shewa; ESH= East= Shewa; NSH= North Shewa; WG =West Gojam; EG=East Gojam; NG=North Gojam; SG= South Gonder; SW=South Wello

In the other sampled zones (North Gondar, South Gondar ad West Shewa) *genospecies* 2A comprised 33-57% of observed diversity (Table 3.3). By contrast, *genospecies* 2A was notably rare in East Gojam, despite sampling of seven strains from this zone. The *Mesorhizobium* strains geographic distribution exhibited the identified strains scattered into 8 major regional zones. Most of the strains (15) clustered in *M. genospecies* 2A (with the *M. plurifarium* species category) originated from North Gondar (Table 3.3). Most of the full diversity of *Mesorhizobium* observed at major chickpea growing regions of a country was re-capitulated in Gondar and Gojam Amhara, regional zone. While the least species composition was found in Oromia, regional zone (Figure 3.5).

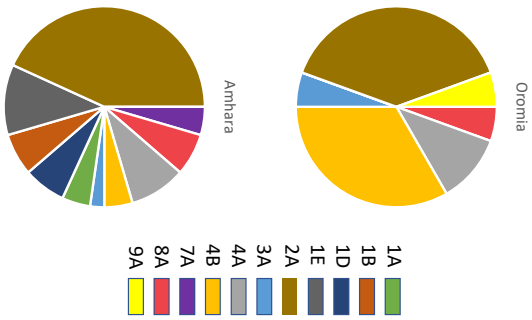


Figure 3. 5. *Mesorhizobium* genospecies clustering distribution at major chickpea growing regions.

In the highland of Northern Ethiopia, we observed well supported phylogenetic groups, three of which contain the majority of sampled strains with Ethiopian center of origin North Gonder. Wondwosen Tena *et al*., (2017) showed the existence of native chickpea nodulating isolates in southern Ethiopia, but this study indicated of high occurrence in the other parts of the country as well in North Gonder. Parallel to these, diverse populations of chickpea rhizobia also reported from different localities, such as in Portugal (Alexandre *et al*., 2009), Ethiopia, India, Turkey and Morocco (Greenlon *et al*., 2019). Most of the strains observed in Northen Ethiopia share their highest affinity with *Mesorhizobium*

species not previously known to nodulate chickpea, including *M. genospecies* 2A (related to *Mesorhizobium plurifarium)*.

The dominance of *M. plurifarium* related strains and the evolution of new or distinct chickpea nodulating rhizobia in some regions of Ethiopia. That might link to acquisition of the symbiosis gene from the co-occurring natural chickpea symbionts including *M. ciceri* and could permit chickpea affiliation with native microsymbionts well-adapted to the local soil environments (Greenlon *et al*., 2019; Tassew Sirage, 2019).

Based on these patterns in local symbiont diversity, we seek to evaluate the hypothesis that symbiotic performance of identified bacterial taxa varies across soil types, climates and geographic position. To understand the extent to which genetic diversity is structured by geographic distance, we conducted pairwise comparisons of the 64 strains in terms of their genetic distance and the geographic distance between samples (Figure 3.7).

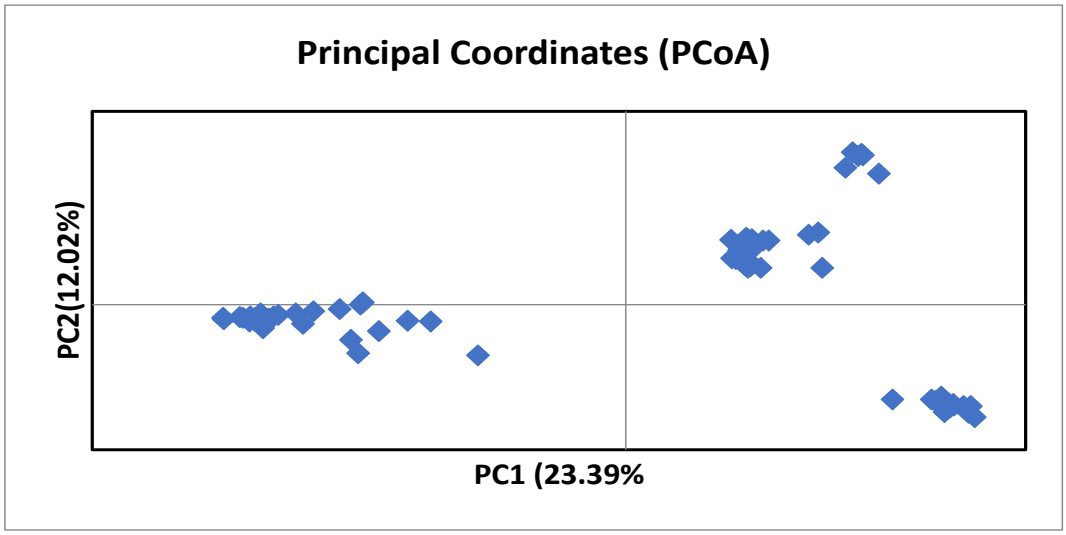


Figure 3.6. Principal coordinates analysis (PCoA) of 64 *Mesorhizobium* based on genospecies groups of strains in each sampling sites.

Each sample represented by colored dotted, indicated principal components analysis of genetic relationships among *Mesorhizobium* strains across overall *Mesorhizobium* diversity (Figure 3.6). The massive dotted indicate the largest loadings of variation explained in the first two axes (PC1 and PC2) in the strain's diversity with cumulative

variation of (35.41%). The intermidiate grouping pattern irrespective of the strain's geographic locations indicated the possibility of a commonly linked gene within and between *Mesorhizobium* strains. Several sample strains load heavily on PC1 (23.39%).

Distribution of rhizobia isolates might not correspond to the geographical locations due to homogeneity of the isolates to the ribosomal gene recombination within and between rhizobia strains (Koskey *et al*.,2018). In addition, Biodiversity and biogeography of rhizobia associated with lateral gene transfer of symbiotic genes among different strains of nitrogen-fixing bacteria reported by (Wang *et al*.,2016; Atsede Muleta, 2020). This analysis is consistent with how important diversity of *Mesorhizobium* strains where chickpea is commonly growing as *Mesorhizobium* phylogenetic community diversity by environmental factors shown (Greenlon *et al*., 2019).

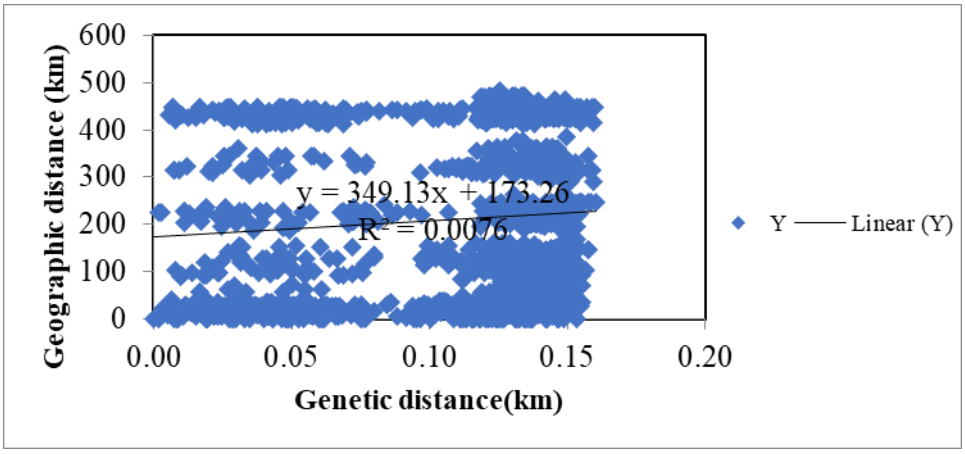


Figure 3.7. The strains distribution relative to genetic distance versus geographic distance.

The geographic distribution of *Mesorhizobium* phylogenetic divergence clades revealed existence of symbiont diversity in local provenance across sample collection regions of the country (Figure 3.7). The results demonstrated that distinct *Mesorhizobium* groups have distinct geographic ranges, so the correlation between genetic distance and geographic distance may to some extent reflect differences in the geographic ranges. This analysis reveals significant ($p < 0.009$) correlations between genetic distance and geographic distance for each strain. The magnitude of correlation is less ($r=0.0076$) between genetic

distance and geographic distance. Generally, strains diversity slightly increased as geographic distance increased. The of analysis relationship between phylogeny and geography showed limited variability among distant. This might be originated from the same genetic background combined with geographic movement of phylogenetic groups of *Mesorhizobium* strains among locations by means of human activities and cropping system (Ismail et al., 2013; Atsede Muleta, 2020)

3.3.6. Soils characteristics of *Mesorhizobium* strains geographic origin

The soil analysis result reveals most phylogenetically identified strains group are distributed into moderately acidic (pH 5.6-6.0) to strongly alkaline (pH 8.5-9.0).

Table 3.4. Strains soil origins and species occurance associated with soil constituent

No.	Soil chemical parameters	Number of *Mesorhizobium* genospecies under different soils										
		2A	**8A**	**1B**	**1E**	**4A**	**4B**	**3A**	**1A**	**1D**	**7A**	**9A**
1	**Soil pH**											
	Alkaline >7.4	2	0	0	1	0	2	0	1	0		
	Neutral 6.6-7.3	10	4	0	0	4	2	0	0	1		1
	Acidic <6.5	11	2	1	3	2	3	1	1	2		
2	**Nitrogen(N)**											
	High>14	3	1	0	0	1	2	1	0	0		
	Medium 10-14	1	0	1	1	0	2	0	0	1		
	Low <10	19	5	0	3	5	3	0	2	2		
3	**Phosphorus(P)**											
	High>16	1	1	0	0	0	1	1	1	1		
	Medium 11-16	3	3	0	1	0	1	0	0	0		
	Low <11	19	2	1	3	6	5	0	1	2		
4	**Potassium(K)**											
	High>140	23	6	0	4	6	7	1	2	2		
	Medium 91-140	0	0	1	0	0	0	0	0	1		
	Low <91	0	0	0	0	0	0	0	0	0		
5	**OM (%)**											
	High>5.17	3	0	0	2	0	1	0	0	0		
	Medium 2.59-5.17	20	6	1	2	6	6	1	1	3		
	Low <2.59	0	0	0	0	0	0	0	1	0		
6	**CEC (%)**											
	High>25	23	5	1	4	6	5	0	1	3		
	Medium 12-25	0	1	0	0	0	2	1	1	0		
	Low <12	0	0	0	0	0	0	0	0	0		
7	**Calcium**											
	High>2000	23	6	1	4	6	7	1	2	3		
	Medium 1001-2000	0	0	0	0	0	0	0	0	0		
	Low <1001	0	0	0	0	0	0	0	0	0		
8	**Magnesium(mg)**											
	High>350	23	6	1	4	6	7	1	2	3		
	Medium 151-350	0	0	0	0	0	0	0	0	0		
	Low <151	0	0	0	0	0	0	0	0	0		

The other characterized soil elements as summarized (Table 3.4) and presented in (appendix 4) for each strain. The soil chemical parameters showed the presence of soil variables seemed to have significant impact on the diversity and distribution of chickpea *Mesorhizobium* populations in the study areas. The influence of main soil factors on the distribution of chickpea rhizobia are shown in (Figure 3.8). The arrows represent soil factors and the longer the arrow showed the greater the influence. The longest arrows indicate the largest loadings of a factor into the amount of variation explained in the first two axes (PC1 and PC2) of the multivariate variation in the soil (Figure 3.8).

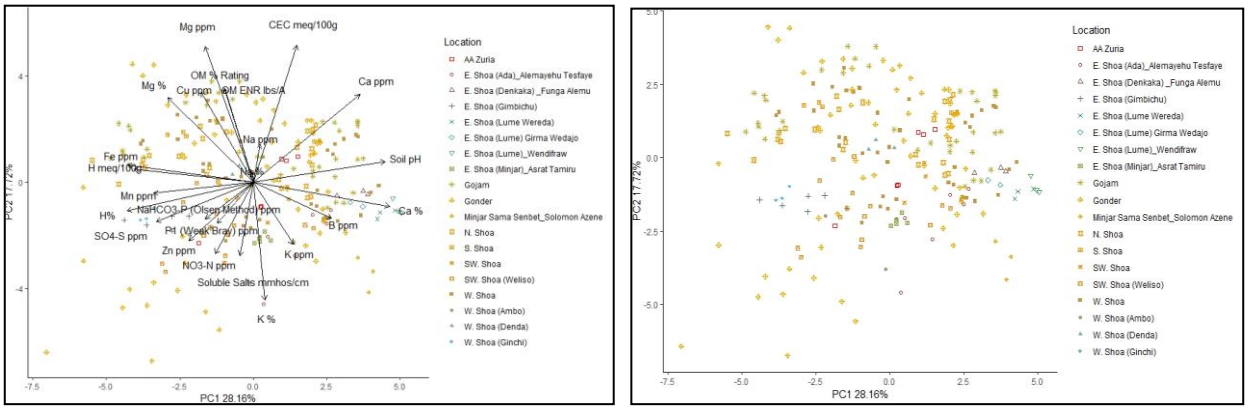


Figure 3.8. Principal coordinates analysis of soil factors on geographic distributions of strains

pH and several factors driven by pH, such as P, Fe, Mn, Zn, and Ca, all load heavily on PC one. pH being the main axis of variation in the soils we sampled. The second has a higher loading from cation exchange capacity, soluble salts, N, Cu, as a multivariate estimate of fertility. Accordingly, studies by (Tassew Sirage, 2019) showed the greater influence of available phosphorus on chickpea rhizobial population distribution and a significant effect of pH of the soil for species distribution. This analysis is consistent with how important soil pH is across the soil types where chickpea is common in Ethiopia

and justifies our emphasis on it in the scope of the larger umbrella of our strain's local adaptation.

3.3.7. Greenhouse authentication

As shown in (Figure 3.8) all strains were able to nodulate cultivated chickpea under greenhouse conditions. Genospecies 7A, particularly the two available strains, formed the highest overall nodules. 7A a *M. ciceri* species, nodulates *C. echinospermum* in its wild habits and it is the most common species used in commercial inoculants. All other groups produced a range of nodule numbers, including some strains that formed as many nodules as those of group 7A. There are two important asssumptions to these data. First, the number of strains tested in each genospecies was generally low, and the validation test was not replicated precluding the ability to make confident assertions about nodulation patterns. Nevertheless, an important observation that all of the tested strains possess the ability to nodulate chickpea was made.

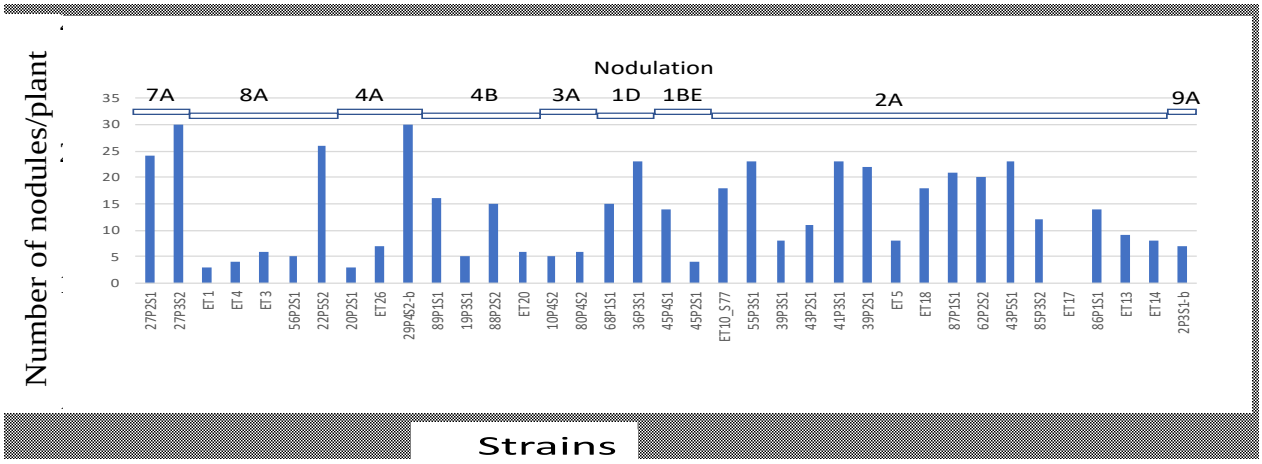


Figure 3.9. Nodule formation status of strains as assessed in UCD greenhouse experiment

In conclusion, the geographic and genomic survey reveals significant and unexpected genomic diversity among chickpea symbionts throughout the two chickpea growing regions of Ethiopia. It is notable that both pairwise average nucleotide identities and core genome phylogenetic trees produced congruent strain assignments to eleven distinct

genospecies of *Mesorhizobium*, with eight novel genospecies that represent 53% of sampled diversity.

In cases where these Ethiopian strains were conspecific with strains previously sampled from legumes, only one of the genospecies 7A (*M. ciceri*, representing 2 of 64 strains (~3%).

M. ciceri is of interest both because it is the species nodulating wild *C. echinospermum* in its native range and *M. ciceri* is the species most commonly used as the basis of commercial inoculants. The other two previously observed species, including the most numerous *genospecies* 2A, were previously observed on distantly related legumes (*Acacia* and *Astragalus*), sampled from Senegal and Eritrea, respectively.

The presence of diverse chickpea endemic *Mesorhizobia* throughout the two regions representing the wider chickpea growing regions of Ethiopia may derive in part from movement of the chickpea-specific IC element that characterizes chickpea symbionts. It is interesting, therefore, to contrast the recent origin of the symbiotic ICE, with the discordant phylogenies of two key symbiosis genes (*nodC* and *nifH)* that are carried within the ICE. *NodC* and *nifH* not only have histories that are dissimilar to one another, but both of their respective histories are distinct from the largely coherent history of the core genome deduced from a set of 400 conserved bacterial proteins.

Thus, within Ethiopia and over a period of not more than three thousand years, the chickpea symbiotic ICE has apparently spread throughout populations of endemic *Mesorhizobia* and then been subject to repeated rounds of horizontal gene transfer within the IC element itself, creating a surprisingly standing variation within the chickpea symbiont community. High symbiont diversity could permit local adaptation to soil and microbial factors and it could impart variation in symbiotic performance. The question of functional consequences of this variation is the topic of work reported elsewhere in this thesis to understand inoculant efficacy at the field level.

Chapter 4

4. Screening *Mesorhizobium* strains from Ethiopian soils for eco-physiological competence, plant growth promoting and symbiotic performance on Chickpea (*Cicer arietinum* L.) under greenhouse condition

Zehara Mohammed Damtew[1*], Douglas R. Cook[3]., Alex Greenlon[4]., Asnake Fikre[2]., Eric J. B.von Wettbergg[6]., Edward marques[5]; Kassahun Tesfaye[1]; Noelia Carrasquilla-Garcia[3] and Fassil Assefa[1]

[1]Department of Microbial Cellular and Molecular Biology, College of Natural Sciences, Addis Ababa University, Addis Ababa, 32853 Ethiopia; [2]Debre Zeit Agricultural Research Center, Ethiopian Institute for Agricultural Research, Bishoftu; [3]Department of Plant Pathology, University of California, Davis, CA 95616;[4]University of California, Berkeley, Environmental Science, Policy and Management; [5]Department of Biological Sciences, Florida International University, Miami, FL 33199; [6]Department of Plant and Soil Science, University of Vermont, Burlington, VT 05405;

Abstract

Chickpea (Cicer arietinum L.) is a major legume crop in Ethiopia and provide multiple benefits, due to high nutritive value as well as the ability of the crop to enrich nitrogen poor soils due to biological nitrogen fixation with different strains of endosymbiotic Mesorhizobium spp. However, the effectiveness of the strains varies due to inherent physiological characteristics of the endo-symbionts and the host varieties. This necessitates the screening of the outstanding properties of the endo-symbionts under controlled environment. To this effect, 20 promising strains from genomically identified 64 Mesorhizobium strains were evaluated for in vitro ecological tolerance, nutritional diversity, symbiotic effectiveness and plant growth promoting properties under laboratory and under greenhouse conditions on two Natoli and Arerti Chickpea varieties. There were significant variations in ecological tolerance and nutritional diversity among species and strains within species. A few strains were tolerant to 40^{o}C. pH 4.5 and 4% NaCl. A limited number of strains were able to utilize most of the carbon and nitrogen substrates, and only three strains (15%) of the isolates solubilized inorganic calcium and aluminium phosphate. From the three phosphate solubilizing strains, M. amorphae 80P4S2 produced 118 µg/ml; and 93.33µg/ml phosphorus from inorganic calcium and aluminium phosphates after 8 days of incubation, which was 2-3 times higher than P released from

the other two strains. Interestingly, all isolates were able to produce Indole Acetic Acid (IAA) ranging from 7µg/ml to 28.43µg/ml. M. australicum ET4, M. ciceri, and M. amorphae 90P4S2, and M.sp. LSJ280B00 strain 2P3S1-b produced the highest amount of IAA in the culture. Almost all (85%) of the Mesorhizobium strains were effective and highly effective on either of the Chickpea varieties based on the dry matter accumulation in comparison with their respective N-fertilized control plants. All taken together, M. amorphae 80P4S2 80 M.sp. LSJ280B00 strain 2P3S1-b, M loti 45P4S1, M loti 38P4S2, M. plurifarium 43P2S1, M. plurifarium 46P3S2, M. amorphae ET26 combined the best ecological tolerance and symbiotic effectiveness with a potential to perform well under field conditions. The data provided an important complement to select representative distinct symbiont strains for testing at multi-location field trials to enhance nitrogen fixation activities for chickpea production.

Keywords: Eco-physiological, PGP, substrate, Symbionts, Stress tolerance, symbiotic effectiveness

4.1. Introduction

Chickpea is one of the most important pulse crops used for food and feed in more than 60 countries worldwide mostly in the semi-arid tropics of sub-Saharan Africa and South Asia (Diapari *et al*.,2014), and contributes to more than 20% of world pulse production (Zafar *et al*.,2017). It is integrated in soil cropping systems in the tropics for soil fertility management, for it fixes nitrogen with root nodule bacteria of the genus *Mesorhizobium* (Nour *et al*., 1994). It is estimated that chickpea can produce up to 176 kg N ha-1 annually depending on cultivar, bacterial strain, and environmental factors (Beck *et al*., 1992). Although chickpea is considered for long as very host specific to *M. ciceri* and *M. mediterraneum* (Jarvis *et al*., 1997). Greenlon *et al*., (2019) showed that the *Mesorhizobium* genus encompasses 36 distinct species; of which eight *Mesorhizobium* species were cosmopolitan that nodulate chickpea globally; and 20 chickpea symbionts had not been previously reported. This suggests that representative strains from the large genetic diversity of the rhizobial species can be selected for developing elite rhizobial bioinoculants for precision agriculture (diCenzo *et al*.,2018).

The *Mesorhizobium* spp are saprophytic soil microbiota that have to persist and compete with other microorganisms to survive in the soil so as to effectively nodulate their host. Their survival in the soil and their symbiotic performance are influenced by various abiotic factors such as temperature, soil pH, salinity, nutrients, and environmental pollutants such as heavy metals and antibiotic produced by other microorganisms in the soil (Al-Falih, 2002; Vriezen *et al*., 2007). This necessitates the primary screening of the isolates to test their tolerance to abiotic stresses in the laboratory and their symbiotic effectiveness under greenhouse conditions. Several studies showed tolerance under laboratory conditions and effectiveness in nitrogen fixation corroborate with their performance of chickpea under similar field conditions (Brigido *et al.*, 2007; Ben-Romdhane *et al.*, 2008; Alexander *et al*., 2009).

Apart from effective nitrogen fixation, root nodule bacteria including *Mesorhizobium spp* acquire Plant growth promoting properties (PGPP) that enhance growth and production of the host through direct or indirect mechanisms (Glick, 2012). These include; nutrient acquisition of phosphorus with phosphate solubilization (Velazquez *et al*.,2019);

enhance plant growth by producing phytohormones (Perez-Fernandez and Alexander, 2017) and inhibit phytopathogens through various forms of antagonism (Zafar *et al*.,2017). The use of rhizobial inoculants in agricultural production is aimed at ensuring that the most effective microsymbiont occupies the largest proportion of nodules formed on the target host legume in the field (Thies *et al*.,2001). The consistency of nodulation can be enhanced by the use of effective rhizobial inoculant strains, which has been described as one of the earliest applications of agricultural biotechnology (Lindstrom *et al*., 2010).

Thus, the selection of metabolic, physiological, ecological and plant growth promoting characteristics of rhizobial strains is vital to obtain information about the ecological competence of the rhizobia in the organism's habitat that may beneficially influence plant growth and development of the host plant (Wdowiak-Wrobel *et al*.,2017). Different reports showed that chickpea harbors *Mesorhizobium* spp with dual benefits of N2-fixation and P-mobilization (Peix *et al*.,2001; Rai *et al*.,2012). Brigido *et al*., (2016) reported that the ability to solubilize inorganic phosphate by chickpea rhizobia may be species related and constitute an adaptive mechanism against phosphorous deficiency in acidic and alkaline soils in Portugal. Wani and Khan, (2013) isolated multiple metals and antibiotic-resistant *Mesorhizobium* sp. and their plant growth promoting activity in order to enhance growth and productivity of chickpea.

In Ethiopia, Mulissa Jida and Fassil Assefa (2012) screened the in vitro ecological diversity and plant growth promoting characteristics of 36 chickpea rhizobial isolates of which 44% of the isolates were phosphate solubilizer; while 28% were capable of producing indole-3-acetic acid (IAA) and 19% of the tested isolates showed antagonistic activity against *Fusarium oxysporum* in dual culture assay. Daniel Muleta and Fassil Assefa, (2015) also showed that 33% of chickpea isolates solubilized inorganic phosphate; whereas Wondwosen Tena *et al*., (2017) isolated 42 root nodule bacteria from chickpea growing areas of southern and central Ethiopia, of which few isolates were phosphate solubilizers indicating that the occurrence of phosphate solubilizing rhizobia differs depending upon the environmental variables of the sampling sites.Apart from abiotic factors, host varieties also influence the symbiotic effectiveness of the rhizobia isolates.

To this effect, (Wubayehu Gebremedhin *et al*., 2018) reported that 87 % of the chikpea isolates were able to nodulate Desi seed types variety Natoli and 13% of them nodulate both Desi seed types and Kabuli seeds types variety Habru on pot culture under greenhouse conditions. Subsequently, (Tassew Siraj and Fassil Assefa, 2018) conducted combined evaluation of ecological competitiveness (in vitro) of *Mesorhizobium ciceri* and *Mesorhizobium prulifarium* strains and their symbiotic effectiveness under greenhouse conditions and indicated that 71% of *M. ciceri* and *M. plurifarium* strains nodulated the Natoli variety with high effectiveness compared to 83% of the same strains on Kabuli seed-type variety Arerti.

Most of the hitherto studies in Ethiopia showed that different groups of chickpea rhizobia are tolerant to different abiotic stresses for selection of competitive strains with potential for inoculant production. However, most of them did not genetically identify the strains to their taxonomic groups lacking the information on the relationship between ecological competitiveness and symbiotic effectiveness and the different Mesorhizobium taxa in the country. Therefore, in order to obtain the maximum biological nitrogen fixation (BNF) from legumes, it is necessary to identify compatible rhizobia strains before commercial production and distribution to farmers (Sahgal and Johri, 2003). The objective of this study is to screen the genetically identified selected Mesorhizobium species from Ethiopia on the basis of ecological competence and plant growth promoting capability in vitro and their symbiotic effectiveness on two chickpea varieties under greenhouse conditions.

4.2. Materials and Methods

4.2.1. Source of strains and growth conditions

Twenty strains were selected from the phylogeny of whole genome sequenced 64 strains belonging to six *Mesorhizobium* species which were isolated from root nodules collected from major chickpea growing regions of Ethiopia and compiled with global level population genomics (Greenlon *et al*.,2019). The strains were deposited in culture collection at Plant Pathology Laboratory of Davis, University of California, USA and Addis Ababa University. NCIB assembly accession links presented in (Dataset S1) accompanying studies by (Greenlon *et al*.,2019). Eco-physiological and plant growth

promoting characteristics activities were also performed at Plant Pathology Laboratory of Davis, University of California, USA.

Table 4.1. List of *Mesorhizobium* strains and origin of culture collection for strains used in Phenotypic and symbiotic characterization

No.	Sample strains	Closest relative	Region	Latitude	Longitude	Elevation
1	*M.genospecies* 7A (27P3S2)	*M. ciceri*	A. E. G.	10° 24' 41.7"N	38° 10' 8.4"E	2429
2	*M.genospecies* 9A (2P3S1-b)	*M.sp.*LSJ280B00	O. E. S.	8° 49' 31.7"N	38° 59' 25.4"E	1944
3	*M.genospecies* 3A (80P4S2)	*M. amorphae*	A. S. W.	12° 20' 56.9"N	38° 3' 35.4"E	1906
4	*M.genospecies* 3A (10P4S2)	*M. amorphae*	O. N. S.	8° 53' 46.3"N	39° 23' 56.5"E	1815
5	*M.genospecies* 4B (19P3S1)	*M. amorphae*	O. W. S.	8° 39' 20.4"N	38° 28' 57.5"E	2192
6	*M.genospecies* 4B (ET20)	*M. amorphae*	A.N. G.	12° 15' 16.9"N	37° 15' 51.5"E	1849
7	*M.genospecies* 1B (45P4S1)	*M. loti*	A.N. G.	12° 26' 43.8"N	37° 20' 48.3"E	1934
8	*M.genospecies* 2A (46P3S2)	*M.plurifarium*	A.N. G.	12° 21' 18.9"N	37° 15' 31.4"E	1873
9	*M.genospecies* 2A (29P5S1)	*M.plurifarium*	A. E. G.	10° 42' 47.3"N	38° 10' 30.6"E	2541
10	*M.genospecies* 2A (43P2S1)	*M.plurifarium*	A.N. G.	12° 27' 43.6"N	37° 21' 29.7"E	1960
11	*M.genospecies* 8A (ET1)	*M. australicum*	A. S. G.	11° 27' 58.3"N	38° 12' 46.6"E	2795
12	*M.genospecies* 8A (ET4)	*M. australicum*	O. N. S.	9° 53' 46.8"N	38° 21' 29.6"E	2567
13	*M.genospecies* 8A (23P2S2)	*M. australicum*	A. E. G.	9° 59' 51.2"N	38° 14' 42.9"E	2122
14	*M.genospecies* 4A (ET26)	*M. amorphae*	A.N. G.	12° 27' 34.8"N	7° 48' 23.0"E	1841
15	*M.genospecies* 4A (90P4S2)	*M. amorphae*	O. W. S.	8° 36' 3.4"N	38° 16' 0.8"E	2209
16	*M.genospecies* 4A (22P5S2)	*M. amorphae*	A. E. G.	10° 24' 56.2"N	38° 10' 35.9"E	2429
17	*M.genospecies* 1D (36P3S1)	*M. amorphae*	A. W. G.	11° 13' 22.3"N	37° 35' 42.7"E	2261
18	*M.genospecies* 1A (ET24)	*M. amorphae*	A.N. G.	12° 27' 48.3"N	37° 49' 53.2"E	1754
19	*M.genospecies* 1E (38P4S2)	*M. loti*	A. S. G.	12° 1' 54.3"N	37° 43' 49.3"E	1809
20	10P3S1 (unidentified)	Isolate	O. N. S.	8° 53' 46.3"	39° 23' 56.5"	1815
21	EAL029 (Reference)	-	Commercial	Reference	-	-
22	Ha. Ata (Reference)	-	Tunisia	Reference	-	-
23	USDA-3383(Reference)	-	UcDavis	Reference	-	-

Table legend; A. E. G.=Amhara, East Gojam; O. E. S.= Oromia, East Shewa; A. S. W.=Amhara, South Wollo; Oromia, O. N. S.=North Shewa; O. W. S.=Oromia, West Shewa; A.N. G.=Amhara, North Gondar; A. S. G.=Amhara, South Gondar; A. W. G.=Amhara, West Gojam

4.2.2. Eco-physiological characteristics

Tolerance of the *Mesorhizobia* strains to salinity was tested on YEAM medium supplemented with, 2, 3 and 4 % (w/v) NaCl and that of acidity/alkalinity on the same medium adjusted to pH of 4, 5 and 10 using 1N HCl and NaOH before autoclaving. They were also grown on the same medium and incubated at 35, 37 and 40°C to evaluate their tolerance to heat stress (Laranjo and Oliveira, 2011). The intrinsic antibiotic resistance (IAR) of the strains was performed according (Maatallah *et al*., 2002). The antibiotics were filter sterilized (0.22 µm size membrane filters) antibiotics and prepared at different concentrations (µg/ml); Chloramphenicol (10), Streptomycin (50), Nalidixic acid (10), Erythromycin (10), Neomycin (10), tetracycline (10), all dissolved in water except, Chloramphenicol, Erythromycin, tetracycline that were dissolved in NaOH and ethanol. Resistance to heavy metal was tested on YEMA medium containing; $CoCl_2$ 25, $CuCl_2$ 50, $ZnCl_2$ 50, $AlCl_3$250, $Pb(CH3COOH)_2$ 250 and $NiSO_4$ 100 (µg/ml) of water as (Maatallah *et al*., 2002)

4.2.3. Carbon and nitrogen substrates utilization

The ability of strains to utilize different carbohydrates (1% (w⁄v) as the sole carbon source was tested on basal media containing (g/l); K_2HPO_4(1), KH_2PO_4(1), $FeCl3.6H_2$(0.01), $MgSO_4.7H_2O$ (0.2), $CaCl_2$(0.1; $(NH_4)2SO_4$(1) and agar (15). Heat stable carbon sources such as sucrose and α-cellulose were autoclaved together with the medium and the heat labile sources Trehalose, D-Galactose, D-Xylose and D-Sorbitol were filter sterilized and added to the autoclaved media. Likewise, the ability of the strains to utilize different nitrogen substrates were tested on the same basal medium after replacing ammonium sulfate (1 g/l) and reducing mannitol to a final concentration of 0.5 % (w/v) according to (Amarger *et al*., 1997).

4.2.4. Indole acetic acid and phosphate solubilization properties of *Mesorhizobium* strains

4.2.4.1. Indole acetic acid (IAA) production

Strains were grown on YEMA broth supplemented with filter sterilized L-tryptophan (2g/l) and grown on orbital shaker at 200 rpm at room temperature for 4 days to test their ability to produce IAA (Bric *et al*., 1991). The cultures were centrifuged at 10000 rpm for 15 min from which 2ml of the supernatant was mixed with 100 µl of 10 mM orthophosphoric acid supplemented with Salkowaski reagent (1 m of 0.5 M FeCl3 in 50 ml of 35% HClO4) in a ratio of 2:1, and incubated in the dark for 25 min at room temperature. The detection of pink color in the mixture was positive indicator for IAA and absorbance was measured using spectrophotometer at 530 nm to calculate the amount of IAA produced against astandard curve constructed from known concentrations of IAA.

4.2.4.2. Phosphate solubilization on solid medium

Qualitative estimation of phosphate solubilization was performed using three inorganic phosphate sources, tricalcium phosphate $Ca_3(PO_4)_2$, Aluminum phosphate ($AlPO_4$) and Iron phosphate ($FePO_4$) plate assay according to (Perez *et al*., 2007). Active culture suspension of 10 µl (~10^8 cells/ml) of each strain was spot inoculated on Pikovskaya's agar medium containing tricalcium phosphate. The medium contained g/l; glucose (10), $Ca_3(PO4)_2$ (5), $(NH_4)_2SO_4$ (0.5), $MgSO_4.7H_2O$ (0.1), yeast extract (0.5), NaCl (0.2), $MnSO_4.2H_2O$ (0.002), $FeSO_4.7H_2O$ (0.002) and agar (15). Simultaneously, the strains were inoculated into NBRIP (National Botanical Research Institute's Phosphate) medium to assess their solubilization ability of Aluminum phosphate and iron phosphate. The medium contained the following ingredients in g/l: glucose (10), $AlPO_4$ or $FePO_4$) (5), $MgCl2.6H_2O$ (5), $(NH4)_2SO4$ (0.1), KCl (0.2), $MgSO_4.7H_2O$ (0.25) and agar (15) (Perez *et al*., 2007) The formation of clear halo zone around colonies and the solubilization index (SI) = (colony + halo zone) to the colony diameter in mm was recorded.

4.2.4.3. Phosphate solubilization on liquid medium

Based on their solubilization index, strains were selected to performed quantitative estimation of phosphate solubilization in PKV ($Ca_3(PO_4)_2$ and NBRIP ($AlPO_4$ and $FePO_4$) broth. Each isolate was grown to their exponential phase in a nutrient broth from which 100 µl (~10^8 cells/ml) of the suspension in 100 ml of the respective NBRIP broth in 250 ml Erlenmeyer flask(using Jackson method (Selvi *et al*., 2011). Then the flask were incubated on a rotary shaker at 200 rpm at room temperature for 8 days. From each flask, five ml of the supernatant was taken on the 4th, 8th, days to measured pH with pH meter (InMotion^{TM}Autosampler) and the amount of phosphate released was measured using phosphomolybdate method (Murphy and Riley, 1962). Five ml of the supernatant was centrifuged at 14,000 rpm (Allegra ^{TM} 6KR) for 15 minutes and the amount of phosphate in the clear culture supernatant as well in control (without inoculation) was measured using spectrophotometer (540 nm) (ND-1000 (Nano Drop®) sample holder. The amount of solubilized P (µg/ml) was quantified against a standard curve constructed from known concentrations of Potassium dihydrogen phosphate (KH_2PO_4).

4.2.4.4. Production of hydrogen cyanide (HCN)

Strains were inoculated in YMA Plates supplemented with 4.4 g/l of glycine to test their ability to produce hydrogen cyanide (Chandra *et al*., 2007). A Whatman filter paper no.1 moisturized with picric acid solution (2.5 of picric acid and 12.5 of Na_2CO3 (g/l) in water was placed in the upper lids of the Petri plate. The plates were sealed with parafilm and incubated at 28°C to detect change in colour on the paper. The ones that showed a color change from yellow to light brown was considered as weak, whereas the change to brown and and reddish brown were considered as moderate and strong producers of hydrogen cyanide.

4.2.5. Symbiotic effectiveness screening in the greenhouse

The evaluation of the symbiotic effectiveness of the rhizobial isolates on two chickpea varieties (Natoli and Arerti) was conducted in pot sand culture under greenhouse conditions at Debre Zeit Center, Ethiopian Institute of Agricultural Research (EIAR). The Chickpea seeds of the two varieties were treated with 70% ethanol (30 sec).

Thus, surface sterilized in 2% sodium hypochlorite (3 min) and rinsed five times with sterile water (Somasegaran and Hoben, 1994). Seeds were germinated on 1% (w/v) water agar at 28°C and transplanted into surface sterilized 3-kg capacity pots filled with 95% sulphuric acid (H_2SO_4) washed sand. Each seedling was inoculated with 1ml liquid inoculum (~10^9cells/ml) of each *Mesorhizobium* strain and two reference strains (Ethiopian commercial strain EAL 029, Tunisia strain Ha. Ata) were included as standard check. The experiment was laid out in a Complete Randomized design with three replications. The seedlings were irrigated with N-free nutrient solution (Broughton and Dilworth, 1971) weekly, and with N-fertilized pot at a rate of 70 μg N ml^{-1} KNO_3 solution once a week as a positive control.

