DETERMINATION OF BIODIVERSITY AND SYMBIOTIC EFFICIENCY OF NATIVE RHIZOBIA ISOLATED FROM DIFFERENT REGIONS IN KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree or other awards in any other University.

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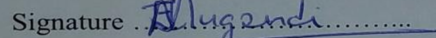
Signature  Date: 30/10/2020

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DEDICATION

I dedicate this work to my first great tutors of life. Firstly, my late father Sh. Mohamed Saied Jalloh. Secondly, I dedicate it to my lovely, adorable and caring mother Haja Fatimata Binta Jalloh who has always been of great motivation to me, your selfless devotion and sacrifice towards my studies and life will always be valued and remembered.

Furthermore, I dedicate this research work and findings to those of us who respect and love unity, peace, reconciliation, reunion and humanity around the entire world.

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ACRONYMS AND ABBREVIATIONS

µL	Microliter
AAS	Atomic Absorption Spectroscopy
ANF	Atmospheric Nitrogen Fixation
ANOVA	Analysis of Variance
BGBD	Below Ground Biodiversity
BLAST	Basic Local Alignment Search Tool
BNF	Biological Nitrogen Fixation
BTB	Bromothymol Blue
CIAT	International Center for Tropical Agriculture
CR	Congo Red Dye
CRD	Complete Randomized Design
DNA	Deoxyribonucleic acid
EPS	Extracellular Polysaccharide
ESI	Euclidian Similarity Indix
FAO	Food and Agriculture Organization
FES	Flame Emission Spectroscopy
gDNA	Genomic Deoxyribonucleic acid
GDP	Gross Domestic Product
GHG	Greenhouse Gas Emission
HSD	Tukey's Honest Significant Difference
LVB	Lake Victoria Basin
MEGA X	Molecular Evolutionary Genetics Analysis X

MLEE	Multilocus Enzyme Electrophoresis
NaClO	Sodium Hypochlorite
NCBI	National Center for Biotechnology Information
NJM	Neighbor Joining Method
PAST	Paleontological Statistics Software Tool
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PGPR	Plant Growth Promoting Rhizobacteria
pH	Potential of Hydrogen
QLAGEN	QIAquick PCR Purification Kit
RCBD	Randomized Complete Block Design
RDA	Redundancy Aanalysis
RNA	Ribonucleic acid
SAS	Statistical Analysis System
SyE	Symbiotic Efficiency
SNF	Symbiotic Nitrogen Fixation
SSA	Sub-Saharan African
TC-LAB KU	Tissue Culture Laboratory at Kenyatta University
UV	Ultra-Violet Radiation
W/V	Weight/Volume
YEMA	Yeast Extract Mannitol Agar
YEMB	Yeast Extract Mannitol Broth

ABSTRACT

Crop production has continued to decline in sub-Saharan Africa due to soil infertility and increased cost of farm inputs. To enhance food security, farmers have adopted the use of both inorganic and organic fertilizers on their farms. The production and use of inorganic chemical fertilizers are not only expensive for family farming systems but also contribute significantly to environmental pollution. Biological nitrogen fixation using rhizobia has proven to be a cost-effective and environmentally friendly alternative to inorganic nitrogen fertilizers. Rhizobia interact with legumes symbiotically, improving soil fertility and legume plants productivity. The present study aimed at determining the morphological and genetic diversity, cross-inoculation ability, and symbiotic efficiency of native rhizobia isolates in sterile and non-sterile soils. Greenhouse experiments were set to trap rhizobia from soil from smallholder farms from Kitui, Embu, and Tharaka Nithi Counties using cowpea as the trap host. The cowpea crops were harvested after one month, and a total of 311 nodule isolates were purified from the crop nodules. Based on morphological characteristics, the nodule isolates were clustered into 42 different groups. The effect of the soil on cowpeas nodulation was determined using redundancy analysis where soil characteristics including P, Zn, Mn, and total organic carbon correlated positively with cowpea nodulation and nodule dry weight. Soil pH and Ca correlated negatively with nodule number and weight. In addition, 53 glycerol stocks nodule isolates (archived samples) from previous studies were revived. Revived cultures were clustered based on morphological characteristics into 11 different groups. The isolates were tested for symbiotic efficiency using common bean, cowpea, green gram and soyabean seeds planted in sterile and non-sterile soils in the greenhouse. Un-inoculated plants were used as controls, while the treatments included the revived native isolates and commercial rhizobia inocula. The experiments were laid out in a completely randomized design. Plants grown in both sterilized and non-sterilized soils and inoculated with the different isolates varied significantly ($p<0.05$) in the shoot, root, and nodule dry weights. Some of the isolates, including; IsAS14, IsAS11, IsAS10, IsAMR6, IsAMR22, IsAMR23, IsAMR27, and IsAGR5 significantly ($P<0.05$) outperformed commercial isolates in influencing the plants growth parameters. Forty-six revived nodule isolates had the ability to infect, induce nodule formation and influence the growth of the non-original host. The best performing native rhizobia isolate IsAS14, IsAMR3, IsAMR27, IsAMR18 and IsAMR22 outperformed the commercial inocula in terms of symbiotic efficiency of 104.97 %, 136.86 %, 136.99 %, 138.88 % and 155.05 %. DNA from representative isolates was extracted using ZYMO research DNA^{TM} extraction Kit. The 16S rRNA region was then amplified using universal primers and sequenced using the same primers. Based on the sequencing of 16S rDNA of representative revived nodule isolates, most of the isolates were rhizobia but clustered with different species with the most dominant cluster being isolates related to *Rhizobium leguminosarum* and *Rhizobium etli*. Nodule number and nodule dry weight were dependent on soil physico-chemical characteristics. Rhizobia isolates also had the ability to form symbiotic interaction with non-original host legume crops. These rhizobia isolates can be used as bio-inoculants to improve the production of the different legumes, and to enhance food security.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

One of the pillars that are usually observed and documented by the International Human Rights is the right to food for humankind (Khoury *et al*., 2014; Riches, 2016). However, the goals laid down by the World Food Summit in 1996 that aimed at decreasing hunger have not offered optimum solutions to food security and problems related to it. It is estimated that about 858 million people globally are suffering from malnutrition, and the most significant percentage are in Africa and Asia (Young, 2016). Agriculture is crucial in ensuring the world's food security (Grafton *et al*., 2015). In Kenya, agricultural crop production plays a vital role in the economy. Crop farming accounts for approximately 29.3 % of the country's Gross Domestic Product (GDP), and three-quarter (3/4) of the Kenyan population depends on agricultural farm products for income and food (FAO, 2014; Bruinsma, 2017; Schmidt *et al*., 2017).

One of the significant crops for the Kenyan population are legumes, with Kenya's annual productions of legume crops being ranked at position eight globally (Akibode and Maredia, 2012). Soyabean (*Glycine max* (L.) Merril), common bean (*Phaseolus vulgaris* L.), green gram (*Vigna radiata* (L.) Wilczek) and cowpea (*Vigna unguiculata* L. Walp) form one of the primary sources of protein for low-income rural populations. These legumes are ranked second (2nd) after maize as the staple food in the country. Legumes are important to human health as they are of nutritional value in the

form of vitamin, lysine, folic acid and insoluble fibre to the diet (Maphosa and Jideani, 2017).

Despite the constant collapse of strategies set in addressing food security, scientists, government policymakers, and family farmers have adopted different plans and strategies that aimed at solving food security, soil fertility and other related problems. Improving legume plants production is among the key steps taken to amend food security (Kang *et al.,* 2017). One such legume plants production mechanism being adopted is the sustainable use of microorganisms, such as rhizobia to improve soil fertility (Muthini *et al*., 2014; Raimi *et al*., 2017; Tanveer *et al*., 2019). Soil microorganisms such as; bacteria, viruses, algae, fungi, and protozoa inhabit a broad range of the soils and make up the below-ground biodiversity (BGBD) (Abhilash *et al*., 2016). The *Rhizobium* species (sp.) and *Bradyrhizobium* sp. are the most prevalent soil microorganisms which provide critical services in the functioning of agricultural ecosystems (Meghvansi *et al*., 2010; Lu *et al*., 2017; Ntambo *et al*., 2017). These groups of bacteria individually add to the soil fertility through cycling of nutrients incuding; phosphorus, carbon, manganese, magnesium, potassium and nitrogen, thereby, improving the production of legume plants and other crops (De Jager *et al*., 2017).

Rhizobia are soil-borne microorganisms that associate symbiotically with legume crops where they take part in biological nitrogen fixation (BNF) (Lu *et al*., 2017). From the association, rhizobia receive nutrients from the legume plants and fix atmospheric nitrogen (N_2) to ammonia via BNF. Rhizobia need host plants for N_2 fixation because

they cannot fix N_2 alone (Martínez-Hidalgo *et al*., 2017). The infection thread in the plant roots and the nitrogenous enzymes play a critical role in nodule formation where fixation of nitrogen occurs (Dunn, 2015; Janczarek *et al*., 2015). Subsequently, the legume benefits from the rhizobia by getting a constant supply of nitrogen (Rashid *et al*., 2015). In return, the plants supply energy, nutrients and house the bacteria. Other mechanisms exist for the conversion of nitrate into ammonia, but the major conversion of nitrates into ammonia is achieved by soil microorganisms through BNF (Olivares *et al.,* 2013). Rhizobia consist of 13 genera classified into 98 species (Weir, 2008), with a large number of these rhizobia belonging to the genera *Mesorhizobium*, *Bradyrhizobium*, *Sinorhizobium* (*Ensifer*), and *Rhizobium.* The diversity of rhizobia changes greatly due to geographical location, with key factors such as soil pH and acid exchangeable (De Castro Pires *et al*., 2018; Diaz *et al*., 2018). Rhizobia biodiversity is mainly found in tropical regions (Van cauwenberghe *et al*., 2015).

The nodulation processes of legume crops by rhizobia include all the diverse genera; *Rhizobium tropici*, *Sinorhizobium melitoli*, *Rhizobium gallicum*, *Rhizobium etli*, *Rhizobium leguminosarum*, *Bradyrhizobium elkanii*, and *Rhizobium phaseoli* (Van Cauwenberghe *et al*., 2015; Rosolem *et al*., 2017; Lu *et al*., 2017). It is hence vital to mention that legume crops production depends mainly on the amount of nitrogen content in the soil. The symbiotic interaction between rhizobia bacteria and the plant roots are more efficacious when environmental conditions are favourable for the rhizobia strains at a temperature between 25 °C - 30 °C (Van Cauwenberghe *et al*., 2015). The rhizobia inocula are believed to be more effective, productive, and

competitive compared to the native rhizobia in the soil (Diouf *et al*., 2019). Nitrogen-fixing bacteria are eco-friendly; they aid in the recovery from environmental degradation and enhance soil biodiversity (Adhikari *et al*., 2013). The requirement of nitrogen in the soil is in higher demand as compared to other soil elements for agricultural productivity and environmental sustainability (Eickhout *et al*., 2006).

In several studies, the inoculation of legume plant roots with rhizobia strains as a biofertilizer has been shown to increase the amount of nitrogen in the soil, reduce environmental instability and increase rhizobia-legumes symbiosis hence improving soil fertility and stability (Muthini *et al*., 2014; Bhardwaj *et al*., 2014; Abhilash *et al*., 2016; Korir *et al*., 2017). According to Nadeem *et al*. (2014), a symbiotic association between legume crops and rhizobia can be more effective if the conditions of the soil, and the environment are favourable for the survival of the bacterial strain. For high efficiency, the bacteria inoculum should be more competitive in the soil and adapted to the environment as compared to the native rhizobia from the soil (Rosolem *et al*., 2017; Diouf *et al*., 2019). Through symbiosis, crops such as; *Phaseolus vulgaris, Glycine max*, *Vigna unguiculata* , and *Vigna radiata* have contributed majorly in improving soil fertility, prevention of nutrient leaching and augmentation of soil organic matter through symbiotic nitrogen fixation (SNF) processes (Kawaka *et al*., 2014; Anglade *et al*., 2015).

1.2 Statement of the problem

There have been re-occurring severe cases of food insecurity in Kenya in the past decades (FAO, 2014; Matusso *et al*., 2014). This phenomenon has been linked to frequent droughts, lack of technical and technology facilities, pest and diseases, declining soil fertility, poor farm practice methods, and decreasing agriculturally productive lands (Gichangi *et al*., 2012). Population growth in Kenya has led to land fragmentation which has exerted pressure on the agriculturally viable lands causing exhaustion of available soil nutrients (Willy *et al*., 2019). To address the food insecurity problems, the Kenyan government, scientists, farmers, and other stakeholders have developed interests in different crops production mechanisms (Alila and Atieno, 2006; Rarieya and Fortun, 2010).

Soil fertility improvement is one such crop production mechanism where the focus has been laid (Andersson and Gabrielsson, 2012). However, the methods used to improve and recover soil fertility, for example, the use of inorganic chemical fertilizers have introduced numerous negative reactions and effects, resulting in the destruction of soil biodiversity and the soil environment as a whole. Some of the effects of the poor family farming systems practices have been further manifested through greenhouse gas emission (GHG), poor water quality, human health problems, waterlogged soil, increasing soil acidity and salinity (Jansa *et al*., 2011; Mutuma *et al*., 2014). These aspects are triggered by the failure of small-scale farmers to access affordable biofertilizers and end up using inorganic fertilizers. Apart from poor farming systems, food security and safety problems in Kenya have been linked to the absence of vital

information and the potential use of BGBD in the production of crops (Mwangi *et al*., 2011). Thus, there is a need to understand the symbiotic efficiency and biodiversity of indigenous rhizobia isolates with their effects on legume crops production.

1.3 Justification

Soil harbours numerous microorganisms (Prasanthi *et al*., 2019), which drive plant diversity, productivity, acquisition of nutrients, increase and enhancement of crop health (Ndungu *et al.*, 2018). Low soil fertility resulting from poor farming systems practices is a critical problem facing farmers in Kenya and sub-Saharan Africa (SSA) (Williams *et al*., 2016). Specifically; soil degradation, land scarity and fragmentation, soil erosion, extreme weathering, poor soil practice methods (overproduction of staple crops) and constant farming are the contributing factors that decrease soil fertility and food insecurity. In addition, frequent droughts is a climatic natural factor that affect food production and BGBD. Rhizobia are important soil nitrogen fixing bacteria (NFB) when utilized well can improve agricultural crop production, soil fertility and soil stability. However, the knowledge of the composition of these NFB is relatively scarce (Ntambo *et al*., 2017). Therefore, the symbiotic efficiency of rhizobia isolates and their genetic diversity need to be assessed.

Knowledge of the symbiotic efficiency of rhizobia isolates in the country is helpful in the optimization of family farming systems. This knowledge can be applied through the use of technology such as rhizobia inoculation. Determining the performance of rhizobia isolates in natural soils is essential to pick out the genotypes of the plant which improve

populations of advantageous rhizobia. The rhizobia may confer defence against pathogens, diseases and abiotic factors (Coleman-Derr and Tringe, 2014; Vargas *et al*., 2017; Volpiano *et al*., 2019). Application of inorganic chemical fertilizers have attracted negative reactions which affect soil biodiversity and the environment as a whole, leading to GHG emission, poor quality of water, human health problem, waterlogged soil, increased soil acidity and salinity (Wall *et al*., 2015; Prasanthi *et al*., 2019). The use of BNF helps to prevent eutrophication in lakes and rivers, formation of acid rain, and overgrowth of agricultural land by non-food crops that is caused by excessive application of conventional nitrogenous fertilizers (Chemining'wa *et al*., 2018). The use of rhizobia inoculum has been hypothesized as one of the intervention measures that can be incorporated into crop production practices to promote soil fertility and improve crop production.

In this regard, the screening for the symbiotic efficiency of rhizobia isolates becomes important. Information regarding rhizobia efficiency can contribute significantly in areas of agriculture and biotechnology, leading to increased crop production, soil fertility and stability. Determination of the native rhizobia biodiversity is also essential as it presents a basis on which biotechnological tools for soil fertility and improved crop productivity can be developed. This study was therefore carried out to determine the biodiversity and the efficiency of rhizobia isolates from different parts of Kenya, so as to come up with information concerning the biodiversity and symbiotic efficiency of native rhizobia in different parts of Kenya.

1.4 Hypotheses

i. Native rhizobia isolated from different regions in Kenya have different morphological and genetic characteristics.

ii. The symbiotic efficiency of indigenous rhizobia isolates collected from different regions in Kenya are significantly different in sterile soils.

iii. Native rhizobia isolates from different regions in Kenya have different effectiveness in nodule formation in non-sterile soils.

iv. Cross-inoculation with native rhizobia bacteria isolates affects growth parameters of common beans, cowpeas and green grams.

1.5 Objectives

1.5.1 General objective

Determination of the morphological and biochemical characterization, genetic diversity, symbiotic efficiency of rhizobia cultures in sterile and non-sterile soils and cross-inoculation in enhancing common bean, cowpea, green gram and soyabean production in Kenya.

1.5.2 Specific objectives

i. To isolate and characterize native rhizobia bacteria from different regions of Kenya.

ii. To determine the symbiotic efficiency of indigenous rhizobia isolates in sterile soils collected from different regions of Kenya.

iii. To determine the effectiveness of native rhizobia isolates in nodules formation in non-sterilized soil.

iv. To determine the effects of cross-inoculation with different native rhizobia isolates on growth parameters of common beans, cowpeas, and green grams.

1.6 Significance of the study

The results of this study will help in improving crop productivity and soil fertility through inoculants that reduce reliance on the use of inorganic chemical fertilizers. High crop production will translate to a higher income for smallholder farmers. Hence, it will contribute to poverty reduction, job availability and enhance food security. Effective native rhizobia strain with higher nodulation, shoot dry weight and SNF efficiencies will be used for bio-inoculant development and production that can be availed to small-scale farmers in many parts of the country and SSA. This information will help adopt environmentally friendly technologies of crop production that are sustainable and affordable to smallholder farmers. Furthermore, information on the diversity of rhizobia associated with legume crops in Kenya will be added to the data currently available, and the most effective rhizobia isolates will be recommended and documented as biofertilizer. In addition, it will help to decrease chemical fertilizer application in the soils, decreasing GHG emissions, reducing water pollution, improving the ecosystem and providing a better environment. Information of genotypic diversity of rhizobia isolates via 16s rRNA gene sequencing of the native isolates obtained, will be added to National Center for Biotechnology Information (NCBI).

CHAPTER TWO

LITERATURE REVIEW

2.1 Rhizobia diversity

Rhizobia are highly diverse in tropical regions where they thrive in the soil as free-living microorganisms or indigenous inhabitant (Pongslip, 2012). The rhizosphere contains a diverse number of rhizobia that take part in biochemical reactions in the soil (Morgan *et al*., 2005; Prashar *et al*., 2014). Some of the activities include but not limited to toxins removal, organic matter decomposition, nutrients cycling; carbon, sodium, potassium, calcium, phosphorus, iron and as well as nitrogen (Minalku *et al*.,, 2009). Rhizobia are aerobic, Gram-negative, rod-shaped, non-endospore forming, and free-soil living bacteria. They are found in the soil as native inhabitants or introduced in the agricultural soils as inocula. The rhizobia co-exist in the soils with their native host plants (Aserse *et al*., 2012; Alori *et al*., 2017; Koskey *et al*., 2018; Zhong *et al*., 2019).

Th diversity of rhizobia has been proven to differ considerably from one geographical region to another (Anyango *et al*., 1995; Aserse *et al*., 2012). Apart from geographical location, some other vital and key elements, for instance, soil pH, temperature, microelements, and acidity also influence the diversity of the rhizobia. As emphasized by Anyango *et al.* (1995), the distribution of rhizobia nodules varies with soil pH. Also, it has been revealed that soil management practices, in addition to the aforementioned soil properties, alter rhizobia diversity and abundance (Andrade *et al*., 2002). In the soils, rhizobia species can also form a symbiotic association with roots of the host

plants. Their potential to form a beneficial symbiotic relationship with the plants makes them important and essential to agricultural soils and the ecosystem. Indigenous rhizobia diversity has attracted a lot of interest from researchers across the globe due to their importance in increasing soil fertility, crop productivity and other environmental benefits (Agrawal *et al*., 2012).

Various methods of rhizobia diversity identification and enumeration have been developed. Identification and enumeration have been traditionally based on morphological, physiological and biochemical methods. These methods present or led several weaknesses whereby they fail to identify strains within a species. This challenge leads to the need for the development of serological and molecular methods (Berrada *et al*., 2012). These methods have been designed to gather data that is generated from phenotypic and genotypic characteristics. The methods include; classical phenotypic analysis and numerical taxonomy, whole-cell protein analysis (DNA-base composition), multilocus enzyme electrophoresis (MLEE), pulsed field gel electrophoresis (PFGE), analysis of cellular fatty acids- FAME, DNA-DNA reassociation, (insertion sequence) IS typing, restriction fragment length polymorphism (RFLP), genome fingerprinting using amplified fragment length polymorphism(AFLP), rep-PCR, randomly amplified polymorphic DNA (RAPD), arbitrarily primed PCR and DNA amplification fingerprinting (DAF) (Weir, 2008). In spite of the advanced methods, currently, rhizobia enumeration and diversity measurement (Biochemical, physiological and morphological methods) often fail to give an exact description of the species with certain strains (Prasanthi *et al.*, 2019).

In addition, soil factors and the trapping host plants can lead to poor estimation and identification of various rhizobia strains (Somasegaran and Hoben, 1994). To classify and identify rhizobia, morphological features must be noted in relation to colours and shape; border elevation, mucosity, creamy, milky, white, texture, raised, watery colonies with convex elevation, and exo-polysaccharide gum production (Berrada *et al*., 2012; Kawaka *et al.*, 2014; Muthini *et al*., 2014). Identification and characterization methods of rhizobia strains can be done best via molecular approaches, for example, 16S rRNA gene, RAPD, RFLP, and other specific gene primers sequencing (Bloemberg and Lugtenberg, 2001; Majeed *et al*., 2015). However, rhizobia diversity is still not well understood (Savvas *et al*., 2017). Native rhizobia diversity is hypothesized to differ in various parts of the ecosystem. The growth and development of rhizobia inoculants is a possible breakthrough mechanism for the exploitation of these microbes.

2.2 Rhizobia taxonomy

Rhizobia are taxonomically diverse and phylogenetically heterogeneous. They comprise the beta group and the alpha group. The classification order of rhizobia includes: class *Alphaproteobacteria;* family *Rhizobiaceae,* order *Rhizobiales,* genus *Rhizobium* and species types are *Rhizobium phaseoli* (Valdes-Ramirez, 1995; Weir, 2008). Presently, ninety-eight (98) species of rhizobia with the potential to nodulate with legume roots plants have been discovered among thirteen (13) genera; two (2) of the genera belonging to *Betaproteobacteria* (*Burkholderia* and *Ralstonia*), and the remaining eleven (11) are placed in the class *Alphaproteobacteria* (*Rhizobium, Cupriavidus*, *Methylobacterium*, *Devosia*, *Sinorhizobium* (*Ensifer*), *Microvirga*, *Bradyrhizobium*, *Mesorhizobium*,

Allorhizobium, *Shinella*, and *Azorhizobium*) (Gyaneshwar *et al*., 2011). Different genera of rhizobia exhibit different potentials for nitrogen fixation and their ability to infect, induce, and competitively occupy root nodules differs significantly (Archana, 2010; Karimi *et al*., 2019). For example, *Rhizobium* sp. and *Bradyrhizobium* sp. have the potential to fix nitrogen with legume crops (Muthini *et al*., 2014; Koskey *et al*., 2017). *Bradyrhizobium* (*B*) species that nodulate with soyabean crops are; *B. liaoningeense, B. elkanii, B. huanghuaihaiense, B. japonicum,* and *B. diazoefficiens B. dinitrificans, B. cytisi* while, *Burkholderia*, *Rhizobium etli*, *Sinorhizobium meliloti* and *Rhizobium leguminosan* nodulates with cowpea, climbing bean, green gram, alfalfa (*Medicago sati*va L.) and common bean plant roots (Guiñazu *et al*., 2010; and Abaidoo *et al*., 2007).

2.3 *Rhizobium* nitrogen (N_2) fixation

The atmosphere contains approximately 80 % of N_2 gas. This gas exists in the form that cannot be utilized by a large number of living organisms and plants. The N_2 gas is of great importance to biotic life, and its deficiency can lead to the death of both plants and animals (Stein and Klotz, 2016). The gas is a critical component in the synthesis of amino acids, proteins, some nitrogenous organelles and nucleic acids which are vital for both plants and animals (Okumoto and Pilot, 2011). Biological Nitrogen fixation in plants occurs when the legume crop roots are infected by rhizobia which induce the formation of nodules. The bacteria in the nodules then fix atmospheric N_2 to ammonia, providing the crops with the nitrogenous substance via *Rhizobiaceae* or *Alphaproteobacteria* (Mendonça *et al*., 2017). Different genera of rhizobia can fix N_2

differently. They achieve this due to variation in the ability to infect the legume root hairs via infection thread and occupy nodule in different ways (Karimi *et al*, 2019). However, for the process of N_2 fixation to occur, rhizobia sp. most find a suitable niche to multiply and develop. And for exchangeable of signal molecules between the root hairs of the plant and the rhizobia cells (Rosolem *et al*., 2017). The plant roots and rhizobia mutual relationship contributes approximately 2.268 kgs nitrogen per acre (Masson-Boivin and Sachs, 2018).

When inoculated in the seedling, the cells of the rhizobia penetrate and enter the root hairs of the plants during the infection stage. They colonize the roots hair increasing the cell numbers that form nodules. Inside the nodule, the bacteria can convert atmospheric N_2 into a form that can be utilized by the plant (Masson-Boivin and Sachs, 2018). *Bradyrhizobium japonicum* has been considered as the most active strain in N_2 fixation and nodulate best with soyabean roots. This increases the nitrogen content in the soil hence enhancing soyabean productivity (Torres *et al*., 2018). The geographical distribution of rhizobia which nodulate legume plants vary due to climatic conditions, genetic diversity and legume crops varieties (Van Cauwenberghe *et al*., 2015).

Nitrogen supply in the soil needs to be stable in order to attain adequate yields in crop productivity, food security, soil fertility and stability (Mwangi *et al*., 2011). In Kenya, high costs of chemical fertilizer lead to decreasing soil fertility, and reduction in soil farm size are some of the limiting factors to optimum crop productivity by family farming systems (Abaidoo *et al*., 2007). Legume-rhizobia interaction have been shown

to fix approximately 20 million tons of N_2 fixed to agro-ecological soils annually by BNF (Bationo *et al*., 2007). It has therefore been hypothesized that nitrogen fixation can be achieved maximally by the introduction of effective native rhizobia strains either as commercial biofertilizer, inoculants or indigenous soil microbes (Owen *et al*., 2015).

As much as the process of inoculation of bio-inoculants in agricultural soils is not universal, in Kenya and SSA. These has led to poor farm practices, due to the application of different chemical fertilizer which reduce the BGBD and destroy soil composition and texture. (Owen *et al*., 2015; Alori *et al*., 2017). However, field application of native rhizobia strains as biofertilizers for *Vigna unguiculata*, *Vigna radiata*, *Phaseolus vulgaris*, *Glycine max* and other related legume crops in Bangladesh, USA, South American and China has been fruitful and successful, leading to increase in soil nitrogen fertility and food security (Davies and Moore, 2016). It is, therefore believed that the application of rhizobia inocula as biofertilizers in various ecological zones in Kenya could enhance the growth and yield of plants (Ndungu *et al*., 2018).

2.4 Interaction and role of rhizobia-legume root hairs

Rhizobia are some of the most abundant soil microbes in the rhizosphere that can either be beneficial, neutral or have detrimental effects on the plant roots (Kumar *et al*., 2015; Pii *et al*., 2015). The deleterious rhizobia that occur naturally within the rhizosphere soil environment are toxigenic and can’t fix N_2 (Sureshbabu *et al*., 2016). They are therefore categorized as parasites to the plant roots. The plant provides them with nutrients, and the parasites reside within the nodule of the roots for protection (Diaz *et al*., 2018).

These rhizobia sp. are not only parasitic but have the potential to suppress weed growth, compete for nutrients as well as produce metabolites like phytotoxins (Jha *et al.*, 2015). The neutral rhizobia, unlike the deleterious one, have no effect. They live in mutual relationship with the plant roots nodule. The beneficial rhizobia, commonly known as plant growth-promoting rhizobacteria (PGPR) have the ability to enhance and promote the growth of the plant (Dorjey *et al.*, 2017). They achieve this due to variation in the ability to infect the legume root hairs via infection thread and occupy nodule in different ways via BNF (Karimi *et al*, 2019).

2.5 Symbiotic interaction of rhizobia and legumes

Rhizobia have the potential to transform atmospheric N_2 into ammonia (NH_3), enabling plants to use the available form of NH_3 via symbiotic nitrogen fixation (Figure 2.1) (Stajkovic *et al.*, 2011). Rhizobia form symbiotic relationships with legume plant by infecting the root hairs. This form of association contributes immensely to the regain of lost soil fertility (Morad *et al.*, 2013). The root hair curt and epidermal cells root are part of the plant that is infected. Rhizobia form nitrogen-fixing nodules via two nitrogenase enzymes. This entails a complex process that is encoded by *nif* genes (Jida and Assefa, 2011). In the process, N_2 is reduced to NH_3 anions by nitrogenase enzyme and hence converted to nitrogenous form. The nitrogenous forms are then made available to the plants (Lu *et al.*, 2017). Phosphorus availability in the soil plays a key role in BNF (Shamseldin *et al.*, 2007). The rhizobia *nod* genes are switched by plant root-cell exudates; isoflavones and flavones with a different compound that has *nod* ABC genes that are present in all rhizobia (Okazaki *et al.*, 2016).