Plants were uprooted after 45 days of planting to record number of nodules, nodule dry weight and shoot dry weight. Relative effectiveness of strains was calculated using the formula, RE= (inoculated plant shoots dry weight/shoot dry weight of nitrogen supplemented plant) x 100 (Date,1993). Nitrogen fixing effectiveness was classified as highly effective >80%; effective 50 to 80%; low effective 35 to 50% and ineffective <35%. Shoot nitrogen content was determined using modified Kjeldahl Methods (Sahlemedhin Sertsu and Taye Bekele, 2000) at Debre Zeit Soil Science Research Laboratory. The percentage of nitrogen content of the samples was calculated after correcting for the blank as; % N=[(A-B) X N X 0.014 X mcf) /S)] x 100 (i.e. A= ml of the H_2SO_4 used in the titration of the sample, B= ml of the H_2SO_4 used in the titration of the blank test, S= dry Weight of sample in grams, N= normality of H_2SO_4 (0.1N), 0.014= molar mass of nitrogen, in grams per mole, mcf = moisture correction factor).

4.2.6. Data analysis

Values were recorded as mean of triplicate samples. Analysis of variance (ANOVA) was done for comparison between the treatments for; shoot dry weight, nodule number and nodule dry weight using the statistical software SAS version 9.3. The difference among treatment means was compared by high range statistical domain (HSD) using Tukey test at 5% probability level.

4.3. Result and Discussion

4.3.1. Eco-physiological and biochemical characteristics

Almost all strains (91%) were able to grow at 1% NaCl concentration, whereas 50% and 35% of the strains were tolerant to 3% and 4% NaCl, respectively (Table 4.2). Thus, *M. ciceri* 27P3S2, *M. loti* strain 45P4S1, *M. plurifarium* 43P2S1and *M. plurifarium* 46P3S2 showed broad range salt tolerance to different NaCl concentrations.

Table 4.2.Eco-physiological characteristics of chickpea nodulating *Mesorhizobium* strains

strains	Closest relative	NaCl	pH	T^{o}C	IAR	HMR
27P3S2	*M. ciceri*	4	10	37	-	Co, Cu, Zn, Ni
2P3S1-b	*M.sp.* LSJ280B0	3	10	37	Ery, Str, Neo	Co, Cu, Zn, Pb, Ni
80P4S2	*M. amorphae*	3	5,10	40	Nal	Co, Cu, Zn, Ni
10P4S2	*M. amorphae*	2	10	35	Chl, Ery, Tet	-
19P3S1	*M. amorphae*	-	10	-	Ery	Co, Cu
ET20	*M. amorphae*	2	0	37	Ery, Str, Nal	Co, Al
45P4S1	*M. loti*	4	4,5,10	40	Ery, Nal, Neo	Cu
46P3S2	*M.plurifarium*	4	5,10	37	Ery, Str, Nal	Co, Cu
29P5S1	*M.plurifarium*	-	10	37	Str, Nal	-
43P2S1	*M.plurifarium*	4	4,5	40	Ery, Nal, Neo	Co
ET1	*M. australicum*	-	10	35	Str	-
ET4	*M. australicum*	3	10	-	Str, Nal	-
23P2S2	*M. australicum*	4	10	37	Nal	Co
ET26	*M. amorphae*	4	4,5,10	40	Ery, Str, Nal, Neo	Zn
90P4S2	*M. amorphae*	-	10	37	Ery, Neo	Co, Cu Zn, Ni
22P5S2	*M. amorphae*	2	4,5,10	37	Chl, Nal	Co, Ni
36P3S1	*M. amorphae*	2	-	-	Ery, Str, Nal	Co
ET24	*M. amorphae*	3	10	-	Ery, Str, Nal	Cu, Al
38P4S2	*M. loti*	4	4,5,10	-	Ery, Nal, Neo	Co, Cu, Zn, Ni
10P3S1	Unidentified	-	10	37	-	-

Stand for; NaCl= Salt tolerance pH=acidity or alkalinity tolerance, ToC = Temperature tolerance, IAR = intrinsic antibiotic resistance; Chl= Chloramphenicol (10µg/ml), Ery= Erythromycin (10 µg/ml), Str= Streptomycin (50µg/ml), Nal= Nalidixic acid (10µg/ml), Tet= Tetracycline (10 µg/ml), Ne= Neomycin (10 µg/ml), HMR = Heavy metal resistance; Co=cobalt(25 µg/ml), Cu= copper (50 µg/ml), Zn=Zinc(50 µg/ml),, Pb=Lead(250 µg/ml),, Ni=Nickel sulfate (100 µg/ml), Al=Aluminium(50 µg/ml),

The response to salt stress within *Mesorhizobium* species is associated with overproduction of low molecular weight proteins which help the cells to osmotic adjustment to intracellular water (Laranjo and Oliveira, 2011). A previous study in Portugal showed that Chickpea isolates showed a better growth with 1.5% NaCl, but inhibited at 3% NaCl concentrations (Brigido *et al*.,2012). Studies in Ethiopia (Mulisa Jida and Fassil Assefa, 2012) showed 11% of the tested chickpea isolates were tolerant to 5% NaCl and other isolates were even tolerant to higher NaCl concentration of (6%) (Daniel Muleta and Fassil Assefa, 2015).

More strains grew at pH 10 (85%) than at pH 5 (60%), but fewer strains were tolerant to pH 4 (25%) (Table 3.2). Five strains such as M. loti strain 45P4S1, M. amorphae ET26, M. plurifarium 43P2S1, M. amorphae 22P5S2 and M. loti 38P4S2, were tolerant of pH 4. Previous studies in Ethiopia showed that chickpea rhizobial isolates grew in moderately acidic pH5 to alkaline pH 9; but sensitive to pH4 (Mulisa Jida and Fassil Assefa, 2012; Wubayehu Gebremedhin et al., 2018; Tassew Sirage and Fassil Assefa,2018). However, (Kucuk and Kivanc, 2008) showed that almost all chickpea rhizobial isolates from Turkey were tolerant to pH 4. Other studies showed that M. ciceri, M. loti and M. amorphae were tolerant to pH 5 to 9.5 (Brigido and Oliveira, 2013; Ltaief et al.,2007).

About 75% of the strains grew at optimum temperature up to 37°C, while 20% were tolerated 40^{o}C (Table 4.2). M. loti 45P4S1, M. amorphae ET26 and M. amorphae 80P4S2 resemble to and M. plurifarium 43P2S1showed rigorous growth on extreme temperature (40°C). This was much lower than the report of Tassew Sirage and Fassil Assefa, (2018) who found that 50% of the chickpea rhizobia strains isolated from Ethiopia were tolerant to 40^{o}C, while others showed many temperature sensitive chickpea rhizobia (40-45°C) (Daniel Muleta and Fassil Assefa, 2015; Wubayehu Gebremedhin et al., 2018).

Chickpea *Mesorhizobia* tolerant to different temperature levels between 15°C, 37°C were isolated elsewhere (Ogutcu *et al*., 2008; Alexandre and Oliveira, 2013). Laranjo and Oliveira, (2011) identified high temperature tolerant *Mesorhizobium plurifarium* affiliated with tropical geographical origin of a species isolated from *Acacia* species in Senegal (de Lajudie *et al*., 1998), which is associated with the characteristics of bacterial population to produce different types of shock proteins to adapt and survive when they are exposed to

cold and high temperatures (Maleki *et al*., 2016). In general, the overall *in vitro* stress tolerance tests showed that *M. loti*, 45P4S1, *M. plurifarium* 43P2S, *M. amorphae* ET26 and *M. amorphae* 80P4S2 relative to showed wide range of tolerance to salinity, pH and temperature indicating their potential competitiveness under stress conditions. Most of the strain relative to the *M. amorphae* were very sensitive compared to other strains.

Most strains were resistant to high concentrations of nalidixic acid (65%), erythromycin (60%) and streptomycin (40%) (Table 4.3). *M. australicum* ET1, *M. sp.* LSJC280B00 2P3S1-b, *M. loti* and *M. plurifarium* 43P2S were more tolerant to high concentrations of the tested antibiotics (33-44%) than the relatively sensitive *M. amorphae* affiliated strains (11-33%). Several studies showed chickpea isolates were resistant to nalidixic acid and erythromycin (Maatallah *et al.*,2002a, Rai *et al., 2*012) showed 26-84% and 15-17% difference respectively. Wondwosen Tena *et al*., (2017) also reported majority of the tested strains exhibited resistance to erythromycin and nalidixic acid. The tested *Mesorhizobium* strains were sensitive to Tetracycline that is similar to the work of Mulisa Jida and Fassil Assefa, 2012) who reported all of the tested isolates were sensitive to Tetracycline.

Most of the *Mesorhizobium* strains exhibited (60%) resistance to the heavy metal $CoCl_2$, but fewer isolates were tolerant $CuCl_2$(45%), followed by $ZnCl_2$ (30%) and highly sensitive to Al (10%) and Pb (5%). *M. sp.* LSJC280B00 2P3S1-b, *M. ciceri* 27P3S2 and some strains closest relative to *M.* amorphae were highly resistant to most heavy metals (Table 4.3). Tolerance to heavy metal might imply that the strains have adapted to colonize heavy metal rich soils. The resistance of chickpea rhizobial isolates up to 41% and 20-41% to Co and Cu was previously reported (Kuçuk and Kivanc,2008; Maatallah *et al.*,2002b). In Ethiopia (Daniel Muleta and Fassil Assefa, 2015) reported that the chickpea rhizobia isolate resistant Cu (16%) and almost all of the isolates were resistant to Co heavy metals.

The pattern of utilization of carbon substrates showed that most strains (60%) were able to grow using D-Sorbitol and D-Glucose as carbon sources; followed by a number of strains

(50%, 45%) utilizing Sucrose and Trehalose, respectively and none of the strains did not utilize α-cellulose (Table 4.3).

The data also showed that, *M*. amorphae 36P3S1 and *M. australicum* ET4 were highly versatile in carbon utilization (83%); whereas *M. sp.* LSJC280B00 2P3S1-b, *M. plurifarium* and *M. loti* relative strains showed moderate (66%) carbon utilization. Most strains belonging to *M*. amorphae affinity group were limited to few (10P4S2; 80P4S2; 19P3S1) carbohydrate sources. Earlier studies showed that chickpea isolates successful utilizing the carbohydrates 5-100%, 25-75% and 33-93%, (Maatallah *et al*. 2002a, b; Kuçuk and Kivanc,2008, Mulisa Jida and Fassil Assefa, 2012) respectively.

Table 4.3.Nutritional versatility, intrinsic antibiotic resistance and heavy metals pattern of different *Mesorhizobium* species group

Strains	**Closest relative**	**Carbohydrate**						**Amino acid**					
		Sor	**Glu**	**cel**	**Suc**	**Xyl**	**Tre**	**lys**	**Phe**	**Try**	**Leu**	**Arg**	**Gly**
27P3S2	*M. ciceri*	+	+	-	-	-	-	-	+	+	-	+	-
2P3S1-b	*M.sp.* LSJ280B0	+	+	-	+	-	+	+	+	+	+	+	+
80P4S2	*M. amorphae*	+	-	-	-	-	+	+	+	+	+	+	-
10P4S2	*M. amorphae*	-	-	-	-	-	-	-	-	-	-	-	-
19P3S1	*M. amorphae*	-	-	-	+	-	-	-	+	-	+	-	-
ET20	*M. amorphae*	-	-	-	+	-	-	-	-	-	-	-	-
45P4S1	*M. loti*	-	+	-	+	-	-	+	-	-	-	+	+
46P3S2	*M.plurifarium*	+	+	-	+	-	-	-	-	-	-	-	+
29P5S1	*M.plurifarium*	+	-	-	+	-	+	-	+	-	+	-	-
43P2S1	*M.plurifarium*	+	+	-	-	+	+	+	+	-	-	-	+
ET1	*M. australicum*	-	+	-	+	-	-	-	-	-	-	-	-
ET4	*M. australicum*	+	+	-	+	+	+	-	+	-	+	-	-
23P2S2	*M. australicum*	+	+	-	-	+	-	-	-	-	-	-	+
ET26	*M. amorphae*	+	+	-	-	-	+	-	+	-	+	+	-
90P4S2	*M. amorphae*	-	-	-	-	+	-	-	-	+	-	+	-
22P5S2	*M. amorphae*	-	+	-	-	-	-	-	-	-	-	-	+
36P3S1	*M. amorphae*	+	+	-	+	+	+	-	+	-	+	-	-
ET24	*M. amorphae*	+	-	-	-	+	+	-	+	-	-	-	-
38P4S2	*M. loti*	+	+	-	+	+	-	-	+	-	-	-	-
10P3S1	Unidentified	-	-	-	-	+	+	-	+	+	+	+	-
	Total (%)	60	60	-	50	40	45	20	60	25	35	40	30

Table legend; Sor=D-Sorbitol, Glu=D-Glucose, Cel=α-cellulose; Suc=Sucrose, Xyl=D-Xylose, Tre=Trehalose, lys=L-lysine, Phe=L-Phenylanine, Try=L-Tryptophan, Leu=L-Leucine, Arg=L-Argenine, Gly=Glycine

Similarly, the *Mesorhizobium* strains better utilized the amino acids Phenylanine (60%), Leucine (40%) and Argenine (35%). Few strains such as *M.sp.* LSJC280B00 2P3S1-b, *M. amorphae* 80P4S2 and *M. plurifarium* 43P2S1 utilized lysine. Relative strains to *M. plurifarium*, *M.sp.* LSJC280B00 and *M. australicum* utilized Glycine. Failure to Earlier studies on chickpea isolates showed that they failed utilize lysine (Mulisa Jida and Fassil Assefa, 2012), or a few isolates were able to metabolize the same as nitrogen source (Kucuk and Kivanc,2008; Wubayehu Gebremedhin *et al.*,2018). The versatility of strains to utilize different substrates implicate their saprophytic competition in the soil which is a one of the desirable characteristics for inoculant strains selection for effective nitrogen fixation (Hungria *et al.*, 2001).

4.3.2. Indole acetic acid and phosphate solubilization properties of *Mesorhizobium* strains

The twenty different *Mesorhizobium* strains were tested for their ability to solubilize various inorganic phosphate sources, production of indole acetic acid and HCN (Table 4.4). With regard to IAA production, all strains produced the phytohormone ranging from 7.7(µg/ml) to 28.4(µg/ml) (Table 4.4). The *M. australicum* ET4 showed the highest production of IAA (28.4µg/ml), followed by *M. amorphae* 90P4S2(27.0 µg/ml) showing a 4-fold difference between the highest IAA producers and the lowest producer (7.7µg/ml) *M. amorphae* 10P4S2. Other studies showed M. *ciceri* and *M. loti* chickpea isolates displayed high IAA production (47.38, 45.2µg/ml) (Brigido *et al*.,2016). Contrary to this study, only 28% of the Chickpea isolates collected from Ethiopia showed IAA production between (12.3 and 58.0 µg/ml) (Mulisa Jida and Fassil Assefa, 2012)

Only three strains; *M. amorphae* strain 80P4S2, *M. amorphae* strain 22P5S2 and *M. loti* strain 45P4S1 that resemble *M loti* solubilized tricalcium and aluminium phosphates with solubilization indices ranging from 0.34-1.17. No strain solubilized iron phosphate and produced HCN. This shows that the relative distribution of phosphate solubilizing *Mesorhizobium* strains (15%) was much lower than the number (44%) reported by (Mulisa

Jida and Fassil Assefa, 2012) and 30% enumerated by (Daniel Muleta and Fassil Assefa, 2015).

Table 4.4.Plant growth promoting properties of chickpea nodulating *Mesorhizobium* strains

No	strains	Relative species	Ca2(PO4) (SI)	AlPO4 (SI)	IAA (µg/ml)
1	27P3S2	*M. ciceri*			26.13^{abc}
2	2P3S1-b	*M.sp.* LSJ280B00			15.73^{ij}
3	80P4S2	*M. amorphae*	1.16	0.34	20.40^{efgh}
4	10P4S2	*M. amorphae*			7.70^{m}
5	19P3S1	*M. amorphae*			12.44^{jkl}
6	ET20	*M. amorphae*			14.74^{ijk}
7	45P4S1	*M. loti*	1.13	0.7	22.57^{cdef}
8	46P3S2	*M.plurifarium*			16.30^{ij}
9	29P5S1	*M.plurifarium*			17.7^{ghi}
10	43P2S1	*M.plurifarium*			24.87^{abcd}
11	ET1	*M. australicum*			18.13^{ghi}
12	ET4	*M. australicum*			28.43^{a}
13	23P2S2	*M. australicum*			12.36^{jkl}
14	ET26	*M. amorphae*			23.80^{bcde}
15	90P4S2	*M. amorphae*			27.0^{ab}
16	22P5S2	*M. amorphae*	1.14	0.71	21.78^{defg}
17	36P3S1	*M. amorphae*			23.34^{bcde}
18	ET24	*M. amorphae*			18.60^{fghi}
19	38P4S2	*M. loti*			20.43^{efgh}
20	10P3S1	Unidentified			16.41^{hij}

Means followed by the same letter within a column are not significantly different at (P<0.05) level of probability., SI stands for = solubilization index for phosphorus, HCN= no growth

The SI of strains in Ethiopia were slightly lower than recorded from SI (1.42) displayed by *Mesorhizobium ciceri* isolated from Iran (Alikhani *et al*., 2006). The amount of phosphorus released by these strains in a liquid culture showed, *M. amorphae* 80P4S2 was able to release the highest amount of available soluble phosphates from $Ca_3(PO4)_2$ (118.0µg/ml) and $AlPO_4$ (93.3µg/ml) after 8 days of incubation (Table 4.5).The phosphates released by the strains ranged from (29.0 to 118.0 µg/ml) from tricalcium and 41.67-93.3 µg/ml from aluminium phosphate after 8 days of incubation The *M. amorphae*

80P4S2 was the best solubilizer of both inorganic phosphate sources as it released phosphate constantly until 8 days of incubation; whereas *M. amorphae* 80P4S2 slowly increased solubilization after 4 days of incubation. The amount of phosphate released in this study was moderate compared to release from tricalcium was ranged (70 to 295 µg/ml) after 8 days of incubation (Mulisa Jida and Fassil Assefa, 2016).

Table 4.5.Tricalcium and Aluminium phosphate solubilization efficiency of *Mesorhizobium* strains

		$Ca_2(PO_4)$				AlPO4			
		4^{th}day		8^{th}day		4^{th}day		8^{th}day	
Strain	Closest relative	pH	P(µg/ml)	pH	P(µg/ml)	pH	P(µg/ml)	pH	P(µg/ml)
22P5S2	*M. amorphae*	5.0	37.47^{b}	4.8	70.47^{b}	5.8	10.43^{b}	6.0	43.43^{b}
45P4S1	*M. loti*	5.4	29.0^{c}	5.8	29.0^{c}	4.7	8.67^{b}	4.3	41.67^{b}
80P4S2	*M. amorphae*	4.8	85.0^{a}	4.8	118.0^{a}	5.3	60.33^{a}	5.6	93.33^{a}
LSD (0.05)			2.24		2.24		3.66		3.66
CV			2.87		1.62		9.99		3.9

Means followed by the same letter within a column are not significantly different at (P<0.05) level of probability., CV stands for= Coefficient of variation, LSD = least significant difference

4.3.3. Nodulation and symbiotic effectiveness of strains under greenhouse conditions

The tested strains showed significant variation in nodulating the two varieties ranging from (31 nodules per plant up to 62 nodules per plant) (Table 4.6). However, Natoli variety induced more nodules per plant (32-62) than Arerti variety with 31-46 nodules per plant and Strains closest affinity to *M. amorphae* and *M. plurifarium* showed more nodules per plant than the others. This might indicate that the varieties responded differently to individual strains within *Mesorhizobium* genospecies. Study in Ethiopia also showed most isolates (59%) induced more nodules (14-62 nodules) on Desi seed type Natoli (Wubayehu Gebremedhin *et al*.,2018). The strains induced nodule dry weight within the range of 55mg/plant to 557mg/plant on two varieties. The highest nodule dry weight was recorded *M. amorphae* and *M.sp.* LSJC280B00 relative group of strain on Arerti and *M. loti* affinity on Natoli variety. Earlier study on chickpea isolates from

Ethiopian soils showed nodule dry weight variation between 44mg/plant to 497mg/plant (Wondwosen Tena *et al*., 2016).

Table 4.6.Inoculation response on different nodulation traits of chickpea varieties at greenhouse

		NN		NDW (mg)		SDW (gm)		
Strains	Closest relative	Natoli	Arerti	Natoli	Arerti	Natoli	Arerti	SN (%)
27P3S2	*M. ciceri*	41.83^{a-g}	31.67^{d-h}	100.67^{def}	80.67^{d-g}	1.08^{a-e}	0.87^{a-e}	1.16^{a-e}
2P3S1-b	*M.sp.* LSJ280B00	39.50^{a-g}	46.17^{a-g}	84.00^{d-g}	154.33^{cd}	0.88^{a-e}	1.11^{a-d}	1.32^{a}
80P4S2	*M. amorphae*	56.17^{a-d}	23.33^{ghi}	94.67^{def}	104.33^{def}	1.21^{abc}	1.03^{a-e}	1.18^{a-d}
10P4S2	*M. amorphae*	40.50^{a-e}	27.33^{fgh}	123.00^{def}	83.67^{d-g}	1.12^{abcd}	0.69^{cde}	1.14^{a-f}
19P3S1	*M. amorphae*	48.83^{a-f}	37.33^{b-h}	91.00^{def}	557.00^{a}	1.00^{a-e}	1.06^{a-e}	1.12^{b-g}
ET20	*M. amorphae*	62.00^{a}	45.67^{a-g}	107.67^{def}	106.33^{def}	0.81^{a-e}	0.87^{a-e}	1.10^{c-i}
45P4S1	*M. loti*	35.17^{b-h}	29.17^{fgh}	361.67^{b}	64.33^{d-g}	1.17^{abc}	0.80^{b-e}	1.17^{a-d}
46P3S2	*M.plurifarium*	42.50^{a-g}	25.50^{fgh}	137.67^{def}	86.67^{def}	1.10^{a-e}	0.92^{a-e}	1.20^{ab}
29P5S1	*M.plurifarium*	47.67^{a-g}	26.33^{fgh}	130.00^{def}	104.00^{def}	1.22^{abc}	0.98^{a-e}	1.13^{a-f}
43P2S1	*M.plurifarium*	59.50^{ab}	33.50^{c-h}	114.33^{def}	89.67^{def}	1.47^{a}	0.96^{a-e}	1.18^{abc}
ET1	*M. australicum*	46.83^{a-g}	37.17^{b-h}	145.67^{de}	121.00^{def}	1.06^{a-e}	0.94^{a-e}	1.03^{h-k}
ET4	*M. australicum*	46.67^{a-g}	36.83^{b-h}	99.67^{def}	82.00^{d-g}	1.06^{a-e}	0.83^{a-e}	0.97^{k}
23P2S2	*M. australicum*	46.33^{a-g}	27.00^{fgh}	108.00^{def}	55.00^{fg}	1.14^{abc}	0.62^{cde}	1.21^{ab}
ET26	*M. amorphae*	39.00^{a-g}	30.50^{e-h}	124.33^{def}	129.33^{def}	1.03^{a-e}	1.12^{a-d}	1.15^{a-e}
90P4S2	*M. amorphae*	41.50^{a-g}	32.00^{d-h}	150.67^{cd}	56.67^{fg}	1.18^{abc}	0.93^{a-e}	1.15^{a-e}
22P5S2	*M. amorphae*	56.83^{abc}	32.83^{c-h}	153.00^{cd}	234.67^{c}	1.01^{a-e}	0.86^{a-e}	1.19^{abc}
36P3S1	*M. amorphae*	34.17^{c-h}	30.83^{e-h}	104.33^{def}	91.33^{def}	1.04^{a-e}	0.79^{b-e}	1.14^{a-f}
ET24	*M. amorphae*	32.33^{c-h}	28.67^{fgh}	110.33^{def}	92.33^{def}	1.12^{a-d}	0.95^{a-e}	1.06^{f-j}
38P4S2	*M. loti*	54.67^{a-e}	39.67^{a-g}	121.00^{def}	109.00^{def}	1.12^{a-d}	1.08^{a-e}	1.04^{g-k}
10P3S1	Unidentified	54.00^{a-e}	37.67^{a-h}	154.00^{cd}	87.00^{def}	1.18^{abc}	0.69^{cde}	1.09^{d-i}
EAL029	Reference	31.50^{e-h}	24.17^{e-h}	101.33^{def}	74.00^{d-g}	0.96^{a-e}	0.88^{a-e}	1.01^{ijk}
Ha. Ata	Reference	30.50^{e-h}	13.83^{hi}	107.33^{def}	105.67^{def}	0.94^{a-e}	0.58^{de}	1.05^{f-k}
Control	-	0.00^{i}	0.00^{i}	0.00^{g}	0.00^{g}	0.39^{cde}	0.36^{e}	0.40^{jk}
Nitrogen	-	0.00^{i}	0.00^{i}	0.00^{g}	0.00^{g}	1.45^{ab}	1.18^{abc}	1.31^{a}
HSD (5%)		24.52		84.74		0.42		ns
CV		20.82		22.08		19.8		3.75

Means followed by the same letter within a column are not significantly different at ((P<0.05) level of probability., NN=number of nodules per plant, NDW=nodules dry

weight, SDW=shoot dry weight; SN=Shoot nitrogen (%); CV stands for= Coefficient of variation, HSD = high range statistical domain

The Natoli variety produced shoot dry weight in the range of (0.62-1.38g) per plant on both varieties. More shoot dry matter accumulation was recorded from the Natoli variety (0.78-138g) compared to fewer shoot dry matter (0.62-1.18g) formed on Arerti variety. Six strains accumulated more, but not significantly higher, shoot dry matter than the reference and the nitrogen fertilized plants on both varieties. The shoot dry matter in this study did not exceed the values reported (0.6-1.36 g/plant) for chickpea isolates by (Mulisa Jida and Fasil Assefa, 2012).

Symbiotic effectiveness in relation to shoot dry matter by the inoculated plants in reference to the nitrogen fertilized control, 85% of the strains were effective and highly effective on both varieties (Table 4.6 and Table 4.7). Strains close relative to *M. ciceri*, *M. amorphae*, *M. plurifarium*, *M. loti*, and *M. australicum* fall into highest relative symbiotic effectiveness. Study at Ethiopia by (Wubayehu Gebremedhin *et al*., 2018) showed up to 125% symbiotic effectiveness and recently (Tassew Siraj and Fassil Assefa, 2018) reported up to (70%) on Natoli and 21% Arerti symbiotically highly effective and effective isolates.

M.sp. LSJ280B00 strain 2P3S1-b and *M. amorphae* 80P4S2 that showed a wide ranging eco-physiological competence, also were highly symbiotic effective. In relation to PGP, *M. amorphae* 80P4S2 and *M. loti* strain 45P4S1 was able to release the highest amount of available soluble phosphates was found the better strains in symbiotic effectiveness when tested in greenhouse. Significant variation were not observed for shoot nitrogen content (Table 4. 6); *M. amorphae* strain ET26 accumulated relative shoot nitrogen content (1.2%) on both respective varieties. Most strains accumulated low nitrogen content compared to (Mulisa Jida and Fassil Assefa, 2016) were reported (1.34-2.48%) shoot nitrogen concentrations in inoculated chickpea plants.

Table 4.7.Symbiotic effectiveness tested of selected *Mesorhizobium* species on Natoli and Arerti varieties grown for 60 days in sand pot culture under greenhouse condition

		SE rating			
Strains	Closest relative	Natoli	Score	Arerti	Score
27P3S2	*M. ciceri*	74	E	74	E
2P3S1-b	*M.sp.* LSJ280B00	61	E	94	HE
80P4S2	*M. amorphae*	83	HE	87	HE
10P4S2	*M. amorphae*	77	E	58	E
19P3S1	*M. amorphae*	69	E	90	HE
ET20	*M. amorphae*	56	E	74	E
45P4S1	*M. loti*	81	HE	68	E
46P3S2	*M.plurifarium*	76	E	78	E
29P5S1	*M.plurifarium*	84	HE	83	HE
43P2S1	*M.plurifarium*	101	HE	81	HE
ET1	*M. australicum*	73	E	80	HE
ET4	*M. australicum*	80	E	70	E
23P2S2	*M. australicum*	79	E	53	E
ET26	*M. amorphae*	71	E	95	HE
90P4S2	*M. amorphae*	81	HE	79	E
22P5S2	*M. amorphae*	70	E	73	E
36P3S1	*M. amorphae*	72	E	67	E
ET24	*M. amorphae*	77	E	81	E
38P4S2	*M. loti*	77	E	92	HE
10P3S1	Unidentified	81	E	58	E
EAL029	Reference	66	E	75	E
Ha. Ata	Reference	65	E	49	LE

Table legend; HE=highly effective; E=effective; LE=lowly effective;

The study showed the presence of a predominant chickpea endemic *Mesorhizobium* species accompanying tolerance to various stress conditions, nutritional versatility, growth

promoting features and high symbiotic effectiveness in chickpea. Strains such as; *M.sp.* LSJ280B00 2P3S1-b and *M. amorphae* 80P4S2 displayed wide range of eco-physiological tolerance and versatile substrates utilization. Regardless of effectiveness in symbiotic nitrogen fixation on different chickpea varieties under greenhouse conditions, *M. genospecies* 1B strain 45P4S1 closely related to *M. loti*, *M. genospecies* 2A strain 43P2S1 affinity to *M plurifarium* and *M.* genospecies 4A strain ET26 relative to *M. amorphae* revealed pronounced competitiveness.

In general, the tested new *Mesorhizobium* strains showed dominance eco-physiological tolerance, carbon and nitrogen assimilation features, inherent antibiotic and heavy metal resistance and high symbiotic effectiveness. This might provide a greater tolerance to adverse environments and compete against the possible influence of ineffective indigenous rhizobia for nodulation and nitrogen fixation. Thus, the results of this study highlighted the potential of such strains to further evaluate at multi-location field trials for the selection of elite inoculant strains that could presumably enable to applied under different soil and environmental conditions.

Chapter 5

5. Symbiotic performance of elite *Mesorhizobium* species isolated from Ethiopian soils on chickpea (*Cicer arietinum* L.) grown in the field conditions

Zehara Mohammed Damtew[12]., Douglas R. Cook[3]., Alex Greenlon[4]., Asnake Fikre[2] and Fassil Assefa[1]

[1]College of Natural Sciences, Addis Ababa University, Addis Ababa, 32853 Ethiopia; [2]Debre Zeit Agricultural Research Center, Ethiopian Institute for Agricultural Research, Bishoftu; [3]Department of Plant Pathology, University of California, Davis, CA 95616;[4]University of California, Berkeley, Environmental Science, Policy and Management

Abstract

The use of chickpea (*Cicer arietinum* L.) as a source of protein rich food and feed and its integration in crop rotation to improve soil fertility in low input agriculture is due to its symbiotic association with nitrogen fixing bacteria of mainly the genus *Mesorhizobium.* With the goal of testing symbiotic effectiveness within the strain collection, 20 diverse strains (two from each clade) were assessed for the ability to enhance plant performance in greenhouse experiments. Thus, 10 of the best performing symbiont strains were tested in multi-location field trials. The results showed that the number of nodule and nodule dry weight showed major difference between the low nodulating and high nodulating strains, and most of the nodulation values fit in with the yield related parameters (shoot dry weight, biological productivity, seed yield). In general, *M plurifarium* 43P2S1 showed the best performance in yield of all strains, followed by *M.sp.* LSJ280B00 strain 2P3S1-b, that showed significant difference in grain yield (19-31%) and biological productivity (24-29%) increase over the uninoculated control plant and better than N-fertilized plants. Although the above two elite strains performed well at all sites, *M. loti* strain 45P4S1, *M. amorphae* strain 80P4S2, *M. ciceri* strain 27P3S2, *M. australicum* ET1 and *M. amorphae* ET26 showed significant variation in grain yield 1.83-2.91t/ha depending upon site. Whereas, these second group of strains were showed similar pattern of difference and limited grain yield at all sites; indicating other nutrient sources and factors contributing to grain yield increase other than nitrogen fixation. The difference was some strains such as *M. amorphae* 22P5S2, *M. loti* 38P4S2 and *M. amorphae* 90P4S2 showed poor nodulation and grain yield improvement at all sites. Therefore, this finding provides results that

support promotion of these Mesorhizobium species inoculant production for distribution to smallholder farmers.

Keywords/phrases: *Biological productivity, grain yield, multi-location, shoot dry weight, strains, Symbiotic N_2-fixation*

5.1. Introduction

Chickpea (*C. arietinum* L) is widely grown in different regions of Ethiopia for food and feed production., and is an important component of crop rotation to enhance its yield and succeeding crops. The main form of nitrogen supply for realizing growth requirement of the crop is related with increasing access to atmospheric nitrogen through a symbiotic relationship in their root system with a group of soil born bacteria collectively rhizobia, specifically for chickpea the genus *Mesorhizobium* which induce the expansion of nitrogen fixing nodules on the roots (Pampana *et al*., 2018). It is estimated that the crop requires about 13 to 41 kg /ha inputs of nitrogen for growth and development from which it derives 70% of its N through symbiotic N_2 fixation and improves the N content of soil which is available for the subsequent crop (Aslam *et al*., 2010; Rasool *et al*., 2015; Khaitov and Abdiev,2018). The amounts of nitrogen fixed by chickpea has been estimated that 60 kg/ha under regular precipitation and 19-24 kg/ha at drought stress conditions (Abi-Ghanem *et al*., 2012).

However, the potential of the crop in fixing appropriate nitrogen depends on both symbiotic partners, the ability of crop varieties to nodulation and their response to incorporate symbiosis, variability of rhizobia in capable of inducing nitrogen fixation in different host varieties, existence and range of indigenous rhizobia diversity in the native soil origin (Hefny *et al*.,2001; Khattak *et al*., 2006; Wielbo *et al*.,2015). Previous studies have revealed that the Ethiopian soil harbor 60-80% effective chickpea rhizobia (Daniel Muleta and Fassil Assefa, 2015, Wubayehu Gebremedhin *et al*, 2018), indicating the vigor of chickpea rhizobia that is related to the natural ecosystem servicing of the legume in the low in put cropping systems in the country. However, these works were limited to the phenotypic features of the chickpea rhizobia and to screening of their symbiotic effectiveness under controlled greenhouse experiments.

Phenotypic features do not clearly indicate taxonomic affiliation of the species nodulating the host, and neither does effectiveness in a controlled experiment necessarily reflect the performance of the strains under field conditions (Ben Romedhane *et al*, 2007).
Wondwosen Tena *et al*., (2016) tested the symbiotic effectiveness of local strains under field conditions and found that grain yields were increased by 50, 28 and 33% above the control by inoculation with Cp41, CpSK and Cp8, respectively. Similarly, Tassew Siraj and Fassil Assefa (2018) reported that *M. plurifarium, M. ciceri, M. abyssinicae, M. gobiense, M. hawasiense, M. shonense* and *M. amorphae* which are the main chickpea nodulating species among which M. plurifarium and M. ciceri were most effective in symbiotic nitrogen fixation. However, all *M. ciceri* and *M. plurifarium* were highly effective on both chickpea varieties (SE values 80-100%), under greenhouse conditions.

Recently, Endalkachew W/Meskel *et al*., (2018) conducted on-farm trials evaluating chickpea varieties; Natoli, Arerti and Habru to inoculation using strains Cp41, and Cp029 alone or/and phosphorus application in Central and Southern parts of the country. They showed that inoculation with elite strains increased grain yield by 21%; Assefa Funga *et al*., (2016) made a field trial on inoculation of EAL029 (Ethiopian standard commercial strain) and ICRE-025 on Natoli and Teketay varieties and showed significant difference in increases in shoot dry matter and level of symbiotic effectiveness compared to the uninoculated trial in Debre Zeit and Wolayta Sodo, central and Southern parts of the country. However, they did not determine the grain yield and biological productivity so as to show the real picture of the contribution of inoculation to total biomass.

Most of the hitherto studies involved evaluation of symbiotic effectiveness based on phenotypic grouping under greenhouse or field conditions. The inoculation trials were undertaken by properly un identified endosymbionts and commercial strains known for a long time on field trials. This necessitate a comprehensive study involving intensive screening of cognate isolates of *Mesorhizobium* from diverse chickpea growing areas to understand adaptation and competitiveness of endemic chickpea symbionts. Evidences relied on such principal hypothesis has been used to determine the response of the crop to symbiosis through effective inoculation by expending native *Mesorhizobium* strain and identify effective broad host range inoculants development at multiple environments.

In this study, comprehensive sampling was used to screen new rhizobia isolate that represent different regions of the country. Genomic tool application to understand the presence of a predominant genomic diversity of endemic strains of chickpea's symbiont has identified six species of *Mesorhizobium* that nodulate chickpea in Ethiopia, compared to>20 species of *Mesorhizobium* that nodulate chickpea globally. The position of our *Mesorhizobium* species were compiled with those of global level population genomics and their differential effects of geography and phylogeny on horizontal gene transfer in soil bacteria as reported (Greenlon *et al*.,2019).

The current field experiment was conducted to identify efficient *Mesorhizobium* species in the symbiosis. The strains comprised new *Mesorhizobium* species unknown earlier in chickpea rhizobia study in other country. Those *Mesorhizobium* strains showed persistent characteristics in vitro test was also evaluated at different types of soils, climate conditions and predict host varieties that yield optimal nitrogen fixation. The research has been executed in broad environment with two host varieties against representative subset of *Mesorhizobium* species.

5.2. Materials and Methods

5.2.1. The *Mesorhizobium* strains

The inoculants (strains) were selected from 64 strains genomically identified strains belonging to six *Mesorhizobium* spp collected from Ethiopia (Greenlon *et al*.,2019). They were the best symbiotic effective strains with a wide range of stress tolerance under *in vitro* conditions (Zehara *et al.*, 2020). The strains were obtained from the culture collection at Plant Pathology Laboratory of Davis, University of California, USA and Addis Ababa University. The strains have NCBI assembly accession (Greenlon *et al*.,2019). List of the *Mesorhizobium* strains geographic origin and geographic position and are presented (Table 5.1). Two commercial strains; one commercial strain (EAL 029 from Menagesha Biotech Ethiopia) and another, Ha. Ata (Tunisian strains from N_2Africa, International Institute of Tropical Agriculture (IITA) were included as standard checks.

Table 5.1.List of *Mesorhizobium* strains and origin of culture collection for strains used in Field

No	Sample strains	Relative species	Region	Latitude	Longitude	Elevation
1	43P2S1 (*M. genospecies* 2A)	*M.plurifarium*	Amhara, North Gondar	12° 27' 43.6"N	37° 21' 29.7"E	1960
2	90P4S2 (*M. genospecies* 4A)	*M. amorphae*	Oromia, West Shewa	8° 36' 3.4"N	38° 16' 0.8"E	2209
3	22P5S2 (*M. genospecies* 4A)	*M. amorphae*	Amhara, East Gojam	10° 24' 56.2"N	38° 10' 35.9"E	2429
4	2P3S1-b (*M. genospecies* 9A)	*M.sp.*LSJ280B00	Oromia, East Shewa	8° 49' 31.7"N	38° 59' 25.4"E	1944
5	ET1 (*M. genospecies* 8A)	*M. australicum*	Amhara, South Gondar	11° 27' 58.3"N	38° 12' 46.6"E	2795
6	38P4S2 (*M. genospecies* 1E)	*M. loti*	Amhara, South Gondar	12° 1' 54.3"N	37° 43' 49.3"E	1809
7	ET26 *(M. genospecies* 4A)	*M. amorphae*	Amhara, North Gondar	12° 27' 34.8"N	7° 48' 23.0"E	1841
8	45P4S1 (*M. genospecies* 1B)	*M. loti*	Amhara, North Gondar	12° 26' 43.8"N	37° 20' 48.3"E	1934
9	80P4S2 (*M. genospecies* 3A)	*M. amorphae*	Amhara, South Wollo	12° 20' 56.9"N	38° 3' 35.4"E	1906
10	27P3S2 (*M. genospecies* 7A)	*M. ciceri*	Amhara, East Gojam	10° 24' 41.7"N	38° 10' 8.4"E	2429
11	EAL029 (Commercial)	-	Commercial	Reference	None	None
12	Ha.Ata (Reference)	-	Tunisia	None	None	None
13	Control (Untreated)	-	Uninoculated	None	None	None
14	Nitrogen (Fertilizer)	-	Nitrogen	None	None	None

5.2.2. Source of chickpea varieties

Chickpea varieties were Natoli and Arerti obtained from Debre Zeit Agricultural Research Center. Their agronomic and phenologic characteristics are shown in (Table 5.1.1). The Natoli variety representing Desi type and Arerti representing Kabuli type); the varieties were chosen based on higher yield, well adapted and widely grown by smallholder farmers and market preferable. Natoli varieties has short to medium maturity (88-150 days) while the Arerti has relatively long duration (105-155 days).

Table 5.2.Selected chickpea varieties growth habit and yield situation at different environment

Variety/Genotype	Chickpea Type	Adaptation/ elevation	Maturity days	Seed color	Grain yield (t/ha)	
					Potential	On farm
Arerti	Kabuli	1900-2600	105-155	white	2.6-4.6	2.0-3.2
Natoli	Desi	1800-2700	88-142	Light golden	1.1-4.6	3.5-3.7

Adopted from Debre Zeit Agricultural Research unpublished report and MoARD, (2009)

5.2.3. Experimental procedures and seed inoculation

The on-farm field trials were performed from 2017-2019 for three years and at three agroecological sites located at Chefedonsa on latitude, Longitude, (8° 57' 15.6"N, 39° 16' 2.2"E), Altitude 2435, Genda Gorba (8° 48' 34.7"N, 39° 14' 8.4"E,), Altitude 1895asl and Alem Tena (8° 18' 32.5"N, 39° 57' 8.8"E) and Altitude 1640, which could possibly represent high, medium and low altitude of chickpea growing agro-ecologies within the central highlands of Ethiopia, respectively. The field sites did not have any history of rhizobial inoculation.

The climatic data such as rainfall, maximum and minimum temperature were recorded at meteorological observatory, main agriculture research station, Debre Zeit during the growing season 2017 (Figure 1); while the latter year meteorological observation incomplete. During that time the area received high amount of rainfall from June to August of the growing season in which the highest amount (416.80 mm) was obtained in July at Chefedonsa followed by Alem Tena (370.6 mm) and no rain on October at Genda Gorba, with average minimum (7.1°C) and maximum (29.3°C) temperatures during the cropping season

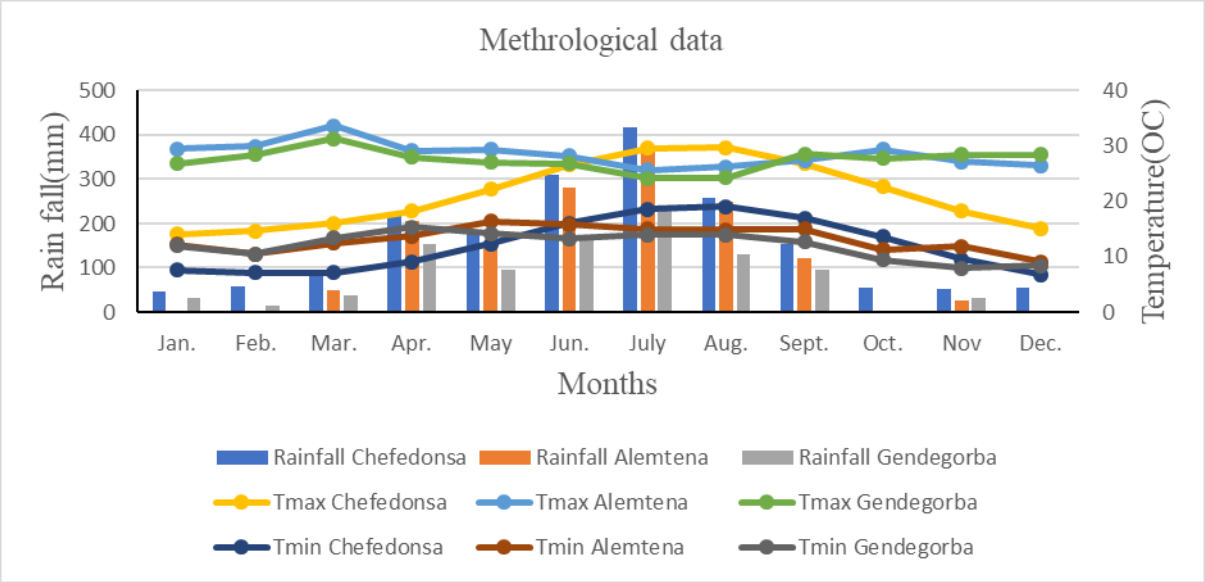


Figure 5.1.Monthly mean min and max temperature (°C) and total rainfall (mm) data of the experimental sites for 2017 cropping season

The experimental design was laid out in a split plot design with three replications. Varieties (two) were assigned as main plots and strains (Twelve strains; ten selected strains; and two reference strains) as sub plots. The size of each experimental plot was 3 m x 1.8 m (5.4 m^2) consisting of six rows at 30 cm spacing, with a total of 84 sub plots. The distance between plots was 1m, blocks was 2m and 10 cm intra- row spacing were kept at seed sowing rate of 120 kg/ha (Debre Zeit Agricultural Research unpublished report). The *Mesorhizobium* inoculants for seed treatment were prepared following the standard procedure of the Menagsha Biotech; they were activated and grown into 100 ml sterilized YEMA broth in Erlenmeyer flask on orbital shaker at 200 rpm at room temperature for 72 hours to inoculums size of approximately (~10^9cells/ml). At sowing time in the field, 80 seeds were mixed with the *Mesorhizobium* inoculants at the rate of 2 g of prepared inoculum for each respective rhizobial strains and 10% sugar solution dissolved in distilled water to serve as an adhesive.

The inoculation was carried out using finalized lignite carrier products provided from National soil testing center of Ethiopia at Debre Zeit Agricultural Research Center Biotechnology Laboratory. In order to determine nitrogen derived from fixation (Biological Nitrogen Fixation, BNF) by the host, non nodulating chickpea isoline variety PM233 was included **(**Davis *et al*.,1985; Gemechu Keneni, 2012). In addition, one treatment fertilized with 18 kg N/ha as positive control and another uninoculated control treatment were included as negative control. All plots were fertilized with phosphorus 20kg/ha as Triple super phosphate (TSP) form uniformly (Eshete, 1994).

5.2.4. Data collection and plant tissue analysis

5.2.4.1. Plant sample analysis

Five plants were carefully up rooted from each plot of the two rows after 45 days from planting to record nodule number, nodule and shoot dry weight after drying in oven at 70°C for 48 hours. At physiological maturity another five plants were collected from each plot to collect data on plant height, pod bearing branches, pods number and seeds. The

shoots were separated from grain and dried at 70°C for 48 hours to a constant weight for estimating nitrogen content (%) according to Kjeldahl Methods (Sahlemedhin Sertsu and Taye Bekele, 2000).

At the harvest stage, plants were manually harvested from the middle four rows for estimation of above ground dry biomass, seeds weight, seed yield and harvesting index (the ratio of seed yield to total above ground dry biomass).

5.2.4.2. Soils sample analysis

Pre-palnting soil were taken from each plot at the depths of 0-20 cm using soil Auger and composited into one for individual experimental sites. Each composited soil sample was dried and passed through a 2 mm sieve and homogenized. Samples were analysed at Debre Zeit Agricultural Research Centre (DZARC) soil laboratory for soil physicochemical analysis. The soil samples were analyzed for pH in a 1:2.5 soil-water suspension using a glass electrode as described by Bouyoucos (1962). Soil texture was determined following the method of Bouyoucos (1962) using a hydrometer. Cation exchangeable capacity (CEC) was determined by the neutral ammonium acetate saturation method (Rhoads, 1982) and organic carbon using wet oxidation methods of Walkley and Black (1934). Total nitrogen content was determined following the Kjeldahal method as described by Jackson, (1958). The available phosphorus content of the soil was analyzed using 0.5M sodium bicarbonate extraction solution (pH 8.5) following the method of Olsen (Olsen *et al*., 1954).

5.2.4.3. Data management and statistical analysis

All data were analyzed by SAS version 9.3 statistical software using PROC GLM procedure after checking the compliance of the data with the assumptions of the statistical test (SAS Institute, 2012). Values were given as means for triplicate samples analyses of variance (ANOVA) were performed to determine treatment effects on shoot dry weight, nodule number and nodule dry weight, biological yield, grain yield and shoot total nitrogen percent. Mean separation was carried out using Tukey high range statistical domain (HSD) comparison procedures at 5% probability level. Correlation analysis was done using Pearson's simple correlation coefficients to test the relationships among

different symbiotic and yield parameters. Percentage change (increase or decrease) in the number of nodules per plant, NDW/plant, aboveground biomass (ha), grain yield (ha) of the treated varieties was obtained by the following formula; M1-MU/ MU X 100 (Where M1= Mean of the values of the treated varieties; MU= Mean of the values of the uninoculated varieties).

5.3. Result and Discussion

5.3.1. Overview of nodulation traits

The *Mesorhizobium* inoculants showed significant ($P \leq 0.05$) response in nodulating the two varieties at different planting years from 2017 upto 2019 at Chefe Donsa, Alem Tena and Genda Gerba sites (Table 5.3, Table 5.4, and Table 5). At Chefe Donsa site, the strains of the different *Mesorhizobium* species induced the highest overall mean nodule number in the range of 10-50 nodules/plant on both Natoli and Arerti varieties with mean of 32, 27 and 30 nodules/plant in the years 2017, 2018, and 2019, respectively (Table 5.3). Nonetheless, *M. amorphae* 80P4S2 induced the highest mean of 49 nodules/plant in 2017 and *M. loti* 45P4S1 showed prolific nodulation with 42 nodules/plant and 50 nodules/ plant in 2018 and 2019, respectively. Generally, *M. ciceri* 27P3S2, *M. amorphae*, 80P4S2, *M.sp. LSJ280B00* 2P3S1-b and *M. plurifarium* strain 43P2S1 showed significant variation in nodulation between the two varieties. (Appendix table 8).

The pattern of nodulation showed variation irrespective of the planting years at Alem Tena sites (Table 5.4). On this site, the inoculants produced relatively lower number of nodules ranging from 10-42 nodules/plant with overall mean nodule number of 29, 40, 42 in 2017, 2018 and 2019, respectively. The same strains perform best at Chefe Donsa also displayed overall mean (15-34 nodules) on Natoli and Arerti varieties irrespective of the planting years at Alem Tena (Table 5.4; Appendix table 9). The difference was some strains such as *M. amorphae*, 38P4S2, *M. amorphae* 90P4S2, the reference Tunisian strain, Ha. Ata and the Ethiopian commercial strain EAL 029 showed poor nodulation at Chefedonsa; whereas these strains induced more nodules with mean (11-34 nodules) on the two varieties irrespective of the planting years at Alem Tena sites (Table 5.4).

Table 5.3 Inoculation response on number of nodules, nodule dry weight and shoot total nitrogen percent of chickpea varieties at Chefe Donsa trial sites.

Treatment	Year 2017			Year 2018			Year 2019		
	NNP	**NDW**	**TN**	NNP	**NDW**	**TN**	**NNP**	**NDW**	**TN**
Strain									
43P2S1 (*M. plurifarium*)	31^{a-d}	123.8^{a}	0.60^{ab}	29^{bc}	104.64^{b}	1.34^{bc}	34^{b}	114.45^{bc}	1.70^{a}
90P4S2 (*M. amorphae)*	30^{a-d}	68.4^{ab}	0.49^{c}	17^{de}	48.70^{fg}	0.79^{d}	17^{ef}	54.97^{g}	0.96^{f}
22P5S2 (*M. amorphae)*	26^{bcd}	75.7^{ab}	0.49^{bc}	13^{e}	49.06^{fg}	0.87^{d}	10^{f}	56.79^{g}	1.04^{f}
2P3S1-b (*M.sp.* L SJC280B00)	39^{abc}	98.1^{ab}	0.69^{a}	36^{ab}	115.02^{ab}	1.36^{b}	33^{b}	117.07^{b}	1.69^{ab}
ET1 (*M. australicum*)	24^{bcd}	68.8^{ab}	0.63^{abc}	27^{c}	49.30^{fg}	0.75^{d}	31^{b}	70.92^{efg}	1.48^{bcd}
38P4S2 (*M. loti)*	18^{d}	77.2^{ab}	0.50^{bc}	28^{bc}	44.90^{g}	0.80^{d}	28^{bc}	66.09^{fg}	1.01^{f}
ET26 *(M. amorphae*)	33^{a-d}	114.4^{ab}	0.53^{bc}	16^{de}	44.65^{g}	0.90^{d}	18^{def}	56.42^{g}	1.62^{abc}
45P4S1 (*M. loti*)	26^{bcd}	89.6^{ab}	0.61^{abc}	42^{a}	122.42^{a}	1.41^{a}	50^{a}	143.37^{a}	1.62^{abc}
80P4S2*(M. amorphae*)	49^{a}	150.7^{a}	0.64^{ab}	29^{bc}	75.98^{cd}	1.3^{ab}	33^{b}	92.51^{d}	1.63^{abc}
27P3S2 (*M. ciceri*)	41^{ab}	116.3^{ab}	0.65^{ab}	30^{bc}	87.91^{c}	1.35^{b}	45^{a}	95.51^{cd}	1.66^{abc}
Mean	32	97.3	0.60	27	74.26	1.15	30	86.81	1.49
Ha. Ata (Reference)	30^{a-d}	122.8^{ab}	0.49^{c}	24^{cd}	48.56^{fg}	0.82^{d}	26^{b-e}	81.36^{def}	1.05^{f}
EAL029 (Commercial)	26^{bcd}	138.8^{a}	0.56^{abc}	27^{c}	66.11^{de}	1.03^{cd}	27^{bcd}	86.30^{de}	1.11^{ef}
Control (Untreated)	18^{d}	69.4^{ab}	0.50^{bc}	16^{de}	59.92^{ef}	0.99^{d}	20^{c-f}	72.68^{f}	1.22^{def}
Nitrogen (Fertilizer)	19^{cd}	69.1^{ab}	0.49^{bc}	22^{cde}	69.75^{de}	0.98^{d}	26^{b-e}	84.99^{def}	1.41^{cde}
Variety									
Arerti	21^{b}	74.55^{b}	0.63^{a}	30^{a}	77.87^{a}	1.06^{a}	23^{b}	74.88^{b}	1.29^{b}
Natoli	39^{a}	115.29^{a}	0.49^{b}	20^{b}	63.11^{b}	1.04^{b}	35^{a}	94.89^{a}	1.46^{a}
P -value									
Variety	**	**	**	**	**	*	**	**	**
Strain	**	**	**	**	**	**	**	**	**
V x S	**	**	*	**	**	**	**	**	*
HSD	20.25	85.57	0.27	9.03	13.54	0.27	9.63	19.02	0.32

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., NNP =Number of nodules per plant, NDW=Nodule dry weight per plant(mg/plant), TN= shoot total nitrogen percent(%), S=strain V=variety, V x S= the interaction of variety and strain, **=significant ($P \leq 0.01$), * = significant ($P \leq 0.05$) and ns = non-significant (P> 0.05); HSD = high range statistical domain

The inoculants showed significant difference in nodulation in 2018 and 2019 at mid land Genda Gorba site compared to 2017 planting year (Table 5.5 and Appendix table 10). The results showed that *M. amorphae*, 80P4S2 induced nodulation on the two varieties with number of nodules ranging from (23-32 nodules) in 2018 and 2019, which was similar with the mean nodule number produced by *M. loti* 45P4S1.

The inoculants showed lower number of nodules at Alem Tena and Genda Gorba compared to Chefe Donsa site irrespective of the planting years except the *M. plurifarium* 43P2S1 strain that induced nodulation with mean (24-32 nodules) on both varieties irrespective of the planting years at Genda Gorba.

Table 5.4. Inoculation response on number of nodules, nodule dry weight and shoot total nitrogen percent of chickpea varieties at Alem Tena trial sites

Treatment	**Year 2017**			**Year 2018**			**Year 2019**		
	NNP	**NDW**	**TN**	NNP	**NDW**	**TN**	NNP	**NDW**	**TN**
Strain									
43P2S1 (*M. plurifarium*)	20^{abc}	86.97^{ab}	0.91^{abc}	15de	83.32^{a}	1.02^{a}	17^{fgh}	122.70^{a}	1.59^{abc}
90P4S2 (*M. amorphae)*	24^{abc}	48.00^{b}	0.48^{d}	12^{e}	57.42^{cd}	0.92^{ab}	17^{fgh}	54.33^{h}	1.34^{bc}
22P5S2 (*M. amorphae)*	19^{e}	55.87^{ab}	1.01^{ab}	12^{e}	37.88^{e}	0.97^{ab}	10^{h}	41.80^{h}	1.26^{c}
2P3S1-b (*M.sp.* L SJC280B00)	29^{ab}	74.70^{ab}	0.73^{bcd}	15de	68.38^{bc}	1.03^{a}	27^{cde}	104.89^{b}	1.56^{bc}
ET1 (*M. australicum*)	18^{bc}	54.23^{b}	0.68^{bcd}	15de	50.93^{de}	0.89^{ab}	29^{cd}	81.99^{def}	1.44^{bc}
38P4S2 (*M. loti)*	16^{bc}	48.27^{b}	1.04^{a}	18^{cd}	60.97^{cd}	0.90^{ab}	27^{cde}	74.94^{fg}	1.36^{bc}
ET26 *(M. amorphae*)	25^{abc}	81.97^{ab}	0.76^{a-d}	14de	63.13^{cd}	0.97^{ab}	18^{fgh}	68.90^{g}	1.67^{ab}
45P4S1 (*M. loti*)	18^{bc}	57.37^{ab}	0.75^{a-d}	40^{a}	84.32^{a}	1.01^{ab}	42^{a}	103.35^{bc}	1.93^{a}
80P4S2*(M.* amorphae)	32^{a}	106.7^{a}	0.69^{bcd}	26^{b}	62.58^{cd}	0.98^{ab}	30^{bc}	72.67^{fg}	1.49^{bc}
27P3S2 (*M. ciceri*)	26^{abc}	83.10^{ab}	0.80^{a-d}	22^{bc}	69.62^{bc}	0.90^{ab}	38^{ab}	79.54^{efg}	1.67^{ab}
Mean	23	69.72	0.75	19	63.86	0.96^{ab}	26	80.51	1.53
Ha. Ata (Reference)	22^{abc}	79.93^{ab}	0.58^{d}	16de	60.53^{cd}	0.83^{b}	22^{d-g}	91.84^{cde}	1.42^{bc}
EAL029 (Commercial)	19^{abc}	92.3^{ab}	0.68^{bcd}	17^{cde}	81.12^{ab}	0.91^{ab}	21^{efg}	96.11^{i}	1.56^{bc}
Control (Untreated)	18^{bc}	53.54^{b}	0.62^{cd}	16de	49.63^{de}	0.86^{ab}	25^{c-f}	64.25^{fg}	1.28^{c}
Nitrogen (Fertilizer)	13^{c}	45.73^{b}	0.58^{d}	18^{cde}	68.68^{bc}	0.86^{ab}	27^{cde}	92.95^{bcd}	1.39^{bc}
Variety									
Arerti	14^{b}	53.51^{b}	0.83^{a}	15^{b}	71.23^{a}	0.91^{b}	23^{b}	70.42^{b}	1.43^{b}
Natoli	28^{a}	83.26^{a}	0.59^{b}	21^{a}	60.07^{b}	1.02^{a}	27^{a}	85.63^{a}	1.54^{a}
P -value									
Variety	**	**	*	**	**	*	**	**	**
Strain	**	**	**	**	**	*	**	**	**
V x S	**	**	**	**	**	ns	**	**	**

HSD	13.24	53.06	0.20	5.40	14.62	0.19	7.51	12.62	0.28

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., NNP =Number of nodules per plant, NDW= Nodule dry weight per plant(mg/plant), TN=shoot total nitrogen percent(%), S=strain V=variety, V x S= the interaction of variety and strain, **=significant ($P \leq 0.01$), * = significant ($P \leq 0.05$) and ns = non-significant (P> 0.05); HSD = high range statistical domain

Indeed, the majority of the strains showed variation in nodulating the two varieties ranging from (6-63 nodules) irrespective of the planting years at the different experimental locations. However, Natoli variety harbored more nodules (10-63 nodules) than Arerti variety with fewer nodules (9-50 nodules/ plant (Appendix table 10). In general, *M. loti* 45P4S1, *M. ciceri* 27P3S2, *M. amorphae* 80P4S2 and *M.sp.* LSJ280B00 2P3S1-b induced more nodules on both varieties at all experimental locations except, *M. plurifarium* strain 43P2S1 that produced as many nodules as the other strains on all but at Genda Gorba site, irrespective of the planting years. On the contrary, *M. amorphae* 90P4S2, *M. amorphae* ET26 and *M amorphae* 22P5S2 did not show major difference between the two varieties.

The inoculated plants displayed nodule dry weight ranging from 44.65-150.7mg/plant at Chefe Donsa, 37.88-122.7mg/plant at Alem Tena and 31.47-134.41 mg/plant at Genda Gorba sites, respectively (Tables 5.3, Table 5.4, and Table 5.5). In most cases, they showed the same pattern of increase in nodule dry weight as that of nodule number and the values were in the range of the overall mean of 74 -97mg/plant at Chefe Donsa site 64-81mg/plant at Alem Tena site and 47-91mg/plant at Genda Gorba sites. Although lower mean nodule dry weight was recorded at Genda Gorba site in 2017, the increase in nodule dry weight at the site was as high as the other sites in 2018 and 2019 years. Both nodule number and nodule dry weight were higher at Chefe Donsa than the Alem Tena and Genda Gorba sites which did not show significant difference between the two sites. The inoculants showed intra-strain difference in that *M. plurifarium* 43P2S1displayed the highest nodule dry weight at all sites although it showed a moderately abundant nodule number compared to the other prolific nodulators such as *M.* loti 45P4S1 and *M. amorphae* 80P4S2 and *M. ciceri* 27P3S2

In general, *M. loti* 45P4S1, *M. ciceri* 27P3S2, *M. amorphae* 80P4S2, *M.sp.* LSJ280B00 2P3S1-b and *plurifarium* 43P2S1 displayed major difference in nodule dry weight accumulation between the two varieties irrespective of the planting years indicating the gap on their effectiveness on the host. The nodule dry weight recorded in this study was comparative the number and nodule dry weight compared with previous experiment from Ethiopia (Wondwosen Tena *et al*., 2016). This has been indicated in other study (Aslam *et al*., 2010) that chickpea cultivar together with its compatible endosymbionts should be evaluated for their potential to produce nodules and effectively fix nitrogen fixation.

The optimum concentration of total nitrogen in shoot was observed in *M.sp.* LSJ280B00 2P3S1-b (0.69-1.69%), followed by *M. ciceri* 27P3S2 produced (0.65-1.66%), with overall mean (1.25, 1.22%) of total nitrogen on either of the varieties compared to uninoculated control plants (0.50-1.22%) across planting year at Chefe Donsa (5.6 and Appendix 14). In addition, the *M. loti* strain 45P4S1and *M. plurifarium* strain 43P2S1were able improve shoot nitrogen with mean increased of (1.15-1.28%) and (1.18-1.24%) both varieties respectively, irrespective of planting years. Other strains such as *M. amorphae* strain 80P4S2 performed well irrespective of variety and planting years (Appendix 14). The *M. loti* strain 45P4S1showed good shoot nitrogen performance across planting year (0.75-1.93%) irrespective of varieties compared to uninoculated control at Alem Tena (Table 5.7). *M. ciceri* strain 27P3S2 performed best on Natoli and Arerti variety (1.19,1.17%) respectively, compared to uninoculated control irrespective planting years at Alem Tena (Appendix 15).

Likewise, *M. plurifarium* strain 43P2S1 and *M. ciceri* 27P3S2 were able to increase (0.60-1.87%) and (0.71-1.79%) of the concentration of total nitrogen in shoot of both varieties well irrespective planting years at Genda Gorba (Table 5.8). The most effective strains *M. loti* strain 45P4S1performed better shoot nitrogen with overall mean (1.21-1.24%) across planting years irrespective of varieties (Appendix 16). In addition, the *Mesorhizobium* inoculant such as *M. loti* 45P4S1, *M. plurifarium* 43P2S1, *M. ciceri* 27P3S2, *M.sp.* LSJ280B00 2P3S1-b and *M. amorphae* 80P4S2 increased with overall mean (1.16-1.24%) of total nitrogen on both varieties compared to the uninoculated control (0.94-1.09%) irrespective of the planting years at three locations (Appendix14,15, 16). Relative

performance of shoot total nitrogen percent due to inoculations in chickpea isolates was reported (Carranca *et al*.,1999, McConnell *et al*.,2002 Assefa Funga *et al*.,2016).

Table 5.5.Inoculation response on number of nodules, nodule dry weight and shoot total nitrogen percent of chickpea varieties at Genda Gorba trial sites.

Treatment	**Year 2017**			**Year 2018**			**Year 2019**		
	NNP	**NDW**	**TN**	NNP	**NDW**	**TN**	**NNP**	**NDW**	**TN**
Strain									
43P2S1 (*M. plurifarium*)	23^{ab}	65.33^{b}	0.60^{cde}	30^{b}	98.80^{a}	1.17^{ab}	31^{bc}	134.41^{a}	1.87^{ab}
90P4S2 (*M. amorphae)*	18^{abc}	31.47^{bc}	0.66^{bcd}	15^{g}	57.07^{fg}	0.91^{abc}	17^{ef}	56.55^{h}	1.38^{cde}
22P5S2 (*M. amorphae)*	17^{abc}	39.78^{abc}	0.49^{def}	26^{bcd}	46.63^{g}	1.07^{abc}	29^{c}	65.71^{gh}	1.29^{de}
2P3S1-b (*M.sp.* L SJC280B00)	20^{abc}	44.97^{abc}	0.64^{bcd}	24^{cde}	98.08^{a}	1.08^{abc}	28^{c}	103.72^{cd}	1.81^{abc}
ET1 (*M. australicum*)	13^{bc}	41.63^{abc}	0.62^{cde}	17^{fg}	77.23^{bcd}	1.00^{abc}	22^{de}	107.63^{bc}	1.68^{abc}
38P4S2 (*M. loti)*	14^{abc}	39.4^{def}	0.96^{a}	19^{fg}	74.86^{cde}	0.84^{c}	12^{g}	80.44^{ef}	1.24^{e}
ET26 *(M. amorphae*)	21^{abc}	59.78^{ab}	0.66^{bcd}	15^{g}	65.70^{def}	1.20^{a}	15^{fg}	81.67^{ef}	1.74^{ab}
45P4S1 (*M.* loti)	10^{c}	34.40^{bc}	0.83^{ab}	38^{a}	94.82^{a}	0.96^{abc}	36^{a}	116.23^{bc}	1.99^{a}
80P4S2*(M. amorphae*)	26^{a}	68.47^{a}	0.82^{ab}	28^{bc}	72.50^{cde}	1.06^{abc}	31^{bc}	72.98^{fg}	1.86^{ab}
27P3S2 (*M. ciceri*)	17^{abc}	42.47^{abc}	0.71^{bc}	38^{a}	82.67^{bc}	1.09^{abc}	35^{ab}	86.66^{e}	1.79^{ab}
Mean	18	46.77	0.70	25.0	76.84	1.04	26	90.60	1.67
Ha. Ata (Reference)	17^{abc}	43.80^{abc}	0.43^{ef}	24^{cde}	63.20^{ef}	0.90^{bc}	26^{cd}	91.84^{de}	1.56^{bcd}
EAL029 (Commercial)	16^{abc}	49.87^{ab}	0.39^{f}	21^{ef}	89.04^{ab}	0.90^{bc}	20^{ef}	117.94^{b}	1.75^{ab}
Control (Untreated)	14^{abc}	34.00^{bc}	0.53^{c-f}	23^{de}	55.70^{fg}	0.85^{c}	21^{ef}	71.18^{fg}	1.58^{bcd}
Nitrogen (Fertilizer)	16^{abc}	33.20^{bc}	0.49^{def}	27^{bcd}	74.68^{cde}	0.93^{abc}	29^{bc}	93.31^{de}	1.68^{abc}
Variety									
Arerti	12^{b}	33.84^{b}	0.65^{a}	27^{a}	82.69^{a}	1.01^{a}	24^{b}	82.94^{b}	1.51^{b}
Natoli	22^{a}	55.07^{a}	0.56^{b}	21^{b}	68.02^{b}	0.98^{b}	27^{a}	100.26^{a}	1.80^{a}
P -value									
Variety	**	**	ns	**	**	*	**	**	**
Strain	**	**	**	**	**	**	**	**	**
V x S	**	**	ns	**	**	ns	**	**	ns
HSD	12.45	29.35	0.20	4.56	12.90	0.30	5.31	13.47	0.29

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., NNP=Number of nodule per plant, NDW=Nodule dry weight per plant(mg/plant), TN= shoot total nitrogen percent, S=strain V=variety, V x S= the interaction of variety and strain, **=significant ($P \leq 0.01$), * = significant ($P \leq 0.05$) and ns = non-significant (P> 0.05); HSD = high range statistical domain

The tested host varieties produced shoot dry weight (SDW) in the range of 12.19-23.4g/plant with the overall mean of 13.41 to18.35 gm/plant at Chefe Donsa sites in their respective planting years (Table 5.6 and Appendix 14). *M. loti* 45P4S1 showed best SDW between varieties in 2018 and 2019, with mean SDW increment (8-33%) on Natoli and Arerti varieties over 2017 planting year. Plants inoculated with *M.sp.* LSJ280B00 strain 2P3S1-b, *M. amorphae* 80P4S2, *M. ciceri* strain 27P3S2, *plurifarium* strain 43P2S1 and the nitrogen fertilizer treated performed better than the other treatments irrespective of variety. Likewise, the two varieties produced shoot dry weight in the range of 9.1-21.8g/plant with the overall mean values of 17g/plant with Natoli variety and 16g/plant with Arerti variety at Alem Tena in their respective planting years (Table 5.7; Appendix 15). The inoculants performed best SDW at Chefe Donsa and Alem Tena site that except *M ciceri* 27P3S2 that showed better mean SDW increase (33.94%) on Natoli and Arerti variety increase (22.89%) compared to the uninoculated control plants (Appendix 15).

The inoculated plants showed SDW accumulation in the range of 8.7-21.8gm/plant with mean of 11.3-18.0 gm g/plant on both varieties in the respective of planting years at Genda Gorba site (Table 5.8 and Appendix 16). Likewise, *M.* loti 45P4S1also increased SDW in the range of (13.5-21.8gm/plant) irrespective of varieties. Moreover, the nitrogen fertilizer showed differences in stimulating good shoot dry matter with mean (12.2-12.9gm/plant) between the Natoli and Arerti varieties. The pattern of SDW was similar to the that of nodulation parameters where chickpea accumulated more shoot dry matter on both varieties at Chefe Donsa, followed by Alem Tena and Genda Gorba sites, respectively. The difference in SDW between Alem Tena and Genda Gorba, with a few exceptions, was not as distinct as nodule number and nodule dry weight within two varieties; indicating that the plants exploited the soil nitrogen for growth and biomass accumulation.

Taken together *M. loti* 45P4S1, *M.sp.* LSJ280B00 strain 2P3S1-b, *M. amorphae* 80P4S2, *M. ciceri* strain 27P3S2 and *plurifarium* strain 43P2S1 increased mean SDW by 21-47%,10-32% and 11-48% on the overall performance at Chefe Donsa, Alem Tena and Genda Gorba soils respectively, compared to the uninoculated negative control plants. Under the circumstances, the two varieties produced shoot dry weight in the range of 11-17g/plant with the overall mean values of 12-16g/plant with Natoli variety and 12-17g/plant with Arerti variety. In all cases, the robust *M. loti* 45P4S1 increased SDM by 40% on Arerti variety compared to the Natoli variety (37%) compared to the uninoculated negative control plants irrespective of the planting years at all experimental locations.

Moreover, nitrogen fertilizer application showed an increase of (18-24%) SDW on Natoli and Arerti varieties compared to uninoculated negative control. This create understanding that inoculation provides optimize the growth stage of the varieties to enhance dry matter accumulation (Imen *et al*.,2015; Abdelkader *et al*.,2019). Previous studies in Ethiopia (Assefa Funga *et al*.,2016) showed rhizobium inoculation increased 5.7-16.0% shoot dry weight of Natoli and other chickpea varieties at Debre Zeit and over control, the sites nearer to one of the current experimental sites.

5.3.2. Total above ground biomass and grain yield

Inoculation with different *Mesorhizobium* strains showed significant difference ($P<0.01$) in total above ground biomass yield and grain yield compared to the uninoculated control (Table 5.6; Table 5.7 and Table 5.8). The inoculated plants gave the overall mean aboveground biomass ranging from 2.98t/ha to 7.47t/ha over the three years. However, they showed significant difference at different sites; 5.81t/ha at Chefe Donsa site, and 4.01t/ha and 4.37 t/ha at Alem Tena and Genda Gorba sites, respectively, indicating that inoculation increased the total above ground biomass yield of the three sites by 22%, 20%, and 13%, respectively compared with un-inoculated control (Table 5.6; Table 5.7 and Table 5.8). Although inoculation seems to show no significant difference between Alem Tena (4.01t/ha) and Genda Gorba (4.37t/ha), they showed slight difference in accumulated total above ground biomass yield in comparison with the control (Table 5.7 and Table 5.8).

The data also showed variations in total above ground biomass yield in different years. Thus, the treatments showed the highest total above ground biomass yield of 6.61-7.47t/ha with average of 6.96t/ha in 2017, compared to lower mean biomass yield of 5.16 and 5.32t/ha in 2018 and 2019, respectively, showing an increase of 35% over 2018 and 2019 at Chefe Donsa site (Table 5.6). Likewise, total above ground biomass yield obtained in 2019 was 52% higher than 2017 and 2018 harvest at Alem Tena site (Table 5.7); and there was 41% and 19% higher biomass yield than the years 2017 and 2018, respectively at Genda Gorba site (Table 5.8).

Table 5.6.Inoculation response on shoot dry weight, grain yield and above ground biomass of chickpea varieties at Chefe Donsa trial sites

Treatment	**Year 2017**			**Year 2018**			**Year 2019**		
	SDW	**BY**	**GY**	**SDW**	**BY**	**GY**	**SDW**	**BY**	**GY**
Strain									
43P2S1 (*M. plurifarium*)	17.57^{ab}	7.11^{a}	3.29^{ab}	15.65^{bc}	5.57^{a}	2.11^{bcd}	16.62^{bc}	5.99^{a}	3.21^{a}
90P4S2 (*M. amorphae)*	16.09^{ab}	6.66^{a}	2.95^{ab}	12.91^{cd}	4.84^{cd}	1.90^{d-g}	12.73^{d}	4.73^{hi}	2.52^{fgh}
22P5S2 (*M. amorphae)*	16.67^{ab}	6.90^{a}	2.90^{ab}	13.44^{bcd}	4.92^{bcd}	1.97^{c-f}	14.87^{bcd}	4.99^{gh}	2.44^{ghi}
2P3S1-b (*M.sp.* L SJC280B00)	18.94^{a}	7.47^{a}	3.40^{a}	15.95^{bc}	5.32^{abc}	2.40^{a}	16.36^{bc}	5.61^{cd}	3.03^{abc}
ET1 (*M. australicum*)	18.82^{a}	6.74^{a}	2.88^{ab}	15.46^{bc}	4.97^{bcd}	2.15^{bc}	15.70^{bcd}	4.97^{gh}	2.23^{j}
38P4S2 (*M. loti)*	16.41^{ab}	6.74^{a}	3.03^{ab}	14.01^{bcd}	4.76^{d}	1.86^{efg}	14.20^{bcd}	5.04^{gh}	2.53^{fg}
ET26 *(M. amorphae*)	15.86^{ab}	7.09^{a}	3.33^{a}	12.37^{d}	5.12^{a-d}	1.90^{d-g}	13.62^{cd}	5.39^{c-f}	2.32^{hij}
45P4S1 (*M.* loti)	16.55^{ab}	7.22^{a}	3.27^{ab}	19.05^{a}	5.59^{a}	2.33^{ab}	20.45^{a}	5.95^{ab}	3.13^{ab}
80P4S2*(M.* amorphae)	18.74^{a}	7.09^{a}	3.21^{ab}	15.50^{bc}	5.15^{a-d}	1.77^{fg}	17.03^{b}	5.24^{efg}	2.82^{de}
27P3S2 (*M.* ciceri)	18.34^{a}	6.61^{a}	3.03^{ab}	14.12^{bcd}	5.38^{ab}	2.04^{cde}	15.70^{bcd}	5.29^{d-g}	2.90^{cd}
Mean	17.44	6.96	3.14	14.85	5.16	2.04	15.63	5.32	2.71
Ha. Ata (Reference)	17.89^{ab}	6.87^{a}	3.00^{ab}	13.69^{bcd}	5.05^{bcd}	1.84^{efg}	14.08^{bcd}	5.10^{fg}	2.65^{ef}
EAL029 (Commercial)	17.21^{ab}	6.68^{a}	3.06^{ab}	12.86^{cd}	5.28^{abc}	2.01^{cde}	14.92^{bcd}	5.49^{cde}	2.96^{bcd}
Control (Untreated)	13.87^{b}	5.60^{b}	2.74^{b}	12.20^{d}	4.20^{e}	1.70^{g}	13.20^{d}	4.50^{i}	2.30^{ij}
Nitrogen (Fertilizer)	18.30^{a}	7.11^{a}	3.05^{ab}	15.66^{bc}	5.18^{a-d}	2.18^{abc}	16.28^{bc}	5.67^{bc}	2.93^{cd}
Variety									
Arerti	17.52	6.33	2.62^{b}	15.15^{a}	5.12	2.05	14.51^{b}	5.03^{b}	2.68^{b}
Natoli	16.90	7.37	3.55^{a}	13.84^{b}	5.07	2.04	16.14^{a}	5.62^{a}	2.80^{a}
P -value									
Variety	ns	**	**	**	ns	ns	**	**	**
Strain	**	**	**	**	**	**	**	**	**
V x S	**	**	**	**	**	**	**	**	ns
HSD	4.04	0.99	0.58	2.92	0.49	0.23	3.05	0.32	0.20

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., SDW= Shoot dry weight per plant(mg/plant), BY=Above ground biomass (t/ha), GY= Grain yield (t/ha), S =strain V=variety, V x S= the interaction of variety and strain, **=significant (P ≤ 0.01), * = significant (P ≤ 0.05) and ns = non-significant (P> 0.05); HSD = high range statistical domain

Considering the total above ground biomass yield production of the different sites in relation to the un-inoculated control, it showed the same pattern of increase in 2017 (33%) compared with the individual years of 2018 at Chefe Donsa and 54% increase in 2019 at Alem Tena, and 44% and 22% increase in 2019, 2018 against 2017 at Genda Gorba indicated that the inoculants did not have an influence on the increase as a function of time.