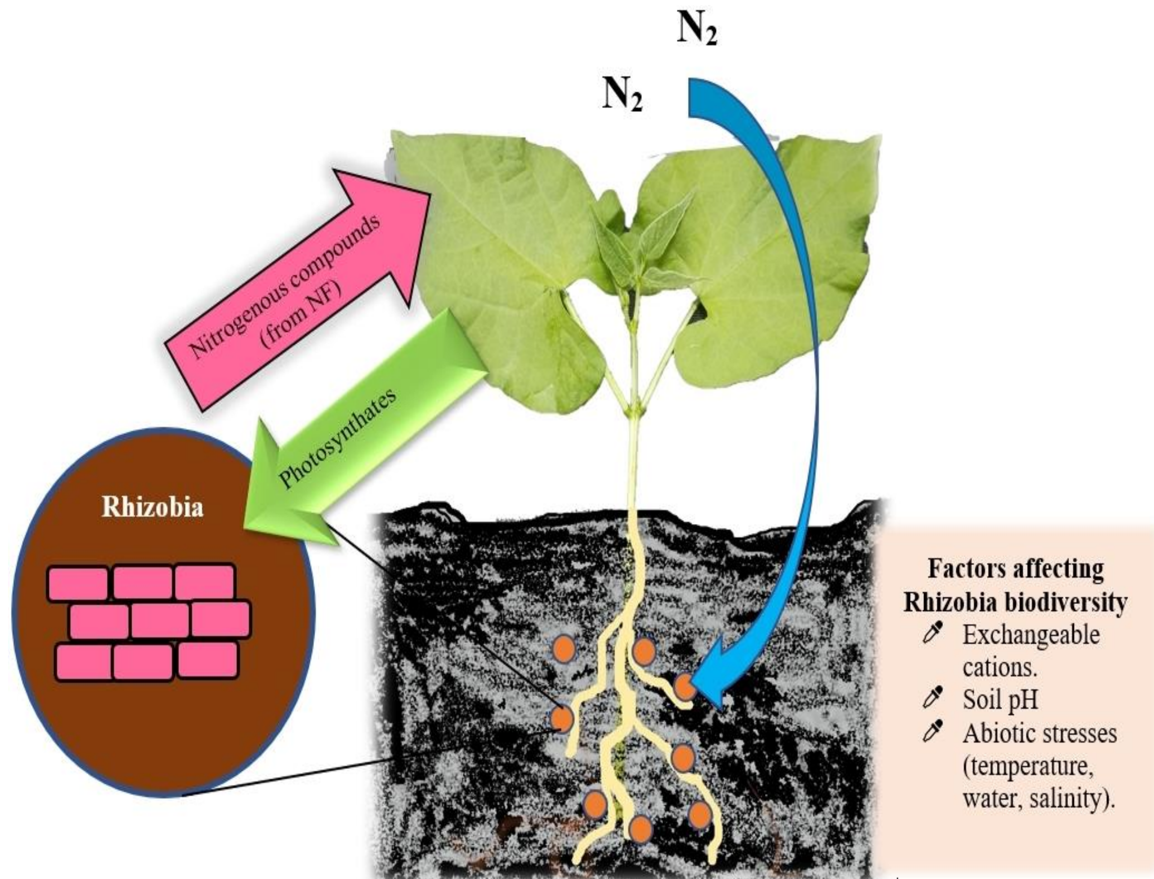

Figure 2.1: Rhizobia-legume symbioses increase soil nitrogen and enable the plants to synthesize nitrogenous compounds. In return, the rhizobia obtain photosynthates from the plants. Native rhizobia biodiversity is largely affected by environmental factors

However, it has been confirmed that rhizobia can fix nitrogen with legume crops and it accounts for approximately 40 % of the nitrogen produced globally (Damiani *et al*., 2016). This process is important and essential in nature. It increase agro-ecosystems soil fertility and sustainability, resistance to abiotic stresses, phosphorus solubilization and enhance food security. In addition, it reduce global warming, environmental pollution and also play a positive role in minimizing chemical fertilizer application by family farming systems (Hérouart *et al.,* 2002, Muthini *et al*., 2014; Kang *et al*., 2017).

2.6 Factors that affect biological nitrogen fixation (BNF)

The mechanism of interaction between rhizobia and the roots of the host is further complicated by other aspects that range from biotic, climatic and edaphic factors. These factors have been shown to significantly affect the process of BNF (Kondo and Yasuda, 2003; and Pii *et al*., 2015).

2.6.1 Edaphic factors

These factors include; drought, excessive soil moisture, phosphorus deficiency, acidity, excess soil nitrogen, waterlogging, and the deficiency of soil minerals (Abubakari and Abubakari, 2015). Water stress in dry season triggers a reduction in the number of potential rhizobia in the soils inhibiting the formation of nodules and N_2 fixation. Persistent drought has been revealed to support the decay of nodules (Anglade *et al*., 2015). Proper development of root hair and nodulation sites are altered during waterlogging seasons. Waterlogging also alters the diffusion of oxygen in the root system of the plant (Smith *et al*., 2019). A soil pH range of 6.0 - 7.0 has been shown to be the optimum pH for rhizobia growth. Any pH above or below the optimum can negatively affect the relationship between the two symbionts (Faoro *et al*., 2010). Low soil pH and other related problems are known to adversely affect nitrogen fixation, nodulation, plant growth and development (Andrade *et al*., 2002). However, some studies have shown the presence of acid-tolerant rhizobia that naturally exists in the soil (Ferguson *et al*., 2013; and Chemining'wa *et al*., 2018). These rhizobia are critical when identified as they can be used in the inoculation of legumes especially where the soil is acidic to enhance plant productivity (Peix *et al*., 2015).

In SSA, deficiency of phosphorus (P) is a common soil characteristic. Low-P in the soil negatively affects nodule formation, N_2 fixation and diminishes crop growth and productivity (Pii *et al*., 2015; Ndungu *et al*., 2018). In addition, inorganic (mineral) nitrogen content in the soil above the optimum has been shown to interfere with the infection process by *Rhizobium* sp. which limits nitrogen fixation (Gicharu *et al*., 2013). This is caused by the impairment of the recognition process caused by the nitrates (NO_3-) as well as the diversion of photosynthates towards the assimilation of nitrates (Rondon *et al.,* 2007).

2.6.2 Climatic factors

Light and temperature significantly affect the process of BNF. Temperature above or below the optimum is known to adversely affect nitrogen fixation. It interrupts the enzymatic process involved during BNF (Kondo and Yasuda, 2003). Comparatively high-root temperatures have been demonstrated to affect rhizobia infection, N_2 fixation potential and plant growth (Faoro *et al*., 2010). It appears to have a great impact on rhizobia strain and cultivar interactions. Inadequate rainfall in agricultural soil may lead to a high temperature which can reduce the number of native rhizobia present in that area. In addition, extreme temperatures above 35 ºC may cause *Rhizobium* sp. and *Bradyrhizobium* sp. to die. The presence of light standardizes the process of photosynthesis, which consequently impacts BNF (Balasubramanian *et al*., 2004). On the contrary, some normal environmental components can significantly increase the number of native rhizobia present in agricultural soils (Prashar *et al*., 2014; De Castro Pires *et al*., 2018). Rainfall is one such component that has been revealed to positively

influence the population of rhizobia in the soil (Roesti *et al*., 2006). The bacteria (rhizobia) survive and grow best in soil temperature between 25 °C to 30 ℃ (Bakhshayeshi *et al.,* 2006).

2.6.3 Biotic factors

The deficiency of essential new (novel) rhizobia species and native strains in the soil is one of the major limitations of the process of BNF. To solve this, specific rhizobia are introduced in the soil through inoculation, especially in cases whereby they are in low numbers or completely missing. However, in cases whereby the rhizobia present in the rhizosphere have the potential to colonize and nodulate with legume roots hair plants but have no potential to fix nitrogen, then it becomes a limitation to the successful exploitation of rhizobia inoculants (Shamseldin *et al*., 2007). This, therefore, informs us that the inoculated rhizobia must be more competitive and aggressive nodulators as compared to the native strains. Insects; pests, birds, nematodes and other diseases caused by microbial pathogens affect the host plants physiology; thus, altering nodule development and functions (Mmbaga *et al*., 2014).

2.7 Legume-rhizobia inoculation

Legume inoculation has become popular as an agricultural practice with a positive influence on soil fertility, nitrogen fixation and plant yield (Muthini *et al*., 2014; Koskey *et al*., 2017; Ndungu *et al*., 2018; Dejene *et al*., 2018; Zhong *et al*., 2019). Inoculation presents a means via which rhizobia isolates carefully selected in the laboratory can be transferred to the plants growing in the field or in the greenhouse. Legume-rhizobia

inoculation is achieved either through the coating of the seeds with sugar during planting or applied directly on the soil or granular, or powder based inoculant. Direct application into the soil via the seedlings is carried out four days after planting. Legume-rhizobia inoculation contributes significantly to induce nodulation, nitrogen fixation, soil fertility, productivity of plant and reduce food insecurity (Rahmani *et al.*, 2011). Inoculating common bean seedlings with rhizobia strains has greatly increased the nodulation, plants health development and yield (Morad *et al*., 2013). In addition, indigenous rhizobia isolate strains were verified and tested for their efficiency and compatibility on the formation of nodules on common beans, cowpeas, green grams, and soyabeans (Oliveira *et al*., 2017; Mathobo *et al*., 2017; Meena *et al*., 2018).

Although legume inoculation is currently believed to be a successful agricultural practice, there are still a number of challenges that face these mechanisms of agricultural plants production. One such challenge that has been documented, is the inability of the inocula applied to influence nodulation. This challenge has been associated with competition between the inocula and the native soil bacteria whereby, the native bacteria have been shown to out-compete the inoculant strains. The inoculants are therefore hindered from occupying a larger part of the root nodule (Meghvansi *et al*., 2010). Elsewhere, factors that include, but not limited to poor management practice, water stress, low soil pH, pests, and diseases have been reported to negatively affect BNF. Low water supply in the soil affects the survival of rhizobia which eventually disrupts the nodule formation process. It is important to mention that deficiency in phosphorus, total organic carbon, potassium, iron, sodium, manganese, and calcium,

which is characteristic of tropical soils, may negatively affect the formation of nodules by most rhizobia species (Mwangi *et al*., 2011). However, family farming systems are encouraged to use bio-fertilizers instead of inorganic fertilizers which has negative adverse effects and reactions on agricultural soils, the environment and causes water pollution.

2.8 Legume crops characteristics

2.8.1 *Phaseolus vulgaris* L.

Phaseolus vulgaris L. (common bean) are legume herbaceous crops, which are classified under the family of *Leguminosae*, which belong to the subfamily *Papilionoideae*, tribe *Phaseoleae*, sub-tribe *Phaseolinae* and under the section of *Phaseolus* (Code, 2008, muthini *et al*., 2014). Common bean is mainly grown and cultivated for edible dry seeds, green pods and commercial purpose for export and consumption worldwide. However, common bean varieties are different in shape, colour, pod fibrousness size and taste (Ssekandi *et al*., 2016). The growth habits of common bean differ greatly in climatic conditions, drought areas, soil infertility, and diseases in both sub-tropical and tropical regions (Darkwa *et al*., 2016). Common bean crop has two plant types mainly; the climbing type (range from 20-60 cm tall) and the herbaceous bush type (around 2 - 5 m long) (Kimani *et al*., 2007). The taproots of a bean crop contain many adventitious roots. Bush beans' stems are slender with numerous branches; also the stem of the climbing beans are prostrate with purple or green trifoliate leaves which have long green petioles. The leaflets of the beans range from 6 - 15 cm long and 3 - 11 cm broad by axillary. Common beans are kidney in

shape, ranging from 0.5 - 2 cm long and with various colours; red, green, grey purple and black based on the different variety of the seeds type (Santos *et al*., 2016).

Common bean seed is rich in proteins, fibre, and starch. The pods and stems have a small amount of protein, about 4 - 8 % dry matter in composition (Dejene *et al*., 2018). The leaves have a high quantity of protein, with about 20 % dry matter. Like many other legume seeds, common bean has a higher nutritional value as a source of protein, minerals (Phosphorus, Calcium, Zinc, and Iron), and low content of fibre. It is, however, important to highlight that some components exist in common bean that remains anti-nutritional. They include; lectins, trypsin-alpha-amylase, saponins, and chymotrypsin. Nevertheless, the activity and composition of these compounds vary in different common bean genotypes (Cominelli *et al.,* 2019).

Common bean grow healthier and better between a temperate environments (25 °C - 30 °C), sub-tropical region, and they can also thrive best in tropical areas. However, under extremely high humid environmental conditions (> 35 °C) the seeds fail to grow and develop, due to diseases such as fungal, pests, bacterial and viral infections, leading to yields reduction and intensification in fibre pods content (Diaz *et al*., 2018; Smith *et al*., 2019). An increase in incidences of diseases in common bean has been associated with high rainfall and extremely hot environment, which causes the pods, leaves and flowers to drop (Mathobo *et al*., 2017). When the environment is cold, a temperature below 10 °C, the growth performance may be affected and may stop at different stages (Diaz *et al*., 2018). During maturation, less humidity can lead to advantageous protection and

preservation of seeds. The suitable soil pH for common bean growth ranges from four to nine (pH 4 - 9), with the soil having high organic matter. However, common bean crops can't tolerate high acidic soil, sandy soil, waterlogged areas and calcareous soils (Wani and Khan. 2010; Muthini *et al*., 2014). The crops are sensitive to certain minerals like; Aluminum, Boron, high amount of Sodium and Manganese. The crop is favoured by clay-loam, silt-loam, and sandy-loam soils (Koskey *et al*., 2017; Wanjala *et al*., 2019).

Common bean plants' (green pods and dry seeds) annual productivity in Kenya is approximately 765,977 (t) metric tons (FAOSTAT, 2018). This can earn up to $199,743, 000 (United States Dollars) according to Wanjala *et al*. (2019). In Kenya, human consumption of common beans is high, ranking 2nd after maize. Also, the use of beans as raw materials for animal feeds in agricultural practices is common (Nanyunja *et al*., 2015). They plant grows well in midlands and highland regions in Kenya, particularly in Embu, Meru, Kericho, Kisii, and the whole of the western region excluding Busia (Ndungu *et al.,* 2018). In addition, common bean plants are also beneficial in fixing atmospheric N_2 when inoculated with active native rhizobia isolates or commercial inocula (Koskey *et al.,* 2017).

2.8.2 *Vigna unguiculata* L. Walp

Vigna unguiculata L. Walp (cowpea) is a legume crop that is nutritionally rich in proteins and amino acids which serve as a source of food for mankind and animals. Cowpea belong to the *Fabaceae* family (annual herbaceous legumes) (Boakye *et al*., 2016; Da Silva Júnior *et al*., 2018). Cowpea farming is more attractive to smallholder

farms in semi-arid regions as it is able to withstand water stress and low nutrients status. Furthermore, its suppresses growth of weeds as well as parasitic nematodes and viruses by acting as a cover crop. The plant grows between 80 cm to 200 cm in height, and its self-pollinated plants. The pods of the cowpea plant are cylindrical, smooth and measuring 3 mm to 12 mm, 2 cm to 6 cm in breadth and length. The seeds varied in colours (pink, black and brown). In Kenya, this plant is cultivated in drier agro-ecological zones, where its substantial productivity provides income and serves as a food diet for small-holder farmers (Ndungu *et al*., 2018). Cowpea are cultivated for it dry seeds, hay and green-leafy vegetables.

Rhizobia are mainly promiscuous to cowpeas in its symbiotic relationship with the roots to form nodulation. The roots of the plants can form symbiotic associations with both genera of rhizobia; (Alpha- and Beta-) *proteobacteria* to form nodules. Cowpeas are also beneficial in N_2 fixation when inoculated with effective native rhizobia strains (Hakim *et al*., 2018). For rhizobia-cowpea inoculant to be successful in improving cowpea plants yield and soil fertility in the agro-ecosystem, the bacteria must adapt well to the environmental conditions (Thomas *et al*., 2015). Failure to adapt to the prevailing environmental conditions, the rhizobia inoculation becomes unsuccessful, and this may lead to poor N_2 fixation and cowpea plants production. Apart from the environment, other aspect factors that arise from both biotic and abiotic factors have been suggested to hinder native rhizobia-cowpea symbiosis and diversity (Ntambo *et al*., 2017). It's significant to understand and know the ecological and geographical distribution and soil physico-chemical requirements so as to enhance rhizobia-cowpea strains survival and

symbiosis. It is thus, important for the maintenance of native rhizobia with cowpea crops for their symbiotic relationship and their effectiveness and ineffectiveness in BNF (Boakye *et al*., 2016).

2.8.3 *Glycine max* L. Merril

Soyabean (*Glycine max* L. Merril) is an oily seeds crop that has a lot of oil content when compared to other legumes of economic important (FAO, 2014). It is nutritional to both animals and humankind (García-Rebollar *et al*., 2016; Xiao *et al*., 2018). Soyabean contains approximately 40 % of protein content, 34 % of carbohydrate approximately, and about 21 % of oil content (Medic *et al*., 2014). Countries with rapid growth of human population have relied on soyabean production as one of the measures to curb food insecurity (Jackson, 2016). Soyabean is a legume crop that associates with rhizobia sp. to convert atmospheric nitrogen into a form that is useable by plants (Vargas *et al*., 2017). The association of soyabean roots with native rhizobia isolates has been revealed to be effective in efforts to reduce the use of inorganic fertilizers for increasing nitrogen content in the agricultural soil so as to enhance productivity and soil fertility (Fatima and Chaudhary, 2007).

According to Azadbakht *et al*. (2012), the seedlings of soyabean emerge slowly within five to fifteen days after planting. Moreover, the unfolding of the cotyledons takes place after three to twelve days, the trifoliolate leaf also unfolds. The environmental conditions can favor the plant to flower within 24 - 74 days after planting the seeds.

They are self-pollinated flowering plants. Hence, the pods' number varies from crop to crop. The plant takes approximately 65 to above 145 days for maturity and harvesting.

Soyabean was introduced in Kenya in 1909 by the British colonists who regarded it as a promising cash crop (Jackson, 2016). Currently, the annual demand for soyabean in Kenya exceeds 100,000 metric tons (MT) (Mutai, 2018) which is the highest in East Africa. However, the total yearly soyabean yield has never gone beyond 5,000 MT (FAO, 2014; FAO, 2017), creating a deficit of approximately 95 %. This deficit is covered through soyabean importation. Soyabeans are grown in two key agro-climatic zones in Kenya; the Western region and Central highlands region (Mburu *et al*., 2016). Comparably, central highland regions produce less soyabeans than the western regions. To address the deficit, soyabean is imported from Uganda (Tinsley, 2009), Malawi, Zambia, Zimbabwe, Argentina, India and recently, Brazil. Processed soyabean products are also imported from USA and China (Chianu *et al*., 2011).

The importation of soyabean and the processed products drain Kenyan's foreign exchange. For instance, Kenya spent US $27.54 Million in 2008 in the importation of Soyabean. This greatly affects the exchange rates and the country's GDP balance of trade hence affecting its macroeconomic stability (FAO, 2017). There is a need to strengthen and improve the productivity of soyabean to increase food production in Kenya (Jackson, 2016; Mutai, 2018). The yield of soyabean plants can be enhanced via inoculation with Bradyrhizobia due to the enhanced nitrogen fixation (Ndungu *et al.,* 2018). In addition, it is vital to identify the genetic diversity of the native rhizobia

strains that nodulates with soyabean plants before inoculation, and ensure that the condition of the environment is eco-friendly to the rhizobia (Masciarelli *et al*., 2014).

2.8.4 *Vigna radiata* L. Wilczek

Green gram (*Vigna radiata* L. Wilczek) is among the earliest and most nutritious of the pulse crops. Green gram are highly rich in carbohydrates (57 %), with an equally significant proportion of protein and dietary fibre at 25 % and 3.2 % respectively. It is also rich in fats accounting for 1.3 % of the total nutrients. This crop can grow in different soil conditions including light soils with minimal irrigation (Begum and Swain, 2018). It is an ideal plant for crop rotation and intercropping. Green gram belongs to *Leguminosae* family and *Papilionaseae* subfamily. It is one of the most important grain crops ranking third among the pulses cultivated in Kenya after bean and soyabean. It is a rich source of protein with high quality of lysine (460 mg g^{-1}), tryptophan (60 mg g^{-1}), ascorbic acid and riboflavin (0.21 mg 100 g^{-1}) (Saren *et al*., 2018). People generally consume green gram sprouts (shoots and leaves) due to their rich nutritional value consisting of proteins, dietary fibre and bio-active phyto-compounds (Huang *et al*., 2014).

Green gram is cultivated on more than six million hectares (ha) globally accounting for 8.5 % of the world pulse area (Nair *et al*., 2013; Murrell, 2016). In Kenya, the legume is cultivated on more than 302, 000 ha (Mburu *et al*., 2016). This is primarily cultivated on family farming systems depending on seasonal rainfall. Almost ninety percent (90 %) of this cultivation is carried out in the drier parts of Embu, Machakos, Tharaka Nithi,

Makueni, Mbeere, and Kitui counties. It is one of the main cash crops in the Kenyan arid and semi-arid lands (ASALs). Notwithstanding, the yield of green grams in these regions has been declining with a recent report recording a drop from 0.50 t/ha in 2013 to 0.49 t/ha in 2017 (Karimi *et al*., 2019).

2.9 Features of legume plant nodules

The preliminary phase of BNF is the development and formation of nodules at the root hair via the multiplication of rhizobia in the cells cortex of plant roots (Parniske, 2018). During two to three weeks after inoculating 1ml of the broth culture of the bacteria to the rhizosphere of the seedling, small nodules appear visible in the form of grey or white as a sign of rhizobia infection process. However, during this process, the nodules size increase and the colour changes to pink or red, which signal that N_2 fixation is taking place. The nodule's pink colour is due to the leg-haemoglobin which regulates the amount of oxygen flowing in rhizobia (Peix *et al*., 2015).

Perennial legume plants nodules with fingerlike form can fix nitrogen throughout the growing period if the environmental conditions for growth are favourable (Tanveer *et al*., 2019). Annual plant nodules are round with a short lifetime, and they are regularly replaced during growing seasons (Ndungu *et al*., 2018). Plant nodules that change to green colour usually fix nitrogen abundantly. The best nitrogen-fixing nodules which are considered to be pink or green in colour must be in large numbers during the middle growing stages of the plants for N fixation to take in large quantities. However, if the white and grey colour nodules increase and dominate the roots, the level of nitrogen

fixation will be little or none at all (Tanveer *et al*., 2019). Soil infertile and ineffective rhizobia strains (isolated from white and grey nodules) can also cause low or no nitrogen fixation in the plants (Raimi *et al*., 2017). Unlike some environmental conditions such as extreme temperature, nutritional soil stress can be controlled and adjusted by adding fertilizer to the crops. Farmers can improve legume plants production by the application of active rhizobia inoculum and regular soil irrigation practices.

2.10 The importance of legumes

The cultivation of legumes has been significantly on the increase across SSA owing to their economic value, nutritional benefits and as a source of food for mankind and livestock (Cullis and Kunert, 2017; Adebayo and Balogun, 2018). Other important aspects of the plants have been discussed in various literature (Muthini *et al*., 2014; Gentzbittel *et al*., 2015; Atnaf *et al*., 2015; Murrrell, 2016; Koskey *et al*., 2017; Ndungu *et al*., 2018; Zhong *et al*., 2019). For instance, it has been shown that common bean can lower cholesterol levels in the human body, and also the fibre content in common bean can prevent the rise in blood sugar content. Some components of these plants, for example, molybdenum, a component of sulfite oxidase enzyme can be found in beans (Foyer *et al.,* 2016). When present, it assists in detoxification of the sulfites from the human body.

The nodulation process of legume crops with rhizobia sp. has the ability to increase soil fertility and stability by fixing atmospheric N_2 to the soil via a SNF (Gouda *et al*., 2018).

The symbiosis enhances plants yield that provides income to small-scale farmers in the rural area in Kenya, SSA and around the Globe, building the worldwide economy. Legume crops are less expensive and the most alternative form of protein source when compared to other foods like fish and other animals (De Jager *et al*., 2017). In addition, legumes are widely consumed worldwide, and therefore they help reduce food insecurity and controlling malnutrition (Maphosa and Jideani, 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

Soil sample collection was carried out at Tharaka Nithi County, Embu County, Kitui County and Kisumu County, Kenya (Figure 3.1). In Tharaka Nithi, collection of soil samples was carried out in farms in South Tharaka mainly Tunyai, Gakurungu and Gachaine (S 0°9'35" E 37°49'30"; S 0°9'42" E 37°50'44"; and S 0°10'11" E 37°49'21", respectively). This region is classified under the lower midland agro-ecological zones (1000-1500 meters above sea leave (m asl)) that experience low rainfall averaging at 787 millimeters (mm) annually; which is unreliable. The temperature during the cold and hot seasons ranges between 11.0 °C - 25.9 °C respectively. The region has well-drained sand-loam soil. This region has a slightly cold, dry and hot climate condition (Koskey *et al*., 2017).

In Embu farms, soil sample collection was carried out in farms in Kyeni South, Karurumo, Kasafari and Kagaari South (S 0°29'0" E 37°41'18"; S 0°28'19" E 37°39'5"; S 0°29'12" E 37°41'26", respectively). These are the lower midland zones (1000-1500 m asl) of the Embu region that have a hot and dry climate with an annual average rainfall of 650 mm. This is a contrast to the upper midland (1500-2000 m asl), a phenomenon that is brought about by the water recycling effect caused by the forests in the region. The temperatures in this region are also hot with an average of 12.0 °C - 23.9 °C

annually. The region's soils can be categorized as having low to very low fertility (Mburu *et al*., 2016).

In Kitui, sample collection was carried out in farms in Mutonguni, Mutanda and Matinyani (S 1°11'22" E 37°50'59"; S 1°19'12" E 37°58'12"; and S 1°11'28" E 37°51'34" respectively) lying at 1000-1500 m asl (lower midland zones). These regions experience two rainy seasons with an average rainfall of between 750 mm and 1000 mm. Annual mean temperatures range between 20.0 °C - 24.0 °C, explaining the hot and dry climatic conditions experienced in the region. The soils are sodic or saline with considerably low fertility (Nyaga *et al*., 2014).

In Kisumu County, Lake Victoria Basin (LVB), soil samples collected from previous studied by Muthini *et al*. (2014) from farms in the Township area along the LVB (S 0°7'9" E 34°49'11"; S 0°6'2" E 34°46'0"; and S 0°6'7" E 34°47'4", respectively) was used to trapped rhizobia. Also archived samples were obtained from Microbiology Laboratory and used to retrived the active native rhizobia strains. The region's is under the lower midland agro-ecological zones (1000-1500 m asl) that experience an average rainfall of 1300 mm annually, with an annual mean temperature of between 20.9 °C - 22.0 °C. This gives its characteristic warm and slightly dry climate. The region has deep sandy loam to sandy clay soils with varying salinity (Muthini *et al*., 2014).

All of the farms and the global positioning system (GPS) coordinates are shown in the maps (Figure 3.1). Laboratory work and greenhouse experiments were carried out at

Kenyatta University, Department of Plant and Microbial Sciences (S 1° 10' 58" E 36° 55' 45"). Studies conducted in these regions over the recent past have confirmed the presence of rhizobia (Mwangi *et al*., 2011; 2013; Muthini *et al*., 2014; Koskey *et al*., 2017). Furthermore, these regions have been classified as high potential agro-ecological zones (Nyaga *et al*., 2014).

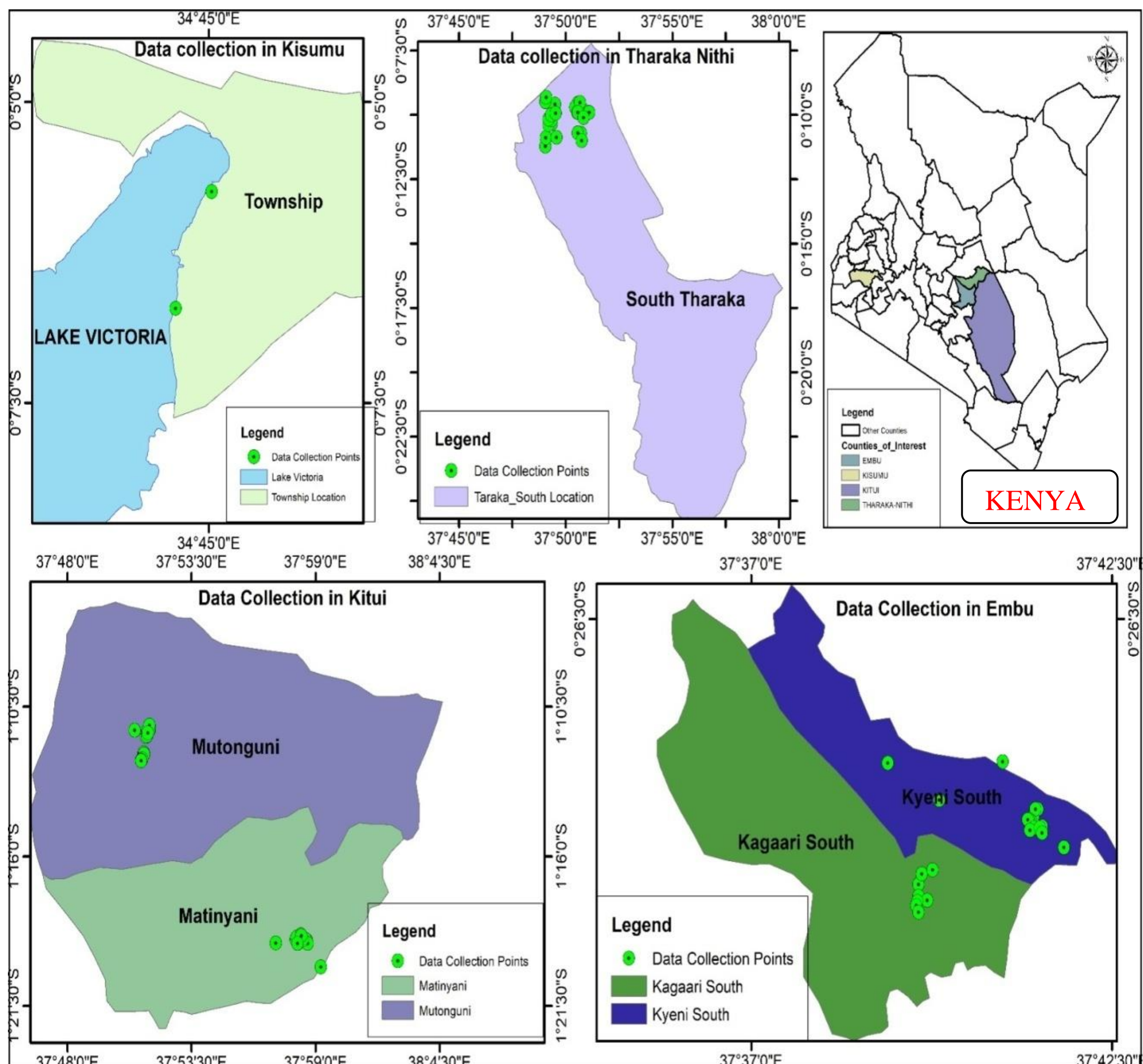

Figure 3. 1: A map of Kenya showing the location of study areas; Embu County, Tharaka-Nithi County, Kisumu County and Kitui County

3.1.1 Study design

The study design used was a split-plot design and randomized complete block design (RCBD) in soil sample collections in the field. In the greenhouse, complete randomized design (CRD) was used to arrange the pots (Mburu *et al*., 2016).

3.2 Soil sampling

A total of 90 soil samples were collected from farms in Embu, Kitui, and Tharaka Nithi Counties. The sample size for the soil sampling sites was calculated using the Snedecor and Cochran statistical formula (1980).

$$n = \frac{4PQ}{L^2}$$

Where; n = sample size, P = proportion in the target population, Q = 1-P, and L = accepted error (5 %). The target population was calculated based on a household baseline survey carried out by the Ministry of Agriculture.

In Embu County, the target household population was 14,500 of legume crops farmers, while the total target population of legume smallholder farmers was 208 farm households. The calculated sample was; P = 208/14,500 = 0.015, Q = 1-0.015 = 0.985, $L^2 = ((0.05)^2 = 0.0025)$

n = (4 x (0.015 1x0.985))/0.0025 = 23.81 = 24 farms

In Tharaka Nithi County, the target household population was 12,915 of legume crops farmers, while the total target population of legume smallholder farmers was 271 farm

households. The calculated sample was; P = 271/12,915 = 0.021, Q = 1-0.021 = 0.979, $L^2 = ((0.05)^2 = 0.0025)$

n = (4 x (0.021x0.979))/0.0025 = 32.894 = 33 farms

In Kitui County, the target household population was 14,590 of legume crops farmers, while the total target population of legume smallholder farmers was 321 farm households. The calculated sample was; P = 321/14,590 = 0.022, Q = 1-0.022 = 0.978, $L^2 = ((0.05)^2 = 0.0025)$

n = (4 x (0.022x0.978))/0.0025 = 34.43 = 34 farms

The samples were collected from 10 points across and diagonally in every farm at an interval length of 5 - 20 meters using a soil auger. A solution of 70 % ethanol was used to sterilize the soil auger to avoid cross-contaminating the soil between the sampling points. This sampling produced five soil sub-samples. In collecting the samples, the organic matter was cleared and the sample taken at a depth of 5 - 30 cm as described by Koskey *et al*. (2017). The samples from each farm were then mixed to make a composite sample from which one kilogram of soil was taken. The 1 kg of soil from each point was then aseptically packed in khaki bags separately and double sealed. They were then taken to Kenyatta University, Microbiology Laboratory and Plant Sciences Laboratory where rhizobia trapping experiments and other analyses were carried out. Soil samples that were not processed immediately were refrigerated at a temperature of 4 °C. This method was adapted from Keith, (2017).

3.3 Soil characteristics

The sampled soils were subjected to physico-chemical tests before the greenhouse experiments. A pH meter was used to determine the soil pH in a ratio of 1:2:5 H_2O suspension (Wani and Khan. 2010). Further analysis of the soil texture and soil organic matter was done through the hydrometer method and Walkley and black oxidation methods, respectively. Walkley and black oxidation method involves a wet combustion of the organic acid with a mixture of potassium dichromate ($K_2Cr_2O_7$) and suphuric acid (H_2SO_4). The excess $K_2Cr_2O_7$ were titrated against ferrous sulphate ($FeSO_4$) (Okalebo *et al.*, 2002).

In addition, total soil N_2 and the available phosphorous (P) were determined through the macro-Kjeldahl digestion, distillation and titration method and the Olsen extraction method, respectively (Okalebo *et al.*, 2002; Pérez-Brandan *et al.*, 2019). Exchangeable cations were determined after leaching the soils with ammonium acetate ($NH_4CH_3CO_2$), and the amounts of calcium ions (Ca^{2+}) and magnesium ions (Mg^{2+}) in the 25-leachate analyzed using atomic absorption spectroscopy (AAS). To measure the amount and quantity of soil microelements such as, zine (Zn), sodium (Na), iron (Fe), copper (Cu), magnesium (Mg) and manganese (Mn) were digested in a mixture of selenium (Se), salicylic acid ($C_7H_6O_3$) and hydrogen peroxide (H_2O_2). The digestion and absorption were aspirated into a flame and detected through an atomic absorption spectrophometer (AAS). While, potassium ions (K^+) and sodium (Na^+), were determined using flame emission spectroscopy (FES) (Pérez-Brandan *et al.*, 2019).

3.4 Planting materials

Certified, healthy and pre-treated planting seeds of common beans (rosecoco) and green grams (Ks-20) were obtained from Simlaw Seed Company, located on Kijabe street Nairobi-City County. Cowpea seeds (K-80) were obtained from the Kenya Agricultural and Livestock Research Organization (KALRO), located in Katumani, Machakos County. Soyabeans (SB-126) were supplied by the International Crops Research Institute (ICRI) for the Semi-Arid Tropics from Kakamega County. These seeds had been pre-treated and had a germination percentage of above 80 %. Rhizobia nodules isolates were trapped from the soils (section 3.6 and 3.7) and used as native isolates inocula. For the control expirements, commercial rhizobia references inocula (CIAT110, USDA889 and USDA3456) and vermiculite were obtained from MEA fertilizers Ltd. Nakuru County, in Kenya.

3.5 Pre-treatment and germination of seeds

Vermiculite was put in a Kilner jar and moistened with sterilized distilled water, covered with aluminium paper foil and autoclaved at 125 °C for 15 mins at 15 psi pressure to remove all possible microbes, nematodes and fungi contaminants before pre-germination of the seeds. Soyabean of uniform size, colour and shape of soyabeans were selected; 70 % C_2H_5OH (ethanol) was used to surface-sterilize the seeds for 30 secs, followed by 3 % NaClO (Sodium hypochlorite) for 1 min. The Kilner jars and vermiculite were allowed to cool, a hole was made with a sterilized spatula, and the sterilized seeds of soyabean (BS-126) were planted immediately (plate 3.1). The seeds in the Kilner jar were incubated at room temperature for four days so that uniform

germination of the seeds was abtained. Three seedlings with radicles of the same uniform size were transplanted to the pots assemblies in the greenhouse with four replicated, the radicle and the rooting system facing downward. The set up was done in the greenhouse and arranged in a complete randomized design (CRD). During inoculation, thinning was done to leave two seedlings in each pot. Yeast Extract Mannitol Broth (YEMB) culture media was prepared (appendix II) and placed in the incubator at 28 °C for 2 days, after which the seedlings were inoculated at the base with 1ml of the culture.

Plate 3.1: Pre-gremination of soyabean seeds in sterile vermiculite in the growthroom

Healthy seeds of common beans (rosecoco), cowpeas (k-80) and green grams (Ks-20) of the same size were put into a beaker, and washed with detergent and tap water to clean the seeds, remove the waxy, and the chemicals from the seeds. The seeds were then surface-sterilized for 30 secs with 70 % ethanol and 3 % chlorine for 1 min. Sterile forceps were used to dig a hole of one centimetre (1 cm) in each pot, containing sterile

soils, and four seeds were planted, after one week of germination two seedlings were uprooted, and the remaining seedlings were maintained and watered daily, they were inoculated with different rhizobia isolate strains inoculum (section 3.7). This method was as described by Jaiswal *et al*. (2011).

3.6 Experimental trapping of rhizobia at the greenhouse

In the greenhouse, soil samples collected from Embu, Tharaka Nithi, and Kitui Counties, were used for experimental trapping of rhizobia. The soil was put in different pots that were sterilized and transported in the greenhouse and arranged in CRD with four replication. Cowpea seeds were used as the host for trapping rhizobia. The seeds were separately washed for 30 secs in 70 % ethanol and then rinsed two times using distilled water. After that, 3 % NaClO (Sodium hypochlorite) was used to separately sterilize the surface of the seeds for 1 min followed by rinsing six times using sterilize distilled water to remove the entire chemical sterilant. Each seed was then pre-germinated on a nutrient-free agar media before planting.

After transplanting the seedlings in the pots, watering was done at an interval of one day following the water holding capacity levels of the soils. Plant nodules assessment, harvesting, and carefully washing off the roots to obtain clean root nodules was carried out after thirty days (four weeks) of planting. The root nodules were detached and separated from the plant roots, and placed in a paper tissue and newspaper to dry and were stored at room temperature (Koskey *et al*., 2017). In addition, root nodules from earlier studies conducted from Kisumu, Embu and Tharaka Nithi Counties stored in

Kenyatta University were also used for isolation of native rhizobia isolates (Muthini *et al*., 2014).

3.7 Rhizobia isolation

Nodules from previous studies in; Embu, Tharaka Nithi and Kisumu Counties stored in the Microbiology and Tissue Culture laboratory at Kenyatta University were also used to isolate rhizobia. Healthy, firm and unbroken root nodules collected from previous studies' trap experiments were obtained and rinsed in distilled water (section 3.7). These root nodules were then imbibed in distilled water for two hours. After that, the nodules were immersed in 70 % ethanol for a half-min to eliminate air bubbles that could have been trapped in the tissue and also to decrease the surface tension. A solution of 3 % (w/v) NaClO was used to surface-sterilized the nodules for 1 min. This was then followed by repeatedly washing the nodules in sterile distilled water six times on different plates. With the addition of 1 ml of sterilized-distilled water, a glass rod was used to crushing the nodules, and a full loop of the crushed suspension streaked on yeast extract mannitol agar (YEMA) plate (appendix I) containing 0.025 mg/L (w/v) Congo red (CR) dye were incubation. The inoculated plates was done in an inverted position for four days at 28 °C. The cultures were checked after every one day (twenty-four hours) for the growth of rhizobia colonies (Muthini *et al*., 2014).

3.7.1 Morphological and biochemical identification of rhizobia

Morphological characteristics such as colour change, opacity, colony elevation, consistency, texture, shape, size, exo-polysaccharide gum, border, transparency and

mucosity were used for presumptive identification of the rhizobia isolates that were isolated from section 3.6 and 3.7. For biochemical identification, Bromothymol Blue (BTB) Test, CR dye, and Gram staining procedures were carried out. For CR dye test, the media was incubated in the dark, rhizobia showed little or no CR absorption and formed colonies that were white, opaque, or sometimes pink; other bacterial species absorb the red dye Somasegaran and Hoben (1994). For BTB test, the isolates' ability to produce acid or alkali was tested by culturing them on YEMA with BTB (appendix I) indicator at a pH of 6.8. These cultures were grown at a temperature of 28 °C in a rotating orbital shaker for a maximum of 5 days (Muthini *et al*., 2014). After the incubation, the isolates were characterized as acid-producers, alkali producers or neutral, based on the observable colour changes in the media. Isolates that produced acid turned the media to yellow from green. The media turned into blue when alkali producers grew in it, while the colour remained green for rhizobia that produced neither acid nor alkali (neutrals) (Mwangi *et al*., 2011).

Beck *et al*. (1993) method of Gram staining was used to characterize and identify the rhizobia strain that was twenty-four hours old. Further characterization was done on the growth of the isolates based on emergence rate of the colonies on YEMA media (appendix I) incubated at a temperature of about 28 °C. Slow growing and fast-growing rhizobia were identified and described as evolved after 3 to 4 days and 7 to 10 days after inoculation, respectively.

3.8 Isolate authentication

Isolate authentication was carried out following the procedure outlined by Somasegaran and Hoben (1994). A 1mL of the cultured bacteria isolates were inoculated into the pre-germinated seedlings of common bean and soyabean in Leonard jars filled with sterile vermiculite and a nutrient solution that was deficient of Nitrogen. The control experiments were set up by using plants that were not inoculated. The experiment was made up of four replicates, arranged in CRD in the greenhouse. The grown plants of common bean and soyabean were harvested 1½ months after planting, and nodulation determined. Harvesting was done by washing off the vermiculite gently with a stream of water from a hosepipe in order to expose the nodules and roots. The nodules were then carefully detached, enumerated and stored in labeled McCartney bottles having silica gel for later isolation of rhizobia. The color of the root nodules are pink, red and white. A seedling was considered positive in nodulating if it bore one or more nodules. Nodulation was scored negative when the seedling bore no nodule.

3.9 Symbiotic efficiency of specific representative isolates of rhizobia in greenhouse

Somasegaran and Hoben, (1994) procedure was carried out to assess the symbiotic efficiency of a particular indigenous (native) rhizobia characteristic strain of isolates. Common bean, green gram, soyabean, and cowpea seeds were selected, sterilized and planted in Leonard jars and pots that contain sterilized soil. The soil was sterilized in an oven (Schutzart DIN 40050-IP20; memmert 854 Schwabach, W-Germary, Tpy U 50) at 70 °C for 72 hours to kill all forms of microorganisms and spores present. Four seeds were planted in each pot. After germination, two seedlings in each pot were maintained

for five days and rearranged in CRD before the inoculation with the rhizobia strains isolates in YEMB. The revived glycerol stocks rhizobia isolates (archived samples), and commercial reference strains (CIAT899, USDA100 and USDA3456) were also cultured in a YEMB (appendix II) for two days before inoculation. A 1mL of every broth was inoculated on the rhizosphere around the seedlings root of the study plants. The CIAT899, USDA100 and USDA3456 isolates were also inoculated in four different pots individually and considered as positive controls (commercial inoculants). Four different pots were not inoculated with any rhizobia strain and were labelled as un-inoculated negative plant controls. Each treatment had four replicates arranged in CRD. The plants were watered after every twenty-four hours following the H_2O logging capacity level.

The positive control (commercial inocula) were compared with the plants that were treated with the native rhizobia isolate strains and the un-inoculated negative control plants. After six weeks of growth under a temperature ranging between 26 ºC - 30 ºC in the greenhouse. Harvesting was carried out after forty-five days of planting, as proposed by Beck *et al*. (1993). The plant roots and nodules were carefully washed with tap water to remove and clean all the soil around the roots; later the nodules were rinsed in distilled water. The shoots, roots, and nodules were stored at room temperature to dry. Each plant was scored and recorded for the absence or presence of nodules. The plants' performance based on the various types of treatment was assessed based on nodules number (NodNo), dry nodule weight (NodDW), shoot dry weight (ShtDW), root dry weight (RtDW) and Symbiotic efficiency (SyE).

Symbiotic efficiency was calculated, and determined by dividing the total shoot dry weight of the native rhizobia isolate inoculated plants with the total shoot dry weight of the commercial strain inoculated plants control multiplied by 100 %. These were done after the shoot, and root of the plants had dried to obtain uniform biomass and to determine nitrogen fixation effectiveness (Yadegari *et al*., 2010).

$$\mathbf{SyE} = \Delta \text{TshootN}/\text{TshootC} \times 100.$$

Where; SyE = Symbiotic efficiency, TshootN = total shoot dry weight of the native rhizobia isolate inoculated plants, TshootC = total shoot dry weight of the commercial inoculated plants control.

3.10 Greenhouse experiment of the legume plants

Two different types of experiments were set up. In the first experiment, soil samples collected from the agriculture farm at Kenyatta University were used. The samples were obtained and combined to make a homogenous sample. The soil was sterilized in an oven for approximately seventy-two hours at 70 ℃ to kill any form of spores and living microorganisms present. This was carried out at Kenyatta University, Plant Science laboratory. After sterilization, the soil was aseptically transported to the greenhouse, where it was put in different sterilized pots. Common bean (rosecoco), soyabean (SB-126), green gram (Ks-20) and cowpea (K-80) were used to set up the experiment. The seeds were selected based on uniform sizes and shapes. They were then washed in distilled water, followed by 70 % ethanol for 30 secs. The seeds were rinsed in distilled sterile water, and 3 % solution of Sodium hypochlorite was then applied to sterilize the

surface of the seeds for 1 min. The seeds were then rinsed 6 times using distilled sterilize water. After sterilization, the seeds were planted in sterilized soils.

In the second experiment, samples previously collected from the agricultural farms in Kitui, Embu, and Tharaka Nithi Counties were used. The samples were obtained and mixed to make a homogenous sample. Cowpea (K-80) seeds were planted to trap the indigenous rhizobia from the soils. Harvesting of cowpea plants was done 30 days after planting. At the same time, stored rhizobia cultures from previous studies (Muthini *et al*., 2014) were revived and purified to obtain pure isolates. To revive the isolates, a droplet of sterilized distilled water was put in a stored rhizobia culture and kept in an incubator for two days for the cultures to imbibe the water. A full loop was picked from these cultures and streaked on the YEMA plate (appendix I), stored in an incubator at 28 ºC for two days and formation of colonies observed. The revived rhizobia cultures were sub-cultured severally to obtain a pure colony isolate.

The pure isolates were put in each universal jar that contained YEMB (appendix II) and transported to the greenhouse for inoculation. The revived glycerol stocks isolates (archived samples) were inoculated on common bean, green gram, soyabean, and cowpea grown in sterilized soil. These would provide data about the biomass, nodulation rate and the number of native effective rhizobia populations. The plants were harvested after 45 days of inoculation and allowed to dry to obtain maximum biomass, and later weighed for ShtDW, NodDW, NodN, RtDW and SyE was calculated (section 3.9). The biomass of the plants from the commercial strain inocula was then compared

to that of the plants treated with native rhizobia isolates. The rhizobia isolates that outcompeted the commercial references strains were purified and again inoculated in non-sterile soils using common bean, cowpea and green gram plants. This was done to see if the inoculated native rhizobia isolates could survive better in non-sterile soils and significantly improve legume plants production in the presence of native microbes (Muthini *et al*., 2014).

3.10.1 Cross-inoculation

The ability of the native rhizobia isolates and commercial strains to induce nodulation and efficiently fix nitrogen in non-original host plants of common bean, cowpea and green gram was assessed by cross-inoculation via in vivo inoculation technique (Granada *et al*., 2014). Native rhizobia isolates from common bean root nodules were cross-inoculated with soyabean seedlings. Also, native rhizobia isolated from cowpea root nodules were cross-inoculated with common bean seedlings in the greenhouse. Additionally, native rhizobia isolates that were isolated from common bean root nodules were also cross-inoculated with green gram plants. The experiments were set using native rhizobia isolates, two commercial inocula and the un-treated negative control plants with four replication for each isolate arranged in CRD as described in section 3.10. The plants were maintained for 45 days, after which, the ability of each isolates to fix nitrogen and induce nodulation was evaluated (section 3.9). If the plants presented nodules and leaves with dark green color, the isolates were considered as efficient nitrogen fixer for non-original host legume plants (Granada *et al*., 2014; Lu *et al*., 2017).

3.11 Molecular characterization of rhizobia nodulating roots legume plants

Rhizobia isolated from nodulating roots plants from non-sterile soil were further subjected to characterization by molecular methods, based on their 16S rDNA (16S rRNA gene region) sequencing. In this process, four steps were adapted; genomic DNA extraction, polymerase chain reaction (PCR), Gel electrophoresis, and 16S rRNA gene region sequencing.

3.11.1 Genomic DNA extraction

Zymo quick DNA^{TM} min prep kit (Zymo Research, USA) was used for genomic DNA (gDNA) extraction as per the manufacturer's instructions (Mechtersheimer *et al*., 2018). In the procedure, freshly cultured rhizobia of 2 days old colonies were suspended in 500 microliters (µL) of normal saline (sodium chloride) in Eppendorf tubes. The contents in the tube were then vortexed for 10 secs and thereafter centrifuged for 3 mins at 13, 000 revolutions per min (rpm). The normal saline was then discarded, and 400 µL of genomic lysis buffer was added to the remaining contents of the tube and vortexed for another 10 secs. The contents in the Eppendorf tubes were then incubated for 30 mins at room temperature and later transferred into zymospin column. The column and its contents were placed in collection tubes and centrifuged at 13,000 rpm for 1 min. To the contents, 200 µL DNA pre-wash buffer was added to the spin column and centrifuged at 13,000 rpm for 1 min. A 500 µL gDNA wash buffer was then added and the column centrifuged for 1 min at 13,000 rpm. The contents were then transferred into sterilized Eppendorf tubes. A 50 µL of DNA Elution buffer was then added and the contents incubated at ambient temperature for 10 mins. Centrifugation was then carried out at

13,000 rpm for 30 secs to elute DNA. The eluted DNA was then stored at -20 °C awaiting further analysis.

3.11.2 Polymerase Chain Reaction (PCR) amplification of 16S rRNA gene

Polymerase Chain Reaction was performed in a 30 µL reaction volume, containing 15.0 µL, Taq mix, 0.3 µL of 10 µm 27 Forward primers, 0.3 µL of 10 micrometers (µm) 1492 Reverse primer, 1.0 µL of DNA template and 13.40 µL of PCR water. The PCR reaction were carried out using Techne thermocycler (made in UK, by Techne (Cambridge) Ltd. with serial No 120090-4), with the following conditions: 95 °C within 2 min for initial denaturation, 35 cycles at 95 °C within 45 secs for denaturation, 54 °C for 45 secs for primer annealing, 72 °C for 2 mins for extension followed by 72 °C for 5 mins for the final extension. The amplicons were thereafter kept at -20 °C for further analysis (Simmon *et al*., 2004).

3.11.3 Agarose gel electrophoresis

The PCR products were stained with SYBR green (BioLab, USA) and separated by gel electrophoresis in 1.0 % (w/v) agarose gel in 0.5X Tris Borate EDTA buffer at 80 volts (V) for 30 mins. The gel was then visualized using Ultra Violet trans-illuminator (Seriennumber: UV 31208/070200004). A 100 base pair (1bp DNA) ladder was used to estimate the molecular sizes of the bands obtained. The gel image was then photographed using a digital camera (Simmon *et al*., 2004).

3.11.4 16S rRNA gene sequencing

Before sequencing, the obtained PCR products were purified using a QIAquick PCR purification kit (Qiagen, Tiangen, China) according to the manufacturer's instruction. For the sequencing procedure, 27F (forward primer; 5′-AGAGTTTGATCATGGCTCAG-3′) and 1492R (reverse primer; 5′-GGTTACCTTGTTACGACTT-3′) were used. PCR reaction was conducted using five picomole (5pmol) of forward and reverse primers (Applied Biosystems, USA) in a 20 µl column with 2.5 µl of DNA template (Gentzbittel *et al*., 2015). This was followed by 500 bp sequencing after the PCR product purification. The purified PCR products were then shipped to Macrogen Europe Laboratory in Amsterdam-Netherlands where they were sequenced using the Sanger sequencing Method, the automated sequencer ABI 3700 DNA with Big Dye Terminator Kit V. 3.1 (Applied Biosystems, USA) where used (Ghyselinck *et al.,* 2013). Sequencing reactions were performed based on the manufacturer's instructions (Ismail *et al*., 2013).

3.12 Data analyses

Morphological (phenotypic) data for rhizobia isolates characteristics was coded numerically and cluster analysis was drawn. Phenograms were drawn based on a hierarchical cluster analysis using the Euclidean distance similarity and single linkage (nearest-neighbor) procedures using General statistics (GENSTAT) computer software version (V) 10.0 (VSN International, 2011). Morphological diversity indices were calculated using paleontological statistics software tool (PAST) computer V 3.2 (Hammer *et al*., 2001). All the data were normalize (log(x-1)) before any analysis was

done. Data on the nodules number (NodN), root dry weight (RtDW), dry nodule weight (NodDW), and shoot dry weight (SHtDW) were analyzed using analysis of variance one way (ANOVA) using statistical analysis system (SAS) computer software V 9.22. Tukey's Honest Significance Difference (HSD) at 5 % probability level was used to separate the means (Dangkulwanich *et al*., 2014).

Base calling for molecular sequences data was done using FinchTV software V 1.4. Consense were created using Genestudio software V 2.2, after which the sequences obtained were compared with the sequences in the National Centre for Biotechnological Information (NCBI) GeneBank database using Local Arrangement Search Tool (BLAST) program (Code, 2008). The sequence identities that were closely related to the sequences of the study native rhizobia isolates were retrieved from the NCBI sequence database, respectively. Molecular sequence data obtained for rhizobia isolates were edited, aligned to form contigs using clustal W (Curk *et al*., 2015). Dendrograms (evolutionary phylogenetic tree) for genetic distance data were drawn by neighbor joining method (NJM) using Molecular Evolutionary Genetics Analysis (MEGA X software) V 5.1. The sequence isolates were added into the NCBI GeneBank database manually, https://www.ncbi.nlm.nih.gov/nuccore/?term=MT825113:MT825140[accn]. The soil physico-chemical parameters comparing the biomass of the harvested plants (NodN, RtDW, NodDW, and SHtDW) were analyzed using SAS computer software 2^{ed} V 5.0 to obtain a Redundancy analysis (RDA) graph (Hakim *et al*., 2018).

CHAPTER FOUR

RESULTS

4.1 Soil characteristics

There were variations in soil's physical and chemical properties among the farms in Embu County, Kitui County, and Tharaka Nithi County.

4.1.1 Physico-chemical parameters of soil's from farms in Embu region

In Embu County, soils from farm EM10 were the most acidic with a pH of 5.11, while soil from farm E15 recorded the most alkaline pH of 7.04. Total nitrogen (TN) and total organic carbon (TOC) ranged between 0.10 to 0.21 and 0.89 to 2.36, respectively. Phosphorus (P), Potassium (K), calcium (Ca), magnesium (Mg), and manganese (Mn) contents for all the farms ranged between 3 ppm to 120 ppm, 0.38 cmol kg^{-1} to 1.88 cmol kg^{-1}, 2.0 % to 10.6 %, 1.86 to 3.95 cmol kg^{-1} and 0.50 to 1.23 in that order. The farm's soil textures were classified as sandy-clay, sandy-clay-loam, and sandy-loam. The highest copper (Cu), iron (Fe), zinc (Zn), and sodium (Na) contents were obtained in farms EM11 (1.93), EM15 (98.7), E6 (12.2), and E11 (1.22 cmol kg^{-1}), respectively.

4.1.2 Interactive effect of soil on cowpea plant (K-80) roots nodulation and shoot enhancement

The soils that were collected from the thirty (30) farms (E1 – E15, and EM1 – EM15) in Embu County were used to trap rhizobia using cowpea seeds as the trapping host, (Table 4.1). The growth performance of the plants varies, there was a significant difference

($P<0.0001$) in nodules number (NodN) of the plants. The NodN ranged from 8.33±1.763 plant^{-1} to 50.33±5.16 plant^{-1} (average±standard error). Farms E1 and EM14 showed the highest NodN, whereas E6 and E9 revealed the lowest NodN. All the farm soils had indigenous rhizobia that induced formation of nodules. There was a significant difference ($P<0.0001$) in nodule dry weight (NodDW) whereby, the NodDW ranged from 5.00±2.00 mg plant^{-1} to 90.00±19.00 mg plant^{-1} (average±standard error). Farms E1 and EM14 also had the highest NodDW when compared to other farms E13 and EM6. There was a significant difference ($P<0.0001$) in shoot dry weight (SHtDW). The highest SHtDW of 2.19±0.19 g plant^{-1} (average±standard error) was recorded in EM10 whereas, farms; E1, E5, E10, E12, E13, E14, EM7, and EM14 had SHtDW above 1.80±0.05 g plant^{-1} when compared to other farms. The lowest SHtDW of 0.47±0.05 g plant^{-1} was recorded in E8. There was a significant difference in the root dry weight (RtDW) of the various farms at $p\leq0.05$, (Table 4.1). E1 had the highest RtDW of 0.23±0.02 g plant^{-1}, whereas the lowest RtDW of 0.04±0.01 g plant^{-1} was recorded in EM8.

Plate 4.1: Trapping indigenous rhizobia in the greenhouse from the rhizospheric soils of Embu County using cowpea (K-80) as a trapping host plants

Table 4.1: Average nodule number (NodN plant^{-1}), nodule dry weight (NodDW mg plant^{-1}), shoot dry weight (SHtDW g plant^{-1}) and root dry weight (RtDW g plant^{-1}) of cowpea plants from the greenhouse experiment

Soils sample	NodN(plant^{-1})	NodDW(mg plant^{-1})	SHtDW(g plant^{-1})	RtDW(g plant^{-1})
E1	50.00±6.18^{a-b}	90.00±19.00^{a}	1.90±0.12^{a-b}	0.23±0.02^{a}
E2	38.00±3.03^{a-d}	56.00±10.00^{a-e}	1.06±0.13^{d-i}	0.10±0.01^{c-f}
E3	34.83±5.53^{a-d}	69.00±13.01^{a-c}	1.09±0.07^{d-i}	0.13±0.02^{c-e}
E4	37.83±4.64^{a-d}	67.00±13.00^{a-c}	1.26±0.07^{c-i}	0.17±0.02^{a-e}
E5	36.83±4.33^{a-d}	56.00±12.00^{a-e}	1.87±0.05^{a-b}	0.22±0.02^{a-c}
E6	24.50±1.57^{c-g}	46.00±9.00^{c-f}	1.46±0.15^{b-g}	0.22±0.02^{a-b}
E7	27.00±4.44^{b-g}	47.00±10.00^{c-f}	0.94±0.09^{g-i}	0.12±0.03^{a-f}
E8	32.83±1.85^{a-e}	45.00±11.00^{b-f}	1.00±0.07^{e-i}	0.10±0.00^{b-f}
E9	36.50±5.48^{a-d}	48.00±10.00^{b-f}	1.87±0.05^{a-b}	0.21±0.02^{a-d}
E10	30.83±6.83^{a-g}	49.00±9.00^{b-f}	1.96±0.21^{a}	0.20±0.04^{a-d}
E11	32.50±3.18^{a-f}	44.00±7.00^{c-f}	0.90±0.08^{g-i}	0.14±0.02^{a-f}
E12	35.83±3.77^{a-d}	47.00±12.00^{b-f}	1.89±0.03^{a-b}	0.17±0.01^{a-e}
E13	31.00±2.67^{a-g}	5.00±2.00^{c-f}	1.91±0.05^{a-b}	0.21±0.01^{a-c}
E14	35.50±3.01^{a-d}	43.00±11.00^{c-f}	1.85±0.05^{a-b}	0.19±0.02^{a-e}
E15	37.50±4.15^{a-d}	56.00±15.00^{c-f}	1.18±0.17^{d-i}	0.21±0.03^{a-d}
EM1	34.50±4.17^{a-e}	55.00±12.00^{b-f}	0.99±0.07^{f-i}	0.13±0.02^{a-f}
EM2	30.17±3.38^{a-g}	44.00±10.00^{c-f}	0.85±0.09^{h-j}	0.09±0.01^{d-f}
EM3	17.33±1.87^{d-g}	34.00±9.00^{c-f}	0.87±0.05^{h-j}	0.11±0.02^{a-f}
EM4	30.33±3.37^{b-g}	45.00±4.00^{b-f}	1.22±0.16^{d-i}	0.17±0.02^{a-e}
EM5	21.67±4.31^{d-g}	33.00±4.00^{c-f}	1.48±0.14^{b-g}	0.15±0.05^{a-f}
EM6	9.50±2.22^{f-g}	12.00±5.00^{f}	0.72±0.07^{i-j}	0.09±0.02^{d-f}
EM7	45.17±5.56^{a-c}	64.00±15.00^{c-f}	1.84±0.03^{a-b}	0.18±0.01^{a-e}
EM8	11.50±4.37^{e-g}	23.00±4.0^{e-f}	0.47±0.05^{j}	0.04±0.01^{a-f}
EM9	8.33±1.76^{g}	23.00±5.00^{a-e}	0.79±0.09^{i-j}	0.08±0.02^{e-f}
EM10	40.33±5.44^{a-d}	55.00±10.00^{b-f}	2.19±0.19^{a}	0.22±0.01^{a-c}
EM11	24.33±4.28^{c-g}	45.00±8.0^{c-f}	1.39±0.13^{-g}	0.20±0.02^{a-e}
EM12	29.50±2.05^{a-g}	40.00±7.00^{c-f}	1.58±0.11^{b-e}	0.22±0.02^{a-c}
EM13	22.83±6.23^{c-g}	34.00±10.00^{c-f}	1.52±0.17^{b-f}	0.15±0.02^{a-f}
EM14	50.33±5.16^{a}	89.00±17.00^{a-b}	1.80±0.05^{a-b}	0.16±0.03^{a-e}
EM15	22.50±5.58^{c-g}	41.00±13.00^{c-f}	1.13±0.22^{d-i}	0.13±0.03^{a-f}
P values	<0.0001	<0.0001	<0.0001	<0.0001

Key: NodN, nodules number; NodDW, nodules dry weight; SHtDW, shoot dry weight; RtDW, root dry weight; g plant^{-1}, gram per plant; mg plant^{-1}, milligram per plant. Values followed by the same letters within the column are not significantly different according to Tukey`s Honest Significant Difference at 5 % level.