However, strains varied significantly increasing total above ground biomass yield where M. plurifarium strain 43P2S1, M.sp. LSJ280B00 2P3S1-b and M. loti strain 45P4S1 showed increases by 29%, 24%, and 24% (Table 5.6; Table 5.7 and Table 5.8). The Ethiopian commercial strain EAL 029, the Tunisian Ha. Ata strain also performed as good as the elite indigenous strains and the Nitrogen fertilized control plants with 17%, 20% and 22% aboveground biomass increase over the un-inoculated control plants, respectively (Table 5.6; Table 5.7 and Table 5.8).

The two varieties did not show major difference in total above ground biomass yield with overall mean at all sites, except Alem Tena site with an increase in the range of 21%-47% and overall mean of 25% (Table 5.6, Appendix 14; Table 5.7, Appendix 15 and Table 5.8, Appendix 16). Although there was no difference in the overall mean of above ground biomass in the two sites, individual inoculants such as *M. plurifarium* strain 43P2S1, *M.sp.* LSJ280B00 strain 2P3S1-b and *M. amorphae* strain 80P4S2 induced effective nodulation on Natoli variety with 10%-47% more total above ground biomass yield over the Arerti variety at all sites. Interestingly, the Natoli variety showed significant difference (15-18%) to inoculation with the local reference strain, EAL029.

The other strains showed significant difference depending upon the site, except *M. loti* strain 38P4S2 and *M. amorphae* strain ET26 that did not show any significant difference in above ground biomass between varieties at all sites. Given that the Natoli variety accumulated 13-24% more total above ground biomass yield than the Arerti variety,

indicates that the increase may be more related to the inherent genetic difference between the two varieties, except a few cases with inoculation response to inoculation with *M. plurifarium* strain 43P2S1and *M.sp.* LSJ280B00 strain 2P3S1-b, that displayed higher above ground biomass (39, 47%) than the other strains at Alem Tena site. Study conducted (Elkoca *et al*.,2007; Zaheer *et al*., 2019) showed chickpea plant treated with bacterial inoculations attained higher values of total biomass than that of control. This implying the new indigenous strains enhance the plant growth.

The inoculated plants showed similar trend in that produced the higher grain yield at Chefe Donsa within a range of 2.04-3.03t/ha with an overall mean of 2.66t/ha over the three years trial compared to the overall mean grain yield of 1.75t/ha and 1.95 t/ha at Alem Tena and Genda Gorba sites, respectively (Table 5.6; Table 5.7 and Table 5.8).

Table 5.7.Inoculation response on shoot dry weight, grain yield and above ground biomass of chickpea varieties at Alem Tena trial sites

Treatment	**Year 2017**			**Year 2018**			**Year 2019**		
	SDW	**BY**	GY	SDW	BY	GY	SDW	**BY**	**GY**
Strain									
43P2S1 (*M. plurifarium*)	12.98^{abc}	4.19^{a}	1.99^{ab}	11.34^{c-f}	3.39^{ab}	1.64^{ab}	14.93^{b-e}	5.33^{bcd}	1.94^{a-d}
90P4S2 (*M. amorphae)*	11.36^{abc}	2.98^{c}	1.44^{d}	10.74^{d-g}	3.10^{ab}	1.40^{cd}	12.73^{ef}	4.73^{ef}	1.78^{d}
22P5S2 (*M. amorphae)*	12.21^{bc}	3.12^{bc}	1.46^{cd}	13.99^{ab}	3.27^{ab}	1.62^{abc}	11.22^{def}	4.88^{de}	1.93^{a-d}
2P3S1-b (*M.sp.* L SJC280B00)	13.94^{a}	4.18^{a}	1.91^{abc}	12.48^{c-f}	3.60^{ab}	1.63^{ab}	16.36^{b}	5.09^{cde}	2.10^{abc}
ET1 (*M. australicum*)	13.91^{a}	3.44^{abc}	1.67^{a-d}	9.67^{g}	3.98^{a}	1.61^{abc}	13.62^{def}	5.82^{a}	1.98^{a-d}
38P4S2 (*M. loti)*	11.68^{abc}	2.98^{c}	1.43^{d}	11.34^{c-g}	3.06^{ab}	1.31^{d}	15.50^{bcd}	5.39^{bc}	1.90^{bcd}
ET26 *(M. amorphae*)	11.03^{abc}	3.41^{abc}	1.67^{bcd}	10.55^{efg}	3.80^{ab}	1.65^{ab}	13.73^{c-f}	5.22^{cde}	1.89^{cd}
45P4S1 (*M.* loti)	11.77^{abc}	3.60^{abc}	2.02^{a}	11.50^{c-g}	3.42^{ab}	1.48^{bcd}	15.93^{bc}	5.42^{ab}	1.74^{d}
80P4S2*(M.* amorphae)	13.83^{ab}	3.49^{abc}	1.96^{ab}	13.58^{abc}	3.71^{ab}	1.67^{ab}	16.44^{b}	5.41^{ab}	2.19^{ab}
27P3S2 (*M.* ciceri)	13.51^{abc}	3.20^{abc}	1.56^{bcd}	15.84^{a}	3.96^{a}	1.74^{a}	20.45^{a}	5.29^{cd}	2.19^{ab}
Mean	12.62	3.50	1.66	12.10	3.53	1.58	15.09	5.26	1.96
Ha. Ata (Reference)	13.10^{abc}	3.98^{abc}	1.71^{a-d}	10.13^{ff}	3.84^{ab}	1.58^{abc}	13.44^{def}	4.91^{de}	1.94^{a-d}
EAL029 (Commercial)	12.64^{abc}	4.11^{ab}	1.74^{a-d}	11.93^{b-g}	3.58^{ab}	1.33^{d}	13.76^{c-f}	4.99^{cde}	1.89^{cd}

Control (Untreated)	10.58^{c}	3.07^{bc}	1.40^{d}	10.12^{fg}	2.80^{ab}	1.49^{bcd}	12.62^{f}	4.32^{f}	1.81^{cd}
Nitrogen (Fertilizer)	12.62^{abc}	3.88^{abc}	1.78^{a-d}	12.96^{b-e}	3.63^{ab}	1.61^{abc}	15.05^{bc}	5.18^{cde}	1.95^{a-d}
Variety									
Arerti	12.81^{a}	2.85^{b}	1.36^{b}	12.51^{a}	3.15^{b}	1.64^{a}	14.46^{b}	5.14^{b}	1.82^{b}
Natoli	12.00^{b}	4.25^{a}	2.04^{a}	11.66^{b}	3.87^{a}	1.47^{b}	15.35^{a}	5.41^{a}	2.08^{a}
P -value									
Variety	*	**	**	**	**	**	**	**	**
Strain	**	**	**	**	**	**	**	**	**
V x S	*	ns	ns	ns	**	**	**	**	**
HSD	3.52	1.04	0.46	4.93	1.15	0.23	2.29	0.50	0.30

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., SDW= Shoot dry weight per plant(mg/plant), BY=Above ground biomass (t/ha), GY= Grain yield (t/ha), S =strain V=variety, V x S= the interaction of variety and strain, **=significant ($P \leq 0.01$), * = significant ($P \leq 0.05$) and ns = non-significant (P> 0.05); HSD = high range statistical domain

This shows the overall yield at Chefe Donsa over the three year trials was 52% and 36% higher than the Alem Tena and Genda Gorba sites respectively. Although the yield at Chefe Donsa was high, the yield increase was only 17% compared to the 11% and 18% increase over the un-inoculated control plants obtained from the Alem Tena and Genda Gorba sites. It may well be that inoculants did not indicate the significance difference of their overall symbiotic performance, but the difference may be related with some environmental and edaphic factors that favor plant growth at Chefe Donsa site.

The reference strains from Tunisia induced nodulation with 11% grain yield increase at all sites. However, the local commercial strain EAL029 showed yield increases of 19% (Chefe Donsa) and 21% (Genda Gorba sites, respectively without showing any significant difference at Alem Tena site. The nitrogen fertilized chickpea plants showed higher grain yield ranging from 21%-31% at all sites, especially at Alem Tena site with the highest yield increase of 31%.

The data indicated the overall mean of grain yield of 1.75-2.66t/ha with the inoculated plants with the selected local *Mesorhizobium* strains, the grain yield of some strains was higher than the overall mean yield. Thus, *M. plurifarium* strain 43P2S1and *M.sp.* LSJ280B00 2P3S1-b, showed higher grain yield of 2.87-2.94t/ha at Chefe Donsa site, 1.86-1.88t/ha at Alem Tena site, and 1.97-2.14t/ha at Genda Gerba site, respectively,

showing 19-31%, increase over the uninoculated control plants (Table 5.6; Table 5.7 and Table 5.8).

Although the above two elite strains performed well at all sites, *M. loti* strain 45P4S1, *M. amorphae* strain 80P4S2, *M. ciceri* strain 27P3S2, *M. australicum* ET1 and *M. amorphae* ET26 showed significant variation in grain yield 1.83-2.91t/ha depending upon site. Thus, *M. loti* 45P4S1 performed best at Chefe Donsa (29%) and Genda Gorba sites (22%); but did not show any difference from others at Alem Tena site. Similarly, *M. amorphae* 80P4S2 produced good grain yield (1.94t/ha) at Alem Tena and 2.09t/ha at Genda Gorba without any significant difference at Chefe Donsa site. Similarly, *M. ciceri* 27P3S2 performed best at Alem Tena site with grain yield of 1.83t/ha; whereas *M. australicum* ET1 and *M. amorphae* ET26 increased yield by 2.01t/ha at Genda Gerba site. In general, high grain yield was obtained from inoculation with *M. plurifarium* 43P2S1, M sp 2P3S1-b, and *M loti* 45P4S1 at Chefe Donsa site;

wheras *M. plurifarium* 43P2S1, *M.sp.* LSJ280B00 2P3S1-b, *M. amorphae* 80P4S2 and *M. ciceri* 27P3S2 did best at Alem Tena site. It is interesting to note that at Genda Gorba site more versatile in response to inoculation with six inoculants to increase grain yield (19-27%) over un-inoculated control plants.

Table 5.8.Inoculation response on shoot dry weight, grain yield and above ground biomass of chickpea varieties at Genda Gorba trial sites.

Treatment	**Year 2017**			**Year 2018**			**Year 2019**		
	SDW	BY	GY	SDW	BY	GY	SDW	BY	**GY**
Strain									
43P2S1 (*M. plurifarium*)	10.41^{a-d}	4.06^{a}	1.95^{a}	14.39^{b}	4.94^{a}	2.03^{ab}	15.09^{bc}	5.86^{a}	2.44^{a}
90P4S2 (*M. amorphae)*	9.56^{bcd}	3.38^{b-e}	1.63^{b-e}	13.42^{bc}	4.07^{abc}	1.83^{a-e}	12.12^{c}	4.39^{c}	2.13^{bc}
22P5S2 (*M. amorphae)*	9.78^{cd}	3.41^{cde}	1.33^{f}	13.25^{bc}	3.84^{c}	1.88^{a-e}	14.07^{bc}	4.66^{bc}	2.12^{bc}
2P3S1-b (*M.sp.* L SJC280B00)	12.73^{ab}	3.78^{abc}	1.80^{abc}	13.22^{bc}	4.11^{abc}	1.87^{a-e}	15.84^{b}	5.68^{a}	2.23^{ab}
ET1 (*M. australicum*)	13.08^{b}	3.78^{abc}	1.82^{abc}	12.95^{bc}	4.60^{abc}	1.90^{a-d}	13.64^{bc}	5.32^{ab}	2.29^{ab}
38P4S2 (*M. loti)*	8.74^{d}	3.11^{e}	1.51^{def}	12.31^{bc}	4.05^{abc}	1.66^{cde}	14.06^{bc}	4.27^{c}	2.20^{ab}
ET26 *(M. amorphae*)	11.13^{abc}	3.85^{ab}	1.88^{ab}	11.39^{c}	3.86^{c}	1.92^{a-d}	13.83^{bc}	5.12^{abc}	2.23^{ab}
45P4S1 (*M.* loti)	13.50^{a}	3.82^{ab}	1.80^{a-d}	18.35^{a}	4.64^{abc}	2.01^{ab}	21.84^{a}	5.13^{abc}	2.26^{ab}
80P4S2*(M.* amorphae)	12.59^{ab}	3.67^{a-d}	1.74^{a-e}	14.09^{b}	4.92^{ab}	2.16^{a}	14.00^{bc}	5.54^{ab}	2.36^{ab}

27P3S2 (*M.* ciceri)	10.28^{a-d}	3.64^{a-e}	1.57^{c-f}	11.11^{c}	3.96^{bc}	1.73^{b-e}	15.98^{b}	5.31^{ab}	2.22^{abc}
Mean	11.49	3.65	1.7	13.30	4.30	1.90	15.05	5.13	2.25
Ha. Ata (Reference)	10.58^{a-d}	3.69^{abc}	1.78^{a-e}	12.43^{bc}	3.79^{c}	1.61^{de}	12.38^{c}	5.13^{abc}	2.09^{bc}
EAL029 (Commercial)	11.33^{abc}	3.81^{ab}	1.83^{abc}	12.61^{bc}	4.27^{abc}	1.98^{abc}	13.21^{bc}	5.10^{abc}	2.16^{abc}
Control (Untreated)	10.04^{a-d}	3.20^{de}	1.50^{ef}	12.04^{bc}	3.78^{c}	1.55^{e}	12.24^{c}	4.60^{bc}	1.90^{c}
Nitrogen (Fertilizer)	12.62^{ab}	3.86^{a}	1.79^{a-d}	13.00^{bc}	3.98^{abc}	1.97^{abc}	12.74^{bc}	5.38^{ab}	2.43^{a}
Variety									
Arerti	11.12^{a}	3.06^{b}	1.42^{b}	14.13^{a}	4.42^{b}	1.96^{a}	13.96^{b}	4.95^{b}	2.14^{b}
Natoli	10.37^{b}	4.23^{a}	2.00^{a}	12.24^{b}	3.98^{a}	1.77^{b}	14.76^{a}	5.27^{a}	2.30^{a}
P -value									
Variety	*	**	**	**	**	**	**	**	**
Strain	**	**	**	**	**	**	**	**	**
V x S	*	**	**	**	**	**	**	**	**
HSD	3.49	0.48	0.29	2.49	0.97	0.35	3.44	0.87	0.28

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., SDW= Shoot dry weight per plant(mg/plant), BY=Above ground biomass (t/ha), GY= Grain yield (t/ha), S =strain V=variety, V x S= the interaction of variety and strain, **=significant ($P \leq 0.01$), * = significant ($P \leq 0.05$) and ns = non-significant (P> 0.05); HSD = high range statistical domain

Related studies in Ethiopia (Gemechu Keneni *et al*., 2012; Assefa Funga *et al*., 2016; Wondwosen Tena *et al*., 2016a;), Morocco (Abdelkader *et al*.,2019), Pakistan (Khattak *et al*., 2006; Aslam *et al*., 2010; Zaheer *et al*., 2019), Turkey (Elkoca *et al*.,2007), Tunisia (Romdhane *et al*.,2008; Imen *et al*.,2015) and Saskatchewan (Kyei-Boahen *et al*., 2002) were reported comparative grain yield advantage of chickpea due to *Mesorhizobium* inoculation. Nitrogen fixation revealed better fixations in some varieties in short seasons and gradual increments in the other varieties (Wielbo *et al*., 2015), since symbiotic nitrogen fixation may stabilize nitrogen source available during the whole period of plant growth and it may be favorable for obtaining a better grain yield.

Greater yield variation between Desi than Kabuli might be related to phenotypic, genotypic, differences in maturity dates (Walley *et al*., 2005). Relatedly, grain yield response to rhizobia inoculation and their variability may be related *Mesorhizobium* species ability to initiate fixation and responsive character of chickpea varieties to inoculation. Khaitov and Abdiev, (2018) described the effectiveness of bioinoculants in improving nutrient acquisition through stimulated root absorption zone and transfer nutrients to the plant. This study suggesting the new *Mesorhizobium* species for further

researches. Generally, the field trial provided an important complement to genetic diversity of chickpea's rhizobia association with symbiotic nitrogen as well as these strains potential for enhancing chickpea yield

5.3.3. Number of pod and seed per plant

The chickpea plants attained highest number of pods in the range of 35-49, with 10% average difference compared to uninoculated negative control irrespective of planting years at Chefe Donsa (Table 5.9). The majority of the strains showed an increasing number of seeds per plant ranging from 4-20% at three experimental locations irrespective of three planting years (Table 5.9, 5.10, 5.11).

Table 5.9.Inoculation response on Harvesting index, Number of pods per plant and Number of seeds per plant of chickpea varieties Chefe Donsa trial site

Treatment	**Year 2017**			**Year 2018**			**Year 2019**		
	HI	NPP	NSP	HI	NPP	NSP	HI	NPP	NSP
Strain									
43P2S1 (*M. plurifarium*)	0.46^{abc}	52.03^{a}	54.07^{a}	0.38^{b-e}	43.65^{a}	41.55^{a}	0.54^{abc}	48.78^{ab}	46.68^{a}
90P4S2 (*M. amorphae)*	0.44^{bc}	44.07^{b}	44.90^{b-e}	0.39^{b-e}	40.33^{abc}	38.27^{abc}	0.53^{abc}	44.10^{bcd}	42.03^{abc}
22P5S2 (*M. amorphae)*	0.42^{c}	45.40^{ab}	43.53^{cde}	0.40^{a-d}	41.28^{abc}	38.38^{abc}	0.49^{cd}	43.70^{cd}	40.80^{bc}
2P3S1-b (*M.sp.* L SJC280B00)	0.46^{abc}	49.30^{ab}	50.80^{ab}	0.45^{a}	40.48^{abc}	37.75^{abc}	0.54^{ab}	46.42^{abc}	43.68^{ab}
ET1 (*M. australicum*)	0.43^{bc}	45.70^{ab}	47.03^{a-e}	0.43^{ab}	40.30^{abc}	37.37^{abc}	0.45^{de}	44.70^{a-d}	41.77^{abc}
38P4S2 (*M. loti)*	0.45^{abc}	42.50^{b}	42.67^{de}	0.39^{b-e}	38.38^{bc}	36.22^{bc}	0.50^{bc}	43.28^{cd}	41.12^{bc}
ET26 *(M. amorphae*)	0.47^{ab}	46.87^{ab}	48.90^{a-d}	0.37^{cde}	37.93^{c}	35.27^{c}	0.43^{e}	40.20^{d}	37.53^{c}
45P4S1 (*M.* loti)	0.45^{bc}	46.80^{ab}	48.50^{a-e}	0.42^{a-d}	38.62^{bc}	36.68^{bc}	0.53^{abc}	44.58^{a-d}	42.62^{ab}
80P4S2*(M.* amorphae)	0.45^{abc}	47.43^{ab}	49.63^{a-d}	0.34^{e}	40.43^{abc}	38.67^{abc}	0.54^{ab}	45.60^{abc}	43.83^{ab}
27P3S2 (*M.* ciceri)	0.46^{abc}	42.37^{b}	41.27^{e}	0.38^{b-e}	42.68^{ab}	39.92^{ab}	0.55^{a}	49.28^{a}	46.52^{a}
Mean	0.45	46.25	47.27	0.40	40.41	38.01	0.51	45.06	42.66
Ha. Ata (Reference)	0.44^{bc}	47.03^{ab}	48.57^{a-d}	0.36^{de}	40.77^{abc}	38.10^{abc}	0.52^{abc}	45.22^{abc}	42.55^{ab}
EAL029 (Commercial)	0.46^{abc}	49.20^{ab}	50.70^{abc}	0.38^{b-e}	42.72^{ab}	39.82^{abc}	0.54^{ab}	46.63^{abc}	43.73^{ab}
Control (Untreated)	0.49^{a}	44.47^{ab}	45.47^{b-e}	0.40^{a-d}	40.22^{abc}	37.88^{abc}	0.51^{abc}	46.07^{abc}	43.73^{ab}
Nitrogen (Fertilizer)	0.43^{bc}	43.73^{b}	44.87^{b-e}	0.42^{abc}	41.55^{abc}	39.28^{abc}	0.52^{abc}	44.68^{a-d}	42.42^{abc}
Variety									

Arerti	41.40[b]	45.75	46.85	39.46	41.43[a]	38.91[a]	53.26[a]	44.39[b]	41.88[b]
Natoli	48.12[a]	46.66	47.57	39.71	39.91[b]	37.54[b]	49.80[b]	46.07[a]	43.70[a]
P -value									
Variety	**	ns	ns	ns	**	**	**	**	**
Strain	**	**	**	**	**	**	**	**	**
V x S	**	ns	ns	**	ns	ns	ns	ns	ns
HSD	4.44	7.57	7.24	5.60	4.93	4.63	4.94	4.91	5.01

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., HI= grain harvest index (%), NPP=Number of pod per plant, NSP= Number of seed per plant, S=strain V=variety, V x S= the interaction of variety and strain, **=significant ($P \leq 0.01$), * = significant ($P \leq 0.05$) and ns = non-significant (P> 0.05), HSD = high range statistical domain

The pattern of pods and seed per plant was similar to that of symbiotic and agronomic performance parameters where chickpea accumulated highest grain yield on both varieties at Chefedonsa followed Genda Gorba and Alem Tena irrespective of planting years. *Rhizobium* inoculation of chickpea at Turkey at Ethiopia (Wondwosen Tena *et al*., 2016a), Turkey (Togay *et al*.,2008), Pakistan (Aslam *et al*., 2010), Iran (Namvar *et al*.,2011) and South Africa (Ogola, 2015) were showed the effect of inoculation performance on number of pod and seed of chickpea plant.

Table 5.10.Inoculation response on Harvesting index, Number of pods per plant and Number of seeds per plant of chickpea varieties Alem Tena trial site

Treatment	**Year 2017**			**Year 2018**			**Year 2019**		
	HI	NPP	NSP	HI	NPP	NSP	HI	NPP	NSP
Strain									
43P2S1 (*M. plurifarium*)	0.47[bc]	33.53[a]	34.37[a]	0.48[a]	35.82[a-d]	32.56[a-d]	0.36[a-d]	41.59[a]	38.32[a]
90P4S2 (*M. amorphae)*	0.49[b]	25.30[b]	26.20[b]	0.45[ab]	32.79[cd]	30.02[cd]	0.38[a-d]	38.36[ab]	35.59[abc]
22P5S2 (*M. amorphae)*	0.47[bc]	24.90[b]	27.47[ab]	0.50[a]	34.89[a-d]	32.39[a-d]	0.40[ab]	37.14[b]	34.64[abc]
2P3S1-b (*M.sp.* L SJC280B00)	0.46[bc]	31.53[ab]	31.73[ab]	0.45[ab]	34.14[a-d]	30.57[bcd]	0.41[a]	37.86[ab]	34.29[bc]
ET1 (*M. australicum*)	0.49[b]	30.70[ab]	30.20[ab]	0.40[ab]	34.29[a-d]	31.56[a-d]	0.34[bcd]	38.94[ab]	36.21[abc]
38P4S2 (*M. loti)*	0.48[bc]	23.83[b]	25.90[b]	0.43[ab]	32.91[cd]	29.91[cd]	0.35[d]	36.37[b]	33.37[c]
ET26 *(M. amorphae*)	0.49[bc]	29.60[ab]	28.77[ab]	0.44[ab]	31.96[d]	29.22[d]	0.36[a-d]	36.46[b]	33.72[bc]
45P4S1 (*M.* loti)	0.56[a]	30.37[ab]	30.00[ab]	0.44[ab]	36.54[abc]	33.94[abc]	0.32[cd]	38.59[ab]	35.99[abc]
80P4S2*(M.* amorphae)	0.56[a]	31.47[ab]	32.27[ab]	0.45[ab]	35.41[a-d]	32.24[a-d]	0.41[ab]	38.41[ab]	35.24[abc]
27P3S2 (*M.* ciceri)	0.49[b]	27.17[ab]	30.33[ab]	0.44[a]	38.49[a]	35.69[a]	0.42[a]	39.42[ab]	36.62[abc]
Mean	0.49	28.84	29.72	0.45	34.72	31.81	0.37	38.31	35.40

Ha. Ata (Reference)	0.43^{de}	26.40^{ab}	26.63^{b}	0.41^{ab}	33.84^{bcd}	31.21^{a-d}	0.39^{cd}	38.52^{ab}	35.89^{abc}
EAL029 (Commercial)	0.42^{e}	28.07^{ab}	29.47^{ab}	0.37^{b}	37.47^{ab}	34.74^{ab}	0.38^{abc}	40.19^{ab}	37.46^{ab}
Control (Untreated)	0.46^{cde}	25.73^{ab}	26.97^{b}	0.53^{a}	34.24^{a-d}	31.67^{a-d}	0.42^{a}	36.74^{b}	34.17^{bc}
Nitrogen (Fertilizer)	0.46^{bcd}	24.67^{b}	26.63^{b}	0.44^{ab}	34.24^{a-d}	31.44^{a-d}	0.38^{a-d}	39.61^{ab}	36.81^{abc}
Variety									
Arerti	47.60^{b}	27.11^{b}	28.09^{b}	53.82^{a}	34.77^{a}	31.95^{a}	35.49^{b}	38.60	35.78
Natoli	48.27^{a}	29.22 ^{a}	30.05^{a}	38.53^{b}	34.80^{a}	31.93^{a}	38.81^{a}	38.28	35.40
P -value									
Variety	*	*	*	**	ns	ns	**	ns	ns
Strain	**	**	**	**	**	**	**	**	**
V x S	**	ns	ns	**	*	*	*	*	*
HSD	3.21	8.13	7.26	14.66	4.41	4.52	6.62	3.84	3.75

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., HI= grain harvest index (%), NPP=Number of pod per plant, NSP= Number of seed per plant, S=strain V=variety, V x S= the interaction of variety and strain, **=significant ($P \leq 0.01$), * = significant ($P \leq 0.05$) and ns = non-significant (P> 0.05), HSD = high range statistical domain

Table 5.11.Inoculation response on Harvesting index, Number of pods per plant and Number of seeds per plant of chickpea varieties Genda Gorba trial site

Treatment	**Year 2017**			**Year 2018**			**Year 2019**		
	HI	NPP	NSP	HI	NPP	NSP	HI	NPP	NSP
Strain									
43P2S1 (*M. plurifarium*)	0.48^{a}	29.27^{a}	29.30^{a}	0.41	39.73^{a-d}	38.57^{ab}	0.42^{b}	45.50^{a}	44.33^{a}
90P4S2 (*M. amorphae)*	0.48^{a}	25.03^{a}	25.30^{a}	0.45	36.70^{cd}	36.10^{b}	0.49^{ab}	42.27^{ab}	41.67^{abc}
22P5S2 (*M. amorphae)*	0.39^{c}	26.03^{a}	26.83^{a}	0.49	38.80^{a-d}	37.53^{ab}	0.46^{ab}	41.05^{b}	39.78^{bc}
2P3S1-b (*M.sp.* L SJC280B00)	0.48^{a}	25.93^{a}	25.50^{a}	0.45	38.05^{a-d}	36.48^{ab}	0.39^{b}	41.77^{ab}	40.20^{bc}
ET1 (*M. australicum*)	0.48^{a}	28.63^{a}	28.57^{a}	0.41	38.20^{a-d}	37.00^{ab}	0.43^{b}	42.85^{ab}	41.65^{abc}
38P4S2 (*M. loti)*	0.48^{a}	26.40^{a}	26.10^{a}	0.41	36.82^{cd}	35.75^{b}	0.52^{a}	40.28^{b}	39.22^{c}
ET26 *(M. amorphae*)	0.49^{a}	24.23^{a}	23.43^{a}	0.51	35.87^{d}	35.20^{b}	0.44^{b}	40.37^{b}	39.70^{bc}
45P4S1 (*M.* loti)	0.47^{ab}	24.43^{a}	25.03^{a}	0.43	40.45^{abc}	39.22^{ab}	0.44^{b}	42.50^{ab}	41.27^{abc}
80P4S2*(M.* amorphae)	0.47^{a}	26.93^{a}	26.57^{a}	0.44	39.32^{a-d}	38.08^{ab}	0.43^{b}	42.32^{ab}	41.08^{abc}
27P3S2 (*M.* ciceri)	0.43^{bc}	26.50^{a}	26.20^{a}	0.43	42.40^{a}	40.67^{a}	0.42^{b}	43.33^{ab}	41.60^{abc}
Mean	0.47	26.34	26.28	0.44	38.63	37.46	0.44	42.22	41.05
Ha. Ata (Reference)	0.48^{a}	24.50^{a}	25.47^{a}	0.42	37.75^{bcd}	37.22^{ab}	0.41^{b}	42.43^{ab}	41.90^{abc}
EAL029 (Commercial)	0.48^{a}	26.23^{a}	25.90^{a}	0.46	41.38^{ab}	40.72^{a}	0.42^{b}	44.10^{ab}	43.43^{ab}
Control (Untreated)	0.47^{ab}	24.20^{a}	24.33^{a}	0.41	38.15^{a-d}	37.48^{ab}	0.41^{b}	40.65^{b}	39.78^{bc}

Nitrogen (Fertilizer)	0.43^{ab}	25.13^{a}	25.47^{a}	0.49	38.15^{a-d}	37.62^{ab}	0.45^{ab}	43.52^{ab}	42.98^{abc}
Variety									
Arerti	47.10	25.21	25.12^{b}	44.86	38.68	37.56	43.32^{b}	42.52	41.40
Natoli	46.50	26.72	26.88^{a}	44.97	38.71	37.81	44.53^{a}	42.19	41.29
***P* -value**									
Variety	ns	ns	*	ns	ns	ns	*	ns	ns
Strain	**	ns	ns	**	**	**	**	**	**
V x S	ns	ns	ns	*	*	*	**	*	*
HSD	4.04	9.27	7.06	13.80	4.41	4.34	10.16	3.84	3.79

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., HI= grain harvest index (%), NPP=Number of pod per plant, NSP= Number of seed per plant, S=strain V=variety, V x S= the interaction of variety and strain, **=significant ($P \leq 0.01$), * = significant ($P \leq 0.05$) and ns = non-significant (P> 0.05), HSD = high range statistical domain

Table 5.12.Combined analysis of variance (ANOVA) for eight symbiotic and agronomic performance of two chickpea varieties tested at three experimental locations irrespective of three planting years.

Parameters	**Mean Square**						
	Location (L)	**Strain (S)**	**Variety (V)**	**L * V**	**L * S**	**L * S* V**	**CV (%)**
Grain yield	**	*	**	**	**	**	19.71
Above ground dry biomass	**	*	**	**	**	**	18.74
Harvesting index	**	ns	ns	**	*	**	14.55
Number of nodules per plant	**	**	**	**	**	**	37.7
Nodule dry weight per plant	**	**	**	**	**	**	42.79
Shoot dry weight per plant	**	**	**	**	**	**	16.98
Number of pods per plant	**	*	**	**	**	**	16.22
Number of seeds per plant	**	**	**	**	**	**	16.08
Shoot total nitrogen percent	**	**	**	**	**	ns	43.31

Table legend; CV= Coefficient of variation, **=significant ($P \leq 0.01$), * = significant ($P \leq 0.05$) and ns = non-significant (P> 0.05)

5.3.4. Correlation among common nodulation and grain yield traits

The correlation analysis indicated highly significant positive correlation among nodulation, grain yield and yield traits (Table 5.12). Nodulation parameters such as nodule

number, nodule dry weight and shoot dry weight were strongly correlated with most of the grain yield components. This strong correlation indicated that nodulation contributes better to attained more nutrient availability, resulting in vigorous plant growth and dry matter accumulation, which in turn resulted in higher grain yield. Parallelly, above ground dry biomass revealed highly positive association with grain yield(r=0.84), implies the higher above ground biomass predicted superior yield behind positive response of inoculation. Number of pods per plant was much correlated with grain yield (r = 0.65) and number of seeds per plant with grain yield (r = 0.71). The results also indicate that shoot nitrogen concentration was more closely related to different yield parameter except seed yield, but insignificant correlations were exited to seed yield. In this regard, the strong association between nodulation and grain yield are useful in selecting rhizobia as inoculants to promote nodulation, plant growth and seed yields in enhancing grain yield of chickpea.

Table 5.13.Correlation coefficients for common nodulation and yield component traits

	GY	AGBY	HI	NNP	NDW	SDW	NPP	NSP	TN
GY	1.00								
AGBY	0.84***	1.00							
HI	0.27*	-0.28*	100						
NNP	0.49**	0.46**	0.05ns	1.00					
NDW	0.47**	0.44**	0.05ns	0.71***	1.00				
SDW	0.55**	0.58**	-0.02ns	0.47**	0.46**	1.00			
NPP	0.65**	0.69***	-0.05ns	0.37*	0.41**	0.55**	1.00		
NSP	0.71***	0.72**	-0.02ns	0.38*	0.43*	0.57**	0.96***	1.00	
TN	0.02ns	0.10*	0.12ns	-0.21*	0.22**	0.24*	0.34*	0.24*	1.00

GY=Grain yield, AGBY =Above ground dry biomass, HI=Harvesting index, NNP =Number of nodules per plant, NDW =Nodules dry weight per plant, SDW= Shoot dry weight per plant, NPP =Number of pods per plant, NSP =Number of seeds per plant, TN= shoot total nitrogen percent.

5.3.5. Soil characteristics

Preplanting soil analysis for 2017 at the Chefe, Alem Tena and Genda Gorba sites are summarized in (Table 10.14). Chefedonsa site display Vertisol soil type at texture composition (clay 63%, silt 23%, sand 12%), Alem Tena site contain Andosol (sand 38%, silt 34% and clay 28%)) texture characteristics. Whereas as Genda Gorba site was Vertisol soil (sand 54%, silt 20% and clay 63%) soil texture observed. The analysis revealed that the Chefedonsa soils has alkaline (8.2) and a low phosphorus concentration of 10.19, while the Alem Tena soil has closer a neutral soil pH of 7.3 and a medium phosphorous concentration of 14.06 and Genda Gorba soil has a neutral soil pH of 7 and a highest phosphorous concentration of 24.

The total nitrogen and OM concentrations at Chefedonsa were in the highest range of 0.08 and 2.26 respectively. At Alem Tena, the NT and OM values were in the low range of 0.14 and 2.23, respectively. Similarly, at Genda Gorba, the NT and OM values were in the low range of 0.09 and 2.03, respectively. The extractable K and CEC were high at Genda Gorba but medium in Alem Tena sites (Tekalign, 1991; Benton,2003). The field trial provided an important complement to genetic diversity of chickpea's rhizobia associated with symbiotic nitrogen fixation efficiency.

In addition, those ecologically competent indigenous rhizobia strain in vitro tests could also permit local adaptation under normal soil environments (Chefe Donsa, Genda Gorba) as well as moisture stress conditions at Alem Tena site. Such strains also impart variation in forming an effective N2-fixing potential with different chickpea varieties. Parallely, results from field evaluation indicated that these new indigenous symbionts typically have higher effectiveness in symbiotic nitrogen fixation than current commercial chickpea inoculants, even out-performing mineral nitrogen. Generally, the present study revealed these strains possess the ability to induce effective nodules, fix nitrogen and competitive in the presence of other native rhizobia in the soils; suggesting the potential of such strains as source of biofertilizer to enhance chickpea yields and benefit small holder farmers

Chapter 6

6. Conclusion and Recommendation

6.1. Conclusion

The present study showed significant genomic diversity of *Mesorhizobium* species throughout major chickpea growing zones of Ethiopia. Comprehensive native nodules sampling and intensive characterization of isolates from varies farmer fields allows us detailed understanding of the distribution of chickpea rhizobia diversity at primary chickpea production regions. Initially whole genome diversity metrics and average nucleotide identify analyses resolved eleven distinct genospecies of *Mesorhizobium*. Thus, further analyses phylogenetic trees produced congruent strain assignments to eight novel genospecies.

The finding showed clearly a relationship between the genetic diversity of this collection and either the strains' geographic separation or the chemical characteristics of soil at their sites of origin. The results, therefore, suggest that diversity of widely distributed. It is possible that the generally broad geographic distribution of strains is related to the long history of chickpea cultivation and therefore movement of agricultural products both intentionally and unintentionally throughout the country.

The results also revealed that Ethiopian *Mesorhizobium* strains typically have high eco-physiological and nutritional variability that are vital to local adaptation. Most of the strains showed IAA production that could enhance root development for the host plants. Three strains have multiple growth promotion properties; solubilization of phosphates from calcium and aluminium phosphates that have the capacity to mobilize phosphorus in the soil. It was interesting to note that more than 85% of the strains showed effective and highly effective symbiosis on both Natoli and Arerti varieties interms of relative shoot dry matter accumulation by the inoculated plants in reference to the nitrogen fertilized control plants.

The overall eco-physiological competence and symbiotic effectiveness indicated 10 strains (*M. plurifarium* strain 43P2S1; *M. amorphae* strain 90P4S2, *M. amorphae* strain 22P5S2, *M. amorphae* strain ET26 and *M. amorphae* strain 80P4S2; *M.sp.* LSJC280B00 strain 2P3S1-b, *M. australicum* strain ET1, *M. loti* strain 38P4S2, *M. loti* strain 45P4S1 and *M. ciceri* strain 27P3S2) should be evaluated at multi-location field trials prior to their application as inoculant for enhancing nitrogen fixation activities in chickpea production.

The response of chickpea varieties to *Mesorhizobium* inoculation lead to confirmed detectable variation among *Mesorhizobium* strains in competence in favored of grain yield improvement in chickpea. The *M.sp.* LSJC280B00 2P3S1-b revealed more pronounced response in nodulation, yield and yield contributing traits of chickpea. *M plurifarium* 43P2S1, not previously known to nodulate chickpea, revealed major differences on both host varieties across three experimental sites compared to the national commercial reference strain EAL029 and exotic Tunisian Ha. Ata strain.