4.1.3 Correlation coefficient of soil physico-chemical properties on cowpea plant parameters

Redundancy analysis (RDA) and correlation of the soil physico-chemical properties of Embu County, and the greenhouse tests on cowpea plants growth parameters indicated that, there is a positive correlation between NodDW and NodN with Mn, K, P, Cu, TN, TOC and Zn. In addition, RTDW positively correlated with Ca, pH, and Na (Figure 4.1). However, TN and TOC negatively correlated with the SHDW. The soil pH and Ca indirectly correlated with NodN and NodDW, respectively.

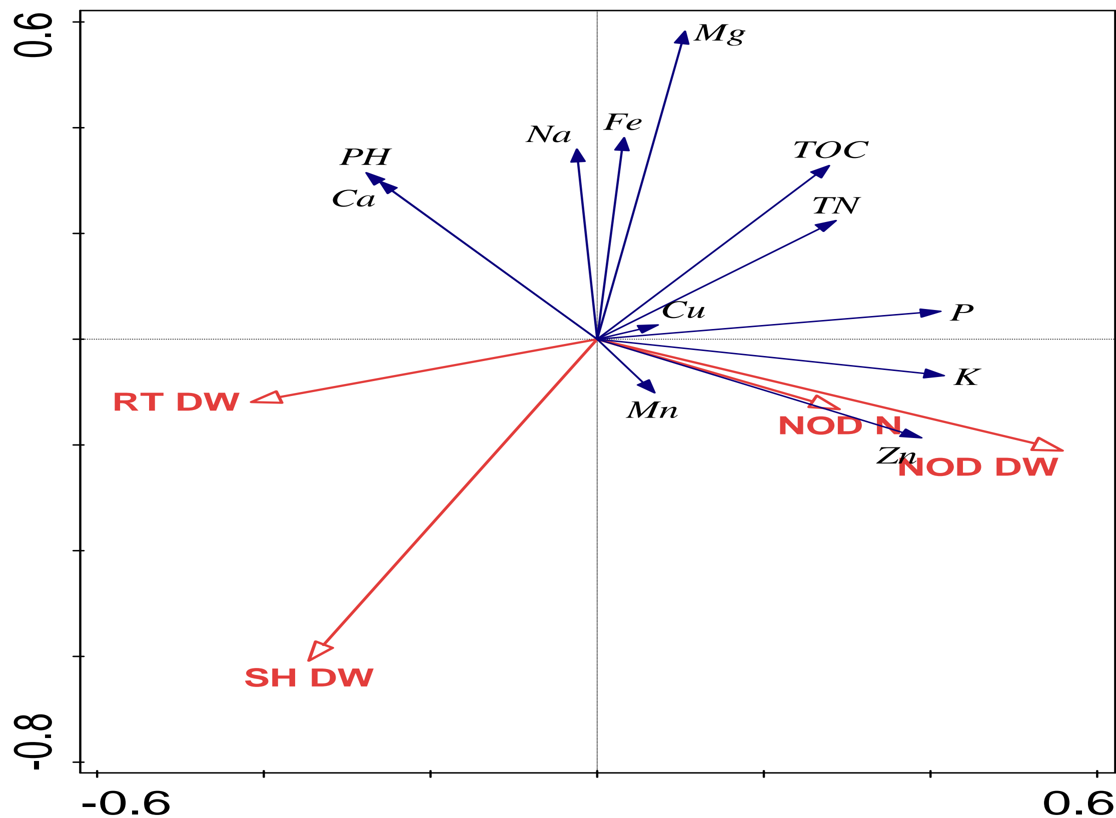

Figure 4.1: RDA comparing soil physico-chemical properties from Embu County family farms and greenhouse experiment on *Vigna unguiculata* planted in the same soils

Key: RTDW, root dry weight; SHDW, shoot dry weight; NODN, nodule number; NODDW, nodule dry weight; pH, soil potential of hydrohen ions; TN, total nitrogen; TOC, total organic carbon; P, % of phosphorus; K, % of potassium; Ca, % of calcium;

Mg, % of magnesium; Mn, % of manganese; Cu, % of copper; Fe, % of iron; Zn, % of zinc; Na, % of sodium.

Summary for figure 4.1 above:

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigen values	0.1015	0.058	0.0244	0.0015
Explained disparity (cumulative)	10.15	16.04	18.48	18.62
Pseudo-canonical correlation	0.5296	0.458	0.3752	0.2888
Explained fitted variation (cumulative)	54.51	86.10	99.22	100.00
Permutation Test Results: On All Axes	pseudo-F=1.4, P=0.154.			

4.1.4 Morpho-cultural characteristics of the trapped native rhizobia isolates from Embu region

A total of 94 native rhizobia isolates were isolated from the roots nodules obtained from the soils collected in Embu County. The isolates were placed into 12 different groups, designated I - XII, based on their differences in morphological and biochemical traits (Table 4.2). Isolates from all the farms were represented in each of the morpho-cultural groupings. Nonetheless, the majority of the isolates were grouped under VIII with a total percentage of 13.8 %, while group XII carried the least number of the total isolates (1.1 %). In addition, all of the isolates are Grain negative bacteria (pink in color). The colonies size of the isolates ranged between 1.0 - 5.6 mm in length. All of the isolates absorbed Congo red dye and turned Bromo-thymol blue (BTB) media plates from green to yellow (Plate 4.2).

Table 4.2: Morphological characteristics of Embu isolates

Group of isolates	Isolates characteristics											
	BTB	Margin	Congo red absorption	Color	Elevation	Gram stain	Size (mm)	Texture	Colony shape	Transpa-rency	Nature of growth	% of isolates
I	ye	S	crn	crw	co	-ve	5.3	sg	c	tr	fgr	10.6
II	ye	S	crn	ww	cv	-ve	5.6	fg	c	op	fgr	19.1
III	ye	sc	crn	mw	cv	-ve	4.3	fig	r	tr	fgr	17
IV	ye	S	crn	ww	do	-ve	2.3	g	Ir	tr	fgr	5.3
V	ye	sc	crn	w	cv	-ve	4.0	g	c	tr	fgr	3.2
VI	ye	swm	crn	mw	do	-ve	1.3	sg	r	trpm	sgr	6.4
VII	ye	s	crn	crw	ra	-ve	5.0	fig	r	tr	fgr	7.4
VIII	ye	s	crn	crw	cv	-ve	2.0	g	c	op	sgr	13.8
IX	ye	s	crn	crw	cv	-ve	2.0	sg	r	trpm	sgr	10.6
X	ye	s	crn	ww	cv	-ve	3.0	fig	ir	tr	fgr	3.2
XI	ye	s	crn	cm	do	-ve	1.0	sf	r	tr	sgr	2.1
XII	ye	s	crn	w	cv	-ve	1.0	sf	c	tropc	sgr	1.1

Key: mw, milky white; ww, watery white; crw, cream white; w, white; cm, cruded milky; s, smooth; sc, smooth clear; wsm, white spotted in the middle; do, doment; cv, converx; tr, translucent; op, opaque; trpm, translucent with white spotted in the middle; tropc, translucent with opaque center; ra, raised; c, circular; r, rod shape; ir, irregular shape; sg, soft gummy; g, gummy; fg, flowing gummy; fig, firm gummy; fgr, fast growing rhizobia within two days; sgr, slow growing rhizobia within two days; -ve, Gram negative (pink in colour); BTB, Bromo-thymol blue; ye, btb reaction turned yellow; crn, congo red non-absorbing; mm, millimeter; %, percentage of isolates. A total of 94 native rhizobia isolates.

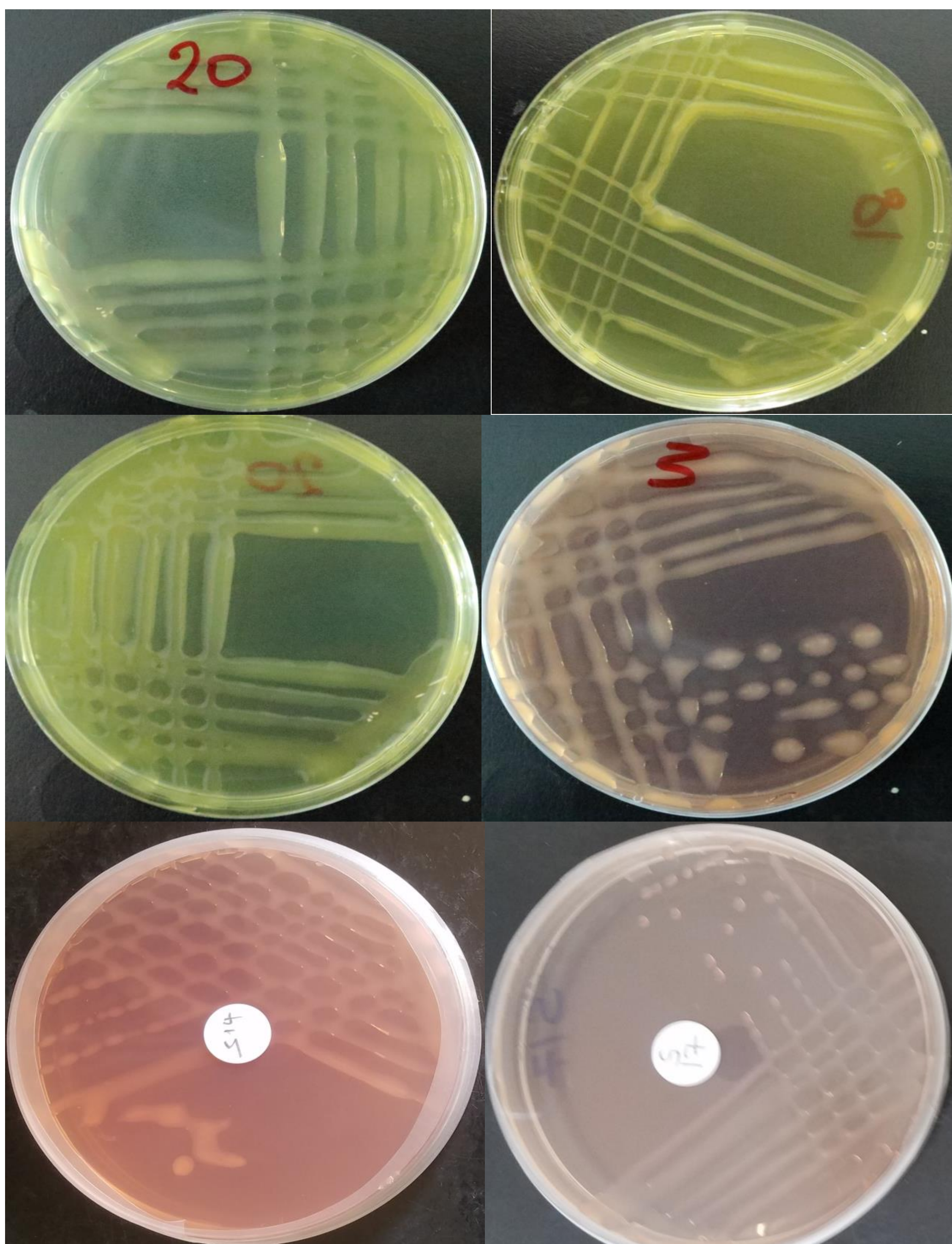

Plate 4.2: Morpho-cultural characterization of the native rhizobia isolates
Isolates 20 and 18 are examples of fast growing rhizobia on YEMA-BTB media, turning it from green to yellow (acid producers). In contrast, isolates 3, 14 and 17 are grown on YEMA-CrD media (congo red non-absorbing).

4.1.5 Cluster analyses of the trapped nodules rhizobia isolates from soil farms in Embu Country based on morphological and biochemical characteristics

Based on cluster analysis of the trapped rhizobia isolates from Embu County, it was established that there were three main phenotypic clusters (Cluster A, B and C) (Figure 4.2). Phenotypic cluster A had the majority of the trapped rhizobia isolates. It was further divided in two sub-clusters and consisted of group XII, group VIII, group II, group IX, group VI and group XI. Phenotypic cluster B had two sub-clusters consisting of group V, group III, group X, and Group IV. Cluster C had only group VII and group I. The evolutionary tree Euclidian Similarity Index (ESI) was calculated using the Neighbor Joining Method (NJM). The bootstrap values ranged from 31 - 51 and replicated 1,000 times to estimate the clusters W analysis values.

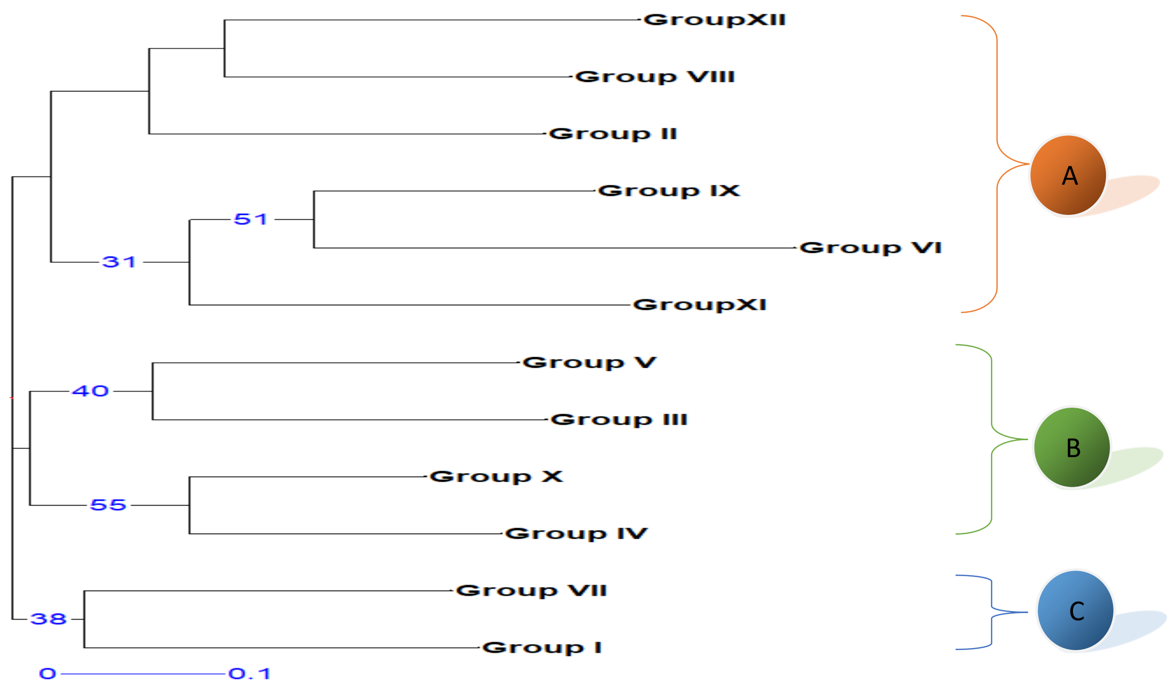

Figure 4.2: Phenotypic tree based on NJM connections and ESI displaying morpho-cultural grouping of the trapped rhizobia isolates from Embu County

4.1.6 Physico-chemical parameters of soil's from Kitui family farms region

In Kitui County, the soil pH of the selected thirty farms ranged from 5.66 to 6.74. The lowest total nitrogen content (TN) was recorded in farm KT8 with a value of 0.04, while the highest TN content was recorded in farms K10 and K6 with a value of 0.13. The soil texture was classified as sandy-clay-loam, sandy-loam and loamy-sandy. The highest total organic carbon (TOC) of 1.18 was detected in farm K15. The study recorded phosphorus (P) levels of between 20 ppm and 250 ppm. Farms KT14 and KT15 recorded the highest potassium (K) level of 1.54 Cmol kg^{-1}, while farms K12, K11 and K6 registered the highest levels for calcium (Ca), magnesium (Mg), manganese (Mn) and copper (Cu) of 28 %, 5.43 Cmol kg^{-1}, 0.58 % and 6.8 %, respectively. Farms K14 and K15 showed the highest concentration of iron (Fe) at 107. Interestingly, farm K6 showed the highest concentration for both manganese and copper, and at the same time showed the highest total nitrogen content. Farms K14 and K15 registered the highest levels of potassium and iron.

4.1.7 Interactive effect of soil on cowpea plants (K-80) nodulation, root and shoot enhancement

The soils that were collected from the thirty farms (K1 - K15, and KT16 - KT30) in Kitui County, were used to trap rhizobia using *Vigna unguiculata* seeds as the trapping host (Table 4.3). The growth performance of the plants varied, there was a significant difference ($P<0.0001$) in nodules number (NodN) of the plants. The NodN ranged from 8.17±0.70 $plant^{-1}$ to 61.50±7.00 $plant^{-1}$ (average±standard error). Farm K5 showed the highest NodN, whereas, KT26 revealed the lowest NodN. All the farms had indigenous

rhizobia in the soils which, induced nodules formation. In addition, there was a significant difference (P<, 0.0001) in nodule dry weight (NodDW) whereby, the NodDW ranged from 9.00±1.00 mg $plant^{-1}$ to 94.00±21.00 mg $plant^{-1}$ (average±standard error). Farms with K5, KT17, KT22, KT25, KT28, and KT30 had the highest NodDW >80.00 mg $plant^{-1}$.

There was a significant difference (P<0.0001) in SHtDW. The highest shoot dry weight (SHtDW) of 2.30±0.09 g $plant^{-1}$ (average±standard error) was recorded in KT22 whereas, the lowest SHtDW of 0.76±0.08 g $plant^{-1}$ was recorded in K4. In addition, K10, KT19, KT24, and KT29 also had high SHtDW when compared to other farms. There was a significant difference in the root dry weight (RtDW) of the various farms at p≤0.05 (Table 4.3). KT24 had the highest RtDW of 0.36±0.03 g $plant^{-1}$ whereas, the lowest RtDW of 0.08±0.01 g $plant^{-1}$ (average±standard error) was recorded in KT26.

Table 4.3: Average nodule number (NodN plant^{-1}), nodule dry weight (NodDW mg plant^{-1}), shoot dry weight (SHtDW g plant^{-1}) and root dry weight (RtDW g plant^{-1}) of cowpea plants from the greenhouse experiment

Soil samples	**NodN(plant^{-1})**	**NodDW(mg plant^{-1})**	**SHtDW(g plant^{-1})**	**RtDW(g plant^{-1})**
K1	31.67±2.92^{b-f}	16.00±8.00^{a-d}	1.41±0.12^{e-i}	0.23±0.02^{b-f}
K2	30.00±3.86^{b-f}	15.00±7.00^{a-d}	1.47±0.08^{d-h}	0.24±0.05^{a-e}
K3	29.17±2.17^{b-f}	45.00±3.00^{a-d}	1.28±0.07^{f-l}	0.17±0.03^{c-j}
K4	15.83±2.82^{e-f}	18.00±2.00^{d-e}	0.76±0.08^{n}	0.11±0.02^{h-j}
K5	61.50±7.00^{a}	92.00±11.00^{a}	1.25±0.09^{f-m}	0.22±0.01^{b-h}
K6	27.67±4.29^{b-f}	53.00±10.00^{a-d}	1.01±0.06^{i-n}	0.12±0.01^{f-j}
K7	34.83±4.02^{b-e}	58.00±6.00^{a-d}	1.50±0.06^{d-g}	0.20±0.01^{b-i}
K8	34.17±5.40^{b-f}	44.00±8.00^{b-e}	1.00±0.03^{i-n}	0.13±0.02^{f-j}
K9	31.33±4.10^{b-f}	58.00±17.00^{a-d}	1.33±0.12^{e-j}	0.13±0.02^{d-j}
K 10	38.00±4.34^{a-e}	48.00±7.00^{a-d}	2.19±0.05^{a-b}	0.27±0.03^{a-c}
K11	19.00±4.85^{d-f}	27.00±5.00^{c-e}	1.33±0.10^{e-j}	0.18±0.01^{c-j}
K12	43.00±1.51^{a-d}	64.00±7.00^{a-d}	1.10±0.10^{g-n}	0.16±0.02^{c-j}
K13	48.00±4.56^{a-c}	79.00±13.00^{a-c}	1.52±0.09^{d-g}	0.16±0.021^{-j}
K14	34.50±7.62^{b-f}	55.00±7.00^{a-d}	0.85±0.04^{k-n}	0.12±0.02^{f-j}
K15	20.50±4.26^{d-f}	34.00±11.00^{b-e}	1.37±0.13^{e-j}	0.12±0.02^{f-j}
KT16	42.50±3.16^{a-d}	75.00±11.00^{a-d}	1.16±0.08^{g-n}	0.11±0.01^{f-j}
KT17	43.33±4.48^{a-d}	87.00±16.00^{a-b}	1.30±0.06^{f-k}	0.13±0.02^{f-j}
KT18	44.50±8.77^{a-d}	72.00±11.00^{a-d}	1.36±0.10^{e-j}	0.16±0.01^{c-j}
KT19	42.50±8.26^{a-d}	73.00±14.00^{a-d}	2.04±0.04^{a-c}	0.25±0.02^{a-e}
KT20	29.50±2.93^{b-f}	49.00±8.00^{a-d}	1.75±0.08^{b-e}	0.21±0.01^{b-i}
KT21	25.83±4.35^{c-f}	35.00±6.00^{b-e}	0.84±0.06^{l-n}	0.13±0.02^{e-j}
KT22	53.83±7.61^{a-b}	93.00±14.00^{a}	2.30±0.09^{a}	0.30±0.04^{a-b}
KT23	33.67±5.25^{b-f}	63.00±13.00^{a-d}	1.34±0.14^{e-j}	0.18±0.01^{c-j}
KT24	35.83±5.80^{a-e}	66.00±15.00^{a-d}	2.14±0.05^{a-b}	0.36±0.03^{a}
KT25	34.67±3.49^{b-f}	83.00±15.00^{a-c}	0.92±0.04^{j-n}	0.10±0.01^{i-j}
KT26	8.17±0.70^{f}	9.00±1.00^{e}	0.82±0.07^{n-m}	0.08±0.01^{j}
KT27	37.33±3.55^{a-e}	61.00±2.00^{a-d}	1.62±0.12^{c-f}	0.20±0.03^{b-j}
KT28	44.83±4.30^{a-d}	84.00±9.00^{a-b}	1.04±0.07^{h-n}	0.13±0.02^{d-j}
KT29	38.17±6.28^{a-e}	94.00±21.00^{a}	2.12±0.10^{a-b}	0.31±0.04^{a-b}
KT30	36.83±3.49^{a-e}	55.00±6.00^{a-d}	1.88±0.04^{a-d}	0.23±0.02^{b-g}
P values	<0.0001	<0.0001	<0.0001	<0.0001

Key: NodN, nodules number; NodDW, dry nodules weight; SHtDW, dry shoot weight; RtDW, dry root weight; g plant^{-1}, gram per plant; mg plant^{-1}, milligram per plant. Values followed by the same letters within the column are not significantly different according to Tukey`s Honest Significant Difference at 5 % level.

4.1.8 Correlation coefficient of soil physico-chemical properties on cowpea plants growth parameters

Redundancy analysis comparing the soil physico-chemical parameters of Kitui County farms, and the greenhouse experiment on cowpea plants growth parameters indicated that, there is a direct correlation between NodDW, NodN, SHDW and RTDW with Fe, K, Mg and Mn. However, there is also an indirect correlation between TOC and TN with NodDW and NodN. pH, Na, and Ca correlate negatively with SHDW, NodDW and NodN (Figure 4.3). In addition, RTDW positively correlated with Mn, TN, and TOC.

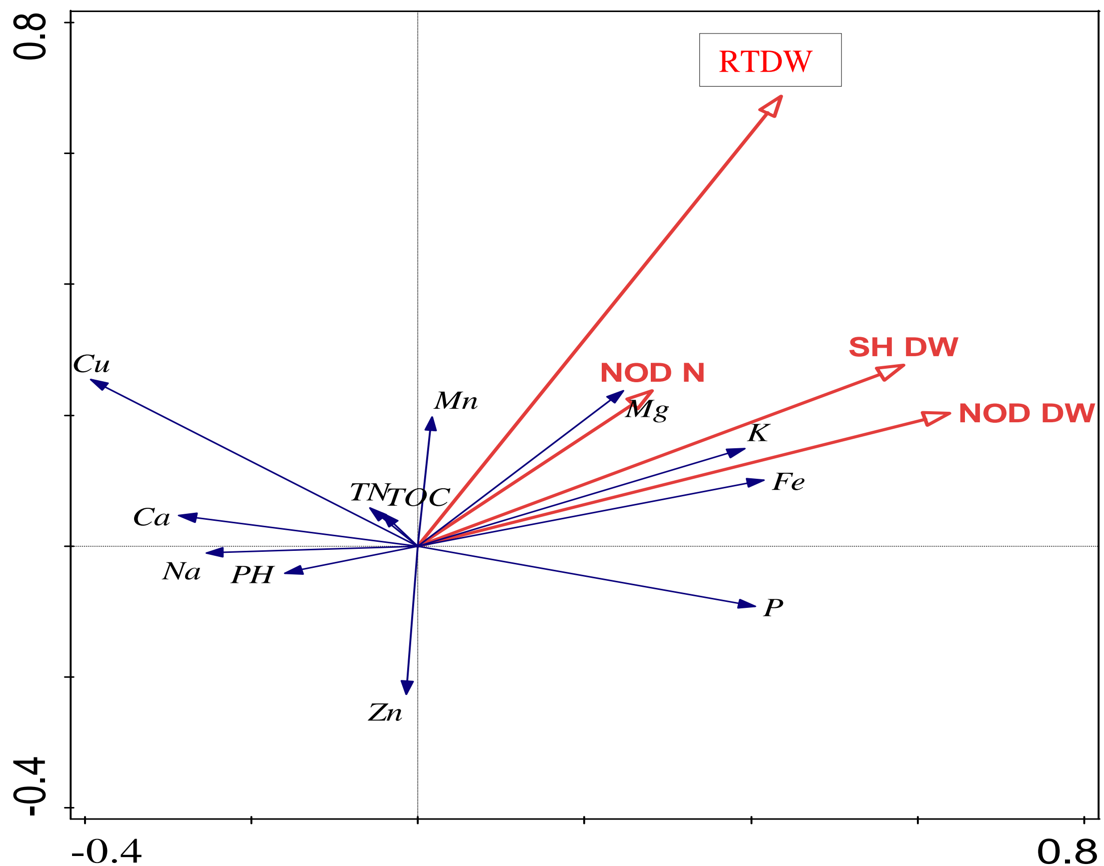

Figure 4.3: RDA analysis showing relationship between soil properties from Kitui County family farms and greenhouse experiment on cowpea plant parameters

Key: RTDW, root dry weight; SHDW, shoot dry weight; NODN, nodule number; NODDW, nodule dry weight; pH, soil potential of hydrohen ions; TN, total nitrogen;

TOC, total organic carbon; P, % of phosphorus; K, % of potassium; Ca, % of calcium; Mg, % of magnesium; Mn, % of manganese; Cu, % of copper; Fe, % of iron; Zn, % of zinc; Na, % of sodium.

Summary for figure 4.2 above:

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigen values	0.1448	0.0153	0.0088	0.0023
Explained variation (cumulative)	14.48	16.01	16.89	17.13
Pseudo-canonical correlation	0.4601	0.4386	0.3174	0.3083
Explained fitted variation (cumulative)	84.53	93.49	98.64	100.00
Permutation Test Results: On All Axes	pseudo-F=1.3, P=0.			

4.1.9 Morpho-cultural characteristics of the trapped native rhizobia isolates from soils in Kitui region

In Kitui County, a total of 96 rhizobia isolates were obtained from the nodules**.** The isolates were morphologically and biochemically placed into 13 distinct groups, and designated I - XIII, (Table 4.4). Group VII had the highest percentage of the isolates (17.7 %) whereas, groups VI and XI carried the least percentage of the total isolates (2.1 %). All of the isolates absorbed Congo red dye and turned Bromo-thymol blue (BTB) media plates from green to yellow (ye). In addition, all of the isolates are Grain negative bacteria (pink in color). The colonies size of the isolates ranged between 1 mm - 5.1 mm in length.

Table 4.4: Morphological characteristics of Kitui County isolates

Group of isolates	Isolates characteristics										
	BTB	Margin	Congo red absorption	Color	Elevation	Gram stain	Size (mm)	Texture	Colony shape	Transpa -rency	Nature of growth	% of isolates
I	ye	s	crna	crw	do	-ve	5.1	sg	c	tr	fgr	5.2
II	ye	sc	crna	ww	cv	-ve	4.6	fg	c	op	fgr	10.4
III	ye	s	crna	mw	cv	-ve	3.3	fig	Ir	tr	fgr	15.6
IV	ye	s	crna	ww	cv	-ve	3.3	g	r	tr	fgr	5.2
V	ye	sc	crna	w	cv	-ve	3.0	g	c	tr	fgr	3.1
VI	ye	s	crna	mw	cv	-ve	4.0	sg	c	tr	fgr	2.1
VII	ye	sc	crna	crw	ra	-ve	2.0	fg	r	tr	sgr	17.7
VIII	ye	s	crna	cm	cv	-ve	5.0	g	ra	op	fgr	8.3
IX	ye	s	crna	crw	cv	-ve	2.1	sg	c	trsm	sgr	6.3
X	ye	s	crna	ww	cv	-ve	3.0	fg	ir	tr	fgr	12.5
XI	ye	s	crna	cm	ra	-ve	1.0	sf	c	tr	sgr	2.1
XII	ye	s	crna	wc	do	-ve	1.0	sf	c	tropc	sgr	3.1
XIll	ye	sc	crna	mw	cv	-ve	4.0	g	c	tr	fgr	8.3

Key: mw, milky white; ww, watery white; crw, cream white; w, white; cm, cruded milky; s, smooth; sc, smooth clear; do, doment; cv, convex; tr, translucent; trsm, translucent with spotted in the middle; op, opaque; tropc, translucent with opaque center; ra, raised; c, circular; r, rod shape; Ir, irregular shape; sg, soft gummy; g, gummy; fg, flowing gummy; fig, firm gummy; fgr, fast growing rhizobia within two days; mm, millimeter; sgr, slow growing rhizobia within two days; -ve, Gram negative bacteria (pink in color); BTB, Bromo-thymol blue; ye, BTB reaction turned yellow; crna, congo red non-absorbing; %, percentage of isolates. A total of 96 native rhizobia isolates.

4.1.10 Cluster analyses of the trapped native rhizobia nodule isolated from farms in Kitui County based on morpho-cultural characteristics

Using the NJM and ESI, the trapped isolates obtained from cowpea (K-80) plants root nodules were grouped into three main clusters (Cluster 1, 2 and 3). The trapped rhizobia isolates were clustered based on their morpho-cultural characteristics. Cluster 1 had four isolates groups, while cluster 2 had most of the trapped nodule isolates groups (six groups), and Cluster 3 represented only three rhizobia isolates groups (Figure 4.4). The representative clusters of the different trapped rhizobia isolates depended on the neighboring phenotypic characteristics, with bootstrap values ranging from 30 - 83 and replicated 1,000 times.

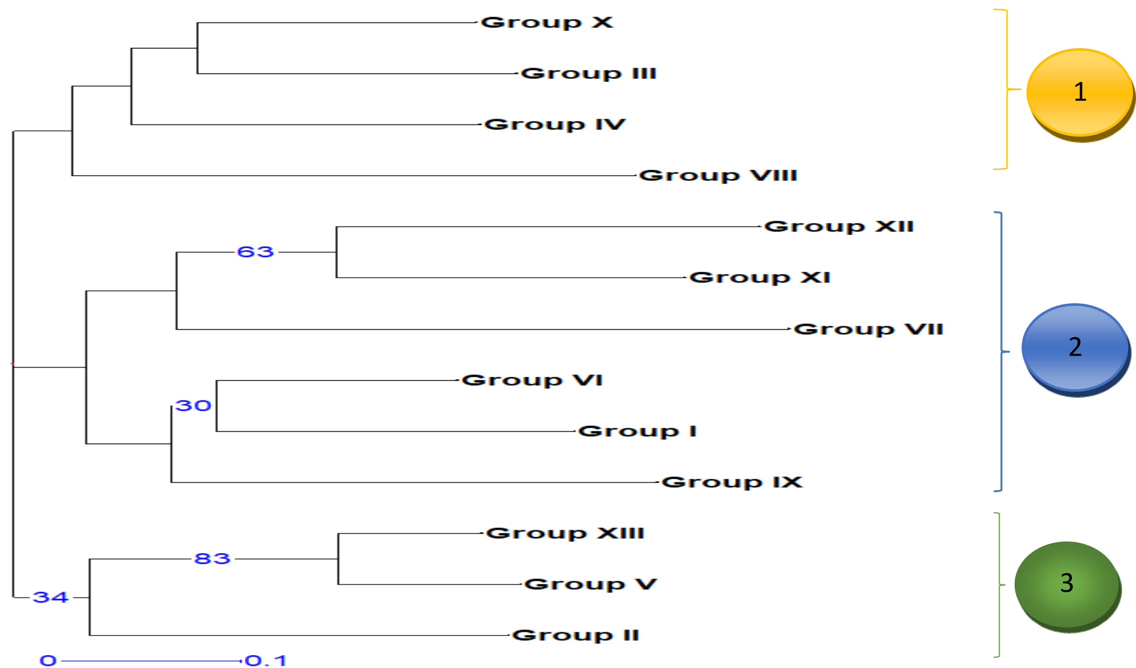

Figure 4. 4: Cluster analysis based on NJM and ESI showing morphological connection of the trapped cowpea nodule isolates from Kitui County

4.1.11 Physico-chemical parameters of soil's collected from family farms in Tharaka Nithi region

In Tharaka Nithi County, the highest pH of the soils (7.25) which was recorded in farm TH2. The lowest pH (5.36) was recorded in farm TH13. The total nitrogen (TN) and total organic carbon (TOC) contents ranged between (0.10 to 0.18) and (0.86 to 2.07), respectively. Mineral levels for the farms ranged from (5.00) in T11 to (250.00) in T6. For phosphorus (P), (0.24 ppm) in farm TH7 to (1.24 ppm) in farm TH4 for potassium (K), (1.00 cmol kg^{-1}) in farm TH12 to (38.00 cmol kg^{-1}) in farm T5 and for calcium (Ca) also. The textural classification of the soils were; sandy-clay-loam, sandy-loam, sandy-clay, loamy-sandy, and clay. The highest content of magnesium (Mg), (5.30 %); manganese (Mn), (3.00 cmol kg^{-1}); copper (Cu), (4.04); iron (Fe), (98.0); zinc (Zn), (7.52); and sodium (Na), (5.93) were recorded in farms TH14, T9, TH15, TH7, T10 and T11.

4.1.12 Interactive effect of soil on cowpea (K-80) plants root nodulation and shoot improvement

The growth performance of the plants varied from the soils collected in Tharaka Nithi County, there was a significant difference ($P<0.0001$) in nodules number (NodN) of the plants. The NodN ranged from 4.18±1.17 $plant^{-1}$ to 70.33±9.18 $plant^{-1}$ (average±standard error) (Table 4.5). Farm TH21 showed the highest NodN, whereas TH30 revealed the lowest NodN. All the farms had indigenous rhizobia in the soils which, induced the formation of nodules with the legume plants. There was a significant difference ($P<0.0001$) in nodule dry weight (NodDW) whereby the NodDW ranged

from 6.00±1.00 mg $plant^{-1}$ to 96.00±13.00 mg $plant^{-1}$ (average±standard error). Farm TH21 had the highest NodDW when compared to other farms TH30. There was a significant difference ($P<0.0001$) in shoot dry weight (SHtDW). The highest SHtDW of 2.23±0.14 g $plant^{-1}$ was recorded in T6 whereas, the lowest SHtDW of 0.53±0.04 g $plant^{-1}$ (average±standard error) was recorded in TH23. In addition, TH16, TH18, TH21, and TH25 also had high SHtDW (>2) when compared to other farms like TH11, TH12, TH17, TH23, and TH28. There was a significant difference in the root dry weight (RtDW) of the various farms at $p\leq0.05$, (Table 4.5). Farm T7 had the highest RtDW of 0.28±0.02 g $plant^{-1}$ whereas, the lowest RtDW of 0.07±0.01 g $plant^{-1}$ (average±standard error) was recorded in TH17 and TH23.

Table 4.5: Rhizobia nodules trapped from soils collected from various farms in Tharaka Nithi County, to determine the average nodule number (NodN plant^{-1}), nodule dry weight (NodDW mg plant^{-1}), shoot dry weight (SHtDW g plant^{-1}) and root dry weight (RtDW g plant^{-1}) of cowpea plants from the greenhouse experiment

Soil samples	NodN(plant^{-1})	NodDW(mg plant^{-1})	SHtDW(g plant^{-1})	RtDW(g plant^{-1})
T1	53.00±5.35^{a-d}	77.00±9.00^{a-c}	1.98±0.06^{a-c}	0.26±0.03^{a-b}
T2	21.33±2.12^{f-k}	23.00±3.00^{f-i}	0.83±0.03^{e-f}	0.10±0.01^{g-h}
T3	38.17±4.50^{b-h}	56.00±8.00^{b-g}	1.91±0.08^{a-c}	0.23±0.02^{a-d}
T4	33.67±4.22^{c-i}	37.00±6.00^{c-g}	1.87±0.03^{a-c}	0.22±0.02^{a-e}
T5	57.50±3.66^{a-b}	83.00±7.00^{a-c}	1.98±0.01^{a-c}	0.21±0.02^{a-f}
T6	60.83±5.96^{a-b}	88.00±12.00^{a-b}	2.23±0.14^{a}	0.22±0.03^{a-c}
T7	54.33±5.43^{a-c}	77.00±9.00^{a-c}	1.82±0.07^{b-c}	0.28±0.02^{a}
T8	20.83±2.19^{f-g}	19.00±3.00^{h-g}	1.24±0.17^{c}	0.11±0.01^{f-h}
T9	52.67±4.73^{a-d}	79.00±7.00^{a-c}	1.87±0.05^{a-c}	0.21±0.02^{a-f}
T10	54.83±7.02^{a-c}	73.00±7.00^{a-d}	1.75±0.09^{c}	0.23±0.02^{a-d}
T11	22.17±2.09^{f-k}	22.00±2.00^{h-g}	0.90±0.07^{d-f}	0.14±0.02^{d-h}
T12	13.83±1.58^{h-k}	22.00±2.00^{h-g}	0.88±0.03^{d-g}	0.13±0.03^{e-h}
T13	29.00±5.69^{d-j}	31.00±5.00^{e-i}	1.91±0.05^{a-c}	0.21±0.01^{a-e}
T14	39.50±3.57^{b-g}	47.00±8.00^{c-g}	1.89±0.05^{a-c}	0.20±0.01^{a-f}
T15	49.33±6.76^{a-e}	87.00±11.00^{a-b}	1.99±0.05^{a-c}	0.23±0.02^{a-e}
TH16	44.67±3.90^{b-f}	80.00±7.00^{a-c}	2.07±0.07^{a-c}	0.23±0.02^{a-e}
TH17	9.83±0.70^{i-k}	8.00±1.00^{i}	0.70±0.07^{f-g}	0.07±0.01^{h}
TH18	45.17±7.60^{b-f}	78.00±11.00^{a-c}	2.00±0.01^{a-c}	0.25±0.03^{a-c}
TH19	38.83±3.05^{b-g}	55.00±4.00^{b-g}	1.99±0.01^{a-c}	0.19±0.01^{a-f}
TH20	34.00±3.05^{c-i}	18.00±2.00^{h-g}	1.74±0.08^{c}	0.08±0.01^{h}
TH21	70.33±9.18^{a}	96.00±13.00^{a}	2.01±0.03^{a-c}	0.21±0.02^{a-e}
TH22	57.00±7.12^{a-c}	79.00±11.00^{a-c}	1.82±0.03^{b-c}	0.20±0.01^{a-f}
TH23	7.83±2.09^{j-k}	8.00±2.00^{i}	0.53±0.04^{g}	0.08±0.03^{h}
TH24	56.67±6.78^{a-c}	82.00±12.00^{a-c}	1.94±0.02^{a-c}	0.17±0.01^{a-h}
TH25	17.00±1.77^{f-g}	29.00±4.00^{e-i}	2.19±0.01^{ab}	0.22±0.01^{a-e}
TH26	26.83±0.70^{e-k}	48.00±3.00^{c-g}	1.18±0.16^{d-e}	0.20±0.02^{a-f}
TH27	42.67±3.54^{b-f}	62.00±8.00^{a-f}	1.91±0.06^{a-c}	0.22±0.02^{a-e}
TH28	9.33±0.76^{i-k}	10.00±2.00^{h-i}	0.81±0.07^{e-g}	0.08±0.01^{h}
TH29	41.67±2.73^{b-g}	63.00±14.00^{a-d}	1.07±0.03^{d-f}	0.15±0.03^{c-h}
TH30	4.18±1.17^{k}	6.00±1.00^{i}	0.73±0.09^{f-g}	0.09±0.03^{h}
P values	<0.0001	<0.0001	<0.0001	<0.0001

Key: NodDN, nodules number; NodDW, dry nodules weight; SH DW, dry shoot weight; RT DW, root dry weight; g plant^{-1}, gram per plant; mg plant^{-1}, milligram per plant. Values followed by the same letters within the column are not significantly different according to Tukey`s Honest Significant Difference (HSD) at 5 % level.

4.1.13 Correlation coefficient of soil physico-chemical properties on cowpea plants growth parameters

Redundancy analysis (RDA) were done to relate the soil physical and chemical properties and the greenhouse experiment on cowpea plants growth parameters indicated that, there is a direct correlation between SHDW and K. Ca and pH correlated directly with NodDW, RTDW, and NodN. TN, Mn, P, TOC and K correlate negatively with NodN and NodDW (Figure 4.5). Interestingly, Na, Mg, Fe and Zn correlated indirectly (negatively) with SHDW.

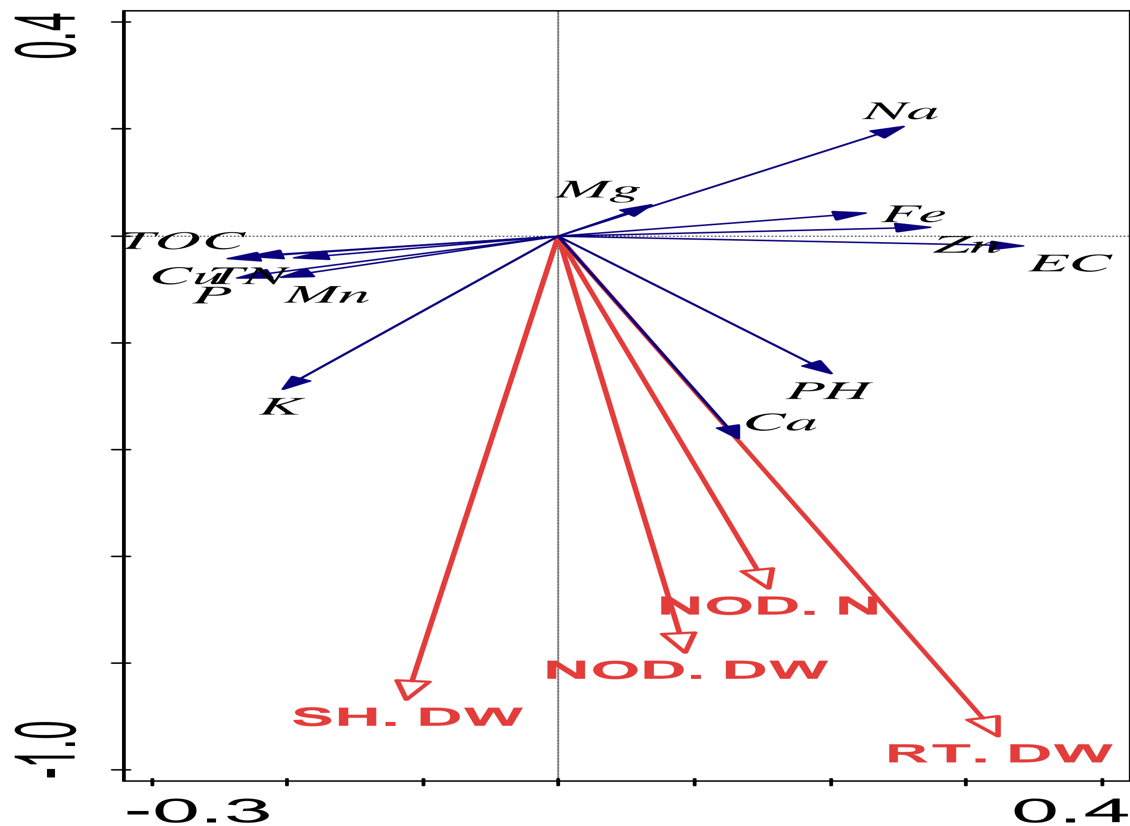

Figure 4.5: RDA analysis comparing physico-chemical properties of soils from Tharaka Nithi County and greenhouse experiment on cowpea plants parameters

Key: RT.DW, root dry weight; SH.DW, shoot dry weight; NOD.N, nodule number; NOD.DW, nodule dry weight; pH, soil potential of hydrohen ions; TN, total nitrogen;

TOC, total organic carbon; P, % of phosphorus; K, % of potassium; Ca, % of calcium; Mg, % of magnesium; Mn, % of manganese; Cu, % of copper; Fe, % of iron; Zn, % of zinc; Na, % of sodium.

Summary of the above figure 4.3:

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigen values	0.0483	0.0348	0.0101	0.0038
Explained difference (cumulative)	4.83	8.32	9.33	9.71
Pseudo-canonical correlation	0.4957	0.4136	0.1897	0.1991
Explained fitted variation (cumulative)	49.79	85.65	96.07	100.00
Permutation Test Results: On All Axes	pseudo-F=0.7, P=0.			

4.1.14 Morpho-cultural characteristics of the trapped native rhizobia isolates collected from Tharaka Nithi County farm soils

A total of 121 isolates were collected from the root nodules obtained from the soil from farms in Tharaka Nithi County. The isolates were placed into 17 separate groups, designated I - XVII, based on their differences in biochemical and morphological characteristics (Table 4.6). Isolates from all the farms were represented in each of the morphological groupings. Group XIII had the highest percentage of the isolates (12.5 %) whereas, groups XVI and XVII carried the least number of the total isolates (1.7 %). All of the isolates absorbed Congo red dye and turned Bromo-thymol blue (BTB) media plates from green to yellow (ye) (Plate 4.12). Furthermore, all of the isolates are Grain negative bacteria (pink in color). The colonies size of the isolates ranged between 0.8 - 8.0 mm in length.

Table 4.6: Morphological characteristics of Tharaka Nithi rhizobia isolates

Group of isolates	**Isolates characteristics**											
	BTB	Margin	Congo-red absorbing	Color	Elevation	Gram stain	Size (mm)	Texture	Colony shape	Transpa-rency	Nature of growth	% of isolates
I	ye	s	cnb	w	do	-ve	5.0	fg	r	tr	fgr	7.4
II	ye	sc	cnb	crw	ra	-ve	6.0	fg	c	tr	fgr	10
III	ye	s	cnb	crw	co	-ve	5.6	sf	c	tr	fgr	7.4
IV	ye	s	cnb	miw	co	-ve	1.3	fg	r	tr	sgr	4.1
V	ye	s	cnb	wm	co	-ve	3.0	fig	c	op	fgr	8.3
VI	ye	sc	cnb	cm	do	-ve	2.6	sg	c	op	fgr	7.4
VII	ye	s	cnb	mw	do	-ve	1.0	g	r	op	sgr	3.3
VIII	ye	s	cnb	ww	do	-ve	4.0	g	c	tr	fgr	3.3
IX	ye	sc	cnb	crw	do	-ve	5.3	sg	c	tr	fgr	11.6
X	ye	S	cnb	cm	co	-ve	2.0	sg	c	op	sgr	5.8
XI	ye	S	cnb	miw	co	-ve	2.5	fig	c	tr	sgr	5
XII	ye	S	cnb	cm	do	-ve	8.0	fg	r	op	fgr	2.5
XIII	ye	sc	cnb	crw	ra	-ve	3.2	fg	c	tr	fgr	12.5
XIV	ye	S	cnb	ww	do	-ve	2.0	Fig	ir	tr	fgr	5.8
XV	ye	Sc	cnb	miw	co	-ve	2.0	sg	c	tr	fgr	2.5
XVI	ye	Sc	cnb	wcm	co	-ve	0.8	sg	c	tr	sgr	1.7
XVII	ye	Sc	cnb	wc	do	-ve	4.6	g	c	op	fgr	1.7

Key: miw, milky white; ww, watery white; crw, cream-white; w, white; cm, crude milky; s, smooth; sc, smooth clear; wsm, white-spotted in the middle; do, doment; do, convex; ra, raised; tr, translucent; op, opaque; c, circular; r, rod shape; Ir, irregular shape; sg, soft gummy; g, gummy; fg, flowing gummy; Fig, firm gummy; fgr, fast-growing rhizobia within two days; sgr, slow-growing rhizobia within two days; -ve, Gram negative bacteria; ye, BTB (bromo-thymel blue reaction) turned yellow; cnb, congo red non-absorbing; cra; %, percentage of isolates. A total of 121 native rhizobia isolates.

4.1.15 Cluster analyses of the trapped nodules rhizobia isolates from soil farms in Tharaka Nithi County based on morphological and biochemical characteristics

Rhizobia isolates obtained from cowpea (K-80) plants root nodules had three main phenotypic clusters (Cluster A, B and C). Phenotypic cluster A represented two sub-clusters and had the majority of the trapped rhizobia morpho-cultural isolates grouped into groups (X, V, III, XI, IV, XVI and XV) (Figure 4.6). Phenotypic cluster B had two sub-clusters which are grouped into groups (XVII, VII, XII, I, XIV, and VIII). However, cluster C had the minority of the groups (XIII, II, IX and VI). The evolutionary tree ESI was calculated using the NJM. The bootstrap values ranged from 43 - 99 and replicated 1,000 times to estimate the clusters analysis values.

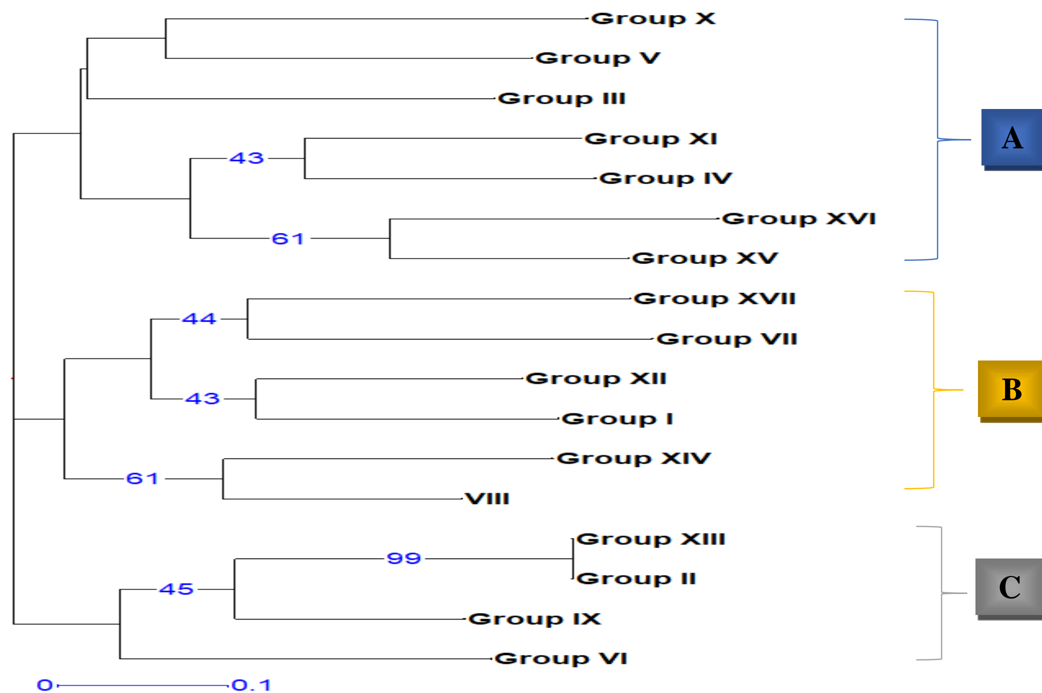

Figure 4.6: Dendrogram based on NJM connections of the morpho-cultural grouping of trapped rhizobia isolates that were isolated from soil collected from farms in Tharaka Nithi County

4.2 Morphological characteristics of the native rhizobia revived isolates

In this study, a total of 53 revived rhizobia cultures (archived samples) were isolated out of which 13 were from soil obtained in family farms from Tharaka Nithi County, 13 isolates were from soils obtained from small-holder farms in Embu County and 27 rhizobia isolates were collected from LVB in Kisumu County. The isolates were placed into 11 different groups, designated as (A - K), based on their differences in morphological and biochemical traits (Table 4.7). Isolates from all the Counties were represented in each of the morpho-cultural groupings. Group H had the highest percentage of the revived rhizobia isolates (17.6 %) whereas, group I carried the least number of the total isolates (1.3 %). All of the isolates cultured in BTB media plates turned the media from green to yellow (y), indicating that the isolates were fast growing rhizobia and acid producers (Plate 4.3). In addition, all of the 53 revived isolates are Grain negative bacteria (pink in color when viewed under the microscopy). The size of the colonies ranged from 0.3 mm - 5.0 mm, all of the isolates did not absorbed Congo red dye when incubated at 28 ^{o}C (Plate 4.4).

Plate 4.3: Rhizobia isolates obtained from the stored revived glycerol stocks clututes
Key: (A) Embu isolate (IsAMR25) on YEMA-Congo red media, isolate did not absorbed the dye, (B) Tharaka-Nithi isolate (IsAMR14) on YEMA- Congo red media

media, the colonies did not absorbed the dye and (C) Kisumu isolate (IsAG3) on YEMA-BTB media, turning the media yellow.

Table 4.7: Morphological grouping of the revived rhizobia cultures (archived samples) obtained from Kisumu, Tharaka Nithi and Embu Counties

Isolates characteristics	Group of isolates characteristics										
	A	B	C	D	E	F	G	H	I	J	K
Gram stain	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Color	miw	ww	ww	cm	w	w	cm	miw	miw	w	cw
Margin	s	s	s	s	sc	s	s	sc	sc	s	sc
Elevation	do	cv	cv	do	cv	do	cv	do	cv	cv	do
Transparency	tr	tr	tr	tr	op	tr	tr	op	op	tr	tr
Size (mm)	5.0	3.0	0.4	4.0	3.5	1.0	2.7	4.0	2.5	0.3	4.5
Colony shape	c	c	ir	r	r	c	c	C	r	r	c
Texture	sg	sg	g	fg	g	fig	fig	fig	sg	g	sg
BTB reaction	y	y	y	y	y	y	y	Y	y	y	y
Congo red	crn	crn	crn	crn	crn	crn	crn	crn	crn	crn	crn
Nature of growth	flg	flg	slg	flg	flg	slg	flg	Flg	flg	slg	flg
% of isolates	10.5	12.2	3.8	9.6	10.2	14.8	6.8	17.6	1.3	4.8	8.5

Key: miw, milky white; cm, crude milky; ww, watery white; cw, cream-white; w, white; s, smooth; sc, smooth clear; do, doment; cv, convex; tr, translucent; op, opaque; c, circular; r, rod shape; ir, irregular shape; sg, soft gummy; g, gummy; fg, flowing gummy; fig, firm gummy; flg, fast growing; slg, slow-growing, -ve, gram-negative; BTB, Bromo-thymol blue reaction; y, BTB reaction turned yellow; crn, congo red non-absorbing; and, mm, millimeter.

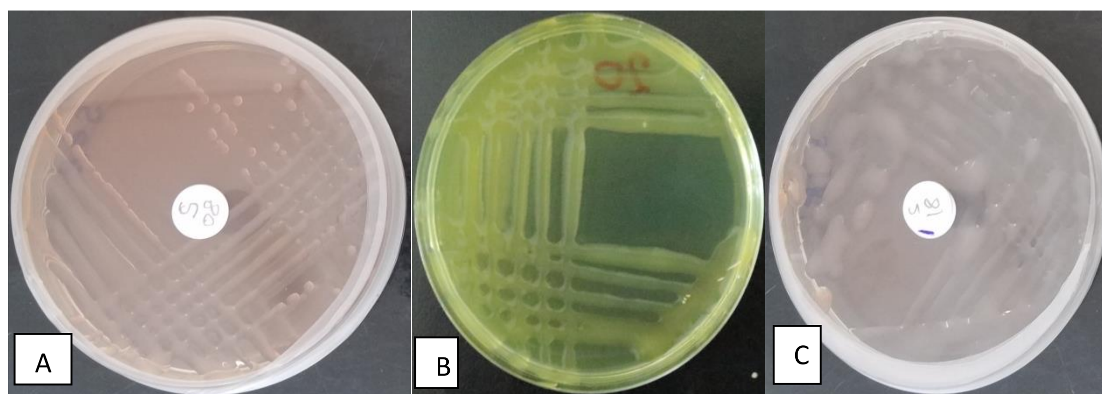

Plate 4.4: Rhizobia isolates morphological and biochemical characterized (A) Congo red non-absorbing, (B) BTB media, and (C) YEMA media without Congo red dye

4.2.1 Cluster analyses of the revived glycerol stocks native rhizobia isolates (archived samples) based on morphol-cultural characteristics

The clustered rhizobia isolates for the entire archived samples established that there were only two phenotypic clusters (Cluster 1 and 2) in (Figure 4.7). Phenotypic cluster 1 represented only one cluster grouped into group K and group H. Phenotypic cluster 2 had the majority of the rhizobia morphological isolates, and they were sub-clustered into two sub-clusters (2a, and 2b). Sub-cluster 2a are sub-divided into (2ai, and 2aii). Sub-cluster (2ai) had group C, group J, group F, and Group G. Whereas, sub-cluster (2aii) had group D, group E, and group I. Sub-cluster 2b had only group A and group B. The representative clusters of the different native rhizobia isolates depended on the neighbouring phenotypic characteristics (Figure 4.7). The evolutionary tree similarity distance index were calculated using the NJM. The bootstrap valves ranged from 8 - 100 and replicated 1,000 times to estimate the clusters W analysis values.

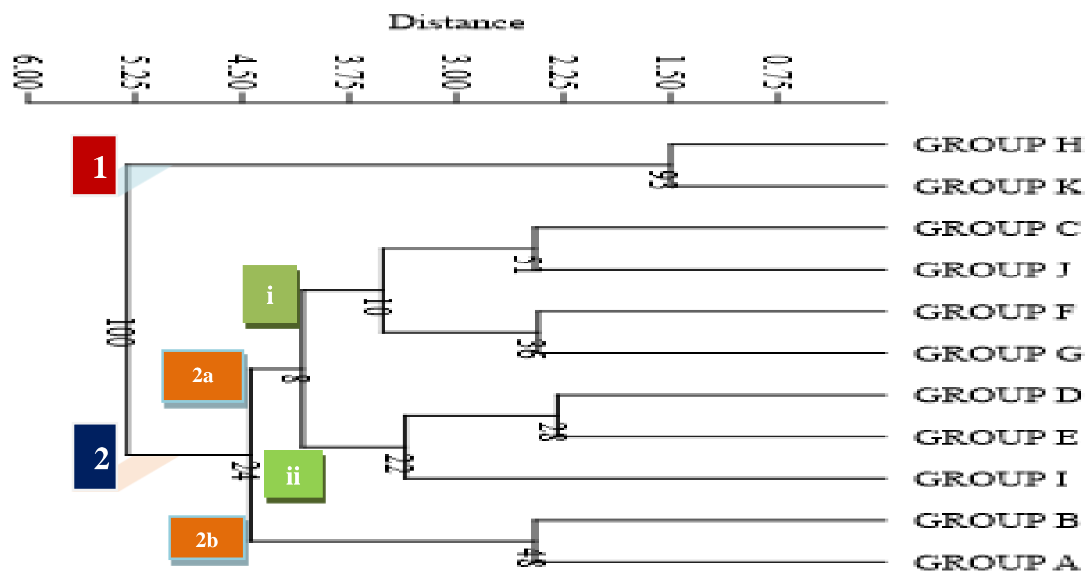

Figure 4.7: Dendrogram displaying the morphological connections of the entire revived rhizobia isolates (archived samples) from the stored glycerol stocks cultures

4.3 Authentication of Isolates

Authentication was done based on nodulation. In this study, the authenticated isolates had the characteristics and ability to induce the formation of nodules with common beans, soyabeans, green grams, and cowpeas under sterilized soils conditions in the greenhouse. In addition, a seedling was considered positive in nodulating if it bore one or more nodules. Nodulation was scored negative when the seedling bore no nodule. Out of the 53 revived stored rhizobia isolates (archived samples) used during symbiotic efficiency screening under environmentally-controlled conditions, only seven (7) isolates designated as IsAS03, IsAS12, IsAS21, IsAS24, IsAGR11, IsAGR17, and IsAGR5 did not nodulate under the tested legume crops used. However, forty six (46) native rhizobia isolates had charactesristics and ability of forming nodulates on common bean, cowpea, soyabean and green gram plant roots.