It is notable that new strains typically have best level of nodulation and yield on Natoli. The variation was greater in highland vertisols of Chefedonsa compared to mid altitude Genda Gorba vertisols and modest at lowland moisture stress area Alem Tena Andosols type. The finding revealed the suitability these *Mesorhizobial* inoculants on different chickpea cultivars over three seasons under three experimental sites with different soil conditions for better symbiotic nitrogen fixation in chickpea.

6.2. Recommendation

Molecular characterization of the chickpea *Mesorhizobia* showed the existence of high genetic diversity in the two major producing regional states of Amhara and Oromia. While future research should include the low and recent growing areas for identifying more new chickpea-nodulating symbionts that reveal a more accurate picture on the diversity of chickpea *Mesorhizobia* in Ethiopia.

In the present study more chickpea *Mesorhizobium* genomes become available. Future research should include detailed detection on taxonomic accuracy, construction of the pangenome, consisting of genes conserved across the group (the core genome) and genes

that are variable by strain (the accessory genome) specific to these Ethiopian strains. This helps to strength the current characterization of the genetic diversity amongst isolates and allow identify the roles of both accessory and highly variable common genes with respect to niche adaptation.

Eco-physiological characterization of the chickpea *Mesorhizobium* strains showed their in vitro ability to metabolize a wide range of respiratory substrates, inherent resistance to different antibiotics, heavy metal and tolerance to environmental factors such as acidity (pH), salinity (salt) and temperature. Including these strain in salt and acid tolerance field evaluation will give a more clear picture on ecological fitness of *Mesorhizobium* strains in the soil for establishing N2-fixing symbiosis under adverse conditions.

The results of phenotypic diversity also revealed Ethiopian soils contain diverse chickpea *Mesorhizobium*, which are endowed with different plant growth promoting attributes and phosphate solubilizing ability and symbiotic effectiveness. This requires additional characterization of PGP traits able to stimulate plant growth and field testing on selected P solubilizing *Mesorhizobium* strains under acidic soil conditions to realize multiple benefits of such inoculants in chickpea production.

The field evaluation results promote that, strains such as *M. plurifarium* 43P2S1, *M.sp. LSJC280B00* 2P3S1-b, *M. amorphae* strain 80P4S2 and *M. ciceri* strain 27P3S2 could be used as a candidate for inoculant production. Thus, these strains need more validation trials whether the inoculated *Mesorhizobium* are those causing nodulation on different chickpea varieties within or beyond a single season under different agroecological conditions.

Inoculation may benefit the crop during the season as a short-term management option; while over time, pre-existing rhizobia adapted to that soil will likely outcompete the inoculant. Thus, there is a need to integrate the success of inoculant strains in nitrogen-fixation with adaptation to the intended soil environment to support the development of a long-term solution.

The present field study relied on effectiveness of inoculation by measuring its effects on nodulation, yield and yield contributing traits of chickpea. But analysis of plant tissue nitrogen content may be required in order to display straw nitrogen content, straw nitrogen yield, grain nitrogen content, grain nitrogen yield, grain protein content, total nitrogen fixed and exploit additional fixation advantage.

6.3. Brief summary from all chapters and future research directions

The use of rhizobial inoculants in agricultural production is aimed at ensuring that the most effective microsymbiont occupies the largest proportion of nodules formed on the target host legume in the field. The consistency of nodulation can be enhanced by the use of effective rhizobial inoculant strains, which has been described as one of the earliest applications of agricultural biotechnology. Therefore, in order to obtain the maximum out of biological nitrogen fixation from legumes, it is necessary to properly identify rhizobia and their compatible legume host varieties before they are made commercially available for field applications. Thus, with the goal of enhancing capacities for commercial inoculum production in chickpea at Ethiopia; a large portion of this work was performed with chickpea activities centered Debre Zeit, EIAR, Addis Ababa University and University of California Davis from 2014/15 to 2020 with support of USAID Chickpea Innovation Lab. During that time, we surveyed, collected and characterized numerous strains of *Mesorhizobium* from Ethiopian major chickpea growing regions.

The genomic survey reveals unexpected genomic diversity among chickpea symbionts throughout chickpea growing zones of Amhara and Oromia regions of Ethiopia **paper I** (Greenlon *et al*., 2019). These chickpea endemic *Mesorhizobium* species accompanying tolerance to various stress conditions, nutritional versatility, growth promoting features and moderate phosphate solubilization performance. The results from greenhouse experiments also indicated high symbiotic effectiveness in chickpea and unique capacities in the new strains compared to previous studies **paper II** (Zehara *et al*.,2020).

Three years field evaluation in three seasons provided an important complement to genetic diversity of chickpea's rhizobia associated with symbiotic nitrogen fixation efficiency. In addition, those ecologically competent indigenous rhizobia strain in vitro tests could also permit local adaptation under normal soil environments (Chefe Donsa, Genda Gorba) as well as moisture stress conditions at Alem Tena site. Such strains also impart variation in forming an effective N2-fixing potential with different chickpea varieties. Parallely, results from field evaluation indicated that new indigenous symbionts typically have higher effectiveness in symbiotic nitrogen fixation than current commercial chickpea inoculants, even out-performing recommended NP.

The findings suggesting the potential of such strains as source of biofertilizer to enhance chickpea yields and benefit small holder farmers. With these multi-seasons recorded result, further "Verification of chickpea (*Cicer arietinum* L.) nodulating *Mesorhizobium strains*" have been continued in 2020/21-2021/22 cropping season. The experiment comprised two location, two varieties and on twelve farmers field at Central Ethiopia. With the goal of integrating the success of *Mesorhizobium* inoculant strains in nitrogen-fixation with the intended soil environment and realizing their application as inoculant for enhancing nitrogen fixation activities in chickpea production. Further "Promotion of effective Chickpea (*Cicer arietinum* L.) *Mesorhizobium* strains for commercial inoculum production in Ethiopia" will be proposed in 2021/22 at different regions of the country.

15. Reference

Abdelkader, M., Btissam, B. M., Omar, Z., Laila, N. and Jamal, I. (2017). Response of Chickpea to the Rhizobia Inoculation in Different Region of Morocco. *Canadian Journal of Agriculture and Crops*. 2(1):1-10.

Abi-Ghanem, R., Carpenter-Boggs, L., Smith, J. L. and Vandemark, G. J. (2012). Nitrogen Fixation by US and Middle Eastern Chickpeas with Commercial and Wild Middle Eastern Inocula. ISRN Soil Science. 1-5. doi:10.5402/2012/981842

Adeleke, R. A., Nunthkumar, B., Roopnarain, A. and Obi, L. (2019). Applications of Plant Microbe Interactions in AgroEcosystems *In*: Kumar, V., Prasad, R., Kumar, M. and Choudhary, D.K(ed.), Microbiome in Plant Health and Disease, PP 1-34. Springer Nature Singapore Pte Ltd.

Ahmed, T., Shahid, M.,Noman, M.,Hussain, S., Khan, M.A., Zubair, M.,Ismail,M., Manzoor,S., Shahzad,T. and Mahmood,F.(2019). Plant Growth Promoting Rhizobacteria as Biological Tools for Nutrient Management and Soil Sustainability. *In:* Kumar, A. and Vijay Singh Meena, V.S. (ed.), Plant Growth Promoting Rhizobacteria for Agricultural Sustainability, pp 94-110. Springer Nature Singapore Pte Ltd.

Alexandre, A. and Oliveira, S. (2013). Response to temperature stress in rhizobia. *Critical Reviews in Microbiology*. 39(3): 219-22

Alexandre, A., Brigido, C., Laranjo, M., Rodrigues, S. and Oliveira, S. (2009). Survey of Chickpea Rhizobia Diversity in Portugal Reveals the Predominance of Species Distinct from *Mesorhizobium* ciceri and *Mesorhizobium mediterraneum*. *Microbial Ecology*.58(4):930-941.

Alexandre, A., Laranjo, M and Oliveira, S. (2006). Natural Populations of Chickpea Rhizobia Evaluated by Antibiotic Resistance Profiles and Molecular Methods. *Microbial Ecology*. 51(1):128-136.

Al-Falih, A.M.K., (2002). Factors affecting the efficiency of symbiotic nitrogen fixation by Rhizobium. *Pak. J. Biol. Sci*. 5, 1277-1293. http://dx.doi.org/10.3923/pjbs.2002. 1277.1293.

Alikhani, H.A., Saleh-Rastin, N. and Antoun, H. (2006). Phosphate solubilization activity of

Amarger, N., Macheret, V. and Laguerre, G. (1997). *Rhizobium gallicum sp*. nov. and *Rhizobium* giardinii sp. nov., from Phaseolus vulgaris nodules. *International Journal of Systematic Bacteriol*. 47:996-1006.

Andrews, M., De Meyer, S., James, E. K., Stepkowski, T., Hodge, S., Simon, M. F. and Young, J. P. W. (2018). Horizontal Transfer of Symbiosis Genes within and Between Rhizobial Genera: Occurrence and Importance. *Genes*. 9(321):1-24. doi:10.3390/genes9070321(Andrews *et al*,2018).

Aslam, M., Ahmad. H. K., Ullah. H., Ayaz. M., Ahmad. E., Sagoo. A. G., Ullah. I., Hussain. A. and Manzoor. M. (2010). Nodulation grain yield and grain protein contents as affected by rhizobium inoculatioin and fertilizer placement in chickpea cultivar bittle-98. Sarhad Journal. Agriculture. 26(4): 467-474

Aserse, A.A., Woyke, T., Kyrpides, N. C., Whitman, W.B. and Kristina Lindstrom, K. (2017). Draft genome sequence of type strain HBR26 and description of *Rhizobium*. *Standards in Genomic Sciences*. 12(14):1-16

Assefa Funga., Ojiewo, C.O., Turoop, L. and Mwangi, G. S. (2016). Symbiotic Effectiveness of Elite Rhizobia Strains Nodulating Desi Type Chickpea (*Cicer arietinum* L.) Varieties. *Journal of Plant Sciences*. 4(4): 88-94

Assefa Funga. (2016). Symbiotic Effectiveness of Elite *Rhizobia* Strains on Productivity of Improved Chickpea (*Cicer arietinum* L.) Varieties. Case of Debre Zeit and Wolayta Sodo, Ethiopia. M.sc. thesis, Jomo Kenyatta University of Agriculture and Technology, Kenya. pp 1-84.

Atsede, Muleta. (2020). Genomic diversity, plant growth promoting properties and symbiotic effectiveness of Mesorhizobum spp. nodulating chickpea (Cicer arietinum L.) on acidic soils of Ethiopia: Implication for inoculant development to enhance production under low inputs Agriculture. PhD thesis, Addis Ababa University, Ethiopia (pp 1-178).

Azcarate, M.O., Gonzalez, A. M. and Santalla, M. (2011). Natural rhizobial diversity helps to reveal genes and QTLs associated with biological nitrogen fixation in common bean. *AIMS Microbiology*. 3(3): 435-466.

Bankevich, A., Nurk, S., Antipov, D., Gurevich,A.A., Dvorkin, M., Kulikov,A.S. and Lesin,V. M. (2012). SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *J Computational Biology*. 19(5):455-477.

Beck, D.P. (1992). Yield and nitrogen fixation of chickpea cultivars in response to inoculation with selected rhizobial strains. *Agronomy*. Journal. 84: 510-516.

Bekuma, A. A. (2017). Symbiotic Effectiveness, Phylogeny and Genetic Stability of Biserrula pelecinus-nodulating Mesorhizobium sp. isolated from Eritrea and Ethiopia. PhD thesis, Murdoch University, Australia (pp1- 133)

Benjelloun, I., Alami, I. T., Douira, A. and Udupa, S. M. (2019). Phenotypic and Genotypic Diversity Among Symbiotic and Non-symbiotic Bacteria Present in Chickpea Nodules in Morocco. *Frontiers in Microbiology*. 0:1885:1-19. doi: 10.3389/fmicb.2019.01885

Ben Romdhane, S., Aouani, M. E. and R. Mhamdi, (2007b). Inefficient nodulation of chickpea (Cicer arietinum L.) in the arid and Saharan climates in Tunisia by *Sinorhizobium meliloti* biovar medicaginis. *Annals of Microbiology*. 57(1): 15-20

Romdhane, S.B., Tajini, F., Trabelsi, M., Aouani, M.E. and Mhamdi, R. (2007a). Competition for nodule formation between introduced strains of *Mesorhizobium ciceri* and the native populations of rhizobia nodulating chickpea (*Cicer arietinum*) in Tunisia. *World J. Microbiol. Biotechnol*. 23:1195-1201.

Ben Romdhane, S., Aouani, M.E., Trabelsi, M., De Lajudie, P. and Mhamdi, R. (2008). Selection of high Nitrogen-fixing Rhizobia nodulating chickpea (*Cicer arietinum*) for semi-arid Tunisia. *Journal of Agronomy and Crop Science*. 194: 413-420

Benton, J.J. (2003). Agronomic Handbook: Management of Crops, Soils and Their Fertility, CRC Press, Boca Raton, FL.

Berger, J.D. and Turner, N. C. (2007) The Ecology of Chickpea. In: Yadav, S.S., Redden, R.J., Chen, W., Sharma, B.(ed.), Chickpea Breeding and Management, pp 47-71. CABI, Oxfordshire, United Kingdom,

Bolger, A. M., Lohse, M. and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 30:2114-2120.

Bonello, E. R. (2018). Characterisation of the symbiosis ICE and accessory plasmid of *Mesorhizobium ciceri* CC1192. PhD thesis, Murdoch University, Australia (pp 1-93)

Bouyoucos, G. J. (1962). Hydrometer method improved for making particle size analyses

Bric, J.M., Bostoc, R.M. and Silverstone, S.E. (1991). Rapid in situ assay for indole acetic acid production by bacteria immobilized on a nitrocellulose membrane. *Appl. Environ. Microbiol*. 57: 534-538.

Brigido., Alexandre, A., Laranjo, M. and Oliveira, S. (2007). Moderately acidophilic mesorhizobia isolated from chickpea. *Lett Applied Microbiol*. 44:168-74.

Brigido, C. and Oliveira, S. (2013). Most Acid-Tolerant Chickpea *Mesorhizobia* Show Induction of Major Chaperone Genes upon Acid Shock. *Microbe. Ecology*.65:145-153

Brigido., C. Alexandre., A. and Oliveira, S. (2012). Transcriptional analysis of major chaperone genes in salt-tolerant and salt-sensitive mesorhizobia. *Microbiological Research*. 167:623-629

Brigido, C., Glick, B. R. and Oliveira, S. (2016). Survey of Plant Growth-Promoting Mechanisms in Native Portuguese Chickpea *Mesorhizobium* Isolates. *Microbial Ecology*. 73(4):900-915.

Broughton., W. J. and Dilworth., M. J. (1971) Control of Leghaemoglobin Synthesis in Snake beans. *Biochemistry*. *Journal*.125:1075-1080.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K. and Madden, T. L. (2009). BLAST+: architecture and applications. BMC Bioinformatics.10(1):1-9

Carranca, c., de Varennes, A. and Rolston, D. (1999). Biological nitrogen fixation by fababean, pea and chickpea, under field conditions, estimated by the 15N isotope dilution technique. *European Journal of Agronomy*. 10: 49-56

Chandra, S., Choure, K., Dubey, R. C., and Maheshwari, D. K. (2007). Rhizosphere competent *Mesorhizobium loti* MP6 induces root hair curling, inhibits *Sclerotinia sclerotiorum* and enhances growth of Indian mustard (*Brassica campestris*). *Brazilian Journal of Microbiology*. 38(1):124-130.

Clua, J., Roda, C., Zanetti., M. E. and Blanco, F. A. (2018). Compatibility between Legumes and Rhizobia for the Establishment of a Successful Nitrogen-Fixing Symbiosis. *Genes*. 9(125):1-21

Coba de la Pena, J.J. T. José J. Pueyo J. J. (2012). Legumes in the reclamation of marginal soils, from cultivar and inoculant selection to transgenic approaches. *Agronomy. Sustainable. Development*. 32:65-91. DOI 10.1007/s13593-011-0024-2

Compant,S., Duffy,B., Nowak,J., Clement,C. and Barka,E.A.(2005). Use of Plant Growth-Promoting Bacteria for Biocontrol of Plant Diseases: Principles, Mechanisms of Action, and Future Prospects. *Applied and Environmental Microbiology*.41(9):4951-4959.

Corbin, E., J. Brockwell and R. Gault. (1977). Nodulation studies on chickpea (Cicer arietinum). Australian Journal of Experimental Agriculture 17: 126-134. Mesorhizobium ciceri strain CC1192

CSA (Central Statistical Agency). (2018). Agricultural sample survey 2017/2018. Report on area and production of major crops. The Federal Democratic Republic of Ethiopia, Addis Ababa, Statistical Bulletin, no. 585

Daniel Muletta and Fassil Assefa (2015). Screening for symbiotically effective and ecologically competitive chickpea rhizobial inoculants from Ethiopian soils. *Ethiopian. Journal. Biological. Science*. (14): 1-18.

Dar., J.S, Pushpa., Rehmani, M.I.A., Abbassi, Z. A. and Magsi., A. G. (2016) Effect of starter nitrogen on yield and yield components of chickpea (Cicer arietinum L.) at Dokri, Larkana. *Pure and Applied Biology*. 5(4):1296-1303.

Darling, A.E., Jospin, G., Lowe, E., Matsen, F.A., Bik, H.M. and Eisen, J.A. (2014). PhyloSift: phylogenetic analysis of genomes and metagenomes. Peer J 2:e243.

Date, R.A. (1993). Assessment of Rhizobial status of the soil. In: Nitrogen Fixation in Legumes, pp. 85-94 (Vincent, J.M., ed.). Academic Press, Sydney.

de Lajudie, P., Willems, A., Nick, G., Moreira, F., Molouba, F., Hoste, B., Torck, U., Neyra, M., Collins, M. D., Lindstrom, K., Dreyfuslt, B. and Gillis, M. (1998). Characterization of tropical tree rhizobia and description of *Mesorhizobium plurifarium* sp. *International Journal of Systematic Bacteriology*. 48: 369-382

Desbrosses, G. J. and Stougaard, J. (2011). Root Nodulation: A Paradigm for How Plant-Microbe Symbiosis Influences Host Developmental Pathways. *Cell Host and Microbe*.10(4): 348-358

Diapari, M., Sindhu, A., Bett, K., Deokar, A., Warkentin, T. D. and Taran, B. (2014). Genetic diversity and association mapping of iron and zinc concentrations in chickpea (*Cicer arietinum* L.). *Genome*. 57(8): 4594-68. doi:10.1139/gen-2014-0108

Di Benedetto, N.A., Corbo, M. R., Campaniello, D., Cataldi, M. P., Bevilacqua, A., Sinigaglia, M. and Flagella, Z. (2017). The role of Plant Growth Promoting Bacteria in improving nitrogen use efficiency for sustainable crop production: a focus on wheat. *AIMS Microbiology*. 3(3): 413-434.

diCenzo, G. C., Zamani, M., Checcucci, A., Fondi, M., Griffitts, J., Finan, T.and Mengoni, A. (2018). Multi-disciplinary approaches for studying rhizobium legume symbioses. *Canadian Journal of Microbiology*. 65:1-33.

Diouf, F., Diouf, D., Klonowska, A., Le Quere, A.,Bakhoum, N., Fall, D., Neyra,M., Parrinello,H., Diouf, M., Ndoye, I. and Moulin, L. (2015) Genetic and genomic diversity studies of Acacia Symbionts in Senegal reveal new species of Mesorhizobium with a putative geographical pattern. PLoS One 10, e0117667.

Dos Santos, P. C., Fang, Z., Mason, S. W., Setubal, J. C., and Dixon, R. (2012). Distribution of nitrogen fixation and nitrogenase-like sequences amongst microbial genomes. *BMC Genomics*. 162(13):1-12. doi:10.1186/1471-2164-13-162

Dwivedi, S. L., Sahrawat, K.L., Upadhyaya, H. D., Mengoni, A., Galardini, M., Bazzicalupo, M., Biondi, E.G., Mariangela Hungria, M., Kaschuk, G., Blair, M. W. and Ortiz, R. (2015). Advances in Host Plant and Rhizobium Genomics to Enhance Symbiotic Nitrogen Fixation in Grain Legumes. *Advances in Agronomy*. 129:1-116

Elias, N. V. and Herridge, D. F. (2015). Naturalized populations of *Mesorhizobia* in chickpea (*Cicer arietinum* L.) cropping soils: Effects on nodule occupancy and productivity of commercial chickpea. *Plant Soil*. 387: 233-249.

Elkoca, E., Kantar, F. and Sahin, F. (2007). Influence of Nitrogen Fixing and Phosphorus Solubilizing Bacteria on the Nodulation, Plant Growth, and Yield of Chickpea. *Journal of Plant Nutrition*. 31(1): 157-171, DOI: 10.1080/01904160701742097

Endalkachew Wolde-Meskel., van Heerwaarden, J., Abdulkadir, B.,Kassa, S., Aliyi, I.,Degefu, T.,Wakweya, K., Kanampiu, F. and Giller, .K.E.(2018). Additive yield response of chickpea (*Cicer arietinum* L.) to rhizobium inoculation and phosphorus fertilizer across smallholder farms in Ethiopia. *Agriculture, Ecosystems and Environment*. 261:144-152

Eshete, M. (1994). Chickpea and lentil agronomy research. In: T., Asfaw *et al*., (eds.) Cool-season Food Legumes of Ethiopia. p. 230–251

FAOSTAT. (2015). Retrieved from ttp://faostat3.fao.org/download/Q/QC/E Date: Accessed Wed Oct 26/2016

Fauvart, M. and Michiels, J. (2008). Rhizobial secreted proteins as determinants of host specificity in the rhizobium legume symbiosis. *FEMS Microbiology Letters*. 285(1): 1-9. doi:10.1111/j.1574-6968.2008. 01254.x

Gaby, J.C. and Buckley, D.H. (2014). A comprehensive aligned nifH gene database: a multipurpose tool for studies of nitrogen-fixing bacteria. Database doi:10.1093/database/bau001.

Geletu Bejiga and Ketema Daba (2006). Breeding chickpea for wide adaptation. In: Food and forage legumes of Ethiopia progress and prospects, pp 59-66, (Kemal Ali, Gemachu keneni, Seid Ahmed, Malhotra, R., Beniwal, S., Makkouk, K. and Halila, M. H. eds), ICARDA, Addis Ababa, Ethiopia.

Gemechu Keneni, Endashaw Bekele., Fassil Assefa, Imtiaz, M., Tolessa Debele., Kifle Dagne and Emana Getu (2012). Evaluation of Ethiopian chickpea (*Cicer arietinum* L.) germplasm accessions for symbio-agronomic performance. *Renewable Agriculture and Food Systems*: 28(4):338-349

Gibson, K.E., Kobayashi, H. and Walker, G. C. (2008). Molecular Determinants of a Symbiotic Chronic Infection. *Annual Review Genet*. 42: 413-441.

Glick, B.R. (1995). The enhancement of plant growth by free living bacteria. *Can. J. Microbiol.* 41: 109- 117.

Glick, B. R. (2012). Plant Growth-Promoting Bacteria: Mechanisms and Applications. *Scientifica*.1-15. doi:10.6064/2012/963401

Goris, J., Klappenbach, J. A., Vandamme, P., Coenye, T., Konstantinidis, K. T. and Tiedje, J. M. (2007). DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *International Journal of Systematic and Evolutionary Microbiology*.57(1): 81-91.95

Goswami, D., Thakker,J.N. and Dhandhukia, P. C. (2016). Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. *Cogent Food and Agriculture*.2:1-19

Greenlon, A. (2018). Global Diversity and Function of Bacteria Associated with Wild and Domesticated Chickpea Root Nodules. PhD thesis, University of California, Davis, USA (pp1-164).

Greenlon, A., Chang, P., Damtew, Z.M., Muleta, A., Kim, D., Nguyen, H., Sudheer Yadav, S. J Patil, J.S., Udupa, S., Yasin, M., Patil, B., Singh, S., Sarma, B., von Wettberg, E., Kharaman, A., Bukun, B., Assefa, F., Tefsaye, K., Carrasquilla-Garcia, N. and Douglas Cook, D. (2019). Global-level population genomics reveals differential effects of geography and phylogeny on horizontal gene transfer in soil bacteria. *Proceeding of the National Academy of Science of the United states of America*. 116(30):15200-15209

Gupta, A., Gopal, M. and Tilak, K. V. B. (2000). Mechanisms of plant growth promotion by *rhizobacteria. Indian. Journal of Experimental. Biology*. 38:856-862.

Gupta G, Parihar, S.S., Ahirwar, N.K., Snehi, S.K., and Singh, V. (2015). Plant Growth Promoting Rhizobacteria (PGPR): Current and Future Prospects for Development of Sustainable Agriculture. *Journal of Microbe Biochem*.7: 096-102. doi:10.4172/1948-5948.1000188

Harish, S., Parthasarathy, S., Durgadevi, D., Anandhi, K. and Raguchander, T. (2019). Plant Growth-Promoting Rhizobacteria: Harnessing Its Potential for Sustainable Plant Disease Management. *In*: Kumar, A. and Meena, V.S. (ed.), Plant Growth Promoting Rhizobacteria for Agricultural Sustainability, pp 150-187. Springer Nature Singapore Pte Ltd.

Harlan., J. R. (1969). Ethiopia: A Center of Diversity. *Economic Botany*. 23(4): 309-314

Haskett, T. L., Terpolilli, J. J., Bekuma, A., O'Hara, G. W., Sullivan, J. T., Wang, P., … Ramsay, J. P. (2016). Assembly and transfer of tripartite integrative and conjugative genetic elements. *Proceedings of the National Academy of Sciences*:113(43):12268-12273

Haskett, T. L., Ramsay, J. P., Bekuma, A. A., Sullivan, J. T., O'Hara, G. W. and Terpolilli, J. J. (2017). Evolutionary persistence of tripartite integrative and conjugative elements. *Plasmid*. 92: 30–36. doi: 10.1016/j.plasmid.2017.06.001

Hazelton, P. and Murphy, B. (2007). Interpreting soil test results: What do all the numbers mean? 2nd Edition. *CSIRO Publishing*. 152p.

Hefny, M., Doliñski, R. and Małek., W. (2001). Variation in symbiotic characters of alfalfa cultivars inoculated with Sinorhizobium meliloti strains. *Biol Fertil Soils*. 33:435–437

Hungria, M., Chueire, L. M. de. O., Coca, R. G. and Megl'as, M. (2001). Preliminary characterization of fast growing rhizobial strains isolated from soyabean nodules in Brazil. *Soil Biol. Biochem*. 33: 1349-1361.

Illumina. (2012). Illumina, Nextera XT DNA Sample Preparation Guide

Imen, H., Neila, A. A., dnane, B., Manel, B., Mabrouk, Y., Saidi, M. and Bouaziz, S. (2015). Inoculation with Phosphate Solubilizing Mesorhizobium Strains Improves the Performance of Chickpea (*Cicer arietinum* L.) Under Phosphorus Deficiency. *Journal of Plant Nutrition*. 38:1656-1671

Imran, A., Mirza, M. S., Shah, T. M., Malik, K. A. and Hafeez, F. Y. (2015). Differential response of Kabuli and desi chickpea genotypes toward inoculation with PGPR in different soils. *Frontiers in Microbiology*. 6:859. doi:10.3389/fmicb.2015.00859

Ipek, M., Arikan, S., Pirlak, L. and Essitken, A. (2019). Sustainability of Crop Production by PGPR Under Abiotic Stress Conditions. In: Kumar, A. and Vijay Singh Meena, V.S. (ed.), Plant Growth Promoting Rhizobacteria for Agricultural Sustainability, pp 292-314. *Springer Nature Singapore* Pte Ltd.

Ismail, M., El-zanatay, A. M., Eissa, R. A., and Hewedy, O. A. (2013). Genetic diversity of Rhizobium leguminosarum as revealed by 16S rRNA Gene Sequence. Am. Eurasian J. Agric. Environ. Sci. 13: 797-801.

Jackson, M.L. (1958). Nitrogen determinations for Soil and plant tissue. In soil Chemical Analysis. pp 183-204, Prentice Hall, Inc., Engle Wood Cliffs. New Jersey, USA, 183-204. 2nd ED

Jarvis, B.D.W., Berkum, V. P., Chen, W.X., Nour, S.M., Fernandez, M.P., Cleyet-Marel, J.C. and Gillis, M. (1997). Transfer of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium mediterraneum* and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. *International Journal of Systematic Bacteriol*. 47:895-898

Jha, C. K. and Saraf, M. (2015). Plant growth promoting Rhizobacteria (PGPR): a review. *E3 Journal of Agricultural Research and Development*.5(2):0108-011

Jordan, D.C. (1984). Rhizobiaceae. *In*: Bergey's Manual of Systematic Bacteriology. pp. 234-242, (Krieg, N.R. and Holt, J.G., (eds.), The Williams and Wilkins, Baltimore.

Jukanti, A. K., Gaur, P. M., Gowda, C. L. L. and Chibbar, R. N. (2012). Nutritional quality and health benefits of chickpea (Cicer arietinum L.): a review. *British Journal of Nutrition*. 108(S1): S11-S26. doi:10.1017/s0007114512000797

Khan, M. N., Ijaz, M., Ali, Q., Ul-Allah, S., Sattar, A. and Ahmad, S. (2019). Biological Nitrogen Fixation in Nutrient Management. *In*: Hasanuzzaman, M. (ed.), Agronomic Crops, pp 127-148. Sher-e-Bangla Agricultural University, Dhaka, Bangladesh,

Khaitov, B. and Abdiev, A. (2018). Performance of chickpea (Cicer arietinum L.) to biofertilizer and nitrogen application in arid condition. *Journal of Plant Nutrition*.41(15): 1980-1987, DOI: 10.1080/01904167.2018.1484134

Khattak, S.G., Khan. D.F., Shah, S. H., Madani, M.S. and Khan, T. (2006). Role of Rhizobial inoculation in the production of chickpea crop. *Soil and Environment*. 25(2): 143-145

Kuçuk, C. and Kivanc, M. (2008). Preliminary characterization of Rhizobium strains isolated from chickpea nodules. *African. Journal of Biotechnology*. 7: 772-775.

Kumar, A., Meena, V. S., Roy, P., Vandana and Kumari, R. (2019). Role of Rhizobia for Sustainable Agriculture: Lab to Land. In: Kumar, A. and Meena, V.S. (ed.), Plant Growth Promoting Rhizobacteria for Agricultural Sustainability, PP 129-149.

Koskey, G., Mburu, S. W., Kimiti, J. M., Ombori, O., Maingi, J. M., and Njeru, E. M. (2018). Genetic Characterization and Diversity of *Rhizobium* Isolated from Root Nodules of Mid-Altitude Climbing Bean (*Phaseolus vulgaris* L.) Varieties. *Frontiers in Microbiology*. 9(968):1-18

Kour, D., Rana, K. L., Yadav, N., Yadav, A. N., Kumar, A., Meena, V. S., Singh, B., Chauhan, V. S., Dhaliwal, H.S. and Saxena, A. K. (2019). Rhizospheric Microbiomes: Biodiversity, Mechanisms of Plant Growth Promotion and Biotechnological Applications for Sustainable Agriculture. In: Kumar, A. and Vijay Singh Meena, V.S. (ed.), Plant Growth Promoting Rhizobacteria for Agricultural Sustainability, pp19-66. Springer Nature Singapore Pte Ltd.

Kyei-Boahen, S., Slinkard, A.E. and Walley, F. L. (2002). Evaluation of Rhizobial Inoculation Methods for Chickpea. *Journal of Agronomy*. 94:851-859.

Kyei-Boahen, S., Giroux, C., Walley, F.L. (2005). Fall vs. spring rhizobial inoculation of chickpea. *Canadian. Journal. of Plant Science*. 85:893-896

Laranjo. M., Alexandre, A. and Oliveira, S. (2014). Legume growth-promoting rhizobia: An overview on the Mesorhizobium genus. *Microbiological Research*. 169 (2014): 2-17

Laranjo, M. and Oliveira, S. (2011). Tolerance of Mesorhizobium type strains to different environmental stresses. *Antonie van Leeuwenhoek*. 99(3): 651-662.

Laranjo. M.,Young, J.P.W. and Oliveira, S. (2012). Multilocus sequence analysis reveals multiple symbiovars within Mesorhizobium species. *Systematic and Applied Microbiology*. 35 (2012) 359-367

Laranjo. M., Alexandre, A., Rivas, R., Velazquez, E., Young, J.P.W., and Oliveira, S. (2008). Chickpea rhizobia symbiosis genes are highly conserved across multiple *Mesorhizobium* species. *FEMS Microbiol Ecol*.66:391-400.

Larrainzar, E., Riely, B. K., Kim, S. C., Carrasquilla-Garcia, N., Yu, H.-J., Hwang, H.-J., … Cook, D. R. (2015). Deep Sequencing of the Medicago truncatula Root Transcriptome Reveals a Massive and Early Interaction between Nodulation Factor and Ethylene Signals. *Plant Physiology*. 169(1): 233-265. doi:10.1104/pp.15.00350

Lebauer, D. L. and Treseder, K. K. (2008). Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*. 89(2):371-379

Letunic and Bork (2019). Interactive Tree Of Life (iTOL) v4: recent updates and new developments Nucl. Acids.Res. 47: 256-259.

Lindstrom, K., Murwira, M., Willems, A., Altier, N. (2010). The biodiversity of beneficial microbe host mutualism: the case of rhizobia. Res. Microbiol, 161: 453- 63.

Ltaief, B., Sifi, B., Gtari, M., Allah, M. Z. and Lachaal, M. (2007). Phenotypic and molecular characterization of chickpea rhizobia isolated from different areas of Tunisia. *Canadian Journal of Microbiol*. 53:427-434

Lugtenberg, B. and Kamilova, F. (2009). Plant-Growth-Promoting Rhizobacteria. *Annual. Reiewv. Microbiol*. 63: 541-556.

Maatallah, J., Berrah, E.B. Sanjuan, J. and Lunch. C. (2002b). Phenotypic characterization of Rhizobia isolated from chickpea (*Cicer arietinum*) growing in Moroccan soils. *Agronomie*. 22: 321-329.

Maatallah. J., Berraho, E.B., Munoz, S., Sanjuan, J. and Lluch, C. (2002a). Phenotypic and molecular characterization of chickpea rhizobia isolated from different areas of Morocco. *Journal of Applied Microbiology*. 93(4):531-540

Maatallah, J., Berrah, E.B. Sanjuan, J. and Lunch. C. (2002b). Phenotypic characterization of Rhizobia isolated from chickpea (*Cicer arietinum*) growing in Moroccan soils. *Agronomie*. 22: 321-329.

Mahato, N. K., Gupta, V., Singh, P., Kumari, R., Verma, H., Tripathi, C., … Lal, R. (2017). Microbial taxonomy in the era of OMICS: application of DNA sequences, computational tools and techniques. *Antonie van Leeuwenhoek*. 110(10): 1357-1371. doi:10.1007/s10482-017-0928-1

Maleki, F., Khosravi, A. Nasser, A., Taghinejad, H. and Azizian, M. (2016). Bacterial Heat Shock Protein Activity. *Journal of Clinical and Diagnostic Research*. 10(3): BE01-BE03

McConnell, J. T., Miller, P. R., Lawrence, R. L., Engel, R. and Nielsen, G. A. (2002). Managing inoculation failure of field pea and chickpea based on spectral responses. *Canadian. Journal of Plant Scince*. 82: 273-282.

MoARD/Ministry of Agriculture and Rural Development, 2009. Crop Variety Register. Issue No. 12. Addis Ababa, Ethiopia.

Mohamed, A.A. and Hassan, M.A. (2015). Evaluation of two chickpea (Cicer arietinum l.) cultivars in response to three Rhizobium strains at River Nile State, Sudan. *Merit Research Journal of Agricultural Science and Soil Sciences*. 3(5):062-069

Mohite, B. (2013). Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *Journal of Soil Science and Plant Nutrition*. 13(3): 638-649.doi:10.4067/s0718-95162013005000051

Mousavi, S. A., Willems, A., Nesme, X., de Lajudie, P., and Lindström, K. (2015). Revised phylogeny of Rhizobiaceae: Proposal of the delineation of *Pararhizobium* gen. nov. and 13 new species combinations. *Systematic and Applied Microbiology*. 38(2):84-90.

Mulissa Jida and Fassil Assefa (2012). Phenotypic diversity and plant growth promoting characteristics of *Mesorhizobium* species isolated from chickpea (*Cicer arietinum* L.) growing areas of Ethiopia. *African Journal of Biotechnology*. 11(29):7483-7493

Mulissa Jida Midekssa and Fassil Assefa. (2016). Phenotypic diversity and plant growth promoting characteristics of Mesorhizobium species isolated from chickpea (*Cicer arietinum* L.) growing areas of Ethiopia. *African. Journal. Biotechnology*. 11: 7483-7493.

Murray, J. D. (2011) Invasion by Invitation: Rhizobial Infection in Legumes: current review. *The American Phytopathological Society*. 24(6): 631-639.

Mus, F., Crook, M. B., Garcia, K., Garcia Costas, A., Geddes, B. A., Kouri, E. D., Paramasivan, P., Ryu M-H, O. G., Poole, P.S., Udvardi, M.K., Voigt, C.A., Ane, J.M. and Peters, J. W. (2016). Symbiotic Nitrogen Fixation and the Challenges to Its Extension to Nonlegumes. *Applied and Environmental Microbiology*. 82(13): 3698-3710.

Musarrat, J., Zaidi, A. and Khan, M. S. (2010). Recent Advances in Rhizobium-Legume Interactions: A Proteomic Approach. *In*: Khan, M. S., Zaidi, A. and Musarrat, J. (ed.), Microbes for Legume Improvement, pp81-101

Nagargade, M., Tyagi, V. and Singh, M. K. (2018). Plant Growth-Promoting Rhizobacteria: A Biological Approach Toward the Production of Sustainable Agriculture. *Role of Rhizospheric Microbes in Soil*. 205-223. doi:10.1007/978-981-10-8402-7-8)

Namvar, A., Shari. R.R. and Khandan, T. (2011). Growth analysis and yield of chickpea (*Cicer arietinum* L.) in relation to organic and inorganic nitrogen fertilization. *EKOLOGIJA*.57(3): 97-108

Nandasena, K.G., O'Hara, G.W., Tiwari, R.P., Willems, A. and Howieson, J.G. (2009) Mesorhizobium australicum sp. nov. and Mesorhizobium opportunistum sp. nov., isolated from Biserrula pelecinus L. in Australia. Int J Syst Evol Microbiol.59(9):2140-7.

Nasr Esfahania, M., Kusano, M., Huu Nguyend, K.H., Watanabee, Y., Ha, C.V., Saito, K., Sulieman, S., Estrellah, L.H. and Tran, L.S. P. (2014). Adaptation of the symbiotic *Mesorhizobium* chickpea relationship to phosphate deficiency relies on reprogramming of whole-plant metabolism. *Proceeding of the National Academy of Science*. 113(32) E4610-E4619. doi:10.1073/pnas.1609440113.