4.4 Genetic diversity of the isolates

The genetic identity and diversity of the studied revived glycerol stocks isolates was confirmed by sequencing the amplified fragment of the 16S rRNA gene region. Molecular characterization with 16S rRNA region confirmed the isolates belong to diverse rhizobia strains. Genomic DNA (gDNA) extracted from the pure rhizobia cultures showed an intact band when loaded on 1 % agarose gel and run at 80 V for about 30 mins. The 1500 bp amplified 16S rRNA region revealed different band intensities, compared to that of gDNA (Plate 4.5a and 4.5b), when visualized in 1 % agarose gel run at 80 V for 60 mins (one hour). At approximately 1500 bp, all PCR

products showed a definite and appropriately sized band in all lanes (Plate 4.6a and 4.6b).

4.4.1 Genomic DNA extraction

Genomic DNA were extracted from the twenty-six native rhizobia isolates and from three commercial inocu (CIAT110, USDA3456 and CIAT899) (Plate 4.5a and 4.5b).

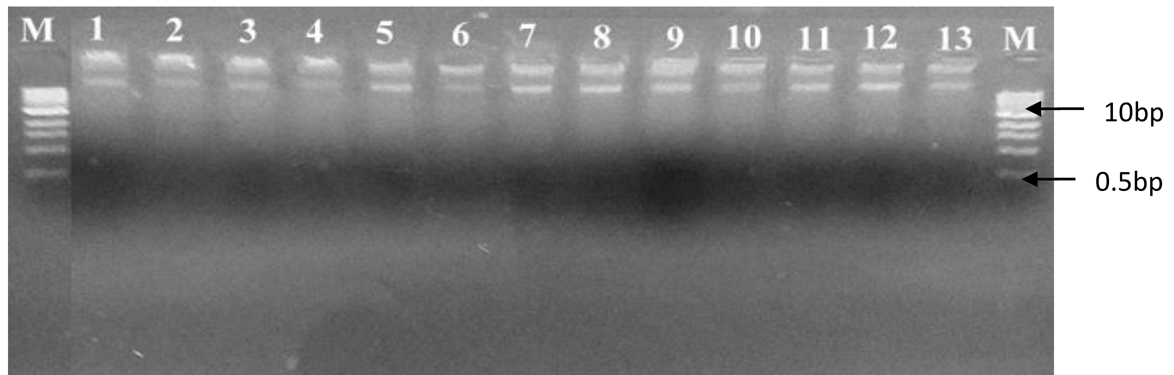

Plate 4.5a: Genomic DNA of the rhizobia isolates produced a double band on 1 % agarose gel and stained on SYBE green: M, DNA ladder; 1, IsAS01; 2, IsAGRP4; 3, IsAMR7; 4, IsAS10; 5, IsAGR10; 6, IsAMR14; 7, IsAS18; 8, IsAMR21; 9, IsAMR23; 10, IsCIAT110; 11, IsCIAT899; 12, IsAMR24; 13, IsAGR81

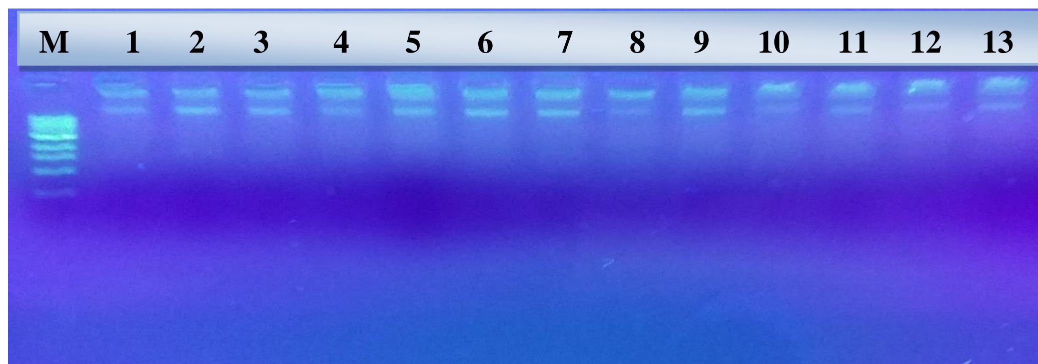

Plate 4.5b: Genomic DNA of the rhizobia isolates produced a double band: M, DNA ladder; 1, IsAS01; 2, IsAGRP4; 3, IsAMR7; 4, IsAS10; 5, IsAGR10; 6,

IsAMR14; 7, IsAS18; 8, IsAMR21; 9, IsAMR23; 10, IsCIAT110; 11, IsCIAT899; 12, IsAMR24; 13, IsAGR81

4.4.2 Polymerase Chain Reaction amplification

PCR amplification was conducted from the twenty-six native rhizobia isolates of common bean, soyabean, cowpea and green gram plants and three commercial inocula strains (CIAT110, USDA3456 and CIAT899). The produced a single band of 1.5kbp size using primers 27f and 1492r (Plate 4.6a and Plate 4.6b). Gel electrophoresis of the PCR product of isolates with SYBR green reaction on 1.0 % agarose gel and M-1kbp ladder DNA from Biolabs.

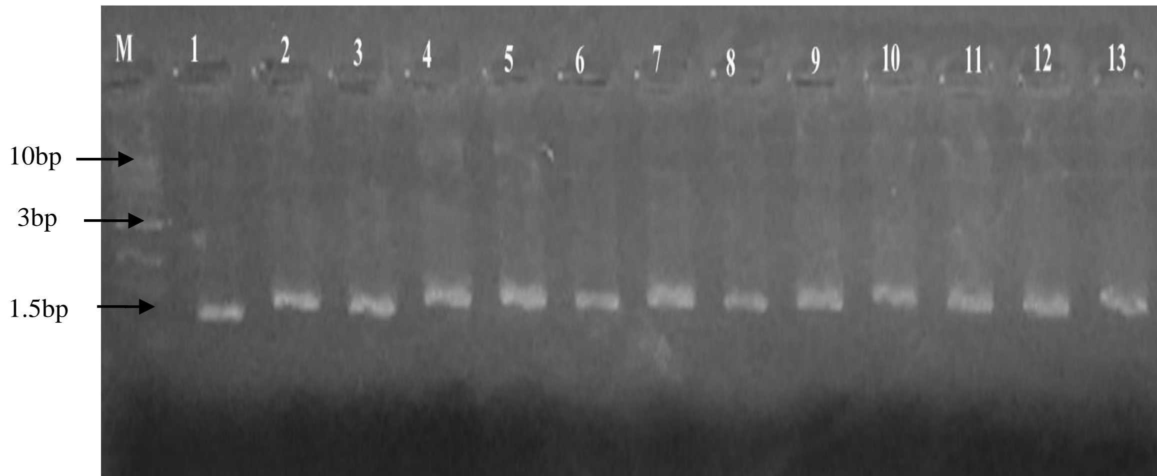

Plate 4.6a: Agarose gel electrophoresis of PCR amplified 16S rRNA gene of rhizobia isolates that were stained with SYBR green and separated on 1 % agarose gel: M=100 pb DNA ladder; 1, IsAS01; 2, IsAGRP4; 3, IsAMR7; 4, IsAS10; 5, IsAGR10; 6, IsAMR14; 7, IsAS18; 8, IsAMR21; 9, IsAMR23; 10, IsCIAT110; 11, IsCIAT899; 12, IsAMR24; 13, IsAGR81

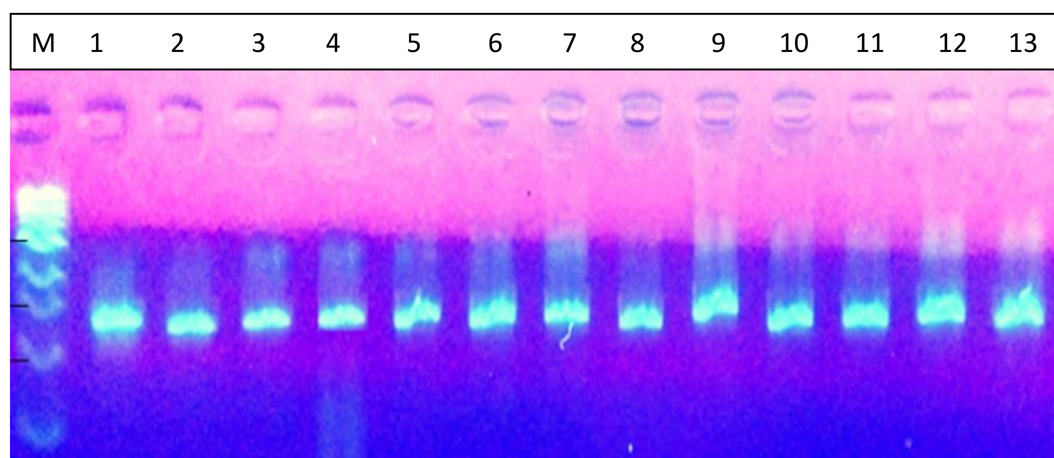

Plate 4.6b: Agarose gel electrophoresis of PCR amplified 16S rRNA gene of rhizobia isolates: M=100 pb DNA ladder; 1, IsAS01; 2, IsAGRP4; 3, IsAMR7; 4, IsAS10; 5, IsAGR10; 6, IsAMR14; 7, IsAS18; 8, IsAMR21; 9, IsAMR23; 10, IsCIAT110; 11, IsCIAT899; 12, IsAMR24; 13, IsAGR81

Based on the phylogenetic clustered tree, the rhizobia isolates were clustered and presented into two main phenotypic clusters mainly; Cluster 1 and 2 (Figure 4.8). Based on the NJM, the twenty-six native rhizobia isolates from the native revived glycerol stocks (archived samples) cultures were clustered closely and tightly with the three commercial inocula strains; CIAT889, *Rhizobium* (*R*) *leguminosarum* MNF-EM-R15; USDA3546, *R. leguminosarum* MNF-EM-R15; and USDA110, *R. etli*.

A total of twenty-nine isolates were sequenced. Phenotypic cluster 1 was subdivided into two different sub-cluster (1a and 1b). Sub-cluster 1a had the majority of the isolates and consisted of sub-clusters (1ai, and 1aii). In addition, isolates AR21, AR7, AR6, AR2, AR17, AR19 and AR29 grouped closely with slightly different *Rhizobium* sp. strains. Whereas, the phenotypic cluster 2 were divided into two different sub-clusters

(2a and 2b). Interestingly, isolates AR25, AR11 and AR10 were identified as *Rhizobium etli* strain based on the NCBI neighbour marched and clustered closely with sub-cluster 2a. However, IsAR8 (commercial inocula strain CIAT889) clustered with isolates AR4, AR9, AR16 and AR23 in sub-clusters 2b, and they matched different *Rhizobium leguminosarum* species. The 16S rRNA gene sequences matched with similar rhizobia strains sequences that were available in the GenBank database through BLAST analysis at NCBI database. The evolutionary tree distances were calculated using the NJM. The bootstrap values range from 68 to 99 and replicated 1,000 times to estimate the clusters W analysis value.

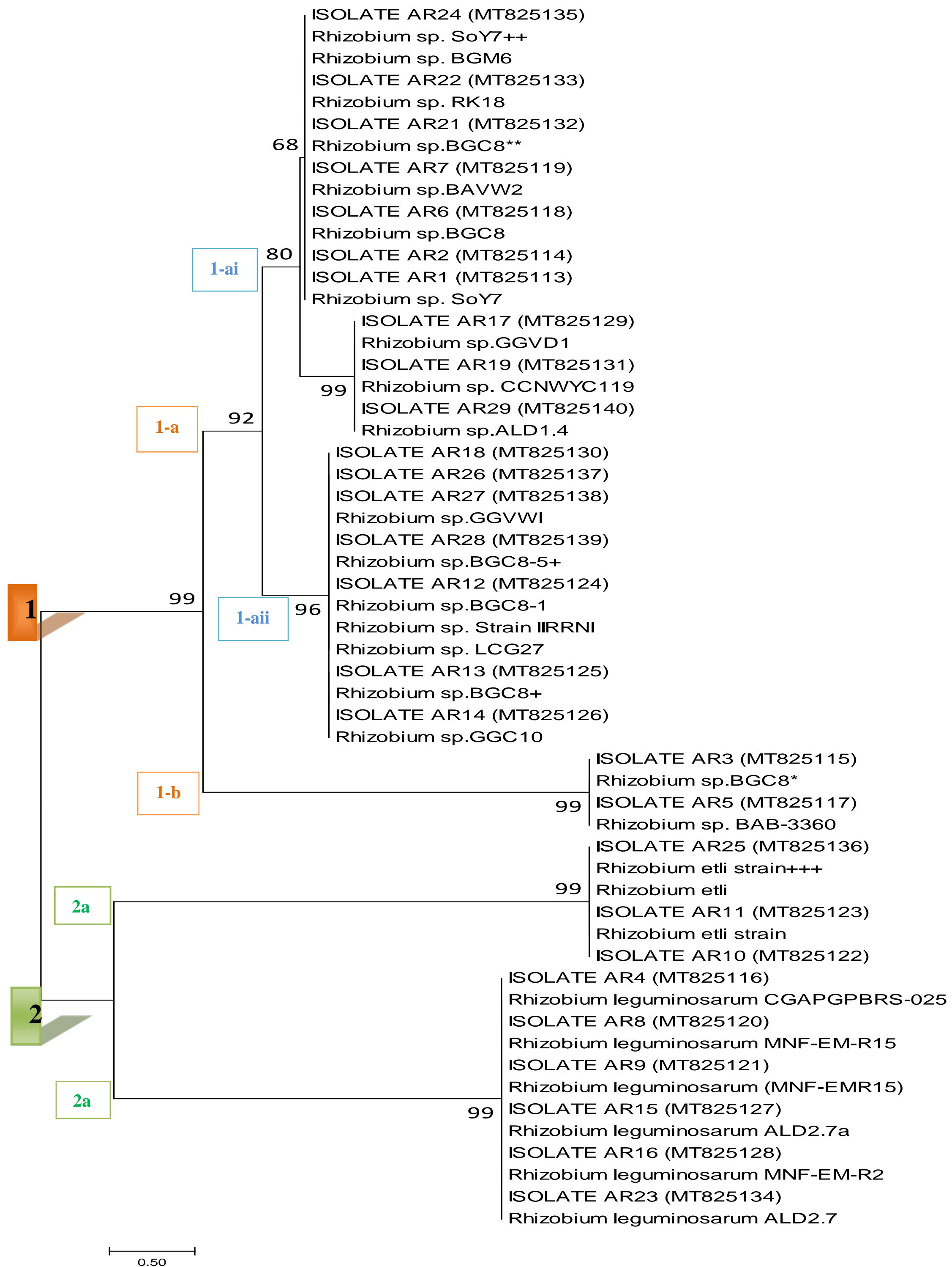

Figure 4.8: Phylogenetic tree connection of the revived glycerol stocks isolates, sequences as shown by the NJM

The genetic diversity of the 29 selected isolates were assessed and evaluated through sequencing partially targeting the 16S rRNA gene and later BLAST to obtain the neighbour names for the closely related species in GenBank (Table 4.8). Giving the highest scores of sequence similarity and match, the scores were obtained and expressed as a percentage. The diversity of the isolates all contained bacteria from the *Rhizobium* genera.This showed high disparity among the isolates from the revived stored cultures.

Table 4.8: Phylogenetic resemblance, match and relationship of the isolates of the partial similar 16S rRNA gene sequences (https://www.ncbi.nlm.nih.gov/nuccore/?term=MT825113:MT825140[accn])

	Isolates sequence	**Host Plants**	**Isolation Sites**	**Similarity (%)**	**GenBank Accession number(s)**	**Most Similar March Published Sequences on NCBI**
1	AR1 (IsAS01)	SB	EC	95	MT825113.1	*Rhizobium* sp. SoY7[#]
2	AR2 (IsAS10)	SB	EC	96	MT825114.1	*Rhizobium* sp. BGC8[*]
3	AR3 (IsAS11)	SB	EC	95	MT825115.1	*Rhizobium* sp. BGC8[*]
4	AR4 (IsAS14	SB	EC	100	MT825116.1	*Rhizobium leguminosarum* CGAPGPBRS-025
5	AR5 (IsAS18)	SB	EC	96	MT825117.1	*Rhizobium* sp. BAB-3360
6	AR6 (IsAS21)	SB	EC	95	MT825118.1	*Rhizobium* sp. BAVW2
7	AR7 (IsAS27)	SB	EC	96	MT825119.1	*Rhizobium* sp. BGC8[*]
8	AR8 (Cm1 889)	CI	MEA	96	MT825120.1	*Rhizobium leguminosarum* MNF-EM-R15[^]
9	AR9 (Cm2 110)	CI	MEA	95	MT825121.1	*Rhizobium leguminosarum* MNF-EM-R15[^]
10	AR10 (Cm3 3456)	CI	MEA	95	MT825122.1	*Rhizobium etli* strain[>]
11	AR11 (IsAMR5)	CB	KC	96	MT825123.1	*Rhizobium etli* strain[>]
12	AR12 (IsAMR6)	CB	KC	96	MT825124.1	*Rhizobium* sp. BGC8[*]
13	AR13 (IsAMR7)	CB	KC	95	MT825125.1	*Rhizobium* sp. GGC10
14	AR14 (IsAMR11)	CB	KC	95	MT825126.1	*Rhizobium* sp. GGVD1^
15	AR15 (IsAMR12)	CB	KC	94	MT825127.1	*Rhizobium leguminosarum* MNF-EM-R2
16	AR16 (IsAMR14)	CB	KC	95	MT825128.1	*Rhizobium leguminosarum* ALD2.7 [α]
17	AR17 (IsAMR18)	CB	KC	91	MT825129.1	*Rhizobium* sp. GGVD1^
18	AR19 (IsAMR22)	CB	KC	90	MT825130.1	*Rhizobium* sp. OCNWYC119
19	AR20 (IsAMR23)	CB	KC	95	MT825131.1	*Rhizobia* sp. ALD1.4
20	AR21 (IsAMR24)	CB	KC	97	MT825132.1	*Rhizobium* sp. RK18
21	AR22 (IsAMR25)	CB	KC	99	MT825133.1	*Rhizobium* sp. BGM6
22	AR23 (IsAGR10)	CB	TNC	94	MT825134.1	*Rhizobium leguminosarum* ALD2.7 [α]
23	AR24 (IsAGR12)	CP	TNC	95	MT825135.1	*Rhizobium* sp. SoY7[#]
24	AR25 (IsAGR14)	CP	TNC	95	MT825136.1	*Rhizobium etli* strain[>]
25	AR26 (IsAGR5)	CP	TNC	100	MT825137.1	*Rhizobium* sp. LCG27
26	AR27 (IsAGRP4)	CP	TNC	95	MT825138.1	*Rhizobium* sp. GGVWI
27	AR29 (IsAGR3)	CP	TNC	96	MT825139.1	*Rhizobium* sp. BGC8[*]
28	AR18 (IsAMR21)	CP	TNC	95	MT825140.1	*Rhizobium* sp. ALD1.4
29	AR18 (IsAMR21)	CP	TNC	91	-	*Rhizobium* sp. Strain IIRRNI

Key: Each symbol represent a similar type of rhizobia species [(#),(^),(>),(α)]. SB, soyabean; CI, commercial inocula; CB, common bean; CP, cowpea; EC, Embu County; KC, Kisumu County; TNC, Tharaka Nithi County; and MEA, Mea company Ltd.

4.5 Symbiotic efficiency of indigenous rhizobia isolates

4.5.1 Effect of native rhizobia isolates from lake Victoria basin on *Phaseolus vulgaris* L. growth parameters, tested in sterilized soils

There was a significant difference ($P<0.0001$) in nodules number (NodN) of the common beans, planted in sterile soil inoculated with different native isolates, as shown in Table 4.9. The NodN ranged from 0.75±0.31 plant^{-1} to 25.88±4.07 plant^{-1} (average±standard error). The common bean (rosecoco) plants inoculated with isolate IsAMR2 showed the highest NodN, whereas, common bean crops inoculated with isolate IsAMR20 had the lowest NodN. Out of the total isolates tested, thirteen (13) isolates; IsAMR27, IsAMR2, IsAMR7, IsAMR11, IsAMR12, IsAMR14, IsAMR17, IsAMR18, IsAMR19, IsAMR22, IsAMR23, IsAMR24, and IsAMR25 induced formation of nodules more than the commercial isolate, CIAT899.

There was a significant difference ($P<0.0001$) in nodule dry weight (NodDW) whereby, the NodDW ranged from 10.00±1.00 mg plant^{-1} - 120.00±17.00 mg plant^{-1} (average±standard error). Plants inoculated with isolates IsAMR2 and IsAMR18 had the highest NodDW when compared to other rhizobia isolates. The NodDW for common bean plants inoculated with isolates; IsAMR27, IsAMR2, IsAMR18, and IsAMR19 was higher when compared to NodDW of common bean plants innoclated with the isolate CIAT899. Interestingly, the results showed no significant difference in NodDW of the un-inoculated control plants (Exp-) compared to the plants inoculated with isolates; IsAMR1, IsAMR5, IsAMR8, IsAMR16, IsAMR20, and IsAMR26 at $P<0.05$.

There was a significant difference (P<0.0001) in shoot dry weight (SHtDW) which ranged from 0.99±0.17 g plant^{-1} in isolate IsAMR8 to 3.07±0.38 g plant^{-1} in isolate IsAMR22 (average±standard error). In addition, isolates IsAMR27, IsAMR2, IsAMR4, IsAMR5, IsAMR6, IsAMR7, IsAMR11, IsAMR12, IsAMR14, IsAMR17, IsAMR18, IsAMR19, IsAMR22, IsAMR23, IsAMR24 and IsAMR25 recorded high SHtDW compared to the CIAT899. The other plants inoculated with IsAMR1, IsAMR8, IsAMR9, IsAMR13, IsAMR15, IsAMR16, IsAMR20, IsAMR21, and IsAMR26 recorded lower SHtDW than the CIAT899. The un-inoculated control plants recorded SHtDW of 1.00±0.18 g plant^{-1}.

There was a significant difference (P=0.0004) in root dry weight (RtDW). The RtDW ranged from 0.23±0.08 g plant^{-1} in isolate IsAMR16 to 0.74±0.10 g plant^{-1} in isolate IsAMR18 (average±standard error). Isolates IsAMR27, IsAMR2, IsAMR7, IsAMR14, IsAMR22, and IsAMR25 registered higher RtDW compared to CIAT899 whereas, isolates IsAMR1, IsAMR4, IsAMR5, IsAMR6, IsAMR8, IsAMR10, IsAMR11, IsAMR12, IsAMR13, IsAMR15, IsAMR16, IsAMR17, IsAMR19, IsAMR20, IsAMR21, IsAMR23, IsAMR24 and IsAMR26 recorded lower RtDW than CIAT899. However, plants that were un-inoculated with native rhizobia isolate had RtDW of 0.25±0.07 g plant^{-1} (Table 4.9). Among the tested native rhizobia isolates, there was a significant difference in symbiotic efficiency (SyE) potential at P<0.0001, where some isolates showed superior SyE performance. Isolates IsAMR3, IsAMR27, IsAMR18 and IsAMR22 had the highest SyE of 136.86 %, 136.98 %, 138.88 % and 155.05 %, respectively. Seventeen native rhizobia isolates had SyE higher than the commercial

inocula (Table 4.9). However, nine rhizobia isolates had SyE lower than the CIAT899. All the inoculated plants had a higher SyE than the un-inoculated control plants, except for plants inoculated with isolate IsAMR8.

Table 4.9: Effect of native rhizobia isolates from lake Victoria basin on *Phaseolus vulgaris* L. growth parameters and nodule formation, tested in sterilized soil

Isolates	NodN (plant^{-1})	NDW (mg plant^{-1})	SHtDW (g plant^{-1})	RtDW (g plant^{-1})	SyE %
Exp-	0.0	0.0	$1.00+0.18^{d}$	0.25 ± 0.07^{b}	50.50^{d}
IsAMR1	1.25 ± 0.82^{ij}	ND	1.76 ± 0.21^{abcd}	0.57 ± 0.14^{ab}	88.88^{abcd}
IsAMR3	13.88 ± 1.54^{bcdefg}	110.00 ± 14.00^{a}	2.71 ± 0.28^{ab}	0.63 ± 0.09^{ab}	136.86^{ab}
IsAMR2	25.88 ± 4.07^{a}	120.00 ± 14.00^{a}	2.59 ± 0.31^{ab}	0.63 ± 0.12^{ab}	130.80^{ab}
CIAT899	12.25 ± 2.25^{bcdefghi}	90.00 ± 13.00^{ab}	1.98 ± 0.30^{abcd}	0.59 ± 0.12^{ab}	100.00^{abcd}
IsAMR4	6.88 ± 1.26^{defghij}	50.00 ± 13.00^{abc}	2.09 ± 0.25^{abcd}	0.54 ± 0.10^{ab}	105.55^{abcd}
IsAMR5	1.00 ± 0.65^{ij}	ND	2.04 ± 0.13^{abcd}	0.55 ± 0.12^{ab}	103.03^{abcd}
IsAMR6	4.25 ± 0.97^{fghij}	28.00 ± 4.00^{de}	2.45 ± 0.35^{ab}	0.52 ± 0.09^{ab}	123.73^{ab}
IsAMR7	16.38 ± 1.96^{abcde}	30.00 ± 9.00^{cde}	2.24 ± 0.25^{abcd}	0.60 ± 0.15^{ab}	113.13^{abcd}
IsAMR8	1.25 ± 0.81^{ij}	ND	0.99 ± 0.17^{d}	0.26 ± 0.03^{b}	50.01^{d}
IsAMR9	5.63 ± 2.55^{efghij}	20.00 ± 4.00^{de}	1.85 ± 0.40^{abcd}	0.42 ± 0.11^{ab}	93.43^{abcd}
IsAMR10	8.94 ± 1.95^{cdefghij}	20.00 ± 4.00^{de}	1.92 ± 0.18^{abcd}	0.49 ± 0.06^{ab}	96.96^{abcd}
IsAMR11	13.88 ± 1.72^{bcdefg}	40.00 ± 6.00^{cde}	2.26 ± 0.12^{abcd}	0.55 ± 0.08^{ab}	114.14^{abcd}
IsAMR12	15.00 ± 1.36^{abcdefg}	40.00 ± 6.00^{cde}	2.44 ± 0.13^{ab}	0.53 ± 0.04^{ab}	123.23^{ab}
IsAMR13	10.38 ± 1.49^{cdefghij}	20.00 ± 2.00^{de}	1.82 ± 0.15^{abcd}	0.45 ± 0.08^{ab}	91.91^{abcd}
IsAMR14	20.00 ± 2.63^{abc}	40.00 ± 6.0^{bcde}	2.57 ± 0.24^{ab}	0.64 ± 0.07^{ab}	131.12^{ab}
IsAMR15	$8.00\pm3.56d^{efghij}$	20.00 ± 2.0^{de}	1.57 ± 0.30^{bcd}	0.36 ± 0.08^{ab}	79.29^{bcd}
IsAMR16	1.63 ± 1.06^{hij}	ND	1.07 ± 0.18^{cd}	0.23 ± 0.08^{b}	54.04^{cd}
IsAMR17	19.62 ± 2.57^{abc}	60.00 ± 11.00^{bc}	2.33 ± 0.36^{abcd}	0.58 ± 0.07^{ab}	117.67^{abcd}
IsAMR18	23.63 ± 4.27^{ab}	120.00 ± 17.00^{a}	2.75 ± 0.38^{ab}	0.74 ± 0.10^{a}	138.88^{ab}
IsAMR19	13.00 ± 2.84^{bcdefgh}	110.00 ± 14.00^{a}	2.31 ± 0.32^{abcd}	0.47 ± 0.10^{ab}	116.66^{abcd}
IsAMR20	0.75 ± 0.31^{ij}	ND	1.54 ± 0.22^{bcd}	0.29 ± 0.06^{ab}	77.77^{bcd}
IsAMR21	6.88 ± 0.81^{defghij}	10.00 ± 1.00^{de}	1.75 ± 0.12^{abcd}	0.30 ± 0.02^{ab}	88.38^{abcd}
IsAMR22	14.75 ± 1.42^{abcdefg}	50.00 ± 10.00^{bc}	3.07 ± 0.38^{a}	0.63 ± 0.05^{ab}	155.05^{abc}
IsAMR23	13.63 ± 3.30^{bcdefg}	30.00 ± 9.00^{cde}	2.23 ± 0.19^{abcd}	0.41 ± 0.06^{ab}	112.62^{abcd}
IsAMR24	15.25 ± 1.12^{abcdef}	30.00 ± 9.00^{cde}	2.19 ± 0.12^{abcd}	0.47 ± 0.05^{ab}	110.62^{abcd}
IsAMR25	17.25 ± 3.47^{abcd}	30.00 ± 8.00^{cde}	2.38 ± 0.21^{ab}	0.62 ± 0.08^{ab}	120.20^{ab}
IsAMR26	3.63 ± 1.37^{ghij}	ND	1.51 ± 0.25^{bcd}	0.44 ± 0.09^{ab}	76.26^{bcd}
IsAMR27	13.88 ± 1.54^{bcdefg}	110.00 ± 14.00^{a}	2.72 ± 0.28^{ab}	0.63 ± 0.09^{ab}	136.98^{ab}
P values	<0.0001	<0.0001	<0.0001	0.0004	<0.0001

Key: NodN, Nodules number; NodDW, nodules dry weight; ShtDW, shoots dry weight; RtDW, roots dry weight; ND, weight not detected; IsCIAT899, commercial isolate

reference strain; Exp-, untreated plants; g plant^{-1}, gram per plant; mg plant^{-1}, milligram per plant; SyE, Symbiotic efficiency. Values followed by the same letters within the column are not significantly different according to Tukey`s Honest Significant Difference (HSD) at 5 % level.