Nour, S.M., Fernandez, M.P., Normand, P. and Cleyet-Maret, J.C. (1994). Rhizobium ciceri sp. nov. consisting of strains that nodulate chickpea (*Cicer arietinum* L). *International Journal of Systematic Bacteriology*. 44(3) 511-522

Nour, S. M., Cleyet-Marel, J. C., Normand, P. and Fernandez, M. P. (1995) Genomic heterogeneity of strains nodulating chickpeas (*Cicer arietinum* L.) and description of Rhizobium mediterraneum sp. nov. *International. Journal. Systematic. Bacteriol*. 45, 640-648.

Ogola, J. B. O. (2015). Growth and yield response of chickpea to Rhizobium inoculation: productivity in relation to interception of radiation. *Legume Research*. 38 (6): 837-843

Ogutcu, H., Algur, O.F., Elkoca. E. and Kantar, F. (2008). The determination of symbiotic effectiveness of Rhizobium strains isolated from wild chickpeas collected from high altitudes in Erzurum. *Turk Journal of Agriculture For*. 32:241-248.

O'hara, G.W., Zilli, J.E., Poole, P.S. and Hungria, M. (2016). Taxonomy and physiology of rhizobia. In: Howieson, J. G. and Dilworth., M.J. (ed.), Working with rhizobia. pp 125-144. Australian Centre for International Agricultural Research.

Oldroyd, G. E. D., Murray, J. D., Poole, P. S. and Downie, J. A. (2011). The Rules of Engagement in the Legume Rhizobial Symbiosis. *Annual Review of Genetics*. 45(1): 119-144.

Olivares, J., Bedmar, E. J. and Sanjuán, J. (2013). Biological Nitrogen Fixation in the Context of Global Change. *Molecular Plant-Microbe Interactions*.26(5):486-494.

Olsen, S. R., Cole C. V., Watanabe, F.S. and Dean, L.A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *USDA Circular*. 939:1-19

Pampana, S., Masoni, A., Mariotti, M., Ercoli, L. and Iduna Arduini.,I. (2018). Nitrogen fixation of grain legumes differs in response to nitrogen fertilization. Experimental Agriculture. 54(1):66-82

Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P. and Tyson, G.W. (2015). CheckM Assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. 25(7):1043-1055.

Peakall, R. and Smouse, P.E.(2006).GenAlEx 6. Genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes, 6: 288-295.

Peix, A., Rivas-Boyero, A.A., Mateos, P.F., Rodriguez-Barrueco, C., Martinez-Molin, E. and Velazquez, E. (2001). Growth promotion of chickpea and barley by phosphate solublizing strain of *Mesorizobium mediteraneum* under growth chamber condition. *Soil Biology. Biochemistry*. 33: 103-110.

Perez-Fernandez, M. and Alexander, V. (2017). Enhanced Plant Performance in Cicer arietinum L. Due to the Addition of a Combination of Plant Growth-Promoting Bacteria. *Agriculture*. 7(40):2-8

Perez, E., Sulbaran, M., Ball, M. M. and Yarzabal, L. A. (2007). Isolation and Characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. *Soil Biology Biochemistry*. 39: 2905-2914.

Pikovskaya, R.I. (1948). Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Mikrobiologiya* 17: 362-370.

Poggio, M., Hepperle, E. and Marsan F.A. (2008). Metals pollutions and human bioaccessibility of topsoils in Grugliasco, Italy. Environmental Pollution. 157: 680-689.

Rai, R., Dash, K.P., Mohapatra, T. and Singh, A. (2012). Phenotypic and molecular characterization of indigenous rhizobia nodulating chickpea in India. *Indian Journal of Experimental. Biology* 50:340-350

Rasool, S., Latef, A. A. H. A. and Ahmad, P. (2015). Chickpea: Role and responses under abiotic and biotic stress. 67-79. doi: 10.1002/9781118917091.ch4

Redden, R.J. and Berger, J. (2007). History and Origin of Chickpea. In: Yadav, S.S., Redden, R.J., Chen, W., Sharma, B.(ed.), Chickpea Breeding and Management, pp 1-13. Oxfordshire, United Kingdom,

Rhoades, J.D., (1982). Cation exchange capacity. In: Page, A.L., Miller R.H. and Keeney, D.R. (Eds.). Methods of soil analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy, Inc. Soil Science Society of America. Inc. Madison, Wisconsin, pp: 149-157.

Richter, M. and Rossello-Mora, R. (2009). Shifting the genomic gold standard for the prokaryotic species definition. *Procceding of National Acadamic Scicience USA.* 106:19126-19131.

Riva, V., Terzaghi, E., Vergani, L., Mapelli, F., Zanardini, E., Morosini, C., Raspa, G., Di Guardo, A. and Borin., S. (2019). Exploitation of Rhizosphere Microbiome Services. *In*: Reinhardt, D. and Sharma, A.K. (ed.), Methods in Rhizosphere Biology Research, pp 105-132. Springer Nature Singapore

Rivas, V., Terzaghi, E., Vergani, L., Mapelli, F., Zanardini, E., Morosini, C., Raspa ,G., Rivas, R., Laranjo, M., Mateos, P. F., Oliveira, S., Martínez-Molina, E. and Velazquez, E. (2007). Strains of *Mesorhizobium amorphae* and *Mesorhizobium tianshanense*, carrying symbiotic genes of common chickpea endosymbiotic species, constitute a novel biovar (ciceri) capable of nodulating Cicer arietinum. *Letters in Applied Microbiology*. 44(4): 412-418. doi:10.1111/j.1472-765x.2006. 02086.x

Sahgal, M. and Johri, B. (2003). The changing face of rhizobial systematics. *Current Science*. 84:43-48

Sahlemedhin Sertsu and Taye Bekele (2000). Procedures for soil and plant analysis. National Soil Research Center. EARO, Ethiopia, Pp.70-76.

SAS Institute, Inc. The SAS System for Windows. Release 9.3; SAS Institute, Inc.: Cary, NC, USA, 2012.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., et al., (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75: 7537–7541.

Seemann, T. (2014) Prokka: Rapid prokaryotic genome annotation. *Bioinformatics*. 30: 2068-2069.

Segata, N., Bornigen, D., Morgan, X.C. and Huttenhower, C. (2013) PhyloPhlAn is a new method for improved phylogenetic and taxonomic placement of microbes. *Nature Communication.* 4(2304). doi:10.1038/ncomms3304.PhyloPhlAn.

Shamseldin, A., Abdelkhalek, A. and Sadowsky, M. J. (2017). Recent changes to the classification of symbiotic, nitrogen-fixing, legume-associating bacteria: a review. *Symbiosis*: 71:91-109

Sharma, S. K., Ramesh, A. and Johri, B. N. (2013). Isolation and Characterization of Plant
Growth- Promoting Bacillus amyloliquefaciens Strain sks-bnj-1 and its Influence on Rhizosphere Soil Properties and Nutrition of Soybean (*Glycine max* L. *Merrill*). Journal of *Virology and Microbiology*. 1-19

Sinclair, T. R. and Nogueira, M. A. (2018). Selection of host-plant genotype: the next step to increase grain legume N2 fixation activity. *Journal of Experimental Botany*. 69(15): 3523-3530. doi:10.1093/jxb/ery115

Siyar, S., Inayat, N. and Hussain, F. (2019). Plant Growth Promoting Rhizobacteria and Plants' Improvement: A MiniReview. *PSM Biol. Res*.4(1):1-5.

Skorupska, A., Wielbo, J., Kidaj, D. and Marek Kozaczuk, M. (2010). Enhancing Rhizobium Legume Symbiosis Using Signaling Factors. *In*: Khan, M. S., Zaidi, A. and Musarrat, J. (ed.), Microbes for Legume Improvement, pp 27-54. SpringerWienNewYork

Selvi, B., Paul, A., Ravindran, A. and Vijaya, V.(2011). Quantitative estimation of insoluble inorganic phosphate solubilization. Int. J. Sci. Nat, 2:292-295.

Somasegaran, P. and Hoben, H.J. (1994). Handbook for Rhizobia: Methods in Legume-Rhizobium Technology. Springer-Verlag New York, Inc. 455p.

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*. 30: 1312-1313

Stonoha-Arther, C. and Wang, D. (2018). Tough love: accommodating intracellular bacteria through directed secretion of antimicrobial peptides during the nitrogen-fixing symbiosis. *Current Opinion in Plant Biology*. 44: 155-163. doi: 10.1016/j.pbi.2018.04.017

Sultan Mohammed., Seid Ahmed., Chemeda Fininsa., Negussie Tadesse., Hamwieh, A., and Douglas R. Cook. (2018). Distribution and factors influencing chickpea wilt and root rot epidemics in Ethiopia. *Crop Protection*. 106:150-155.

Tanveer, A., Ali, H. H. and Ikram, R. M. (2019). Nitrogen Fixation in Nutrient Management. In: Hasanuzzaman, M. (ed.), Agronomic Crops, pp 195-206. Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

Tassew Sirage and Fassil Assefa, (2018). Mesorhizobium ciceri and Mesorhizobium plurifarium are the dominant symbiotically effective strains on Natoli and Arerti chickpea host varieties. *Ethiopian Journal of Biological science*.17:19-35

Tassew Sirage. (2019). Genomic diversity, eco-physiological and symbiotic characteristics of chickpea (*Cicer arietinum* L.) rhizobia isolated from Central and Northern Ethiopia: Implication for inoculant production and application to enhance chickpea production. PhD thesis, Addis Ababa University, Ethiopia (pp 1-128).

Tekalign, Tadese. (1991). Soil, plant, water, fertilizer, animal manure and compost analysis. Working Document No. 13. International Livestock Research Center for Africa, Addis Ababa.

Thies, J. E., Holmes, E. M., and Vachot, A. (2001). Application of molecular techniques to studies in Rhizobium ecology. a review: *Australian Journal of Experimental Agriculture*. 41(3): 299-319.

Thudi, M., Roorkiwal, M., Kudapa, H., Chaturvedi, S.K., Singh, N. P. and Varshney, R. K. (2017). An overview of Chickpea Research: From discovery to delivery. *Pulse India*. 2(5):1-5

Togay, N., Togay., Cimrin, K.M. and Turan, M. (2008). Effects of rhizobium inoculation, sulfur and phosphorus applications on yield, yield components and nutrient uptakes in chickpea (*Cicer arietinum* L.). *African Journal of Biotechnology*.7(6): 776-782

Tulu Degefu., Endalkachew Wolde-meskel and Frostegard, A. (2013). Phylogenetic diversity of Rhizobium strains nodulating diverse legume species growing in Ethiopia. *Syst. Appl. Microbiol*. 36, 272-280.

Unay, J. and Perret, X. (2019). Synthetic Plasmids to Challenge Symbiotic Nitrogen Fixation Between Rhizobia and Legumes *In*: Reinhardt, D. and Sharma, A.K. (ed.), Methods in Rhizosphere Biology Research, pp 3-18 Springer Nature Singapore

Valentine, A. J., Benedito, A. and Kang, Y. (2011). Legume nitrogen fixation and soil abiotic stress: from physiology to genomics and beyond. *Annual Plant Reviews*. 42: 207-248

van der Maesen, L.J.G., Maxted, N., Javadi, F., Coles, S, and Davies, A.M.R. (2007). Taxonomy of the Genus Cicer Revisited. *In*: Yadav, S.S., Redden, R.J., Chen, W., Sharma, B.(ed.), Chickpea Breeding and Management, pp 14-46. Oxfordshire, United Kingdom.

Vargas, L.K., Lisboa, B. B., Giongo, A., Beneduzi, A. and Pereira Passaglia, M. M. (2010). Potential of Rhizobia as Plant Growth Promoting Rhizobacteria. In: Khan, M. S., Zaidi, A. and Musarrat, J. (ed.), Microbes for Legume Improvement, pp 137- 155. SpringerWienNewYork

Varghese, N.J., Mukherjee, S., Ivanova, N., Konstantinidis, K.T., Mavrommatis, K., Kyrpides, N.C., et al. (2015). Microbial species delineation using whole genome sequences. Nucleic Acids Res 43: 6761–6771.

Velazquez, E., Carro, L., Flores-Felix, J. D., MenEndez, E., Ramírez-Bahena, M.H. and Peix, A. (2019). Bacteria Inducing Legume Nodules Involved in the Improvement of Plant Growth, Health and Nutrition. *In*: Kumar,V., Prasad, R., Kumar, M. and Choudhary, D. K. (ed.), Microbiome in Plant Health and Disease, PP 79-104. Springer Nature Singapore Pte Ltd.

Velazquez, E., Garcia-Fraile, P., Rivas, R. and Martinez-Molina, E. (2010). Bacteria Involved in Nitrogen Fixing Legume Symbiosis: Current Taxonomic Perspective. *In*: Khan, M. S., Zaidi, A. and Musarrat, J. (ed.), Microbes for Legume Improvement, pp 1-25. SpringerWienNewYork

Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*. 255: 571-586.

von Wettberg, E. J. B., Chang, P. L., Başdemir,F., Carrasquila-Garcia, N., Lijalem Balcha Korbu., Moenga, S. M., Gashaw Bedada., Greenlon, A., Ken, S., Singh, M. V., Cordeiro, M. A., Noujdina, N.V., Kassaye Negash Dinegde., Sani, S. G.A. S., Tsegaye Getahun., Vance, L., Bergmann, E., Lindsay, D., Bullo Erena Mamo., Emily J. Warschefsky, E.J., Dacosta-Calheiros, E.., Marques, E., Yilmaz, M.A., Cakmak, A., Rose, J., Migneault, A., Krieg, C. P., Saylak, S., Teme, H., Friesen, M. L., Siler, E., Akhmetov, Z., Kholova, O.H. J., Can, C., Gaur, P., Yildirim, M., Sharma, H., Vincent Vadez, H., Kassahun Tesfaye., Asnake Fikre Woldemedhin., Taran, B., Aydogan, A., Bukun, B.R., Penmetsa, V., Berger, J., Kahraman, A., Sergey V. Nuzhdin, S.V. and

Cook, D. R.(2018) Ecology and genomics of an important crop wild relative as a prelude to agricultural innovation. *Nature Communication*. 9:649.

Vriezen, J.A.C., De Bruijn, F.J., Nusslein, K. (2007) Responses of *Rhizobia* to desiccation in relation to osmotic stress, oxygen, and temperature. *Applied Environ Microbiol*. 73:3451-3459

Walkley, A. and Black, C.A. (1934). An examination of different methods for determining determining soil organic matter and the proposed modification by the chromic acid titrationmethod. Soil Sciences. 37: 29-38.

Walley, F. L., Kyei-Boahen, S., Hnatowich, G. and Stevenson, C. (2005). Nitrogen and phosphorus fertility management for desi and kabuli chickpea. *Canadian. Journal of Plant Science*. 85: 73-79

Wang, L., Cao, Y., Wang, E. T., Qiao, Y. J., Jiao, S., Liu, Z. S., et al. (2016). Biodiversity and biogeography of rhizobia associated with common bean (Phaseolus vulgaris L.) in Shaanxi Province. Syst. Appl. Microbiol, 39:211.219.

Wang, Q., Liu, J. and Zhu, H. (2018). Genetic and molecular mechanisms underlying symbiotic specificity in legume-rhizobium interactions.doi.org/10.3389/fpls.2018.00313

Wang, E. T., Tian, C. F., Chen, W. F., Young, J. P. W. and Chen, W. X. (2019). Ecology and Evolution of Rhizobia: Springer Singapore. pp 1-13. 1st Ed. https://doi.org/10.1007/978-981-32-9555-1

Wani, P. A. and Khan, M. S. (2013). Isolation of Multiple Metal and Antibiotic Resistant Mesorhizobium species and Their Plant Growth Promoting Activity. *Research Journal of Microbiology*.8: 25-35

Wdowiak-Wrobel, S., Marek-Kozaczuk, M., Kalita, M., Karas, M., Wojcik, M. and Malek, W. (2017). Diversity and plant growth promoting properties of rhizobia isolated from root nodules of Ononis arvensis. *Antonie van Leeuwenhoek*. 110(8): 1087-1103.

Wielbo, J., Podleśna, A., Kidaj, D., Podleśny, J. and Skorupska, A. (2015). The Diversity of Pea Microsymbionts in Various Types of Soils and Their Effects on Plant Host Productivity. *Microbes Environ*. 30 (3): 254-261

Wondwosen Tena., Endalkachew Wolde-Meskel and Walley, F. (2016a) Response of chickpea (*Cicer arietinum* L.) to inoculation with native and exotic *Mesorhizobium* strains in Southern Ethiopia. *African Journal of Biotechnology*: 15(35): 1920-1929

Wondwosen Tena., Endalkachew Wolde-Meskel. and Fran,W. (2017). Genetic and phenotypic diversity of rhizobia nodulating chickpea (Cicer arietinum L.) in soils from southern and central Ethiopia. *Can. J. Microbiol*. 63: 690-707.

Wubayehu Gebremedhin., Fassil Assefa., Thuita, M. and Masso, C. (2018). Nutritionally Versatile, Abiotic Stress Resistant and Symbiotically Effective Chickpea (*Cicer arietinum* L.) Root Nodulating Rhizobial isolates from Eastern, Southeastern and Southern Ethiopia. *Electronic Journal of Biology*.14(2): 87-99

Xuan, W., Beeckman, T. and Xu, G. (2017). Plant nitrogen nutrition: sensing and signaling. *Current Opinion in Plant Biology*. 39: 5-65.

Yadav, A. S., (2006) Mesorhizobium_ciceri_ca181 was isolated from root nodules of chickpea plant in India.

Zafar, M., Ahmed, N., Mustafa, G., Zahir, Z.A. and Simms, E. L. (2017). Molecular and biochemical characterization of rhizobia from chickpea (*Cicer arietinum*). *Pakistan Journal of Agricultural Sciences*. 54(2): 373-381

Zaheer, A., Malik, A., Sher, A., Mansoor Qaisrani, M., Mehmood, A., Ullah Khan, S., Ashraf, M., Mirza,Z., Karim,S. and Rasool, M. (2019). Isolation, characterization, and effect of phosphate-zinc-solubilizing bacterial strains on chickpea (*Cicer arietinum* L.) growth. *Saudi Journal of Biological Sciences*. 1061-1067.doi: 10.1016/j.sjbs.2019.04.004

Zehara Mohammed Damtew., Douglas R Cook., Greenlon A., Asnake Fikre., von Wettberg E., Marques E., Kassahun Tesfaye., Carrasquilla-Garcia N and Fassil Assefa. (2020). Ecological Competence, Plant Growth Promoting and Symbiotic Characteristics of Different Mesorhizobium Strains Nodulating Chickpea (*Cicer arietinum* L.) from Ethiopia. Journal of Plant Pathology and Microbiology. 11(8): 1-11.

Zhang, J., Guo, C., Chen, W., de Lajudie, P., Zhang, Z., Shang, Y.1 and Wang, E. T. (2018) Mesorhizobium wenxiniae sp. nov., isolated from chickpea (*Cicer arietinum* L.) in China. *International Journal of Systematic Evolution Microbiol*. 68:1930-1936

Zhang, J.J, Liu, T.Y., Chen, W.F., Wang, E.T., Sui, X.H., Zhang, X.X., Li, Y., Li, Y. and Chen, W.X. (2012b). *Mesorhizobium muleiense* sp. nov., nodulating with *Cicer arietinum* L. *International Journal of Systematic Evolution Microbiology*. 62(11):2737-42.

Zhang, S., Yang, S., Chen, W., Chen, Y., Zhang, M., Zhou, X., Fan, G. and Feng, F.Y. (2017). *Rhizobium arenae* sp. nov., isolated from the sand of Desert Mu Us, China. *International Journal of Systematic Evolution Microbiology*. 67:2098-103

16. Appendices

Appendix 1. List of *Mesorhizobium* strains, administrative zone and geographical origin

No.	**Sample strains**	**Region**	**Latitude**	**Longitude**	**Elevation**
1	27P2S1 (*M. genospecies*7A)	Amhara, East Gojam	10° 24' 41.7"N	38° 10' 8.4"E	2429
2	27P3S2 (*M. genospecies*7A)	Amhara, East Gojam	10° 24' 41.7"N	38° 10' 8.4"E	2429
3	ET1 (*M. genospecies*8A)	Amhara, South Gonder	11° 27' 58.3"N	38° 12' 46.6"E	2795
4	ET4 (*M. genospecies*8A)	Oromia, North Shewa	9° 53' 46.8"N	38° 21' 29.6"E	2567
5	ET3 (*M. genospecies*8A)	Oromia, East Shewa	8° 49' 31.7"N	38° 59' 25.4"E	1943
6	56P2S1 (*M. genospecies*8A)	Amhara, South Gonder	11° 55' 9.3"N	37° 52' 36.9"E	1995
7	ET2 (*M. genospecies*8A)	Amhara, South Gonder	11° 29' 21.1"N	38° 12' 45.7"E	2884
8	23P2S2 (*M. genospecies*8A)	Oromia, East Shewa	9° 59' 51.2"N	38° 14' 42.9"E	2122
9	22P5S2 (*M. genospecies* 4A)	Amhara, East Gojam	10° 24' 56.2"N	38° 10' 35.9"E	2429
10	90P4S2 (*M. genospecies*4A)	Oromia, West Shewa	8° 36' 3.4"N	38° 16' 0.8"E	2209
11	29P4S2-a (*M. genospecies* 4A)	Amhara, East Gojam	10° 42' 47.3"	38° 10' 30.6"E	2541
12	20P2S1 (*M. genospecies* 4A)	Oromia, West Shewa	8° 39' 21.1"N	38° 29' 2.8"E	2207
13	ET26 *(M. genospecies*4A)	Amhara, North Gondar	12° 27' 34.8"N	7° 48' 23.0"E	1841
14	29P4S2-b (*M. genospecies* 4A)	Amhara, East Gojam	10° 42' 47.3"N	38° 10' 30.6"E	2541
15	17P2S2 (*M. genospecies* 4B)	Oromia, West Shewa	8° 40' 30.9"N	38° 36' 20.5"E	2101
16	89P1S1 (*M. genospecies* 4B)	Oromia, West Shewa	8° 35' 44.1"N	38° 14' 29.3"E	2237
17	19P3S1 (*M. genospecies* 4B)	Oromia, West Shewa	8° 39' 20.4"N	38° 28' 57.5"E	2192
18	88P2S2 (*M. genospecies*4B)	Oromia, West Shewa	8° 37' 10.6"N	38° 14' 6.2"E	2234
19	ET20 (*M. genospecies* 4B)	Amhara, North Gondar	12° 15' 16.9"N	37° 15' 51.5"E	1849
20	ET27 (*M. genospecies* 4B)	Oromia, West Shewa	8° 46' 10.9"N	38° 39' 5.6"E	2231
21	58P2S1 (*M. genospecies* 4B)	Amhara, South Gonder	11° 29' 16.6"N	38° 12' 45.8"E	2870
22	13P3S2 (*M. genospecies* 4B)	Oromia, West Shewa	8° 46' 10.9"N	38° 39' 5.6"E	2231
23	10P4S2 (*M. genospecies* 3A)	Oromia, North Shewa	8° 53' 46.3"N	39° 23' 56.5"E	1815
24	80P4S2 (*M. genospecies* 3A)	Amhara, South Wollo	12° 20' 56.9"N	38° 3' 35.4"E	1906
25	76P3S1 (*M. genospecies* 1A)	Amhara, North Gondar	12° 20' 59.4"N	37° 11' 57.9"E	1808
26	ET24 (*M. genospecies* 1A)	Amhara, North Gondar	12° 27' 48.3"N	37° 49' 53.2"E	1754
27	68P1S1 (*M. genospecies* 1D)	Amhara, North Gondar	12° 26' 13.2"N	37° 30' 25.3"E	1930
28	36P3S1 (*M. genospecies* 1D)	Amhara, West Gojam	11° 13' 22.3"N	37° 35' 42.7"E	2261
29	43P1S1 (*M. genospecies* 1D)	Amhara, East Gojam	12° 27' 43.6"N	37° 21' 29.7"E	1959
30	36P4S1 (*M. genospecies* 1B)	Amhara, West Gojam	11° 13' 22.3"N	37° 35' 42.7"E	2261
31	ET15 (*M. genospecies* 1B)	Amhara, North Gondar	12° 26' 42.6"N	37° 20' 49.0"E	1932
32	45P4S1 (*M. genospecies* 1B)	Amhara, North Gondar	12° 26' 43.8"N	37° 20' 48.3"E	1934

Appendix 1. Continued

No.	Sample strains	Region	Latitude	Longitude	Elevation
33	38P4S2 (*M. genospecies* 1E)	Amhara, South Gonder	12° 1' 54.3"N	37° 43' 49.3"E	1809
34	ET7 (*M. genospecies* 1E)	Amhara, North Gondar	12° 28' 35.9"N	37° 22' 57.5"E	1988
35	ET19 (*M. genospecies* 1E)	Amhara, South Gonder	12° 5' 20.4"N	37° 45' 17.9"E	1865
36	45P2S1 (*M. genospecies* 1E)	Amhara, North Gondar	12° 27' 43.6"N	37° 21' 29.7"E	1960
37	42P3S1-a (M. genospecies 1E)	Amhara, North Gondar	12° 28' 35.9"N	37° 22' 57.5"E	1988
38	ET10_S77 (*M. genospecies*2A)	Amhara, North Gondar	12° 27' 51.9"N	37° 21' 20.4"E	1948
39	55P3S1 (*M. genospecies* 2A)	Amhara, South Gonder	12° 21' 18.9"N	37° 15' 31.4"E	1873
40	39P3S1 (*M. genospecies*2A)	Amhara, North Gondar	12° 1' 54.3"N	37° 43' 49.3"E	1809
41	43P2S1 (*M. genospecies* 2A)	Amhara, North Gondar	12° 27' 43.6"N	37° 21' 29.7"E	1960
42	41P3S1 (*M. genospecies* 2A)	Amhara, North Gondar	12° 11' 15.4"N	37° 40' 24.8"E	1889
43	39P2S1 (*M. genospecies* 2A)	Amhara, South Gonder	12° 1' 54.3"N	37° 43' 49.3"E	1809
44	37P2S1 (*M. genospecies* 2A)	Amhara, West Gojam	11° 29' 1.9"N	37° 23' 40.0"E	1811
45	ET5 (*M. genospecies* 2A)	Oromia, West Shewa	8° 40' 30.1"N	38° 36' 16.7"E	2100
46	29P5S1 (*M. genospecies* 2A)	Amhara, East Gojam	10° 42' 47.3"N	38° 10' 30.6"E	2541
47	ET18 (*M. genospecies* 2A)	Amhara, North Gondar	12° 23' 37.6"N	37° 7' 42.4"E	1867
48	17P3S2 (*M. genospecies* 2A)	Oromia, West Shewa	8° 40' 30.9"N	38° 36' 20.5"E	2101
49	87P1S1 (*M. genospecies* 2A)	Oromia, West Shewa	8° 43' 6.1"N	38° 15' 53.5"E	2127
50	62P2S2 (*M. genospecies* 2A)	Amhara, South Gonder	11° 23' 43.3"N	38° 14' 44.1"E	2458
51	2P3S1-c (*M. genospecies* 2A)	Oromia, East Shewa	8° 49' 31.7"N	38° 59' 25.4"E	1944
52	43P5S1 (*M. genospecies* 2A)	Amhara, North	12° 27' 43.6"N	37° 21' 29.7"E	1960

		Gondar			
53	16p3S1 (*M. genospecies* 2A)	Oromia, West Shewa	8° 40' 30.5"N	8° 36' 18.4"E	2097
54	85P3S2 (*M. genospecies* 2A)	Oromia, West Shewa	8° 50' 46.3"N	38° 27' 38.8"E	2065
55	86P1S1 (*M. genospecies* 2A)	Amhara, West Gojam	11° 29' 1.9"N	37° 23' 40.0"E	1811
56	86P1S1 (*M. genospecies* 2A)	Oromia, West Shewa	8° 50' 23.4"N	38° 23' 12.4"E	2068
57		Amhara, North			
	46P5S1 (*M. genospecies* 2A)	Gondar	12° 21' 18.9"N	37° 15' 31.4"E	1873
58		Amhara, North			
	65P2S1 (*M. genospecies* 2A)	Gondar	12° 1' 42.4"N	37° 43' 25.0"E	1822
59		Amhara, North			
	46P2S1 (*M. genospecies* 2A)	Gondar	12° 26' 43.8"N	37° 20' 48.3"E	1934
60		Amhara, North			
	46P3S2 (*M. genospecies* 2A)	Gondar	12° 21' 18.9"N	37° 15' 31.4"E	1873
61		Amhara, South			
	38P3S1 (*M. genospecies* 2A)	Gonder	12° 1' 54.3"N	37° 43' 49.3"E	1809
62		Amhara, North			
	ET13 (*M. genospecies* 2A)	Gondar	12° 11' 15.4"N	37° 40' 24.8"E	1889
63		Amhara, North			
	ET14 (*M. genospecies* 2A)	Gondar	12° 11' 14.7"N	37° 40' 24.8"E	1888
64	2P3S1-b (*M. genospecies* 9A)	Oromia, East Shewa	8° 49' 31.7"N	38° 59' 25.4"E	1944

Appendix 2. Root nodule Mesorhizobium strains sampling sites cultivation history

No.	**Sample strains**	**Chickpea variety**	**Crop rotation pattern**	**Desired traits**	**Diseases symptom**
1	27P2S1 (*M. genospecies*7A)	DZ-10-4	Barely-Chickpea	consumption	Low
2	27P3S2 (*M. genospecies*7A)	DZ-10-4	Barely-Chickpea	consumption	Low
3	ET1 (*M. genospecies*8A)	DZ-10-4	Tef-Chickpea	consumption	Low
4	ET4 (*M. genospecies*8A)	Arerti	Tef-Chickpea	consumption	Medium
5	ET3 (*M. genospecies*8A)	Arerti	Tef-Chickpea	consumption	Medium
6	56P2S1 (*M. genospecies*8A)	Landrace	Tef-Chickpea	Market	Low
7	ET2 (*M. genospecies*8A)	Arerti	Tef-Chickpea	consumption	Low
8	23P2S2 (*M. genospecies*8A)	Landrace	Tef-Chickpea	Market	Low
9	22P5S2 (*M. genospecies* 4A)	Landrace	Tef-Chickpea	consumption	Low
10	90P4S2 (*M. genospecies*4A)	Landrace	Tef-Chickpea	consumption	Low
11	29P4S2-a (*M. genospecies* 4A)	Landrace	Barely - Chickpea	consumption	Low

12	20P2S1 (*M. genospecies* 4A)	Landrace	wheat-Chickpea*	consumption	Low
13	ET26 *(M. genospecies*4A)	DZ-10-4	Barely-Chickpea	consumption	Low
14	29P4S2-b (*M. genospecies* 4A)	Landrace	Barely-Chickpea	Market	Low
15	17P2S2 (*M. genospecies* 4B)	DZ-10-4	Tef-Chickpea	consumption	Low
16	89P1S1 (*M. genospecies* 4B)	Landrace	Barely-Chickpea	Market	Low
17	19P3S1 (*M. genospecies* 4B)	Landrace	Tef-Chickpea*	Market	Low
18	88P2S2 (*M. genospecies*4B)	Landrace	Barely-Chickpea	Market	Low
19	ET20 (*M. genospecies* 4B)	Landrace	wheat-Chickpea*	consumption	Low
20	ET27 (*M. genospecies* 4B)	DZ-10-4	Barely-Chickpea	consumption	Low
21	58P2S1 (*M. genospecies* 4B)	Landrace	Sorghum-chickpea	consumption	Low
22	13P3S2 (*M. genospecies* 4B)	Landrace	wheat-Chickpea	consumption	Low
23	10P4S2 (*M. genospecies* 3A)	Habru	Tef-Chickpea*	consumption	Low
24	80P4S2 (*M. genospecies* 3A)	Habru	Sorghum-Chickpea*	consumption	Low
25	76P3S1 (*M. genospecies* 1A)	Landrace	Tef-Chickpea	consumption	Low
26	ET24 (*M. genospecies* 1A)	Landrace	Tef-Chickpea	consumption	Low
27	68P1S1 (*M. genospecies* 1D)	Landrace	Sorghum-Chickpea	consumption	Low
28	36P3S1 (*M. genospecies* 1D)	Mariye	Barely-Chickpea	consumption	Low
29	43P1S1 (*M. genospecies* 1D)	Landrace	Sorghum-Chickpea	Market	Low
30	36P4S1 (*M. genospecies* 1B)	Mariye	Barely-Chickpea	consumption	Low
		Acos		consumption	Low
31	ET15 (*M. genospecies* 1B)	Dube	Tef-Chickpea		
32	45P4S1 (*M. genospecies* 1B)	Landrace	Sorghum-Chickpea*	consumption	Low

Table legend; *Farmer used compost; *Farmer used DA

Appendix 2. Continued

No.	**Sample strains**	**Chickpea variety**	**Crop rotation pattern**	**Desired traits**	**Diseases symptom**
33	38P4S2 (*M. genospecies* 1E)	Landrace	Tef-Chickpea	consumption	Low
34	ET7 (*M. genospecies* 1E)	Landrace	Wheat-Chickpea	consumption	Low
35	ET19 (*M. genospecies* 1E)	Landrace	Barley-Chickpea*	Market	Low
36	45P2S1 (*M. genospecies* 1E)	Landrace	Sorghum-Chickpea*	consumption	Low
37	42P3S1-a (M. genospecies 1E)	Landrace	Sorghum-Chickpea	consumption	Low
38	ET10_S77 (*M. genospecies* 2A)	Habru	Tef-Chickpea*	consumption	Low
39	55P3S1 (*M. genospecies* 2A)	Landrace	Barley-Chickpea	consumption	Low
40	39P3S1 (*M. genospecies*2A)	Landrace	Tef-Chickpea	Market	Low
41	43P2S1 (*M. genospecies* 2A)	Landrace	Sorghum-Chickpea	consumption	Low
42	41P3S1 (*M. genospecies* 2A)	Landrace	Sorghum-Chickpea	consumption	Low

43	39P2S1 (*M. genospecies* 2A)	Landrace	Tef-Chickpea	consumption	Low
44	37P2S1 (*M. genospecies* 2A)	Mariye	Tef-Chickpea	consumption	Low
45	ET5 (*M. genospecies* 2A)	Arerti	Tef-Chickpea	consumption	Low
46	29P5S1 (*M. genospecies* 2A)	Landrace	Barley-Chickpea	consumption	Low
47	ET18 (*M. genospecies* 2A)	Landrace	Tef-Chickpea*	consumption	Low
48	17P3S2 (*M. genospecies* 2A)	DZ-10-4	Tef-Chickpea	consumption	Low
49	87P1S1 (*M. genospecies* 2A)	Landrace	Tef-Chickpea	Market	Low
50	62P2S2 (*M. genospecies* 2A)	Landrace	Sorghum-Chickpea	consumption	Low
51	2P3S1-c (*M. genospecies* 2A)	Arerti	Tef-Chickpea	consumption	Low
52	43P5S1 (*M. genospecies* 2A)	Landrace	Sorghum-Chickpea	consumption	Low
53	16p3S1 (*M. genospecies* 2A)	Habru	Tef-Chickpea	consumption	Low
54	85P3S2 (*M. genospecies* 2A)	Landrace	Tef-Chickpea	Market	Low
55	86P1S1 (*M. genospecies* 2A)	DZ-10-4	Tef-Chickpea	consumption	Low
56	86P1S1 (*M. genospecies* 2A)	Landrace	Tef- Chickpea	consumption	Low
57	46P5S1 (*M. genospecies* 2A)	Landrace	Sorghum-Chickpea	Market	Low
58	65P2S1 (*M. genospecies* 2A)	Landrace	Chickpea-Chickpea	consumption	Low
59	46P2S1 (*M. genospecies* 2A)	Landrace	Sorghum-Chickpea	Market	Low
60	46P3S2 (*M. genospecies* 2A)	Landrace	Sorghum-Chickpea	consumption	Low
61	38P3S1 (*M. genospecies* 2A)	Landrace	Tef-Chickpea	consumption	Low
62	ET13 (*M. genospecies* 2A)	Landrace	wheat-Chickpea	consumption	Low
63	ET14 (*M. genospecies* 2A)	Landrace	Tef-Chickpea	consumption	Low
64	2P3S1-b (*M. genospecies* 9A)	Arerti	Tef-Chickpea	consumption	Low

Table legend; *Farmer used compost; *Farmer used DAP

Appendix 3.*Mesorhizobium* strains genome sequencing characteristics

No.	**Sample strains**	**Coting**	**Scaffolds (No)**	**Genome size**	**Longest Scaffolds**	**ANI_{95}**	**N50**	**GC%**
1	27P2S1 (*M. genospecies*7A)	1001	1001	6986330	108499	7A	13883	62.46
2	27P3S2 (*M. genospecies*7A)	1990	1990	6605632	29525	7A	5213	62.46
3	ET1 (*M. genospecies*8A)	908	905	6588211	69457	8A	12415	62.9
4	ET4 (*M. genospecies*8A)	1143	1143	6677665	120432	8A	9583	62.95
5	ET3 (*M. genospecies*8A)	2129	2129	6331846	28197	8A	4597	63.01
6	56P2S1 (*M. genospecies*8A)	6007	6007	10268787	34205	8A	3973	63.1
7	ET2 (*M. genospecies*8A)	2409	2409	6862475	36448	8A	4362	62.73
8	23P2S2 (*M. genospecies*8A)	2516	2516	6135888	23236	8A	3579	63
9	22P5S2 (*M. genospecies* 4A)	855	855	6788051	93527	4A	13483	63.56
10	90P4S2 (*M. genospecies*4A)	1071	1071	6232807	64721	4A	9581	63.82
11	29P4S2-a (*M. genospecies* 4A)	2477	2477	7197387	60878	4A	7157	63.47
12	20P2S1 (*M. genospecies* 4A)	1445	1445	6698930	40127	4A	7566	63.61

13	ET26 *(M. genospecies*4A)	1578	1577	7011949	73927	4A	7617	63.35
14	29P4S2-b (*M. genospecies* 4A)	4312	4312	6037939	11676	4A	1839	63.59
15	17P2S2 (*M. genospecies* 4B)	761	759	6730316	116842	4B	15239	63.93
16	89P1S1 (*M. genospecies* 4B)	1623	1622	7008229	58256	4B	12554	63.66
17	19P3S1 (*M. genospecies* 4B)	1039	1038	6612936	63315	4B	11047	63.94
18	88P2S2 (*M. genospecies*4B)	2218	2218	6298172	36320	4B	4444	63.92
19	ET20 (*M. genospecies* 4B)	1180	1176	7128838	87353	4B	12512	63.66
20	ET27 (*M. genospecies* 4B)	813	812	6802077	112145	4B	16200	63.83
21	58P2S1 (*M. genospecies* 4B)	2261	2261	6605369	32803	4B	5145	63.88
22	13P3S2 (*M. genospecies* 4B)	4787	4787	5926771	9197	4B	1555	63.56
23	10P4S2 (*M. genospecies* 3A)	2340	2340	6768884	85378	3A	9218	63.21
24	80P4S2 (*M. genospecies* 3A)	2072	2072	6183894	23396	3A	4663	63.12
25	76P3S1 (*M. genospecies* 1A)	4699	4699	8194817	55719	1A	3251	62.7
26	ET24 (*M. genospecies* 1A)	833	833	7413583	91179	1A	17189	62.67
27	68P1S1 (*M. genospecies* 1D)	2773	2772	6766960	41335	1D	4879	63.65
28	36P3S1 (*M. genospecies* 1D)	4363	4363	6249621	12112	1D	1864	63.44
29	43P1S1 (*M. genospecies* 1D)	2603	2603	6966301	25887	1D	4104	63.26
30	36P4S1 (*M. genospecies* 1B)	5665	5663	10506185	84734	1B	4569	63.18
31	ET15 (*M. genospecies* 1B)	4059	4059	6585417	19345	1B	2230	63.03
32	45P4S1 (*M. genospecies* 1B)	2776	2933	7330283	27904	1B	3660	63

Appendix 3. Continued

No.	**Sample strains**	**Coting**	**Scaffolds (No)**	**Genome size**	**Longest Scaffolds**	**ANI_{95}**	**N50**	**GC%**
33	38P4S2 (*M. genospecies* 1E)	2007	2007	7093544	34312	1E	6587	63.16
34	ET7 (*M. genospecies* 1E)	1890	1296	7135397	70565	1E	9647	63.19
35	ET19 (*M. genospecies* 1E)	1822	1821	7217700	128751	1E	7845	63.08
36	45P2S1 (*M. genospecies* 1E)	2933	2776	6992617	40492	1E	3960	63.1
37	42P3S1-a (M. genospecies 1E)	NA	NA	NA	NA	NA	NA	NA
38	ET10_S77 (*M. genospecies* 2A)	679	677	6969280	93671	2A	21061	63.43
39	55P3S1 (*M. genospecies* 2A)	5668	5668	9866558	44337	2A	4041	63.41
40	39P3S1 (*M. genospecies*2A)	1799	1798	7251800	53757	2A	6909	63.23
41	43P2S1 (*M. genospecies* 2A)	2883	2883	6656145	22538	2A	3426	63.44
42	41P3S1 (*M. genospecies* 2A)	2670	2670	7124313	30304	2A	4194	63.16
43	39P2S1 (*M. genospecies* 2A)	4646	4646	5376853	7949	2A	1455	63.22

44	37P2S1 (*M. genospecies* 2A)	3947	3947	2346573	2302	2A	591	62.34
45	ET5 (*M. genospecies* 2A)	531	529	6801026	135609	2A	27384	63.57
46	29P5S1 (*M. genospecies* 2A)	1122	1122	6716983	68095	2A	10383	63.51
47	ET18 (*M. genospecies* 2A)	1513	1512	6957119	86955	2A	7807	63.44
48	17P3S2 (*M. genospecies* 2A)	713	712	6786555	68251	2A	18325	63.53
49	87P1S1 (*M. genospecies* 2A)	2926	2926	6725833	74027	2A	4598	63.55
50	62P2S2 (*M. genospecies* 2A)	5116	5116	7773314	25880	2A	2708	63.38
51	2P3S1-c (*M. genospecies* 2A)	369	710	6447211	87575	9A	17281	62.46
52	43P5S1 (*M. genospecies* 2A)	4068	4068	6840356	16206	2A	2564	63.28
53	16p3S1 (*M. genospecies* 2A)	3925	3925	6484455	31929	2A	2479	63.43
54	85P3S2 (*M. genospecies* 2A)	3399	3399	7047501	31144	2A	3647	63.37
55	86P1S1 (*M. genospecies* 2A)	1299	1890	7114537	50143	2A	5962	63.34
56	86P1S1 (*M. genospecies* 2A)	5979	5978	11152187	53774	2A	3528	63.5
57	46P5S1 (*M. genospecies* 2A)	8377	8377	9314633	12787	2A	1498	63.24
58	65P2S1 (*M. genospecies* 2A)	6431	6431	9890636	36716	2A	2716	63.26
59	46P2S1 (*M. genospecies* 2A)	2501	2501	7302055	47813	2A	4577	63.08
60	46P3S2 (*M. genospecies* 2A)	967	967	7490033	81854	2A	16404	63.13
61	38P3S1 (*M. genospecies* 2A)	2018	2018	7174446	57551	2A	5791	63.21
62	ET13 (*M. genospecies* 2A)	1629	1629	7473343	84202	2A	8575	63.26
63	ET14 (*M. genospecies* 2A)	2873	2872	6991699	39065	2A	3977	63.23
64	2P3S1-b (*M. genospecies* 9A)	711	710	6447211	87575	9A	17281	62.46

Appendix 4 Soil analysis of root nodule *Mesorhizobium* strains sampling sites

No.	**Sample strains**	**OM**	**P1**	**P2**	**K**	**pH**	**CEC**	**N**	**Mg**	**Ca**	**Na**	**SO4 -S**	**Mn**
1	27P2S1 (*M. genospecies*7A)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	27P3S2 (*M.genospecies*7A)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	ET1 (*M.genospecies*8A)	4	36	40	834	6.9	30	4	761	3812	10	3	6
4	ET4 (*M.genospecies*8A)	3.4	14	13	704	6.8	29.8	4	804	4089	13	3	26
5	ET3 (*M.genospecies*8A)	3.4	14	14	615	6.7	29.2	3	757	4007	14	3	12
6	56P2S1 (*M.genospecies*8A)	4.4	1	7	246	5.4	20.7	39	494	5104	13	3	34
7	ET2 (*M.genospecies*8A)	3.4	8	14	431	7.3	30.7	5	704	4757	20	3	5
8	23P2S2 (*M.genospecies*8A)	3.7	2	5	278	6.1	42.2	4	1037	5382	38	4	15
9	22P5S2 (*M.genospecies* 4A)	3.5	5.	6	294	7.1	32.8	4	558	5429	13	3	14
10	90P4S2 (*M.genospecies*4A)	2.6	4	8	464	6.8	29.7	14	666	4420	18	3	13
11	29P4S2-a (*M.genospecies* 4A)	4.3	7	11	263	6.4	33.7	9	785	4703	14	4	19

12	20P2S1 (*M.genospecies* 4A)	3.9	5	6	468	7.3	34	5	432	5485	14	3	12
13	ET26 *(M.genospecies*4A)	4.4	3	7	337	7.3	41.7	7	1012	6501	28	3	13
14	29P4S2-b (*M.genospecies* 4A)	4.3	7	11	263	6.4	33.7	9	785	4703	14	4	19
15	17P2S2 (*M.genospecies* 4B)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
16	89P1S1 (*M.genospecies* 4B)	3.2	3	6	448	6.7	24.8	9	722	3507	30	5	25
17	19P3S1 (*M.genospecies* 4B)	3.5	5	9	561	6.4	34.6	11	706	4844	23	2	22
18	88P2S2 (*M.genospecies*4B)	3.4	3	7	419	6.4	24.6	5	711	3269	20	5	28
19	ET20 (*M.genospecies* 4B)	3.9	3	6	451	7.3	34	5	432	5865	13	2	10
20	ET27 (*M.genospecies* 4B)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
21	58P2S1 (*M.genospecies* 4B)	4.8	13	21	168	6.2	27.4	54	735	3500	33	5	6
22	13P3S2 (*M.genospecies* 4B)	3.9	7	10	508	7.5	33.2	10	463	5170	41	2	13
23	10P4S2 (*M.genospecies* 3A)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
24	80P4S2 (*M.genospecies* 3A)	3.1	19	14	317	6.2	19.1	15	533	2709	9	4	16
25	76P3S1 (*M.genospecies* 1A)	1.5	5	8	193	7.8	24.2	5	723	3521	97	2	4
26	ET24 (*M.genospecies* 1A)	5	55	50	367	6.3	36.8	6	1008	4340	27	4	25
27	68P1S1 (*M.genospecies* 1D)	4.9	7	39	264	6.8	41.8	5	2236	4053	45	5	22
28	36P3S1 (*M.genospecies* 1D)	4.9	3	14	130	6.2	29.8	10	1042	3444	32	5	42
29	43P1S1 (*M.genospecies* 1D)	4.8	1	5	230	6	33.8	7	1222	3620	14	7	55
30	36P4S1 (*M.genospecies* 1B)	4.9	3	14	130	6.2	29.8	10	1042	3444	32	5	42
31	ET15 (*M. genospecies* 1B)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
32	45P4S1 (*M.genospecies* 1B)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table legend; P1 (Weak Bray) and P2 (Olsen Method), plant-available phosphorus; Mg, magnesium; Ca, calcium; Na, sodium; soil sulfur, SO4-S; Mn, manganese; Fe, Iron; Zn, Zinc; Cu, Copper; B, Boron. All values in parts per mission (ppm). NA, Not Available.

Appendix 4. Continued

No.	Sample strains	OM	P1	P2	K	pH	CEC	N	Mg	Ca	Na	SO 4-S	Mn
33	38P4S2 (*M.genospecies* 1E)	5.4	9	13	420	6.2	30	6	983	5050	43	3	13
34	ET7 (*M.genospecies* 1E)	3.9	10	14	509	7.4	38.5	2	490	6633	19	2	7
35	ET19 (*M.genospecies* 1E)	3.5	5	9	561	6.4	34.6	11	706	4844	23	2	22
36	45P2S1 (*M.genospecies* 1E)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
37	42P3S1-a (M.genospecies 2E)	5.3	1	4	239	5.8	37.8	6	1249	3802	15	5	35
38	ET10_S77 (*M.genospecies* 2A)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
39	55P3S1 (*M.genospecies* 2A)	5.5	3	8	145	5.6	29.9	42	1064	2748	10	8	11
40	39P3S1 (*M.genospecies*2A)	3.2	2	8	706	6.2	37.4	4	1243	4590	27	6	17
41	43P2S1 (*M.genospecies* 2A)	4.8	1	5	230	6	33.8	7	1222	3620	14	7	55
42	41P3S1 (*M.genospecies* 2A)	3.8	1	3	209	6.5	37.3	3	1103	4927	66	4	9

43	39P2S1 (*M.genospecies* 2A)	3.2	2	8	706	6.2	37.4	4	1243	4590	27	6	17
44	37P2S1 (*M.genospecies* 2A)	4.7	1	6	180	6.3	36.6	4	1201	4482	19	3	18
45	ET5 (*M.genospecies* 2A)	3.2	7	9	563	8	29.2	8	548	4656	9	4	4
46	29P5S1 (*M.genospecies* 2A)	4.3	7	11	263	6.4	33.7	9	785	4703	14	4	19
47	ET18 (*M.genospecies* 2A)	3.6	4	7	277	6.8	35.8	6	1093	4994	40	3	19
48	17P3S2 (*M.genospecies* 2A)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
49	87P1S1 (*M.genospecies* 2A)	2.7	4	7	253	6.3	36.7	3	998	4793	19	3	34
50	62P2S2 (*M.genospecies* 2A)	3.9	6	9	252	7.1	44.1	4	1755	5752	76	3	9
51	2P3S1-c (*M.genospecies* 2A)	3.4	8	14	431	7.3	30.7	5	704	4757	20	3	5
52	43P5S1 (*M.genospecies* 2A)	4.8	1	5	230	6	33.8	7	1222	3620	14	7	55
53	16p3S1 (*M.genospecies* 2A)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
54	85P3S2 (*M.genospecies* 2A)	4.2	7	9	215	6.6	27.7	8	419	4419	9	3	29
55	86P1S1 (*M.genospecies* 2A)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
56	86P1S1 (*M.genospecies* 2A)	3	11	15	249	6.3	34.3	14	1041	2500	14	6	40
57	46P5S1 (*M.genospecies* 2A)	5	2	4	230	6.7	42.9	5	1674	5820	40	4	18
58	65P2S1 (*M.genospecies* 2A)	3.8	2	6	278	6.5	35.4	4	1345	4181	32	8	27
59	46P2S1 (*M.genospecies* 2A)	5	2	4	230	6.7	42.9	5	1674	5820	40	4	18
60	46P3S2 (*M.genospecies* 2A)	5	2	4	230	6.7	42.9	5	1674	5820	40	4	18
61	38P3S1 (*M.genospecies* 2A)	5.4	9	13	420	6.2	30	6	983	5050	43	3	13
62	ET13 (*M.genospecies* 2A)	3.7	7	9	508	7	31.8	8	553	5170	41	2	13
63	ET14 (*M.genospecies* 2A)	3.7	7	10	552	6.4	35.1	12	729	4904	20	4	23
64	2P3S1-b (*M.genospecies* 9A)	3.4	8	14	431	7.3	30.7	5	704	4757	20	3	5

Appendix 4. Continued

No.	Sample strains	Fe	Zn	Cu	B	No.	Sample Strains	Fe	Zn	Cu	B
1	27P2S1 (*M.genospecies*7A)	NA	NA	NA	NA	33	38P4S2 (*M.genospecies* 1E)	25	0.7	3	0.1
2	27P3S2 (*M.genospecies*7A)	NA	NA	NA	NA	34	ET7 (*M.genospecies* 1E)	6	0.3	1.1	0.2
3	ET1 (*M.genospecies*8A)	14	0.7	2.2	0.2	35	ET19 (*M.genospecies* 1E)	12	0.3	1.5	0.1
4	ET4 (*M.genospecies*8A)	13	0.5	2.2	0.2	36	45P2S1 (*M.genospecies* 1E)	NA	NA	NA	NA
5	ET3 (*M.genospecies*8A)	12	0.4	2.1	0.1	37	42P3S1-a (M.genospecies 1E)	25	0.7	2.1	0.1
6	56P2S1 (*M.genospecies*8A)	28	0.7	0.8	0.1	38	ET10_S77 (*M.genospecies* 2A)	NA	NA	NA	NA
7	ET2 (*M.genospecies*8A)	9	0.4	1.7	0.3	39	55P3S1 (*M.genospecies* 2A)	24	0.4	1.5	0.1
8	23P2S2 (*M.genospecies*8A)	22	0.3	3	0.1	40	39P3S1 (*M.genospecies*2A)	20	0.4	1.9	0.1
9	22P5S2 (*M.genospecies* 4A)	16	0.3	1.9	0.1	41	43P2S1 (*M.genospecies* 2A)	20	0.8	2.1	0.1
10	90P4S2 (*M.genospecies*4A)	14	0.4	1.7	0.2	42	41P3S1 (*M.genospecies* 2A)	17	0.3	1.5	0.1
11	29P4S2-a (*M.genospecies* 4A)	18	0.5	2	0.2	43	39P2S1 (*M.genospecies* 2A)	20	0.4	1.9	0.1
12	20P2S1 (*M.genospecies* 4A)	8	0.3	1.5	0.3	44	37P2S1 (*M.genospecies* 2A)	21	0.4	2.6	0.1
13	ET26 *(M.genospecies*4A)	18	0.2	2.8	0.2	45	ET5 (*M.genospecies* 2A)	4	0.2	1	0.3
14	29P4S2-b (*M.genospecies* 4A)	18	0.5	2	0.2	46	29P5S1 (*M.genospecies* 2A)	18	0.5	2	0.2
15	17P2S2 (*M.genospecies* 4B)	NA	NA	NA	NA	47	ET18 (*M.genospecies* 2A)	17	0.4	1.8H	0.1

16	89P1S1 (*M.genospecies* 4B)	16	0.4	1.8	0.1	48	17P3S2 (*M.genospecies* 2A)	NA	NA	NA	NA
17	19P3S1 (*M.genospecies* 4B)	12	0.3	1.5	0.1	49	87P1S1 (*M.genospecies* 2A)	20	0.4	1.8	0.1
18	88P2S2 (*M.genospecies*4B)	18	0.5	1.8	0.1	50	62P2S2 (*M.genospecies* 2A)	16	0.4	2.3	0.1
19	ET20 (*M.genospecies* 4B)	8	0.3	1.5	0.3	51	2P3S1-c (*M.genospecies* 2A)	9	0.4VL	1.7	0.3
20	ET27 (*M.genospecies* 4B)	NA	NA	NA	NA	52	43P5S1 (*M.genospecies* 2A)	20	0.8L	2.1	0.1
21	58P2S1 (*M.genospecies* 4B)	16	0.4	0.3	0.1	53	16p3S1 (*M.genospecies* 2A)	NA	NA	NA	NA
22	13P3S2 (*M.genospecies* 4B)	13	0.5	2.7	0.2	54	85P3S2 (*M.genospecies* 2A)	16	0.6	2.2	0.2
23	10P4S2 (*M.genospecies* 3A)	NA	NA	NA	NA	55	ET17 (*M.genospecies* 2A)	19	0.7	1.9	0.1
24	80P4S2 (*M.genospecies* 3A)	11	3.4	0.7	0.1	56	86P1S1 (*M.genospecies* 2A)	19	0.7	1.9H	0.1
25	76P3S1 (*M.genospecies* 1A)	6	0.2	1.9	0.3	57	46P5S1 (*M.genospecies* 2A)	16	0.3	2.6VH	0.1
26	ET24 (*M.genospecies* 1A)	38	0.6	3.6	0.1	58	65P2S1 (*M.genospecies* 2A)	20	0.5	1.9	0.1
27	68P1S1 (*M.genospecies* 1D)	31	0.6	3.9	0.1	59	46P2S1 (*M.genospecies* 2A)	20	0.3	2.8	0.1
28	36P3S1 (*M.genospecies* 1D)	15	0.5	1.8	0.1	60	46P3S2 (*M.genospecies* 2A)	20	0.3	2.8	0.1
29	43P1S1 (*M.genospecies* 1D)	20	0.8	2.1	0.1	61	38P3S1 (*M.genospecies* 2A)	25	0.7	3	0.1
30	36P4S1 (*M.genospecies* 1B)	15	0.5	1.8	0.1	62	ET13 (*M.genospecies* 2A)	13	0.5	2.7	0.2
31	ET15 (*M.genospecies* 1B)	NA	NA	NA	NA	63	ET14 (*M.genospecies* 2A)	11	0.4	1.8	0.1
32	45P4S1 (*M.genospecies* 1B)	NA	NA	NA	NA	64	2P3S1-b (*M.genospecies* 9A)	9	0.4	1.7	0.3

Appendix 5. Eco-physiological characteristics of chickpea nodulating rhizobial strains stressful conditions

No	Sample Strains	Closest relative	Salt tolerance			pH tolerance			Temperature		
			2%	3%	4%	pH 4	pH 5	pH 10	35°C	37°C	40°C
1	*M.genospecies* 7A (27P3S2)	*M. ciceri*	+	+	+	-	-	+	+	+	-
2	*M.genospecies* 9A (2P3S1-b)	*M.sp.*LSJ280B00	+	+	-	-	+	+	+	+	-
3	*M.genospecies* 3A (80P4S2)	*M. amorphae*	+	+	-	-	+	+	+	+	+
4	*M.genospecies* 3A (10P4S2)	*M. amorphae*	+	-	-	-	-	+	+	-	-
5	*M.genospecies* 4B (19P3S1)	*M. amorphae*	-	-	-	-	+	+	-	-	-
6	*M.genospecies* 4B (ET20)	*M. amorphae*	+	-	-	-	-	+	+	+	-
7	*M.genospecies* 1B (45P4S1)	*M. loti*	+	+	+	+	+	+	+	+	+
8	*M.genospecies* 2A (46P3S2)	*M.plurifarium*	+	+	+	-	+	+	+	+	-

9	*M.genospecies* 2A (29P5S1)	*M.plurifarium*	-	-	-	-	-	+	+	+	-
10	*M.genospecies* 2A (43P2S1)	*M.plurifarium*	+	+	+	+	+	-	+	+	+
11	*M.genospecies* 8A (ET1)	*M. australicum*	-	-	-	-	+	-	+	-	-
12	*M.genospecies* 8A (ET4)	*M. australicum*	+	+	-	-	+	+	-	-	-
13	*M.genospecies* 8A (23P2S2)	*M. australicum*	+	+	+	-	-	+	+	+	-
14	*M.genospecies* 4A (ET26)	*M. amorphae*	+	-	+	+	+	+	+	+	+
15	*M.genospecies* 4A (90P4S2)	*M. amorphae*	-	-	-	-	-	+	+	+	-
16	*M.genospecies* 4A (22P5S2)	*M. amorphae*	+	-	-	+	+	+	+	+	-
17	*M.genospecies* 1D (36P3S1)	*M. amorphae*	+	-	-	-	-	-	-	-	-
18	*M.genospecies* 1A (ET24)	*M. amorphae*	+	+	-	-	-	+	-	-	-
19	*M.genospecies* 1E (38P4S2)	*M. loti*	-	+	+	+	+	+	-	-	-
20	10P3S1(unidentified)	*M. loti*	-	-	-	-	+	+	+	+	-
	Total (%)		70	50	35	25	60	85	75	65	20

Appendix 6. Intrinsic Antibiotic resistance, heavy metal, carbon and nitrogen utilization pattern of *Mesorhizobium* strains

Characteristics	*M.genospecies* 7A (n=1)	*M.genospecies* 9A (n=1)	*M.genospecies* 3A (n=2)	*M.genospecies* 4B (n=2)	*M.genospecies* 1B (n=1)	*M.genospecies* 2A (n=3)	*M.genospecies* 8A (n=3)	*M.genospecies* 4A (n=3)	*M.genospecies* 1D (n=1)	*M.genospecies* 1A (n=1)	*M.genospecies* 1E (n=1)	10P3S1(unidentified)	Total (%)
Carbohydrate													
D-Sorbitol	1	1	1	0	0	3	2	1	1	1	1	0	**60**
D-Glucose	1	1	0	0	1	2	3	2	1	0	1	0	**60**
α-cellulose	0	0	0	0	0	0	0	0	0	0	0	0	**0**
Sucrose	0	1	0	1	1	2	2	0	1	0	1	0	**50**
D-Xylose	0	0	0	0	0	1	2	1	1	1	1	1	**40**
Trehalose	0	1	1	0	0	2	1	1	1	1	0	1	**45**
Amino acid													
L-lysine	0	1	1	0	1	1	0	0	0	0	0	0	**20**

L-Phenylanine	1	1	1	1	0	2	1	1	1	1	1	1	**60**
L- Tryptophan	1	1	1	0	0	0	0	1	0	0	0	1	**25**
L- Leucine	0	1	1	1	0	1	1	1	1	0	0	1	**35**
L- Argenine	1	1	1	0	1	0	0	2	0	0	0	1	**40**
Glycine	0	1	0	0	1	2	1	1	0	0	0	0	**30**
Antibiotics													
Chloramphenicol	0	0	1	0	0	0	0	1	0	0	0	0	**10**
Erythromycin	0	1	1	2	1	2	0	2	1	1	1	0	**60**
Streptomycin	0	1	0	1	0	3	2	1	1	1	0	0	**50**
Nalidixic acid	0	0	1	1	1	3	2	2	1	1	1	0	**65**
Neomycin	0	1	0	0	1	1	0	2	1	0	1	0	**35**
Tetracycline	0	0	1	0	0	0	0	0	0	0	0	0	**5**
Heavy metals													
CoCl2	1	1	1	2	0	2	1	2	1	0	1	0	**60**
CuCl2	1	1	1	1	1	1	0	1	0	1	1	0	**45**
ZnCl2	1	1	1	0	0	0	0	2	0	0	1	0	**30**
Pb(CH3COOH)	0	1	0	0	0	0	0	0	0	0	0	0	**5**
NiSO4	1	1	1	0	0	0	0	2	0	0	1	0	**30**
AlCl3	0	0	0	1	0	0	0	0	0	1	0	0	**10**

Table legend; n= the number of strains under each species group and the number 3, 2, 1 and 0 represent strains performance level on tested traits.

Appendix 7. Comparative studies on eco-physiological and nutritional properties of indigenous chickpea rhizobia from different chickpea growing areas

	Carbon	Nitrogen	NaCl	pH	T^{o}C	IAR	HR	Reference
1	16-83	16-100	35-100	25-100	20-100	0-66	0-83	This study
2	37-93	73-100	11-100	22-100	17-100	0-75	0-69	Mulisa Jida and Fasil Assefa, 2012
3	40-80	0-100	50-100	50-100	50-100	50-100	0-100	Daniel Muleta and Fassil Assefa, 2015
4	50-100	43-86	0-100	0-89	33-83	12-87	50-100	Wubayehu Gebremedhin *et al*., 2018
5	70-92	40-80	25-67	29-100	25-100	0-80	-	Tassew Sirage and Fassil Assefa,2018
6	-	-	46-100	42-100	9-100	53-89	-	Laranjo and Oliveira, 2011
7	25-75	-	57-100	46-100	64-100	0-89	3-17	Kucuk and Kivanc, 2008
8	5-100	-	11-100	35-100	4-100	11-100	11-100	Maatallah *et al*.,2002a,b
9	-	-	13-100	6-100	16-100	16-100	-	Rai *et al*.,2012

Appendix 8. Inoculation response on number of nodules of chickpea varieties at Chefe Donsa trial sites.

	Year 2017		Year 2018		Year 2019		Average (across years)	
Treatment	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**
43P2S1 (*M. genospecies* 2A)	48.15^{d}	14.57^{jkl}	18.65^{hij}	38.74^{bc}	41.86^{jk}	25.79^{jk}	36.17	26.58
90P4S2 (*M. genospecies* 4A)	38.12^{e}	21.02^{ghi}	12.00^{l}	21.37^{hi}	20.90^{jk}	12.62^{mn}	23.67	18.34
22P5S2 (*M. genospecies* 4A)	40.18^{e}	12.20^{kl}	12.27^{l}	15.53^{n}	11.03^{mn}	9.75^{n}	21.16	12.16
2P3S1-b (*M. genospecies* 9A)	63.06^{b}	14.81^{jkl}	37.36^{bcd}	30.87^{b-e}	33.84^{fg}	31.58^{gh}	44.93	26.75
ET1 (*M. genospecies* 8A)	28.48^{fg}	17.97^{ijk}	20.50^{ij}	32.0^{cde}	34.68^{hi}	27.58^{hi}	28.23	26.15
38P4S2 (*M. genospecies*1E)	11.06^{l}	25.47^{fg}	20.89^{hi}	34.76^{cde}	35.57^{jkl}	19.48^{lm}	22.51	26.57
ET26 *(M. genospecies* 4A)	47.03^{d}	18.63^{hij}	12.26^{kl}	20.10^{ij}	21.14^{jk}	13.83^{lmn}	27.14	17.85
45P4S1 (*M. genospecies* 1B)	25.95^{fg}	25.26^{fg}	34.56^{cde}	50.32^{a}	60.89^{a}	40.16^{de}	40.13	38.58
80P4S2 (*M. genospecies* 3A)	69.98^{a}	27.79^{f}	17.58^{ijk}	40.10^{b}	46.76^{jk}	20.18^{l}	44.77	29.34
27P3S2 (*M. genospecies*7A)	56.45^{c}	25.82^{fg}	27.80^{fg}	31.46^{ef}	52.0^{bc}	38.45^{def}	44.75	31.91
Mean	42	21	21	32	36	23	33	25
Ha. Ata (Reference)	42.99^{de}	17.59^{ijk}	14.80^{jkl}	31.99^{def}	35.76^{klm}	16.26^{lmn}	31.18	21.95
EAL029 (Commercial)	27.41^{fg}	25.43^{fg}	21.62^{hi}	33.08^{de}	32.94^{jkl}	19.9^{ljk}	27.32	26.15
Control (Untreated)	23.4^{ghi}	14.30^{jkl}	13.51^{kl}	19.90^{hij}	26.40^{ijk}	27.20^{jk}	23.30	23.30
Nitrogen (Fertilizer)	22.41^{g-j}	15.12^{jkl}	23.18^{gh}	21.32^{hi}	30.76^{hi}	26.85^{jk}	27.12	22.43
HSD (0.05)	5.72		5.23		5.75			

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., CV stands for= Coefficient of variation, HSD = high range statistical domain; **=significant ($P \leq 0.01$), * = significant ($P \leq 0.05$) and ns = non-significant (P> 0.05

Appendix 9. Inoculation response on number of nodules of chickpea varieties at Alem Tena trial sites

	Year 2017		Year 2018		Year 2019		Average (across years)	
Treatment	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**
43P2S1 (*M. genospecies* 2A)	21.73^{fg}	17.60^{fgh}	15.47^{g-j}	15.30^{g-i}	16.49^{jk}	18.49^{hij}	18.95	17.13
90P4S2 (*M. genospecies* 4A)	34.67^{cd}	13.38^{hij}	9.82^{kl}	14.59^{g-i}	17.22^{jk}	16.77^{jk}	20.57	14.91
22P5S2 (*M. genospecies* 4A)	30.96^{cd}	7.31^{jk}	9.82^{jkl}	13.65^{jk}	9.18^{l}	11.09^{kl}	16.65	10.68
2P3S1-b (*M. genospecies* 9A)	42.97^{ab}	14.21^{gh}	7.66^{l}	21.99^{cd}	27.41^{def}	25.95^{ef}	26.01	20.72
ET1 (*M. genospecies* 8A)	30.25^{cd}	6.66^{k}	10.20^{h-k}	19.60^{jkl}	38.63^{b}	19.90^{g-j}	26.86	15.39
38P4S2 (*M. genospecies*1E)	15.01^{fgh}	17.48^{gh}	14.89^{g-i}	21.40^{c-f}	26.83^{def}	27.40^{def}	18.58	21.76
ET26 *(M. genospecies* 4A)	35.16^{cd}	14.40^{gh}	11.97^{i-l}	16.15^{efg}	17.47^{ij}	17.44^{ij}	21.53	16.00
45P4S1 (*M. genospecies* 1B)	18.96^{fgh}	16.73^{fgh}	37.46^{a}	42.20^{a}	46.34^{a}	38.20^{b}	34.26	32.38
80P4S2 (*M. genospecies* 3A)	45.80^{a}	17.87^{fgh}	21.94^{cd}	29.10^{b}	35.49^{bc}	24.74^{fg}	34.41	23.90
27P3S2 (*M. genospecies*7A)	37.07^{bc}	14.54^{gh}	17.93^{d-h}	26.27^{bc}	43.92^{a}	31.23^{cd}	32.98	24.01
Mean	31	14	15	22	28	23	25	20
Ha. Ata (Reference)	28.53^{de}	14.93^{gh}	13.47^{h-k}	17.97^{d-h}	24.77^{fg}	19.30^{hij}	22.26	17.40

EAL029 (Commercial)	20.28^{fg}	17.73^{fgh}	18.03^{d-h}	16.15^{f-i}	22.57^{f-i}	19.88^{g-j}	20.29	17.88
Control (Untreated)	23.80^{ef}	13.43^{hi}	11.69^{i-l}	19.52^{d-g}	25.75^{ef}	23.34^{fgh}	18.41	17.77
Nitrogen (Fertilizer)	13.21^{hij}	12.53^{hij}	13.53^{h-k}	21.53^{cde}	30.71^{cde}	23.65^{fgh}	19.15	19.24
HSD (0.05)	6.75		5.49		5.19			

Means followed by the same letter within a column are not significantly different at (P >0.05) level of probability., HSD = high range statistical domain; **=significant (P ≤ 0.01), * = significant (P ≤ 0.05) and ns = non-significant (P> 0.05).

Appendix 10. Inoculation response on number of nodules of chickpea varieties at Genda Gorba trial sites

	Year 2017		**Year 2018**		**Year 2019**		**Average (across years)**	
Treatment	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**
43P2S1 (*M. genospecies* 2A)	33.00^{ab}	13.33^{g-l}	34.00^{b}	25.55^{e-h}	33.63^{a-d}	28.61^{d-g}	33.23	22.60
90P4S2 (*M. genospecies* 4A)	25.67^{cde}	10.00^{ikl}	13.50^{op}	16.60^{m-o}	18.27^{i-l}	16.30^{klm}	19.15	14.20
22P5S2 (*M. genospecies* 4A)	28.33^{bc}	6.33^{l}	23.63^{hij}	28.24^{d-g}	30.17^{efg}	28.22^{d-g}	27.70	20.93
2P3S1-b (*M. genospecies* 9A)	31.00^{abc}	6.19^{kl}	20.20^{j-m}	28.41^{def}	32.53^{a-d}	22.15^{g-j}	27.58	19.25
ET1 (*M. genospecies* 8A)	16.77^{efg}	8.67^{jkl}	14.50^{op}	19.63^{lmn}	25.63^{f-i}	18.99^{klm}	18.83	15.43
38P4S2 (*M. genospecies*1E)	11.99^{h-l}	16.31^{f-j}	18.67^{lmn}	18.70^{lmn}	11.50^{m}	11.60^{m}	13.72	15.20
ET26 *(M. genospecies* 4A)	28.67^{cde}	12.67^{h-l}	12.3^{p}	16.91^{mno}	16.49^{lm}	14.16^{mn}	19.16	14.58
45P4S1 (*M. genospecies* 1B)	9.67^{jkl}	11.00^{i-l}	34.31^{b}	41.81^{a}	38.11^{a}	34.31^{abc}	27.03	29.37
80P4S2 (*M. genospecies* 3A)	34.67^{ab}	18.00^{e-i}	23.20^{h-k}	32.89^{bc}	30.37^{b-e}	31.45^{b-e}	29.41	27.45
27P3S2 (*M. genospecies*7A)	23.67^{c-f}	9.33^{jkl}	31.90^{bcd}	43.99^{a}	36.23^{ab}	33.79^{a-d}	31.43	29.04
Mean	24	11	22	28	27	24	24	21
Ha. Ata (Reference)	21.00^{d-g}	12.00^{h-l}	20.27^{j-m}	27.55^{e-h}	28.96^{f-i}	23.06^{h-k}	23.41	20.85
EAL029 (Commercial)	14.33^{g-i}	18.33^{e-j}	19.31^{k-n}	22.87^{jki}	17.31^{k-n}	22.86^{i-m}	16.98	21.35
Control (Untreated)	19.74^{e-i}	9.20^{jkl}	22.33^{j-l}	24.06^{g-j}	22.17^{g-j}	20.84^{ijk}	20.75	18.37
Nitrogen (Fertilizer)	19.77^{efg}	11.41^{i-l}	23.91^{hij}	29.84^{cde}	31.91^{b-e}	26.90^{e-h}	25.20	22.72
HSD (0.05)	7.87		4.30		5.91			

Means followed by the same letter within a column are not significantly different at (P >0.05) level of probability., HSD = high range statistical domain; **=significant (P ≤ 0.01), * = significant (P ≤ 0.05) and ns = non-significant (P> 0.05)

Appendix 11. Inoculation response on nodules weight dry of chickpea varieties at Chefe Donsa trial sites

	Year 2017		**Year 2018**		**Year 2019**		**Average (across years)**	
Treatment	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**
43P2S1 (*M. genospecies* 2A)	172.80^{b}	74.83^{h}	95.69^{de}	113.58^{b}	145.23^{c}	83.68^{hij}	137.91	90.70
90P4S2 (*M. genospecies* 4A)	90.49^{g}	46.25^{j}	42.35^{mn}	55.05^{i-l}	59.01^{lmn}	50.93^{mn}	55.67	43.02
22P5S2 (*M. genospecies* 4A)	108.65^{ef}	42.84^{jk}	44.07^{lmn}	54.05^{i-l}	61.73^{lmn}	51.84^{mn}	62.16	40.24
2P3S1-b (*M. genospecies* 9A)	167.19^{c}	28.92^{l}	99.66^{cd}	130.39^{a}	133.89^{bc}	100.20^{def}	130.69	82.74
ET1 (*M. genospecies* 8A)	97.77^{g}	39.94^{jk}	39.43^{n}	59.18^{hij}	77.60^{h-k}	64.24^{klm}	71.60	54.44
38P4S2 (*M. genospecies*1E)	74.85^{h}	79.53^{ij}	38.66^{n}	51.14^{i-m}	76.91^{h-k}	55.27^{mn}	55.46	55.36
ET26 *(M. genospecies* 4A)	122.11^{d}	106.16^{ef}	39.42^{n}	49.88^{j-n}	65.56^{klm}	47.27^{n}	68.47	61.84
45P4S1 (*M. genospecies* 1B)	92.98^{kl}	86.18^{jk}	108.35^{bc}	136.50^{a}	157.39^{a}	129.35^{c}	160.55	165.68
80P4S2 (*M. genospecies* 3A)	202.49^{a}	98.93^{fg}	57.22^{h-k}	94.73^{de}	99.69^{d-g}	85.33^{f-j}	139.51	102.63
27P3S2 (*M. genospecies*7A)	185.44^{b}	47.24^{j}	85.70^{e}	90.13^{de}	107.03^{d}	83.98^{hij}	122.81	70.36
Mean	134.10	67.74	66.20	85.12	97.71	74.92	99.33	75.93
Ha. Ata (Reference)	131.97^{d}	111.64^{e}	47.06^{k-n}	50.06^{j-n}	90.43^{e-h}	72.29^{i-k}	89.82	78.00

EAL029 (Commercial)	74.85^{h}	201.66^{a}	61.10^{g-j}	71.13^{fg}	101.94^{de}	70.67^{jkl}	82.89	124.17
Control (Untreated)	79.72^{h}	59.08^{i}	57.95h^{-k}	61.88^{f-i}	84.84^{g-j}	64.48^{hij}	61.24	57.43
Nitrogen (Fertilizer)	76.04^{h}	62.17^{i}	66.95^{fgh}	72.54^{f}	86.23^{f-i}	83.75^{hij}	67.97	65.92
HSD (0.05)	10.07		11.40		15.05			

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., HSD = high range statistical domain; **=significant (P ≤ 0.01), * = significant (P ≤ 0.05) and ns = non-significant (P> 0.05)

Appendix 12. Inoculation response on nodules weight dry of chickpea varieties at Alem Tena trial sites

	Year 2017		**Year 2018**		**Year 2019**		**Average (across years)**	
Treatment	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**
43P2S1 (*M. genospecies* 2A)	145.87^{a}	28.07^{g}	75.01^{b-f}	91.65^{a}	131.23^{a}	114.16^{b}	117.4	78.00
90P4S2 (*M. genospecies* 4A)	62.54^{c-g}	33.46^{fg}	51.24^{i-l}	63.60^{e-i}	55.56^{klm}	53.10^{lm}	55.58	49.60
22P5S2 (*M. genospecies* 4A)	80.00^{cde}	31.74^{g}	35.56^{m}	40.20^{lm}	47.03^{mn}	36.57^{n}	53.24	35.79
2P3S1-b (*M. genospecies* 9A)	119.40^{ab}	30.00^{g}	72.86^{-m}	63.89^{ab}	117.19^{b}	92.58^{cd}	95.18	70.14
ET1 (*M. genospecies* 8A)	48.25^{efg}	60.21^{d-g}	44.94^{jkl}	58.16^{h-k}	89.22^{cde}	74.69^{g-j}	60.8	64.4
38P4S2 (*M. genospecies*1E)	48.07^{efg}	48.47^{efg}	58.27^{h-k}	63.67^{e-i}	80.39^{e-h}	69.49^{hij}	62.24	60.54
ET26 *(M. genospecies* 4A)	87.60^{bcd}	76.33^{cde}	61.23^{g-j}	65.03^{d-h}	71.50^{g-j}	66.30^{ijk}	73.45	69.22
45P4S1 (*M. genospecies* 1B)	57.07^{d-g}	57.67^{d-g}	75.63^{b-e}	93.00^{a}	120.17^{ab}	86.53^{c-f}	84.30	79.07
80P4S2 (*M. genospecies* 3A)	145.86^{a}	67.54^{c-f}	49.83^{jkl}	75.33^{b-f}	81.92^{d-g}	63.42^{jkl}	106.21	75.10
27P3S2 (*M. genospecies*7A)	132.93^{a}	33.27^{j}	65.13^{d-h}	73.96^{c-g}	89.67^{cde}	69.41^{hij}	95.94	58.90
Mean	95.60	47.43	56.38	71.33	87.30	71.72	79.76	63.50
Ha. Ata (Reference)	82.60^{cde}	77.27^{cde}	59.20^{hij}	61.87^{f-j}	97.12^{c}	86.55^{c-f}	79.64	75.23
EAL029 (Commercial)	87.04^{bcd}	97.56^{bc}	84.20^{abc}	78.03^{bcd}	108.26^{o}	83.97^{o}	104.86	92.16
Control (Untreated)	50.96^{efg}	56.11^{d-g}	65.96^{d-h}	71.14^{c-h}	75.55^{b}	52.95^{f-i}	50.76	45.52
Nitrogen (Fertilizer)	87.52^{bcd}	51.43^{d-g}	65.87^{d-h}	71.50^{c-h}	97.05^{c}	88.84^{cde}	68.15	64.09
HSD (0.05)	35.17		13.68		11.38			

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., HSD = high range statistical domain; **=significant (P ≤ 0.01), * = significant (P ≤ 0.05) and ns = non-significant (P> 0.05)

Appendix 13. Inoculation response on nodules weight dry of chickpea varieties at Genda Gorba trial sites

	Year 2017		**Year 2018**		**Year 2019**		**Average (across years)**	
Treatment	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**
43P2S1 (*M. genospecies* 2A)	101.75^{a}	28.91^{g-k}	88.42^{c-g}	109.18^{ab}	149.29^{a}	119.54^{c}	113.15	85.88
90P4S2 (*M. genospecies* 4A)	39.67^{efg}	23.27^{i-l}	49.77^{op}	64.38^{j-n}	64.75^{m}	48.34^{n}	47.58	42.48
22P5S2 (*M. genospecies* 4A)	53.60^{c}	25.93^{h-l}	40.20^{p}	53.06^{m-p}	68.40^{lm}	63.02^{m}	50.60	44.14
2P3S1-b (*M. genospecies* 9A)	70.30^{b}	19.63^{jkl}	84.39^{e-h}	111.77^{a}	118.70^{cd}	88.75^{hi}	87.07	73.45
ET1 (*M. genospecies* 8A)	37.39^{e-h}	45.88^{cde}	66.82^{i-l}	87.64^{d-g}	109.11^{de}	106.16^{efg}	71.11	79.89
38P4S2 (*M. genospecies*1E)	41.86^{l}	36.87^{i-l}	67.00^{i-l}	82.73^{fgh}	85.43^{ij}	75.45^{kl}	56.19	60.28
ET26 *(M. genospecies* 4A)	65.92^{b}	53.61^{c}	65.03^{i-m}	66.37^{i-l}	81.28^{hij}	82.05^{ijk}	68.17	64.59
45P4S1 (*M. genospecies* 1B)	32.22^{f-i}	36.58^{e-h}	96.34^{b-e}	93.30^{abc}	123.51^{c}	108.95^{ef}	82.46	80.51
80P4S2 (*M. genospecies* 3A)	91.10^{a}	45.82^{cde}	55.55^{l-0}	89.45^{c-f}	85.70^{i}	60.25^{m}	84.48	68.15
27P3S2 (*M. genospecies*7A)	67.20^{b}	17.73^{kl}	75.81^{g-j}	89.53^{c-f}	97.37^{gh}	75.95^{jkl}	79.52	60.35
Mean	54.99	34.55	68.61	85.07	99.91	83.29	75.42	66.72
Ha. Ata (Reference)	46.47^{cde}	41.13^{def}	61.87^{k-o}	64.53^{j-n}	104.62^{ef}	79.05^{ijk}	70.98	61.57

					g			
EAL029 (Commercial)	44.88^{cde}	54.85^{f-j}	78.03^{f-h}	100.05^{a-d}	136.43^{b}	99.45^{efg}	86.75	84.48
Control (Untreated)	37.30^{efg}	31.00^{f-j}	51.60^{nop}	59.80^{k-o}	79.95^{ijk}	62.41^{m}	56.36	50.89
Nitrogen (Fertilizer)	37.87^{efg}	28.53^{g-k}	71.50^{h-k}	77.86^{f-i}	99.31^{fg}	87.32^{i}	69.56	64.57
HSD (0.05)	11.73		13.08		9.74			

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., HSD = high range statistical domain; **=significant (P ≤ 0.01), * = significant (P ≤ 0.05) and ns = non-significant (P> 0.05).