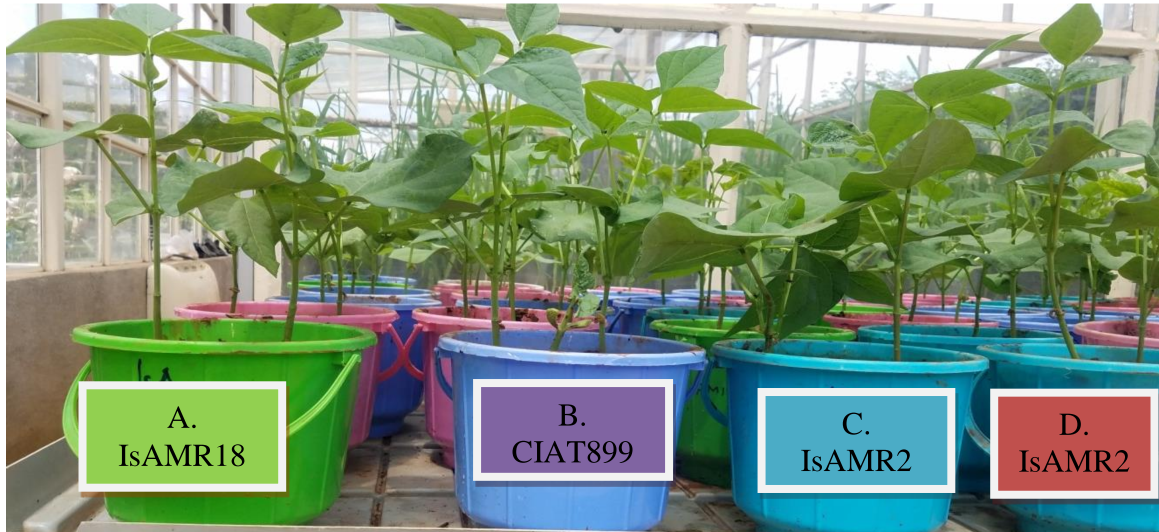

Plate 4.7: Five weeks old common bean planted in sterile soils treated with different native rhizobia isolates, plus a commercial inoculant and un-treated plants pots
Plants in pots A, C, D are treated with native rhizobia isolates, while plants in pot B is inoculated with the commercial inocula.

4.5.2 Effect of native rhizobia isolates from Embu and Tharaka Nithi Counties on Soyabean growth parameters in sterilized soil

There were significant differences in nodule number ($p \leq 0.05$), root dry weight ($p \leq 0.05$), shoot dry weight ($p \leq 0.05$) as well as nodule dry weight ($p \leq 0.05$) of soyabeans when planted on sterilized soils inoculated with the revived rhizobia isolates (Table 4.10). In the study, soyabean planted in sterile soil inoculated with isolate IsAS11 registered the highest nodule number of 16.38±4.56 plant^{-1} whereas, isolate IsAS02 recorded lowest nodule number of 0.38±0.26 plant^{-1} (average±standard error). Interestingly, soyabeans planted in sterilized soils inoculated with isolates; IsAS21, IsAS24, IsAS12 and IsAS03

did not have any nodules. Statistically, when compared among specific isolates, plants inoculated with isolates IsAS01, IsAS10 and IsAS29 showed no significant difference in nodule numbers at $P<0.05$. Furthermore, it was observed that the higher nodule numbers in soyabean plant translated to higher nodule dry weight and shoot dry weight of IsAS11.

The highest nodule dry weight (19.00 ± 5.00 mg plant^{-1}) was recorded in soyabean planted in sterilized soil inoculated with isolate IsAS11 whereas, the lowest nodule dry weight (3.00 ± 2.00 mg plant^{-1}) were recorded in soyabean plants inoculated with isolate IsAS02 (average±standard error). Moreover, when inoculated with isolates; IsAS24, IsAS21, IsAS12, and IsAS03, the harvested plants did not have any nodules. It was also observed that the non-inoculated common bean plants did not have any nodules. Shoot dry weight of soyabean was significantly different ($p \leq 0.05$) in different rhizobia isolates. The highest shoot dry weight of 2.53 ± 0.14 g plant^{-1} was recorded in soyabean planted in sterilized soils inoculated with IsAS14 whereas, the lowest shoot dry weight of 1.19 ± 0.18 g plant^{-1} (average±standard error) was recorded in un-inoculated control plants.

There was a significant difference in the root dry weight of the tested soyabeans at $p \leq 0.05$ (Table 4.10). Soyabeans inoculated with isolate IsAS14 recorded the highest root dry weight of 0.58 ± 0.04 g plant^{-1} whereas, the lowest root dry weight of 0.27 ± 0.06 g plant^{-1} (average±standard error) was recorded in soyabeans planted in sterile soils inoculated with isolate IsAS24. Inoculated isolates such as; IsAS29, IsAS21, IsAS18,

IsAS14, IsAS11, and IsAS10 recorded higher root dry weight than the commercial strain inoculant IsUSDA110. Nodule numbers and nodule dry weights, of all the tested isolates registered a detectable shoot dry weight and root dry weight.

There were significant differences in symbiotic efficiency (SyE) at P<0.0001 among the tested native rhizobia isolates (Table 4.10). The leaves were deep green in several of the treated plants inoculated with the native rhizobia isolates and commercial inocula strains. The un-treated control plants' leaves were yellow in color. The native rhizobia isolate with the highest SyE was IsAS14 (104.97 %), whereas, the rest of the isolates had SyE lower than the commercial inoculant.

Table 4.10: *Glycine max* planted on sterilized soils inoculated with native rhizobia isolates collected from Embu and Tharaka Nithi Counties

Isolates	**NodN ($plant^{-1}$)**	**NodDW (mg $plant^{-1}$)**	**ShtDW (g $plant^{-1}$)**	**RtDW (g $plant^{-1}$)**	**SyE %**
Exp-	0.0	0.0	1.19 ± 0.18^{b}	0.19 ± 0.04^{c}	49.37^{b}
IsAS01	9.63 ± 2.90^{abc}	10.00 ± 3.00^{ab}	2.18 ± 0.29^{a}	0.48 ± 0.09^{ab}	90.46^{a}
IsAS02	0.38 ± 0.26^{c}	3.00 ± 2.00^{c}	1.77 ± 0.10^{ab}	0.43 ± 0.03^{abc}	73.44^{ab}
IsAS03	0.0	0.0	1.73 ± 0.19^{ab}	0.42 ± 0.05^{abc}	71.70^{ab}
IsAS10	6.88 ± 2.45^{abc}	6.00 ± 2.00^{ab}	2.30 ± 0.22^{a}	0.52 ± 0.05^{ab}	95.45^{a}
IsAS11	16.38 ± 4.56^{a}	19.00 ± 5.00^{a}	2.37 ± 0.13^{a}	0.51 ± 0.05^{ab}	98.34^{a}
IsAS12	0.0	0.0	1.71 ± 0.15^{ab}	0.45 ± 0.07^{abc}	70.95^{ab}
IsAS14	5.63 ± 1.81^{bc}	6.00 ± 2.00^{ab}	2.53 ± 0.14^{a}	0.58 ± 0.04^{a}	104.97^{a}
IsAS17	5.63 ± 1.81^{c}	6.00 ± 2.00^{b}	1.72 ± 0.24^{ab}	0.34 ± 0.06^{abc}	71.38^{ab}
IsAS18	12.63 ± 2.67^{ab}	17.00 ± 6.00^{a}	2.37 ± 0.20^{a}	0.51 ± 0.06^{ab}	98.34^{a}
IsAS21	0.0	0.0	1.76 ± 0.21^{ab}	0.44 ± 0.05^{abc}	73.03^{ab}
IsAS24	0.0	0.0	1.63 ± 0.12^{ab}	0.27 ± 0.06^{bc}	67.61^{ab}
IsAS27	4.00 ± 1.93^{bc}	7.00 ± 4.00^{ab}	2.26 ± 0.20^{a}	0.41 ± 0.04^{abc}	93.78^{a}
IsAS29	7.25 ± 2.88^{abc}	10.00 ± 5.00^{ab}	2.33 ± 0.12^{a}	0.53 ± 0.03^{ab}	96.68^{a}
IsUSDA110	11.50 ± 2.78^{ab}	18.00 ± 6.00^{a}	2.41 ± 0.16^{a}	0.44 ± 0.03^{abc}	100.00^{a}
P values	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Key: NodN, Nodules number; NodDW; nodules dry weight; ShtDW, shoots dry weight; RtDW, roots dry weight; g $plant^{-1}$, gram per plant; mg $plant^{-1}$, milligram per plant;

IsUSDA110, commercial inoculant; Exp-, non-inoculated negative control. Values followed by the same letters within the column are not significantly different according to Tukey`s Honest Significant Difference (HSD) at 5 % level.

4.5.3 The potential of native rhizobia isolated from Embu and Tharaka Nithi Counties inoculated on *Phaseolus vulgaris* L. planted in sterilized soil for growth production

The ability of indigenous rhizobia isolated from soils in Embu County and Tharaka-Nithi County to enhance and induce nodulation in *Phaseolus vulgaris* L. when planted in sterile soils was tested. The ability of the tested revived native rhizobia isolates to induce nodulation in the study plants was statistically different at $p \leq 0.05$ (Table 4.11). The native rhizobia isolate IsAGR12 recorded the highest nodule number of 20.13±3.85 plant^{-1} while the lowest nodule number of 0.38±0.15 plant^{-1} (average±standard error) was recorded in sterile soils inoculated with isolate IsAGR2. However, there was no significant difference in nodule numbers between isolates; IsAGR5, IsAGR17, IsAGR11 and Exp- which showed no nodulation at all. The high nodule numbers in common bean translated to higher nodule dry weight.

The nodules' dry weight of common bean plants (rosecoco) was significantly different ($P<0.0001$) across the native rhizobia isolates. The highest nodule dry weight 21.10±5.00 mg plant^{-1} was recorded when sterile soils were inoculated with the commercial isolates (IsCIAT899) whereas, the lowest nodule dry weight of 1.00±0.10 mg plant^{-1} (average±standard error) was observed when the plants were inoculated with isolate IsAGR6. However, comparison among specific isolates revealed that there was

no significant difference ($P<0.05$) between the nodule dry weight of common bean after inoculation with the IsCIAT899 and other native rhizobia isolates; IsAGR12, IsAGR13 and IsAGR10. Further, Tukey's pairwise comparison revealed that there was no significant difference in nodule number and nodule dry weight among the isolates; IsAGR5, IsAGR17, IsAGR11, and un-inoculated control plants.

Unlike the nodule dry weight, there was enhanced shoot dry weight for all the harvested common bean crops when treated with the native rhizobia isolates. The un-inoculated plants also had a shoot dry weight of 0.99±0.10 g $plant^{-1}$ (Table 4.11). A comparison among specific isolates showed that IsAGR81 recorded the highest shoot dry weight of 2.72±0.31 g $plant^{-1}$ (average±standard error). Isolate IsAGR81 performed better than the commercial isolates, IsCIAT899, which recorded shoot dry weight of 2.43±0.21 g $plant^{-1}$. The lowest shoot dry weight of 0.76±.21 g $plant^{-1}$ was realized when soils were inoculated with isolate IsAGR2. However, this weight was less than the one recorded by un-inoculated control plants.

The root dry weight of the harvested plants that were inoculated with different rhizobia isolates was statistically different at $p \leq 0.05$ (Table 4.11). All the tested isolates significantly influenced the root dry weight in all the harvested crops. Isolate IsAGR12 recorded a higher root dry weight of 0.55±0.07 g $plant^{-1}$ than that of the IsCIAT899 of 0.49±0.07 g $plant^{-1}$ (average±standard error). On the other hand, plants inoculated with isolates IsAGR17 and IsAGR2 had lower root dry weights than the un-inoculated control plants (0.15±0.02 g $plant^{-1}$). Based on Tukeys' pair-wise comparison the study

revealed shared significance in the mean root dry weight when the common bean plants were subjected to isolates IsAGR1, IsAGR10, IsAGR14, IsAGR5, IsAGR6, IsAGR26, and IsAGR3 at P=0.0001. Similarly, there was shared significance among root dry weight when common bean was tested with isolates; IsCIAT899, IsAGR13 and IsAGR81 at P=0.0001.

There were significant differences in symbiotic efficiency (SyE) at P<0.0001 among the tested rhizobia isolates. The leaves were deep green in several of the inoculated cowpea plants with the native rhizobia isolates and commercial inocula CIAT899 (Plate 4.8). The un-treated control plants leaves were yellowish in color. The native rhizobia isolates IsAGR1 and IsAGR81 had the highest SyE of 101.65 % and 111.93 %, respectively (Table 4.11). However, the rest of the native rhizobia isolates and the un-treated control plants control (Exp-) had SyE lower than the commercial inoculant.

Plate 4.8: Greenhouse experiment showing three weeks old common bean plants and two weeks old cowpea plants inoculated with differents native rhizobia isolates testing for symbiotic efficiency and nodules formation arranged in CRD design

Key: Isolates inoculated; A, IsAGR12; B, CIAT899; C, IsAGR81; D, IsAGR1; E, IsAGR13; F, IsAGR2; G, IsAGR10; H, IsAGR17; I, Exp-negative control.

Table 4.11: Potential of native rhizobia isolates collected from Embu and Tharaka Nithi Counties on *Phaseolus vulgaris* L. planted in sterilized soil for growth parameters

Isolates	NodN (plant^{-1})	NodDW (mg plant^{-1})	SHtDW (g plant^{-1})	RtDW (g plant^{-1})	SyE %
IsAGR12	20.13±3.85^{a}	21.10±5.00^{ab}	2.39±0.18^{ab}	0.55±0.07^{a}	98.35^{ab}
IsAGR1	8.00±2.82^{bcd}	7.00±3.00^{cd}	2.47±0.24^{ab}	0.38±0.06^{abc}	101.65^{ab}
IsCIAT899	19.75±2.41^{a}	21.00±3.00^{a}	2.43±0.21^{ab}	0.49±0.07^{ab}	100.00^{ab}
IsAGR13	13.38±3.05^{b}	12.00±3.00^{abc}	2.23±0.22^{ab}	0.48±0.08^{ab}	91.78^{ab}
IsAGR81	10.00±3.54^{bc}	9.00±4.00^{bcd}	2.72±0.31^{a}	0.47±0.09^{ab}	111.93^{a}
IsAGR10	10.63±2.74^{bc}	10.00±3.00^{abc}	2.05±0.09^{ab}	0.39±0.06^{abc}	84.36^{ab}
IsAGR14	7.00±1.69^{cd}	5.00±1.00^{cd}	2.14±0.09^{ab}	0.33±0.04^{abc}	88.07^{ab}
IsAGR5	0.0	0.0	1.46±0.18^{bcde}	0.29±0.06^{abc}	60.08^{bcde}
IsAGR6	1.63±0.71^{de}	2.00±0.30^{cd}	1.95±0.19^{abc}	0.37±0.05^{abc}	80.25^{abc}
IsAGR26	2.38±1.58^{de}	2.00±0.30^{cd}	1.79±0.23^{abcd}	0.31±0.08^{abc}	73.67^{abcd}
IsAGR3	2.25±1.49^{de}	2.00±0.20^{cd}	2.16±0.30^{ab}	0.35±0.09^{abc}	88.89^{ab}
IsAGR17	0.0	0.0	0.91±0.26^{de}	0.12±0.04^{c}	37.45^{de}
IsAGR11	0.0	0.0	1.54±0.18^{b}	0.25±0.04^{bc}	63.37^{b}
IsAGR2	0.38±0.15^{de}	1.00±0.10^{cd}	0.76±0.21^{e}	0.13±0.04^{c}	31.28^{e}
Exp-	0.0	0.0	0.99±0.10^{cde}	0.15±0.02^{c}	40.74^{cde}
P values	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Key: NodN, Nodules number; NodDW, nodules dry weight; ShtDW, shoots dry weight; RtDW, roots dry weight; Exp-, un-inoculated negative control; IsCIAT899, commercial rhizobia reference strain; g plant^{-1}, gram per plant; mg plant^{-1}, milligram per plant. Values followed by the same letters within the column are not significantly different according to Tukey`s Honest Significant Difference (HSD) at 5 % level.

4.6 Effectiveness of native rhizobia isolates in non-sterilized soils

4.6.1 Soyabean plants inoculated with native rhizobia isolates planted in non-sterilized soil collected from Embu and Tharaka Nithi Counties for growth parameters

The potential of soyabeans (SB-126) to nodulate with the native rhizobia isolates from Embu and Tharaka Nithi counties was tested in non-sterilezed soil for growth

production. The highest nodule number of 39.75±8.00 plant^{-1} (average±standard error) was recorded when plants growing on non-sterilized soil were inoculated with IsAS11 (Table 4.12). In addition, isolates; IsAS18 and IsAS11 enhanced and induced nodulation higher than the commercial inoculant (IsUSDA110). The lowest nodule number (11.00±4.96 plant^{-1}) was recorded when soyabeans were planted in non-sterile soil inoculated with IsAS27. There was no significant difference in mean nodule numbers for IsAS29, IsAS14, IsAS10, and IsAS01. However, the un-inoculated control plants also nodulated due to the presence of indigenous rhizobia in the soils.

There was a significant difference in nodule dry weight when soyabeans growing in non-sterilezed soil was inoculated with native rhizobia isolates at $p \leq 0.05$ (Table 4.12). The highest nodule dry weight of 39.00±7.00 mg plant^{-1} was observed when plants were inoculated with isolate IsAS11. However, there was no significant difference between the nodule dry weight of soyabean planted in non-sterilezed soils inoculated with rhizobia; IsAS18, IsAS11 and the commercial inoculant. The lowest nodule dry weight of 13.00±6.00 mg plant^{-1} was observed when the soyabeans were inoculated with IsAS27, which was not significant difference from isolates IsAS14, IsAS10, IsAS09, and IsAS29 according to Tukey`s Honest Significant Difference at 5 % level.

There was a significant difference in shoot dry weight of the soyabeans at $p \leq 0.05$ (Table 4.12). Interestingly, the plants inoculated with the commercial inoculant (IsUSDA110) was not significantly different from the rest of the native rhizobia isolates. A comparison among specific isolates showed that IsAS14 recorded the highest shoot dry

weight (2.86±0.11 g plant^{-1}). This value was slightly above those in plants inoculated with IsUSDA110, IsAS11 and IsAS18 which recorded shoot dry weight of 2.74±0.14 g plant^{-1}, 2.57±0.13 g plant^{-1} and 2.33±0.14 g plant^{-1}. The lowest shoot dry weight of 1.64±0.14 g plant^{-1} (average±standard error) was realized when the plants were not inoculated with any rhizobia isolate in the soil. However, there was no significant different between the tested isolates and the commercial inocula in ShtDW.

The root dry weight of the plants growing non-sterilized soils inoculated with rhizobia isolates were statistically different at $p \leq 0.05$ (Table 4.12). However, among specific isolates, there was no significant difference among the soyabeans that were inoculated with all the isolates except for the plants that are inoculated with isolate IsAS27. Most of the soyabeans inoculated with native isolates and CIAT110 in non-sterile soils registered higher root dry weight above that of the un-inoculated negative control plants (0.31±0.04 g plant^{-1}).

There were significant differences in symbiotic efficiency (SyE) at $P<0.0001$ among the tested native rhizobia isolates, commercial inocula (USDA110) and the un-inoculated control plants (Table 4.12). The native rhizobia isolates with the highest SyE were IsAS10 (102.92 %) and IsAS14 (104.38 %). Isolates IsAS01 (91.27 %), IsAS11 (93.80 %), IsAS18 (85.04 %), IsAS27 (90.15 %) and IsAS29 (89.05 %) had SyE lower than the USDA110. However, the un-inoculated control plants (Exp-) had the lowest SyE (59.85%). In addition, the leaves were deep green in several of the treated plants

inoculated with the native rhizobia isolates and commercial inocula strains (Plate 4.9). The un-treated control plants' leaves were yellow in color.

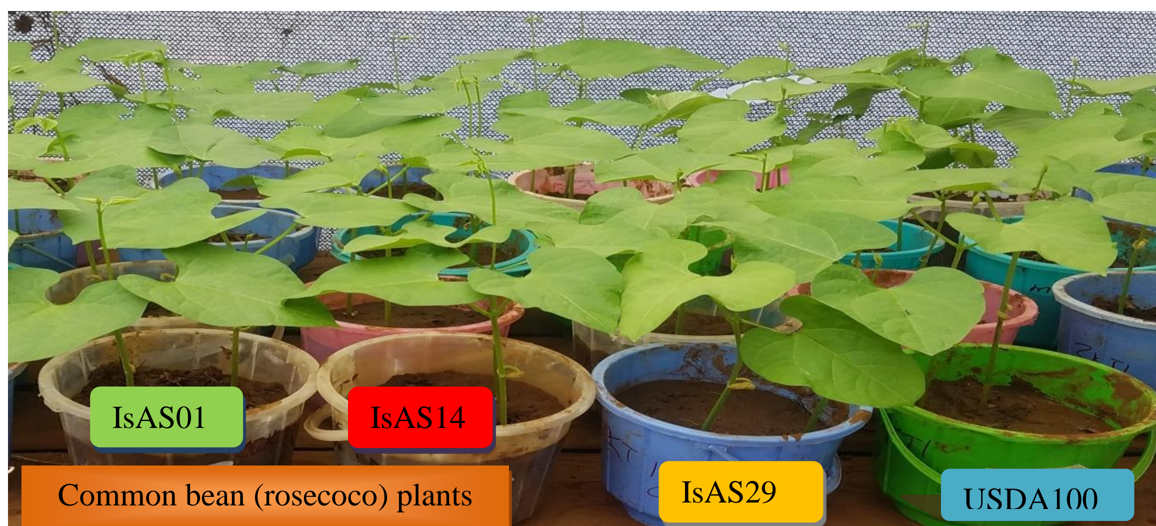

Plate 4.9: Four weeks old plants treated with native rhizobia isolates and commercial inocula in a bacteriological environmental conditions arranged in CRD design

Table 4.12: *Glycine max* plants inoculated with native rhizobia isolates from Embu County and Tharaka Nithi County planted in non-sterilized soil to test for growth parameters

Isolates	NodN (plant^{-1})	NodDW(mg plant^{-1})	ShtDW(g plant^{-1})	RtDW(g plant^{-1})	SyE %
Exp-	2.63±1.40^{d}	3.00±1.00^{c}	1.64±0.14^{b}	0.31±0.04^{b}	59.85^{b}
IsAS01	27.50±7.67^{b}	25.00±7.00^{ab}	2.51±0.13^{a}	0.58±0.05^{a}	91.27^{a}
IsAS10	19.63±5.62^{bc}	24.00±9.00^{ab}	2.82±0.05^{a}	0.58±0.04^{a}	102.92^{a}
IsAS11	39.75±8.00^{a}	39.00±8.00^{a}	2.57±0.16^{a}	0.52±0.03^{a}	93.30^{a}
IsAS14	18.00±4.78^{bc}	20.00±5.00^{ab}	2.86±0.11^{a}	0.61±0.04^{a}	104.38^{a}
IsAS18	32.25±5.65^{ab}	34.00±7.00^{a}	2.33±0.14^{a}	0.53±0.45^{a}	85.04^{a}
IsAS27	11.00±4.96^{c}	13.00±6.00^{b}	2.47±0.13^{a}	0.50±0.04^{ab}	90.15^{a}
IsAS29	21.63±5.29^{bc}	22.00±6.00^{ab}	2.44±0.16^{a}	0.53±0.06^{a}	89.05^{a}
IsUSDA110	31.13±6.82^{ab}	34.00±7.00^{a}	2.74±0.14^{a}	0.51±0.06^{a}	100.00^{a}
P values	0.0011	0.0058	<0.0001	0.0009	<0.0001

Key: NodN, Nodules number; NodDW, nodules dry weight; ShtDW, shoots dry weight; RtDW, roots dry weight; IsUSDA110, commercial inoculant; Exp-, untreated negative

control plants; g plant^{-1}, gram per plant; mg plant^{-1}, milligram per plant. Values followed by the same letters within the column are not significantly different according to Tukey`s Honest Significant Difference (HSD) at 5 % level.

4.6.2 Common beans inoculated with native rhizobia isolates (archived samples) collected from lake Victoria basin Kisumu County tested in non-sterile soil in the greenhouse for growth performance

There was a significant difference (P≤0.05) in *Phaseolus vulgaris* L. (rosecoco) growth performance, tested across different rhizobia isolates using isolates obtaibed from Lake Victoria Basin (LVB) in Kenya (Table 4.13). Isolate IsAMR12 showed the highest nodule number of 77.00±12.36 plant^{-1} while, plants inoculated with IsAMR2 showed the lowest nodule number of 4.38±1.45 plant^{-1} (average±standard error) which were also higher than the plants that is not inoculated with any for of rhizobia isolates (3.13±1.77 plant^{-1}). The commercial reference isolate (CIAT899) recorded nodule number of 66.88±12.01 plant^{-1} which was lower than the one obtained from isolates IsAMR12 and IsAMR14.

The nodule dry weight of the plants was significantly different (p≤0.05) when compared among the inoculants (Table 4.13). The plants inoculated with IsAMR6 recorded the highest nodule dry weight of 126.00±69.00 mg plant^{-1} (average±standard error) whereas, the lowest nodule dry weight of 4.00±2.00 mg plant^{-1} was recorded in plants inoculated with isolate IsAMR2. The shoot dry weight of rosecoco growing in non-sterilized soils inoculated with rhizobia isolates was determined and recorded (Table 4.13). The results showed that there was a significant difference in the shoot dry weight

of the tested common bean plants at $p \leq 0.05$. The plants inoculated with isolate IsAMR22 recorded the highest shoot dry weight of 3.13±0.28 g $plant^{-1}$ whereas, the lowest shoot dry weight of 1.11±0.12 g $plant^{-1}$ was recorded in plants inoculated with isolate IsAMR1.

The root dry weight of the harvested plants that were inoculated with different isolates was statistically different at $p \leq 0.05$ (Table 4.13). All the tested native rhizobia isolates infected the roots hair of all the harvested common bean plants to trap the bacteria. The highest root dry weight of 0.60±0.05 g $plant^{-1}$ (average±standard error) was realized in common bean plants inoculated with isolate IsAMR22 in the non-sterile soil. On the other hand plants inoculatd with isolates; IsAMR2 and IsAMR1 showed root dry weight of 0.23±0.06 g $plant^{-1}$ and 0.21±0.04 g $plant^{-1}$, respectively which was less than the one recorded by the un-inoculated control plants (0.31±0.04 g $plant^{-1}$). Similarly, at $P = 0.0001$ there was shared significance among root dry weight when rosecoco plants were inoculated with isolates IsANR23, IsAMR25 and IsCIAT899.

There were significant differences in symbiotic efficiency (SyE) at $P<0.0001$ (Table 4.13). The leaves were deep green in several of the treated common bean plants inoculated with the isolates. The un-inoculated control plants leaves were yellowish in color. The native rhizobia isolates with the highest SyE were IsAMR6 (105.59 %), IsAMR11 (104.82 %), IsAMR21 (102.06 %), IsAMR23 (107.32 %), and IsAMR24 (104.46 %), whereas, the rest of the isolates had SyE lower than the commercial inocula CIAT899. However, IsAMR12 (100.00 %) had SyE similar to CIAT899.

Table 4.13: *Phaseolus vulgaris* L. plants inoculated with rhizobia isolates collected from lake Victoria basin tested in non-sterile soil in the greenhouse for growth performance

Isolates	NodN (plant^{-1})	NodDW (mg plant^{-1})	ShtDW (g plant^{-1})	RtDW (g plant^{-1})	SyE %
Exp-	3.13 ± 1.77^{e}	4.00 ± 2.00^{d}	1.92 ± 0.23^{def}	0.31 ± 0.04^{cbd}	65.98^{def}
IsCIAT899	66.88 ± 12.02^{ab}	72.00 ± 21.00^{ab}	2.91 ± 0.31^{abc}	0.55 ± 0.04^{a}	100.00^{abc}
IsAMR1	7.50 ± 3.47^{e}	6.00 ± 2.00^{d}	1.11 ± 0.12^{f}	0.21 ± 0.04^{d}	38.14^{f}
IsAMR2	4.38 ± 1.45^{e}	4.00 ± 1.00^{d}	1.21 ± 0.17^{ef}	0.23 ± 0.06^{cd}	41.58^{ef}
IsAMR4	15.00 ± 6.86^{de}	25.00 ± 10.00^{c}	2.18 ± 0.23^{abcde}	0.39 ± 0.04^{abcd}	74.91^{abcde}
IsAMR5	17.13 ± 6.36^{de}	29.00 ± 12.00^{c}	2.55 ± 0.09^{abcd}	0.50 ± 0.04^{ab}	87.63^{abcd}
IsAMR6	47.25 ± 4.93^{abc}	126.00 ± 69.00^{a}	3.07 ± 0.25^{ab}	0.46 ± 0.04^{ab}	105.59^{ab}
IsAMR7	63.75 ± 13.43^{ab}	60.00 ± 11.00^{ab}	2.81 ± 0.21^{abcd}	0.51 ± 0.02^{ab}	96.56^{abcd}
IsAMR10	5.13 ± 2.89^{e}	6.00 ± 3.00^{b}	2.14 ± 0.07^{bcde}	0.46 ± 0.07^{abc}	73.54^{bcde}
IsAMR11	65.13 ± 8.82^{ab}	68.00 ± 11.00^{ab}	3.05 ± 0.20^{ab}	0.47 ± 0.04^{ab}	104.82^{ab}
IsAMR12	77.00 ± 12.36^{a}	115.00 ± 17.00^{a}	2.91 ± 0.12^{abc}	0.51 ± 0.04^{ab}	100.00^{abc}
IsAMR14	71.75 ± 20.11^{a}	108.00 ± 27.00^{a}	2.84 ± 0.19^{abcd}	0.49 ± 0.03^{ab}	97.59^{abcd}
IsAMR17	51.63 ± 14.20^{ab}	72.00 ± 22.00^{ab}	2.77 ± 0.24^{abcd}	0.43 ± 0.04^{abcd}	95.19^{abcd}
IsAMR18	49.25 ± 10.99^{bc}	62.00 ± 15.00^{ab}	2.81 ± 0.23^{abcd}	0.46 ± 0.06^{abc}	96.56^{abcd}
IsAMR19	23.38 ± 7.26^{bcde}	36.00 ± 12.00^{bc}	2.28 ± 0.06^{abcd}	0.40 ± 0.04^{abcd}	78.35^{abcd}
IsAMR20	61.62 ± 11.03^{ab}	62.00 ± 12.00^{ab}	2.97 ± 0.08^{abc}	0.45 ± 0.04^{abc}	102.06^{abc}
IsAMR21	27.88 ± 5.35^{bc}	38.00 ± 8.00^{ab}	2.32 ± 0.14^{abcd}	0.44 ± 0.04^{abcd}	79.73^{abcd}
IsAMR22	46.50 ± 8.41^{bc}	58.00 ± 12.00^{ab}	3.13 ± 0.28^{a}	0.60 ± 0.05^{a}	107.56^{a}
IsAMR23	46.50 ± 12.70^{bc}	58.00 ± 16.00^{ab}	3.04 ± 0.27^{ab}	0.55 ± 0.06^{a}	104.46^{ab}
IsAMR24	17.12 ± 3.92^{cde}	27.00 ± 9.00^{bc}	2.33 ± 0.12^{abcd}	0.43 ± 0.03^{abcd}	80.07^{abcd}
IsAMR25	33.25 ± 7.21^{bcd}	41.00 ± 6.00^{ab}	2.78 ± 0.04^{abcd}	0.55 ± 0.05^{a}	95.53^{abcd}
IsAMR30	28.13 ± 7.02^{bcd}	41.00 ± 12.00^{ab}	1.99 ± 0.19^{cdef}	0.48 ± 0.06^{ab}	68.38^{cdef}
P values	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Key: NodN, Nodules number; NodDW, nodules dry weight; ShtDW, shoots dry weight; RtDW, roots dry weight; IsCIAT899, commercial inoculant; Exp-, untreated negative control; g plant^{-1}, gram per plant; mg plant^{-1}, milligram per plant. Values followed by the same letters within the column are not significantly different according to Tukey`s Honest Significant Difference (HSD) at 5 % level.