Appendix 14. Inoculation response on shoot dry weight of chickpea varieties at Chefe Donsa trial sites

	Year 2017		**Year 2018**		**Year 2019**		**Average (across years)**	
Treatment	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**
43P2S1 (*M. genospecies* 2A)	15.42^{c-f}	19.72^{a-e}	15.69^{-e}	15.60^{b-e}	17.17^{b-e}	16.07^{b-e}	16.10	17.13
90P4S2 (*M. genospecies* 4A)	17.13^{a-f}	15.06^{def}	13.63^{cde}	12.19^{e}	12.49^{e}	12.97^{e}	14.42	13.40
22P5S2 (*M. genospecies* 4A)	17.19^{a-f}	16.15^{b-f}	13.19^{cde}	13.69^{cde}	14.90^{b-e}	14.84^{b-e}	15.09	14.90
2P3S1-b (*M. genospecies* 9A)	19.91^{a-d}	17.96^{a-f}	13.93^{b-e}	17.97^{ab}	18.61^{b}	14.11^{cde}	17.48	16.68
ET1 (*M. genospecies* 8A)	16.35^{a-f}	21.29^{a}	15.23^{b-e}	15.69^{b-e}	16.17^{b-e}	15.23^{b-e}	15.92	17.40
38P4S2 (*M. genospecies*1E)	15.82^{c-f}	17.01^{a-f}	12.97^{cde}	15.06^{b-e}	15.69^{b-e}	12.71^{e}	14.83	14.93
ET26 *(M. genospecies* 4A)	17.20^{ef}	14.52^{a-f}	12.19^{e}	12.54^{de}	14.04^{cde}	13.20^{de}	13.59	14.31
45P4S1 (*M. genospecies* 1B)	15.04^{def}	18.05^{f}	16.62^{bc}	21.48^{a}	23.40^{a}	17.50^{bc}	18.35	19.02
80P4S2 (*M. genospecies* 3A)	17.45^{a-f}	20.03^{abc}	14.53^{b-e}	16.48^{bcd}	17.97^{bc}	16.10^{b-e}	16.65	17.53
27P3S2 (*M. genospecies*7A)	20.84^{ab}	15.84^{c-f}	13.40^{cde}	14.84^{b-e}	16.09^{b-e}	15.31^{b-e}	16.44	15.00
Mean	17.39	16.60	14.15	15.55	16.55	14.71	15.89	16.03
Ha. Ata (Reference)	16.95^{a-f}	18.83^{a-e}	13.50^{cde}	13.88^{cde}	14.97^{b-e}	13.19^{de}	15.14	15.30
EAL029 (Commercial)	17.53^{a-f}	16.89^{a-f}	11.81^{e}	13.91^{cde}	14.83^{b-e}	15.01^{b-e}	14.72	15.28
Control (Untreated)	14.34^{ef}	13.40^{f}	12.15^{e}	12.25^{e}	13.35^{de}	13.05^{e}	13.55	13.15
Nitrogen (Fertilizer)	18.43^{a-e}	18.17^{a-e}	14.87^{b-e}	16.45^{bcd}	17.51^{bc}	15.05^{b-e}	16.94	16.56
HSD (0.05)	4.92		4.04		4.08			

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., HSD = high range statistical domain; **=significant (P ≤ 0.01), * = significant (P ≤ 0.05) and ns = non-significant (P> 0.05)

Appendix 15. Inoculation response on shoot dry weight of chickpea varieties at Alem Tena trial sites

	Year 2017		**Year 2018**		**Year 2019**		**Average (across years)**	
Treatment	Natoli	Arerti	Natoli	Arerti	Natoli	Arerti	Natoli	Arerti
43P2S1 (*M. genospecies* 2A)	13.55^{a-d}	12.41^{a-d}	9.77^{efg}	12.91^{b-g}	16.86^{b-e}	13.00^{jk}	12.22	11.76
90P4S2 (*M. genospecies* 4A)	11.54^{a-d}	11.18^{a-d}	11.21^{b-g}	10.27^{d-g}	12.99^{ijk}	12.46^{jk}	11.91	11.31
22P5S2 (*M. genospecies* 4A)	12.50^{a-d}	11.92^{bcd}	13.23^{b-f}	14.75^{ab}	11.98^{c-j}	10.45^{k}	13.57	12.71
2P3S1-b (*M. genospecies* 9A)	14.42^{abc}	13.46^{a-d}	11.70^{b-g}	13.27^{b-e}	16.14^{c-h}	16.59^{b-e}	14.09	14.44
ET1 (*M. genospecies* 8A)	11.89^{a-d}	15.93^{a}	9.58^{fg}	9.75^{efg}	13.87^{d-k}	13.37^{g-k}	11.78	13.02
38P4S2 (*M. genospecies*1E)	10.59^{bcd}	12.77^{a-d}	11.07^{c-g}	11.61^{b-g}	15.53^{c-i}	15.47^{c-i}	12.40	13.29
ET26 *(M. genospecies* 4A)	9.13^{d}	12.93^{a-d}	10.60^{c-g}	10.50^{d-g}	14.16^{c-k}	13.30^{h-k}	11.30	12.24
45P4S1 (*M. genospecies* 1B)	10.03 ^{cd}	13.51^{a-d}	10.84^{c-g}	12.16^{b-g}	16.38^{b-f}	15.47^{c-i}	12.43	13.71
80P4S2 (*M. genospecies* 3A)	12.66^{a-d}	15.00^{abc}	13.32^{b-e}	13.83^{bcd}	16.69^{bcd}	16.19^{c-g}	14.22	15.01
27P3S2 (*M. genospecies*7A)	15.10^{ab}	11.92^{a-d}	14.17^{abc}	17.50^{a}	21.84^{a}	19.06^{ab}	17.05	16.16
Mean	11.92	12.91	11.55	12.65	15.81	14.53	13.37	13.24
Ha. Ata (Reference)	12.10^{a-d}	14.10^{a-d}	9.46^{g}	10.80^{c-g}	13.73^{e-k}	13.15^{ijk}	11.76	12.69

EAL029 (Commercial)	12.52^{a-d}	12.76^{a-d}	12.13^{b-g}	11.73^{b-g}	13.96^{d-k}	13.55^{f-k}	13.27	13.07
Control (Untreated)	10.55^{bcd}	10.60^{bcd}	10.96^{b-g}	9.28^{b-e}	12.26^{jk}	12.98^{ijk}	12.73	13.15
Nitrogen (Fertilizer)	12.96^{a-d}	12.28^{a-d}	13.14^{b-f}	12.78^{b-g}	15.19^{c-i}	14.89^{b-f}	13.88	13.81
HSD (0.05)	5.00		3.64		2.9			

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., HSD = high range statistical domain; **=significant (P ≤ 0.01), * = significant (P ≤ 0.05) and ns = non-significant (P> 0.05)

Appendix 16. Inoculation response on shoot dry weight of chickpea varieties at Genda Gorba trial sites

	Year 2017		**Year 2018**		**Year 2019**		**Average (across years)**	
Treatment	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**
43P2S1 (*M. genospecies* 2A)	8.21^{bc}	12.61^{ab}	13.62^{b-f}	15.15^{cde}	16.23^{bcd}	13.95^{b-i}	12.69	13.90
90P4S2 (*M. genospecies* 4A)	9.67^{abc}	9.45^{bc}	13.72^{c-f}	13.12^{c-h}	12.99^{c-i}	11.24^{hi}	12.13	11.27
22P5S2 (*M. genospecies* 4A)	9.65^{bc}	8.91^{bc}	12.07^{e-j}	14.43^{bcd}	15.53^{b-f}	12.61^{d-i}	12.26	11.99
2P3S1-b (*M. genospecies* 9A)	12.22^{ab}	13.24^{ab}	10.30^{ij}	16.14^{ab}	16.40^{bc}	15.28^{b-g}	12.98	14.89
ET1 (*M. genospecies* 8A)	11.51^{abc}	14.64^{a}	12.30^{e-i}	13.59^{b-f}	12.23^{e-i}	15.05^{b-g}	12.01	14.43
38P4S2 (*M. genospecies*1E)	8.47^{bc}	9.018^{abc}	12.25^{d-i}	12.36^{e-i}	14.99^{b-h}	13.12^{c-i}	11.94	11.96
ET26 *(M. genospecies* 4A)	9.78^{abc}	12.47^{ab}	10.43^{hij}	12.35^{e-i}	11.66^{ghi}	16.00^{b-e}	10.18	13.00
45P4S1 (*M. genospecies* 1B)	13.55^{c}	13.44^{bc}	18.52^{a}	18.17^{ab}	21.83^{a}	21.84^{a}	16.22	16.75
80P4S2 (*M. genospecies* 3A)	12.28^{ab}	12.90^{ab}	12.88^{c-i}	15.29^{bc}	14.55^{b-i}	13.45^{c-i}	12.46	13.03
27P3S2 (*M. genospecies*7A)	11.35^{abc}	9.21^{bc}	9.30^{j}	12.91^{c-i}	17.52^{b-g}	14.43^{b-i}	11.54	11.21
Mean	10.08	11.43	12.58	13.98	14.82	14.15	12.44	13.24
Ha. Ata (Reference)	10.04^{abc}	11.11^{bc}	11.34^{f-j}	13.52^{b-g}	13.18^{c-i}	11.58^{ghi}	11.40	11.93
EAL029 (Commercial)	11.73^{abc}	10.93^{abc}	10.91^{g-j}	14.31^{b-e}	15.24^{b-g}	11.18^{i}	12.94	12.36
Control (Untreated)	10.90^{abc}	9.18^{bc}	10.78^{g-j}	13.30^{e-g}	10.88^{i}	13.59^{c-i}	12.26	13.64
Nitrogen (Fertilizer)	12.96^{ab}	12.28^{ab}	12.84^{c-i}	13.16^{c-h}	13.45^{c-i}	12.03^{f-i}	14.23	13.51
HSD (0.05)	5.15		2.82		3.78			

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., HSD = high range statistical domain; **=significant (P ≤ 0.01), * = significant (P ≤ 0.05) and ns = non-significant (P> 0.05

Appendix 17. Inoculation response on a bove ground dry biomass of chickpea varieties at Chefe Donsa trial sites

	Year 2017		**Year 2018**		**Year 2019**		**Average (across years)**	
Treatment	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**
43P2S1 (*M. genospecies* 2A)	7.64^{b-e}	6.59^{g-j}	5.63^{ab}	5.52^{abc}	6.38^{a}	5.60^{d-g}	6.55	5.90
90P4S2 (*M. genospecies* 4A)	7.16^{c-g}	6.17^{jk}	4.98^{cde}	4.69^{efg}	5.07^{h-l}	4.39^{nm}	5.74	5.08
22P5S2 (*M. genospecies* 4A)	6.90^{f-i}	6.90^{f-i}	4.86^{def}	4.98^{cde}	5.17^{h-k}	4.82^{jkl}	5.64	5.57
2P3S1-b (*M. genospecies* 9A)	7.92^{ab}	7.01^{e-i}	5.36^{a-d}	5.28^{a-d}	6.08^{abc}	5.14^{h-l}	6.45	5.81
ET1 (*M. genospecies* 8A)	6.40^{ij}	7.08^{d-h}	5.01^{cde}	4.93^{def}	5.21^{g-j}	4.74^{lm}	5.54	5.58
38P4S2 (*M. genospecies*1E)	7.06^{e-i}	6.43^{hij}	4.28^{gh}	5.24^{a-e}	5.20^{h-k}	4.89^{i-l}	5.51	5.51
ET26 *(M. genospecies* 4A)	7.78^{bc}	6.39^{ij}	4.88^{def}	5.36^{a-d}	5.69^{cde}	5.09^{h-l}	6.12	5.61
45P4S1 (*M. genospecies* 1B)	8.50^{a}	5.94^{jk}	5.51^{abc}	5.67^{a}	6.30^{ab}	5.61^{def}	6.77	5.74
80P4S2 (*M. genospecies* 3A)	7.77^{bc}	6.41^{hij}	5.16^{a-e}	5.13^{a-e}	5.40^{e-h}	5.08^{h-l}	6.11	5.54
27P3S2 (*M. genospecies*7A)	7.51^{b-f}	5.71^{kl}	5.34^{a-d}	5.42^{a-d}	5.68^{cde}	4.90^{i-l}	6.18	5.34
Mean	7.46	6.46	5.10	5.22	5.62	5.03	6.06	5.57

Ha. Ata (Reference)	7.52^{b-e}	6.21^{jk}	5.03^{cde}	5.07^{b-e}	5.25^{f-i}	4.95^{i-l}	5.94	5.41
EAL029 (Commercial)	7.76^{bcd}	5.61^{kl}	5.28^{a-d}	5.28^{a-d}	5.87^{cd}	5.12^{h-l}	6.30	5.34
Control (Untreated)	5.98^{jk}	5.22^{l}	4.38f^{gh}	4.02^{h}	4.80^{kl}	4.20^{mn}	5.66	5.02
Nitrogen (Fertilizer)	7.26^{b-f}	6.96^{f-i}	5.28^{a-d}	5.07^{b-e}	5.96^{bcd}	5.38^{e-h}	6.03	5.68
HSD (0.05)	0.68		0.56		0.40			

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., HSD = high range statistical domain; **=significant (P ≤ 0.01), * = significant (P ≤ 0.05) and ns = non-significant (P> 0.05)

Appendix 18. Inoculation response on a bove ground dry biomass of chickpea varieties at Alem Tena trial sites

	Year 2017		**Year 2018**		**Year 2019**		**Average (across years)**	
Treatment	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**
43P2S1 (*M. genospecies* 2A)	5.93^{a}	2.45^{lm}	4.01^{de}	2.78^{i-m}	5.42^{d-g}	5.24^{f-i}	5.12	3.49
90P4S2 (*M. genospecies* 4A)	3.56^{e-i}	2.39^{lm}	3.68^{ef}	2.51^{lm}	4.90^{h-k}	4.56^{klm}	4.26	3.30
22P5S2 (*M. genospecies* 4A)	3.63^{e-h}	2.61^{klm}	3.68^{ef}	2.86^{i-l}	5.03^{f-k}	4.73^{j-m}	4.11	3.40
2P3S1-b (*M. genospecies* 9A)	5.39^{ab}	2.96^{i-l}	4.13^{d}	3.08^{g-j}	5.44^{c-f}	4.74^{j-m}	4.99	3.59
ET1 (*M. genospecies* 8A)	3.80^{e-h}	3.08^{h-l}	4.88^{a}	3.08^{g-j}	6.20^{ab}	5.44^{c-f}	4.96	3.87
38P4S2 (*M. genospecies*1E)	3.43^{f-i}	2.56^{klm}	2.74^{j-m}	3.39^{fgh}	5.56^{a}	5.21^{abc}	3.78	3.59
ET26 *(M. genospecies* 4A)	4.12^{def}	2.70^{j-m}	3.41^{fg}	4.19^{cd}	5.21^{f-j}	5.24^{f-i}	4.25	4.04
45P4S1 (*M. genospecies* 1B)	3.93^{efg}	3.27^{g-k}	4.27^{bcd}	2.57^{klm}	5.51^{c-f}	5.32^{e-h}	4.40	3.55
80P4S2 (*M. genospecies* 3A)	3.92^{efg}	3.06^{i-k}	4.28^{bcd}	3.14^{g-j}	5.88^{a-d}	4.93^{g-k}	4.69	3.71
27P3S2 (*M. genospecies*7A)	4.28^{cde}	2.13^{m}	4.58^{de}	3.34^{g-j}	5.16^{c-h}	5.41^{f-j}	4.50	3.55
Mean	4.20	2.72	3.98	3.11	5.34	5.00	4.51	3.61
Ha. Ata (Reference)	5.01^{bc}	2.95^{i-l}	2.99^{h-k}	4.69^{ab}	5.07^{ab}	4.74^{b-f}	4.54	4.30
EAL029 (Commercial)	4.72^{bcd}	3.49^{f-i}	4.06^{abc}	3.10^{fgh}	4.78^{h-k}	5.21^{f-j}	4.82	4.15
Control (Untreated)	3.71^{e-i}	2.42^{lm}	3.20^{ghi}	2.41m	4.36^{lm}	4.28^{m}	4.26	3.43
Nitrogen (Fertilizer)	4.01^{d-g}	3.76^{e-h}	4.29^{bcd}	2.97^{h-k}	5.26^{f-i}	5.11^{f-j}	4.47	3.90
HSD (0.05)	0.77		0.42		0.49			

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., HSD = high range statistical domain; **=significant (P ≤ 0.01), * = significant (P ≤ 0.05) and ns = non-significant (P> 0.05)

Appendix 19. Inoculation response on a bove ground dry biomass of chickpea varieties at Genda Gorba trial sites

	Year 2017		**Year 2018**		**Year 2019**		**Average (across years)**	
Treatment	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**
43P2S1 (*M. genospecies* 2A)	5.01^{a}	3.10^{fg}	4.85^{b-e}	5.04^{abc}	6.06^{ab}	5.67^{a-d}	5.30	4.60
90P4S2 (*M. genospecies* 4A)	3.72^{ef}	3.03^{fg}	3.91^{g-l}	4.22^{f-j}	4.32^{i}	4.47^{hi}	3.98	3.91
22P5S2 (*M. genospecies* 4A)	3.88^{cde}	2.94^{fg}	3.33^{lmn}	4.35^{d-h}	5.03^{e-h}	4.29^{i}	4.05	3.84
2P3S1-b (*M. genospecies* 9A)	4.37^{b}	3.19^{f}	4.45^{c-g}	3.77^{i-m}	6.06^{ab}	5.31^{c-g}	4.63	3.81
ET1 (*M. genospecies* 8A)	4.39^{b}	3.14^{fg}	3.74^{i-n}	5.47^{a}	5.86^{abc}	4.78^{ghi}	4.80	4.57
38P4S2 (*M. genospecies*1E)	3.27^{ef}	2.94^{fg}	3.15^{n}	4.96^{abc}	3.39^{j}	5.16^{d-g}	3.29	4.38
ET26 *(M. genospecies* 4A)	4.47^{b}	3.23^{f}	3.53^{k-n}	4.19^{f-i}	5.02^{e-h}	5.21^{d-g}	4.34	4.21
45P4S1 (*M. genospecies* 1B)	4.63^{ab}	3.01^{fg}	4.93^{a-d}	4.35^{d-h}	5.27^{d-g}	4.99^{e-h}	4.70	3.91
80P4S2 (*M. genospecies* 3A)	4.20^{bcd}	3.15^{f}	4.46^{c-g}	5.39^{ab}	6.20^{a}	4.88^{gh}	4.95	4.47
27P3S2 (*M. genospecies*7A)	4.26^{bc}	3.02^{fg}	3.69^{j-n}	4.24^{f-j}	5.46^{cde}	5.16^{d-g}	4.42	4.08

Mean	4.27	3.08	3.88	4.49	5.25	4.97	4.47	4.18
Ha. Ata (Reference)	4.37^{b}	3.02^{fg}	3.28^{mn}	4.30^{e-i}	5.45^{c-f}	4.82^{ghi}	4.61	4.37
EAL029 (Commercial)	4.43^{b}	3.19^{f}	4.58^{c-f}	3.96^{g-k}	5.25^{d-g}	4.95^{e-h}	4.83	4.11
Control (Untreated)	3.74^{de}	2.66^{g}	3.68^{j-n}	3.89^{g-l}	4.90^{fgh}	4.31^{i}	4.42	3.88
Nitrogen (Fertilizer)	4.52^{b}	3.20^{f}	4.19^{f-j}	3.78^{i-m}	5.51^{b-e}	5.26^{d-g}	4.74	4.08
HSD (0.05)	0.48		0.60		0.56			

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., HSD = high range statistical domain; **=significant (P ≤ 0.01), * = significant (P ≤ 0.05) and ns = non-significant (P> 0.05)

Appendix 20. Inoculation response on grain yield of chickpea varieties at Chefe Donsa trial sites

	Year 2017		**Year 2018**		**Year 2019**		**Average (across years)**	
Treatment	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**
43P2S1 (*M. genospecies* 2A)	3.79^{abc}	2.79^{f-k}	1.97^{b-h}	2.26^{ab}	3.31^{a}	3.11^{abc}	3.02	2.72
90P4S2 (*M. genospecies* 4A)	3.54^{bcd}	2.35^{jk}	1.96^{b-g}	1.84^{fgh}	2.55^{g-j}	2.48^{h-k}	2.69	2.22
22P5S2 (*M. genospecies* 4A)	3.27^{cde}	2.53^{g-k}	2.03^{b-g}	1.91^{d-h}	2.47^{h-l}	2.41^{i-l}	2.59	2.28
2P3S1-b (*M. genospecies* 9A)	3.81^{ab}	2.99^{e-h}	2.39^{a}	2.42^{a}	3.11^{abc}	2.95^{cde}	3.11	2.79
ET1 (*M. genospecies* 8A)	2.95^{e-h}	2.81^{f-k}	2.16^{a-e}	2.15^{a-f}	2.25^{jkl}	2.20^{kl}	2.45	2.39
38P4S2 (*M. genospecies*1E)	3.42^{b-e}	2.64^{g-k}	1.83^{gh}	1.90^{e-h}	2.55^{g-j}	2.51^{h-k}	2.60	2.35
ET26 *(M. genospecies* 4A)	3.78^{abc}	2.88^{f-j}	1.87^{e-h}	1.93^{c-h}	2.39^{i-l}	2.25^{jkl}	2.68	2.35
45P4S1 (*M. genospecies* 1B)	4.23^{a}	2.30^{k}	2.24^{abc}	2.43^{a}	3.27^{ab}	2.99^{bcd}	3.24	2.58
80P4S2 (*M. genospecies* 3A)	3.83^{ab}	2.59^{h-i}	1.84^{fgh}	1.71^{g}	2.87^{c-f}	2.77^{d-h}	2.85	2.36
27P3S2 (*M. genospecies*7A)	3.68^{bcd}	2.38^{ijk}	2.03^{b-g}	2.04^{b-g}	2.88^{c-f}	2.93^{c-f}	2.86	2.45
Mean	3.63	2.63	2.03	2.06	2.77	2.66	2.81	2.45
Ha. Ata (Reference)	3.42^{b-e}	2.57^{g-k}	1.92^{c-h}	1.76^{fg}	2.66^{e-i}	2.64^{f-j}	2.67	2.32
EAL029 (Commercial)	3.71^{a-d}	2.40^{ijk}	2.05^{b-g}	1.97^{c-g}	3.02^{a-d}	2.91^{c-f}	2.93	2.43
Control (Untreated)	3.00^{efg}	2.48^{ijk}	1.70^{h}	1.70^{h}	2.43^{i-l}	2.16^{l}	2.66	2.59
Nitrogen (Fertilizer)	3.21^{def}	2.89^{f-i}	2.15^{a-f}	2.22^{a-d}	3.04^{a-d}	2.82^{c-g}	2.74	2.38
HSD (0.05)	0.53		0.32		0.31			

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., HSD = high range statistical domain; **=significant (P ≤ 0.01), * = significant (P ≤ 0.05) and ns = non-significant (P> 0.05)

Appendix 21. Inoculation response on grain yield of chickpea varieties at Alem Tena trial sites

	Year 2017		**Year 2018**		**Year 2019**		**Average (across years)**	
Treatment	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**	**Natoli**	**Arerti**
43P2S1 (*M. genospecies* 2A)	2.83^{a}	1.14^{mn}	1.63^{b-e}	1.64^{b-e}	2.01^{c-g}	1.87^{c-h}	2.13	1.52
90P4S2 (*M. genospecies* 4A)	1.73^{f-k}	1.17^{lmn}	1.33^{hi}	1.47^{e-g}	2.06^{b-f}	1.50^{i}	1.71	1.38
22P5S2 (*M. genospecies* 4A)	1.66^{h-k}	1.26^{lmn}	1.51^{d-h}	1.73^{a-d}	2.11^{b-e}	1.76^{e-i}	1.80	1.63
2P3S1-b (*M. genospecies* 9A)	2.41^{b}	1.42^{lmn}	1.55^{c-h}	1.71^{a-d}	2.20^{abc}	2.00^{c-h}	2.00	1.68
ET1 (*M. genospecies* 8A)	1.83^{e-i}	1.51^{i-l}	1.76^{abc}	1.46^{e-g}	1.94^{c-h}	2.02^{c-g}	1.84	1.66
38P4S2 (*M. genospecies*1E)	1.68^{h-k}	1.18^{lmn}	1.19^{i}	1.43^{e-i}	2.06^{b-f}	1.74^{f-i}	1.65	1.45
ET26 *(M. genospecies* 4A)	2.03^{c-f}	1.28^{lmn}	1.57^{b-g}	1.74^{a-d}	1.92^{c-h}	1.85^{d-i}	1.83	1.61
45P4S1 (*M. genospecies* 1B)	2.25^{bc}	1.80^{f-j}	1.33^{hi}	1.65^{b-e}	1.80^{d-i}	1.68^{ghi}	1.64	1.56
80P4S2 (*M. genospecies* 3A)	2.22^{bcd}	1.71^{g-k}	1.59^{b-f}	1.76^{abc}	2.46^{a}	1.92^{c-h}	1.96	1.70
27P3S2 (*M. genospecies*7A)	2.07^{c-f}	1.04^{n}	1.59^{b-f}	1.89^{a}	2.40^{ab}	2.00^{c-h}	1.95	1.57

Mean	2.07	1.35	1.48	1.62	2.06	1.8	1.85	1.58
Ha. Ata (Reference)	2.16^{b-e}	1.25^{lmn}	1.42^{e-h}	1.74^{a-d}	1.94^{c-h}	1.93^{c-h}	1.95	1.70
EAL029 (Commercial)	2.00^{c-h}	1.47^{j-m}	1.30^{hi}	1.36^{f-i}	2.13^{a-d}	1.65^{hi}	1.91	1.57
Control (Untreated)	1.73^{f-j}	1.06^{n}	1.33^{ghi}	1.65^{b-e}	1.95^{c-h}	1.67^{ghi}	1.78	1.54
Nitrogen (Fertilizer)	1.90^{d-h}	1.65^{i-l}	1.43^{e-g}	1.78^{ab}	2.07^{b-f}	1.83^{d-i}	1.80	1.75
HSD (0.05)	0.34		0.24		0.35			

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., HSD = high range statistical domain; **=significant (P ≤ 0.01), * = significant (P ≤ 0.05) and ns = non-significant (P> 0.05)

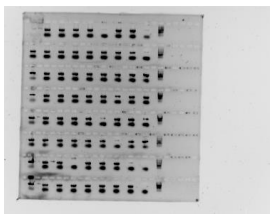
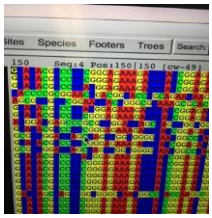
Appendix 22. Inoculation response on grain yield of chickpea varieties at Genda Gorba trial sites

Treatment	Year 2017		Year 2018		Year 2019		Average (across years)	
	Natoli	Arerti	Natoli	Arerti	Natoli	Arerti	Natoli	Arerti
43P2S1 (*M. genospecies* 2A)	2.43^{a}	1.47^{e-h}	1.81^{c-h}	2.26^{a}	2.49^{abc}	2.39^{a-f}	2.24	2.04
90P4S2 (*M. genospecies* 4A)	1.78^{cde}	1.49^{efg}	1.71^{ghi}	1.95^{a-g}	2.20^{b-i}	2.07^{e-j}	1.89	1.84
22P5S2 (*M. genospecies* 4A)	1.55^{d-g}	1.12^{h}	1.95^{a-g}	1.81^{c-g}	2.19^{b-j}	2.06^{f-j}	1.90	1.66
2P3S1-b (*M. genospecies* 9A)	2.10^{abc}	1.50^{d-g}	1.66^{ghi}	2.08^{a-e}	2.32^{a-g}	2.14^{d-j}	2.03	1.91
ET1 (*M. genospecies* 8A)	2.15^{ab}	1.49^{efg}	1.68^{ghi}	2.12^{abc}	2.36^{a-g}	2.23^{a-i}	2.06	1.95
38P4S2 (*M. genospecies*1E)	1.60^{def}	1.42^{e-h}	1.40^{ij}	1.91^{b-g}	2.27^{a-h}	2.14^{d-j}	1.76	1.83
ET26 *(M. genospecies* 4A)	2.20^{ab}	1.56^{d-g}	2.15^{ab}	1.69^{ghi}	2.15^{c-j}	2.31^{a-g}	2.17	1.85
45P4S1 (*M. genospecies* 1B)	2.16^{ab}	1.43^{e-h}	1.93^{b-g}	2.10^{a-d}	2.46^{a-d}	2.05^{f-j}	2.06	1.72
80P4S2 (*M. genospecies* 3A)	2.02^{bc}	1.47^{e-g}	2.15^{ab}	2.17^{ab}	2.55^{a}	2.16^{c-j}	2.24	1.93
27P3S2 (*M. genospecies*7A)	1.86^{bcd}	1.29^{fgh}	1.68^{ghi}	1.73^{fgh}	2.52^{ab}	1.92^{ij}	2.02	1.66
Mean	1.99	1.43	1.82	1.94	2.25	2.15	2.04	1.84
Ha. Ata (Reference)	2.13^{abc}	1.44^{e-h}	1.57^{hij}	1.65^{ghi}	2.16^{b-f}	2.03^{g-j}	2.08	1.84
EAL029 (Commercial)	2.10^{abc}	1.55^{d-g}	1.94^{b-g}	2.03^{a-f}	2.15^{c-j}	2.17^{b-j}	2.07	1.92
Control (Untreated)	2.77^{cde}	1.23^{gh}	1.32^{j}	1.78^{e-h}	1.95^{hij}	1.85^{j}	2.02	1.95
Nitrogen (Fertilizer)	2.11^{abc}	1.49^{efg}	1.79^{d-h}	2.14^{ab}	2.45^{a-d}	2.40^{a-e}	2.12	2.01
HSD (0.05)	0.36		0.31		0.34			

Means followed by the same letter within a column are not significantly different at (P>0.05) level of probability., HSD = high range statistical domain; **=significant (P ≤ 0.01), * = significant (P ≤ 0.05) and ns = non-significant (P> 0.05)

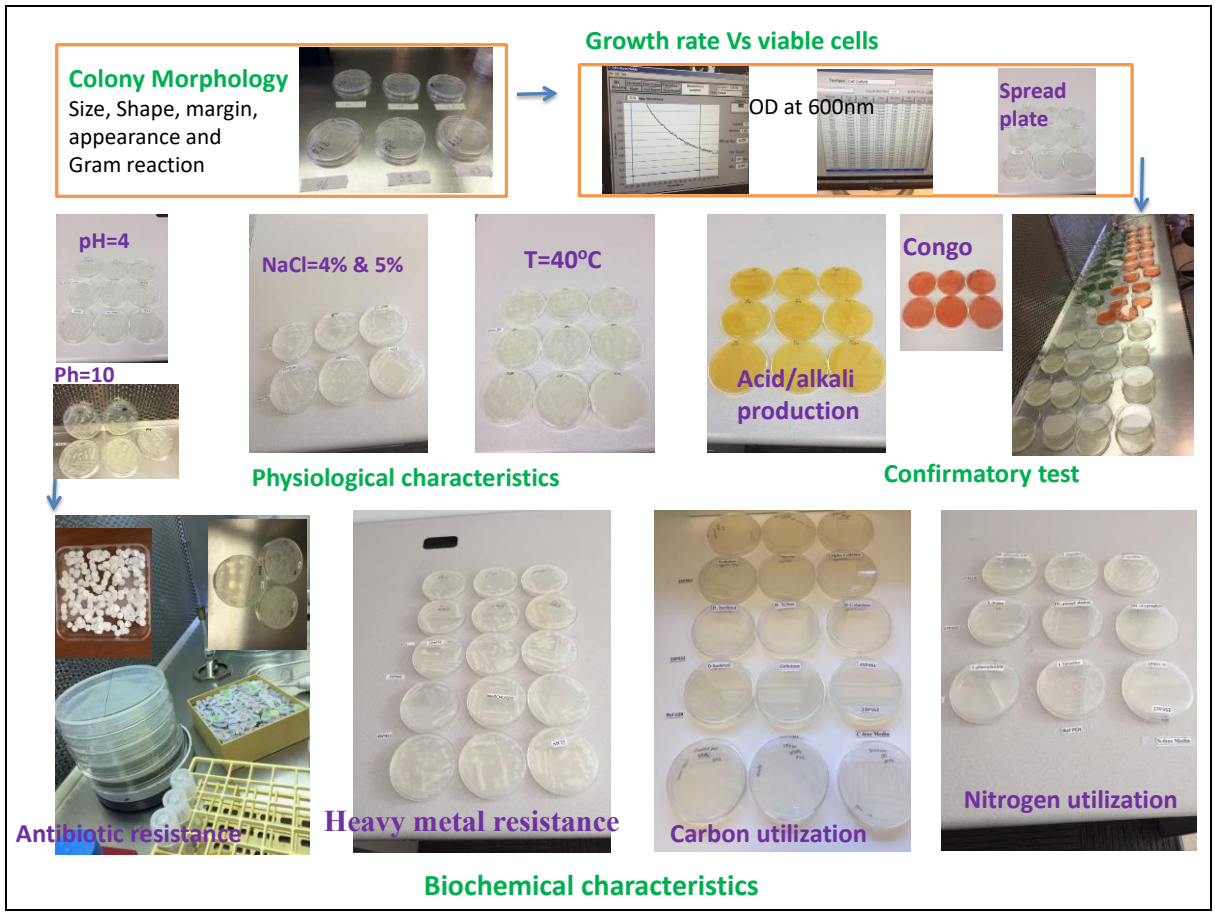
Appendix 23. Collection, genome diversity and symbiont strains practical application as visual presentation






Appendix 24. Eco-physiological competence analysis of diversity on *Mesorhizobium* strains



Appendix 25. Impact of *Mesorhizobium* strains inoculation on crop growth as assessed in split plot organization field trial


3
90P4S2 +P
5
80P4S2 +P
4
45P4S1 +P
1
Untreated
6