4.6.3 Native rhizobia isolates inoculated on cowpeas planted in non-sterile soils from lake Victoria basin to improve growth performance

There was a significant difference in cowpea (K-80) growth parameters across different rhizobia isolates at $p \leq 0.05$. However, the nodule dry weights of the tested isolates were

not significantly different at p≤0.05. The highest nodule number was observed on cowpea plants inoculated with isolates; IsAGR12 and IsAGR10, the numbers were higher than that of the plants inoculated with the commercial inoculant (IsUSDA3456). Furthermore, its was also observed that the plants treated with isolates IsAGR5 and IsAGR12 produce higher nodule dry weight than the plants inoculated with USDA3456. Also IsAGR5, IsAGR12 and USDA3456 were not significantly different in NodDW. Isolate IsAGR5 showed the highest root dry weight as well as shoot dry weight when inoculated with the test crops at p = 0.0001 (Table 4.14). There were significant differences in symbiotic efficiency (SyE) at P<0.0001 among the tested rhizobia isolates, commercial inocula and un-inoculated plants. The un-treated plants leaves were yellow in color, while the inoculated plants leaves were dark green in color (Plate 4.10). Three native rhizobia isolates; IsAGR12, IsAGR10 and IsAGR5 had the highest SyE of 109.26 %, 119.26 % and 122.65 %, respectively which was higher than the USDA3456 100.00 %. However, the USDA3456 outperformed isolates IsAGRP (80.74 %), IsAGR14 (96.72 %) and the un-treated control plants (Exp-) (50.82 %).

Table 4.14: Cowpea plants inoculated with native rhizobia isolates in non-sterilized soils to improve productivity of the crops

Isolates	NodN (plant^{-1})	NodDW(mg plant^{-1})	RtDW(g plant^{-1})	ShtDW(g plant^{-1})	SyE %
IsUSDA3456	38.00±5.95^{b}	84.00±17.00^{a}	0.49±0.03^{ab}	2.44±0.13^{b}	100.00^{b}
Exp-	3.38±2.14^{b}	6.00±4.00^{c}	0.25±0.03^{c}	1.24±0.12^{d}	50.82^{d}
IsAGR10	50.29±5.05^{a}	82.00±12.00a^{b}	0.56±0.04^{a}	2.91±0.06^{a}	119.26^{a}
IsAGR12	53.50±8.09^{a}	84.00±14.00^{a}	0.55±0.04^{a}	2.68±0.16^{ab}	109.84^{ab}
IsAGR14	37.25±9.50^{b}	61.00±19.00^{b}	0.44±0.02^{ab}	2.36±0.12^{bc}	96.77^{bc}
IsAGR5	38.88±6.03^{b}	92.00±14.00^{a}	0.57±0.04^{a}	2.99±0.04^{a}	122.55^{a}
IsAGRP	36.13±6.21^{b}	61.00±10.00^{b}	0.39±0.02^{bc}	1.97±0.07^{c}	80.74^{c}
P values	<0.0001	0.0006	<0.0001	<0.0001	<0.0001

Key: NodN, Nodules number; NodDW, nodules dry weight; ShtDW, shoots dry weight; RtDW, roots dry weight; Exp-, untreated control plants, and pots; $g^{-1,}$ gram per plant; mg $plant^{-1}$; milligram per plant; IsUSDA3456, commercial strain. Values followed by the same letters within the column are not significantly different according to Tukey`s Honest Significant Difference (HSD) at 5 % level.

Plate 4.10: Experiment on native rhizobia isolates inoculated in cowpea plants in non-sterile soils in the greenhouse that were tested for SyE and growth parameters

4.6.4 Rhizobia isolates inoculated on green gram planted in non-sterilized soils to improve growth parameters of the plants from Embu and Tharaka Nithi Counties

The potential of green gram (Ks-20) to nodulate with the native rhizobia isolates from Embu and Tharaka Nithi counties was tested in non-sterilezed soil for growth production and symbiotic efficiency (SyE). The ability of the study isolates to enhance nodulation in green gram was statistically different at $p \leq 0.05$ (Table 4.15). The highest nodulation of 62.25±12.58 $plant^{-1}$ (average±standard error) was obtained on inoculating green gram plants growing in non-sterile soil with IsAS14, while the lowest nodulation

number of 16.50±3.01 plant^{-1} was obtained when green grams were inoculated with IsAS03.

There was a significant difference in nodule dry weight (NodDW), root dry weight (RtSW) and shoot dry weight (ShtDW) when green gram plant were inoculated with rhizobia isolates at $p \leq 0.05$ (Table 4.15). The plants inoculated with isolate IsAS14 had the highest nodule dry weight (83.00±12.00 mg plant^{-1}) and the shoot dry weight (2.88±0.07 g plant^{-1}) whereas, the lowest nodule dry weight (3.00±2.00 mg plant^{-1}) and shoot dry weight (0.89±0.89 g plant^{-1}) were obtained in non-inoculated control plants. On the other hand, the highest root dry weight (0.48±0.01 g plant^{-1}) was obtained when inoculated with isolate IsAS10, whereas, the lowest root dry weight (0.14±0.02 g plant^{-1}) and shoot dry weight (0.89±0.05 g plant^{-1}) was obtained in non-inoculated plants. The lowest shoot dry weight (1.50±0.18 g plant^{-1}) was realized when the non-sterilized soils were inoculated with isolate IsAS29 on green gram plants.

The native rhizobia isolate with the highest SyE above the commercial inocula USDA110 (100.00 %) were IsAS02 (105.21 %), IsAS03 (109.90 %), IsAS10 (114.06 %), IsAS11 (106.25 %), IsAS14 (150.00 %) and IsAS21 (110.94 %) (Table 4.15). Isolate IsAS29 (78.13 %) had the lowest SyE, but this isolate outcompeted the un-inoculated control plants (46.35 %).

Table 4.15: Rhizobia isolates inoculated on *Vigna radiata* planted in non-sterile soils to improve symbiotic efficiency from isolate obtained from Embu and Tharaka Nithi Counties farms soil

Isolates	NodN(plant^{-1})	NodDW (mg plant^{-1})	ShtDW (g plant^{-1})	RtDW (g plant^{-1})	SyE %
Exp-	2.25±1.29^{d}	3.00±2.00^{c}	0.89±0.05^{c}	0.14±0.02^{c}	46.35^{c}
IsAS02	45.63±8.60^{b}	62.00±13.00^{ab}	2.02±0.04^{ab}	0.38±0.012^{b}	105.21^{ab}
IsAS03	16.50±3.01^{c}	30.00±9.00^{bc}	2.11±0.05^{ab}	0.43±0.01^{ab}	109.90^{ab}
IsAS10	52.63±14.16^{ab}	60.00±11.00^{ab}	2.19±0.07^{ab}	0.48±0.01^{a}	114.06^{ab}
IsAS11	17.63±4.49^{c}	33.00±12.00^{bc}	2.04±0.10^{ab}	0.45±0.02^{ab}	106.25^{ab}
IsAS14	62.25±12.58^{a}	83.00±12.00^{a}	2.88±0.07^{a}	0.44±0.02^{ab}	150.00^{a}
IsAS21	23.50±6.89^{bc}	34.00±11.00^{bc}	2.13±0.08^{ab}	0.43±0.03^{ab}	110.94^{ab}
IsAS29	22.75±4.87^{bc}	33.00±9.00^{bc}	1.50±0.18^{bc}	0.16±0.03^{c}	78.13^{bc}
IsUSDA110	34.88±6.04^{b}	52.00±10.00^{ab}	1.92±0.05^{b}	0.40±0.02^{ab}	100.00^{b}
P values	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Key: NodN, Nodules number; NodDW, nodules dry weight; ShtDW, shoots dry weight, RtDW, roots dry weight; g plant^{-1}, gram per plant; mg plant^{-1}, milligram per plant; IsUSDA110, commercial positive strain inoculant; Exp-, un-inoculated negative control plants. Values followed by the same letters within the column are not significantly different according to Tukey`s Honest Significant Difference (HSD) at 5 % level.

4.7 Effects of cross-inoculation with rhizobia isolates on growth parameter of different legume crops

4.7.1 Effects of cross-inoculation of native rhizobia isolated from *Phaseolus vulgaris* L. tested in *Vigna unguiculata* for enhancing productivity of the plants in sterile soil

The non-orginal host of the revived native rhizobia isolated from common bean plants, were further cross-inoculated in cowpea (K-80) plants. There were significant differences in nodule number ($p \leq 0.05$), root dry weight ($p \leq 0.05$), shoot dry weight ($p \leq 0.05$) as well as nodule dry weight ($p \leq 0.05$) of cowpea plants when inoculated with the revived native rhizobia isolates (Table 4.16). In the study, cowpea inoculated with

isolate IsM had the highest nodule numbers of 16.88±2.33 plant^{-1} (average±standard error) whereas, the lowest nodule number of 0.50±0.20 plant^{-1} was realized in the plants inoculated with IsC. Plants inoculated with isolates; IsA, ISE, IsG, un-treated control plants (Exp-) and IsL has no nodules formation. The higher nodule numbers in cowpea plants translated to higher nodule dry weights.

The highest nodule dry weight (23.00±8.00 mg plant^{-1}) was recorded in cowpea plants inoculated with IsM while the lowest nodule dry weight of 1.00±0.10 mg plant^{-1} (average±standard error) was realized in the plants inoculated with isolates IsC. When inoculated with isolates; IsA, IsE, IsG, and IsL, the harvested cowpea plants showed negligible nodule dry weight. Apart from the isolates that did not induced formation of nodules, all the other native isolates showed statistically different in NodDW at $p \leq 0.05$.

There was a significant difference in the shoot dry weight of the cowpea tested ($p \leq 0.05$) (Table 4.16). The highest shoot dry weight of 1.18±0.06 g plant^{-1} (average±standard error) was obtained when the plants were inoculated with isolate IsM, whereas the lowest shoot dry weight of 0.56±0.10 g plant^{-1} was realized when cowpea was not treated with any forms of inoculant or isolate (un-treated control plants). There was a significant difference in root dry weight of the tested plants at $p \leq 0.05$ as presented in Table 4.16. Cowpea plants inoculated with isolate IsD recorded the highest root dry weight of 0.25±0.03 g plant^{-1}, whereas the un-inoculated plants produced the lowest root dry weight of 0.06±0.01 g plant^{-1}.

There were significant differences in cowpea plants growth performance in terms of symbiotic efficiency (SyE) at $P<0.0001$ tested across different rhizobia isolates (Table 4.16). The plants leaves were deep green in several of the treated plants inoculated with the native rhizobia isolates and commercial inocula CIAT899 (Plate 4.11). Native rhizobia isolates with the highest SyE were IsG2 (101.01 %), IsB (106.86 %) and IsM (115.69 %) whereas, the rest of the isolates had SyE lower than CIAT899 (100.00%).

Table 4.16: Effects of non-original host native rhizobia isolated from common beans testde on *Vigna unguiculata* planted in sterilized soils for growth improvement and inducing nodulation

Isolates	**NodN (plant^{-1})**	**NDW (mg plant^{-1})**	**SHtDW (g plant^{-1})**	**RtDW (g plant^{-1})**	**SyE %**
IsA	0.0	0.0	0.65±0.07^{cd}	0.13±0.02^{abc}	63.74^{cd}
IsB	10.38±2.62^{ab}	9.00±3.00^{b}	1.09±0.09^{ab}	0.24±0.02^{a}	106.86^{ab}
IsC	0.50±0.20^{cd}	1.00±0.10^{c}	0.91±0.11^{abc}	0.19±0.04^{abc}	89.22^{abc}
IsD	8.38±2.51^{abc}	8.00±2.00^{b}	0.97±0.08^{abc}	0.25±0.03^{a}	95.09^{abc}
ISE	0.0	0.0	0.66±0.08^{cd}	0.09±0.02^{bc}	64.71^{cd}
IsF	8.88±2.64^{abc}	8.00±3.00^{b}	0.92±0.06^{abc}	0.23±0.03^{ab}	90.20^{abc}
IsG	0.0	0.0	0.71±0.09^{bcd}	0.12±0.03^{abc}	69.61^{bcd}
IsG2	10.25±2.16^{ab}	10.00±2.00^{b}	1.03±0.04^{abc}	0.22±0.02^{ab}	101.01^{abc}
IsH	2.38±1.36^{cd}	2.00±1.00^{c}	0.84±0.06^{bcd}	0.19±0.03^{abc}	82.35^{bcd}
IsI	6.88±2.33^{bc}	6.00±2.00^{b}	0.94±0.08^{abc}	0.16±0.03^{abc}	92.16^{abc}
Exp-	0.0	0.0	0.56±0.10^{d}	0.06±0.01^{c}	54.90^{d}
IsCIAT899	7.38±2.55^{bc}	6.00±2.00^{b}	1.02±0.09^{ab}	0.19±0.04^{abc}	100.00^{ab}
IsL	0.0	0.0	0.78±0.07^{bcd}	0.13±0.02^{abc}	76.47b^{cd}
IsM	16.88±2.33^{a}	23.00±8.00^{a}	1.18±0.06^{a}	0.24±0.04^{a}	115.69^{a}
IsP	2.75±1.61^{cd}	2.00±1.00^{c}	0.95±0.09^{abc}	0.18±0.03^{abc}	93.14^{abc}
P values	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Key: NodN, nodules number; NDW, nodules dry weight; ShtDW, shoots dry weight; RtDW, roots dry weight; commercial inoculant, IsCIAT899; Exp-, untreated control plants; g plant^{-1}, gram per plant; mg plant^{-1}, milligram per plant. Values followed by the same letters within the column are not significantly different according to Tukey`s Honest Significant Difference (HSD) at 5 % level. The original host plants for this isolates were isolated from common bean (rosecoco) plant roots nodules.

Plate 4.11: Harvested six weeks old cowpea plant inoculated with native rhizobia isolate (IsM) in sterile soils that were tested for nodules formation, shoot dry weight, root dry weight and symbiotic efficiency of the isolate

4. 7. 2 *Phaseolus vulgaris* L. inoculated with native rhizobia isolated from soyabean tested in non-sterilized soils in the greenhouse for enhancement of plants production and symbiotic efficiency

The non-original host species of the revived rhizobia isolates obtained from soyabean (SB-126), were tested on common bean (rosecoco) for symbiotic efficiency in non-sterilized soils. There was a significant difference in nodule number ($p \leq 0.05$), nodule dry weight ($p \leq 0.05$), root dry weight ($p \leq 0.05$), and shoot dry weight ($p \leq 0.05$) of *Phaseolus vulgaris* L. inoculated with rhizobia isolates when planted in non-sterile soils (Table 4.17).

The highest nodule number of 79.38±14.15 plant^{-1} (average±standard error) was recorded when common bean plants were inoculated with isolates IsAGR81, whereas

the lowest number of nodules of 6.25±3.03 $plant^{-1}$ of plants was enhanced by isolate IsAGR5. In nodule dry weight, shoot dry weight and root dry weight the highest values were recorded when common bean were inoculated with IsAGR81, IsAGR26 and IsAGR10, respectively. On the other hand, the lowest parameters of the nodule dry weight (7.00±3.00 g $plant^{-1}$), shoot dry weight (2.31±0.13 g $plant^{-1}$) and root dry weight (0.42±0.05 g $plant^{-1}$) were recorded on inoculating the common bean plants with isolates; IsAGR5, IsAGR6 and IsAGR6, respectively. The plants inoculated with isolates; IsAGR10, IsAGR12 and IsAGR81 performed better than plants inocuolated with the commercial inoculant (IsCIAT899) in terms of nodules number, nodule dry weight and shoot dry weight. The un-inoculated plant yielded poorly when compared to the native rhizobia inoculum. Statistically, the IsCIAT899 and IsAGR13 were equal in nodule number and nodule dry weight but performed better in shoot dry weight than IsCIAT899.

There were significant differences in common bean plants growth performance in terms of symbiotic efficiency (SyE) at $P<0.0001$ tested across different native rhizobia isolates, commercial inocula CIAT899 and un-inoculated control plants (Table 4.17). The color of the plants leaf were deep green in several of the treated plants inoculated with the rhizobia isolates. Eight native rhizobia isolates had SyE higher than CIAT899. However, isolates IsAGR5 (97.61 %), IsAGR6 (92.03 %) and the un-inoculated plants (54.18 %) had SyE lower than CIAT899 (100.00%).

Table 4.17: Common beans planted in non-sterile soils in the greenhouse inoculated with non-orginal host rhizobia isolated from soyabean to test for growth parameters

Isolates	NodN ($plant^{-1}$)	NodDW (mg $plant^{-1}$)	ShtDW (g $plant^{-1}$)	RtDW (g $plant^{-1}$)	SyE %
Exp-	1.75 ± 0.98^{de}	4.00 ± 2.00^{d}	1.36 ± 0.14^{ed}	0.26 ± 0.03^{c}	54.18^{ed}
IsCIAT899	51.75 ± 10.67^{ab}	56.00 ± 16.00^{b}	2.51 ± 0.22^{b}	0.59 ± 0.04^{ab}	100.00^{b}
IsAGR1	37.50 ± 10.20^{bc}	57.00 ± 13.00^{b}	2.88 ± 0.09^{ab}	0.59 ± 0.03^{ab}	114.74^{ab}
IsAGR10	66.75 ± 11.93^{a}	105.00 ± 22.00^{a}	3.01 ± 0.06^{a}	0.74 ± 0.05^{a}	119.92^{a}
IsAGR12	62.75 ± 10.42^{a}	76.00 ± 23.00^{ab}	3.01 ± 0.06^{a}	0.64 ± 0.04^{ab}	119.92^{a}
IsAGR13	51.38 ± 12.62^{ab}	58.00 ± 15.00^{b}	3.04 ± 0.04^{a}	0.63 ± 0.02^{ab}	121.12^{a}
IsAGR14	49.63 ± 10.00^{ab}	53.00 ± 12.00^{b}	2.82 ± 0.05^{ab}	0.56 ± 0.03^{ab}	112.35^{ab}
IsAGR26	35.38 ± 11.58^{bc}	46.00 ± 13.00^{b}	3.23 ± 0.16^{a}	0.72 ± 0.03^{ab}	128.69^{a}
IsAGR3	45.75 ± 10.33^{b}	57.00 ± 12.00^{b}	2.93 ± 0.05^{ab}	0.58 ± 0.03^{ab}	116.73^{ab}
IsAGR5	6.25 ± 3.03^{d}	7.00 ± 3.00^{cd}	2.45 ± 0.18^{bc}	0.47 ± 0.05^{b}	97.61^{bc}
IsAGR6	8.75 ± 4.94^{d}	14.00 ± 9.00^{c}	2.31 ± 0.13^{bc}	0.42 ± 0.05^{b}	92.03^{bc}
IsAGR81	79.38 ± 14.15^{a}	115.00 ± 9.00^{a}	3.04 ± 0.07^{a}	0.61 ± 0.06^{ab}	121.12^{a}
P values	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Key: NodN, Nodules number; NodDW, nodules dry weight; ShtDW, shoots dry weight; RtDW, roots dry weight; IsCIAT899, commercial positive inoculant control; g $plant^{-1}$, gram per plant; mg $plant^{-1}$, milligram per plant; Exp-, un-treated negative control rosecoco plants. Values followed by the same letters within the column are not significantly different according to Tukey`s Honest Significant Difference (HSD) at 5 % level.

4.7.3 Potential of the native rhizobia isolates in sterilized soils in increasing *Vigna radiata* plants production

The non-original host species of native rhizobia isolated from common beans (rosecoco) root nodules, were tested on Green gram (*Vigna radiata*) plants for symbiotic efficiency. The plants, green gram (Ks-20) nodule numbers when grown in sterile soils and the roots interacting with native rhizobia strains showed statistical difference ($p\leq0.05$) (Table 4.18). The plants inoculated with isolate IsP had the highest nodules (NodN) of 4.38 ± 2.46 $plant^{-1}$ (average±standard error) whereas, green grams inoculated with IsE, IsK, IsG and IsI recorded the lowest number of nodules (0.63 ± 0.06, 0.77 ± 0.05,

0.80±0.06 and 0.96±0.06 plant^{-1}). Interestingly, it was observed that isolates; IsA, IsB, IsC and IsD influenced and induced higher nodule numbers than green grams inoculated with the commercial reference strains (IsUSDA110 isolate). The un-inoculated control plants showed no formation of nodules.

Nodule dry weight (NodDW) of plants was significantly different ($p \leq 0.05$) when compared among the inoculants (Table 4.18). Green gram inoculated with isolate IsA had the highest NodDW of 403.00±19.00 mg plant^{-1} (average±standard error) whereas, IsE recorded the lowest NodDW of 1.00±0.01 mg plant^{-1}. Of all the plants in the experimental setup, only the un-inoculated plants registered zero NodDW and NodN. Green gram inoculated with isolate IsP had the highest shoot dry weight of 2.64±0.15 g plant^{-1} while the lowest shoot dry weight of 0.77±0.05 g plant^{-1} was obtained in isolate IsK. The RtDW of the inoculated plants with the native rhizobia isolates, commercial isolate and the un-inoculated control plants showed significantly different in RtDW at $p \leq 0.05$.

There were significant differences in symbiotic efficiency (SyE) at $P<0.0001$ between the tested isolates. The native rhizobia isolates with the highest SyE were IsA (100.51 %), IsB (101.43 %) and IsP (125.71 %). The commercial inoculant USDA110 had SyE of 100.00 % (Table 4.18). The plants leaves were deep green in all of the treated plants inoculated with the native rhizobia isolates and CIAT899 (plate 4.12).

Table 4.18: Interaction of native rhizobia isolated from Embu and Tharaka Nithi Counties inoculated with *Vigna radiata* in sterilized soils for growth assessment

Isolates	NodN (plant^{-1})	NodDW (mg plant^{-1})	SHtDW (g plant^{-1})	RtDW (g plant^{-1})	SyE %
IsA	3.38 ± 1.13^{b}	403.00 ± 19.00^{a}	2.11 ± 0.5^{a}	0.34 ± 0.03^{a}	100.51^{a}
IsB	3.75 ± 1.35^{ab}	296.00 ± 17.00^{b}	2.13 ± 0.13^{a}	0.32 ± 0.03^{a}	101.43^{a}
IsC	3.45 ± 1.17^{b}	282.00 ± 16.00^{b}	1.94 ± 0.04^{a}	0.29 ± 0.03^{ab}	92.38^{a}
IsD	3.63 ± 1.25^{ab}	290.00 ± 9.00^{b}	1.83 ± 0.13^{ab}	0.29 ± 0.03^{ab}	87.14^{ab}
ISE	0.63 ± 0.06^{d}	1.00 ± 1.00^{e}	1.02 ± 0.12^{bc}	0.10 ± 0.02^{bcd}	48.57^{bc}
IsF	$1.38\pm0.13b^{c}$	32.00 ± 2.00^{d}	1.07 ± 0.14^{bc}	0.11 ± 0.01^{bcd}	50.95^{bc}
Exp-	0.0	0.0	0.80 ± 0.06^{cde}	0.10 ± 0.02^{bcd}	38.10^{cde}
IsH	1.00 ± 0.06^{d}	92.00 ± 14.00^{c}	1.00 ± 0.06^{bc}	0.09 ± 0.01^{cd}	47.62^{bc}
IsI	0.96 ± 0.07^{d}	142.00 ± 25.00^{bc}	0.96 ± 0.07^{cd}	0.14 ± 0.03^{bc}	45.71^{cd}
IsJ	1.96 ± 0.13^{c}	269.00 ± 30.00^{b}	1.96 ± 0.13^{a}	0.27 ± 0.03^{bc}	93.33^{a}
IsK	0.77 ± 0.05^{d}	72.00 ± 10.00^{cd}	0.77 ± 0.05^{cd}	0.07 ± 0.01^{d}	36.67^{cd}
IsL	1.14 ± 0.13^{c}	111.00 ± 15.00^{bc}	1.14 ± 0.13^{b}	0.11 ± 0.02^{bcd}	54.29^{b}
IsM	1.95 ± 0.03^{c}	288.00 ± 17.00^{ab}	1.95 ± 0.03^{a}	0.29 ± 0.02^{ab}	92.86^{a}
USDA110	2.13 ± 0.11^{bc}	321.00 ± 17.00^{ab}	2.10 ± 0.11^{a}	0.32 ± 0.02^{a}	100.00^{a}
IsG	0.80 ± 0.06^{d}	74.00 ± 9.00^{cd}	0.92 ± 0.09^{cd}	0.07 ± 0.01^{d}	43.81^{cd}
IsP	4.38 ± 2.46^{a}	314.00 ± 16.00^{ab}	2.64 ± 0.15^{a}	0.17 ± 0.03^{bc}	125.71^{a}
P values	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Key: NodN, Nodules number; NodDW, nodules dry weight; ShtDW, shoots dry weight; RtDW, roots dry weight; mg plant^{-1}, milligram per plant; g plant^{-1}, gram per plant; IsUSDA110, commercial positive strain inoculant; Exp-, un-treated negative control plant. Values followed by the same letters within the column are not significantly different according to Tukey`s Honest Significant Difference (HSD) at 5 % level.

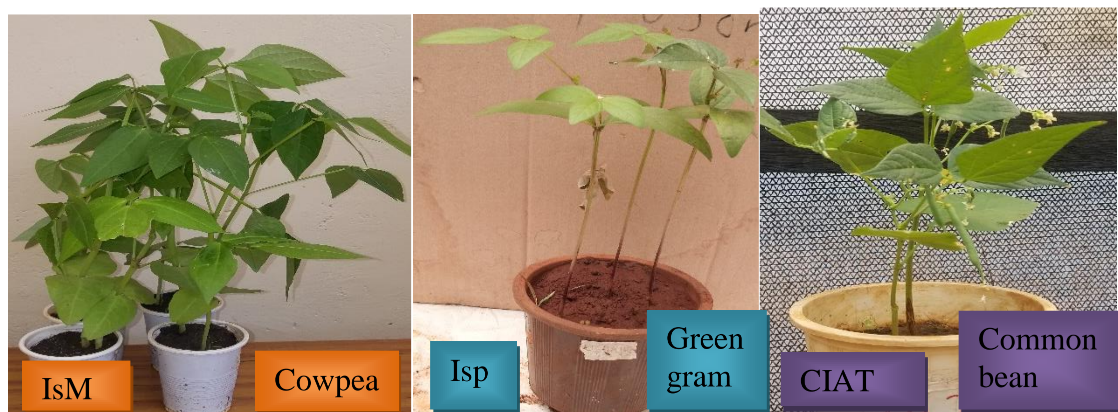

Plate 4.12: Different legume plants inoculated with different non-orginal host of native rhizobia isolates in non-sterile and sterile soils for various growth parameters

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Soil physico-chemical parameters

In this study, various physico-chemical parameters of soils from Kitui, Tharaka Nithi and Embu Counties were determined. These parameters are an essential tool in determining the appropriate soil management methods and practices as these properties determine the germination rate of the crops, plant growth conditions, root growth, nodulation, and plants yield by facilitating various biological and chemical processes (Mutuma *et al*., 2014; Chemining'wa *et al*., 2018; Pérez-Brandan *et al*., 2019).

Majority of the soil samples were reported to be acidic ranging from slightly acidic to highly acidic, with pH values ranging from 5.11 to 7.40. These pH values are unfavorable for optimum productivity as the acidic pH has a buffering capacity, therefore affecting enzyme and microbial activities involved in nitrogen (N_2) mineralization. It has been reported that the optimum pH for bacteria and extracellular enzymes ranges from 6.0 to 7.5 (De Castro Pires *et al*., 2018; Whalen *et al*., 2019).

Changes in the various physico-chemical properties with time due to traditional family farming practices have affected soil fertility, structure and productivity, and gradual soil degradation (Arévalo-Gardini *et al*., 2015). Very vital chemical properties such as Total organic carbon (TOC) and Nitrogen (N_2) are indicators of soil health. Soil samples from

Tharaka Nithi, Embu and Kitui showed very low total Nitrogen (TN) with the lowest recorded in Kitui at 0.05. The three regions recorded moderate to low TOC with the lowest being recorded in Kitui. A study carried out by Willy *et al.* (2019) on soils in Kitui showed that agricultural land use and practice has indeed led to the decline in soil properties such as TOC and TN over the years. Soil management systems have a direct effect on TN, and TOC as well as soil's organic matter (Arévalo-Gardini *et al.*, 2015).

In Tharaka Nithi County, there was a positive correlation between shoot dry weight (SHtDW), nodule dry weight (NodDW) and nodule number (NodN) with the soil pH and calcium (Ca) content. This agrees with a study by Wani and Khan. (2010) who stated that microorganisms are greatly affected by soil pH, therefore, pH may have a direct effect on the NodN and NodDW. There was a direct correlation of potassium (K), manganese (Mn), copper (Cu), and phosphorus (P) with SHtDW, NodDW and NodN (Morgan *et al*., 2005; Pérez-Brandan *et al*., 2019).

In Embu County, there was a significant direct correlation between SHtDW and Mn levels in the soil. Microelements such as Mn, Fe, and Cu are essential and vital in the symbiotic fixation of atmospheric nitrogen (N_2) (Dorjey *et al*., 2017). This was also supported by Morgan *et al.* (2005), that these microelements are important in regulating metabolic functions as well as protecting plants against stressors. They also stated that increased dry matter might be related to the increased enhancement of nutrient availability, nodulation, nitrogen fixation and growth performance of the plants.

In Kitui County, the situation was slightly different with factors such as pH, Na, Ca, and Zn having a negative correlation to SHtDW, NodDW, and NodN. However, there was a significant positive correlation of Mn, Mg, K, Fe, and P to plant growth parameters. Areas with low TOC, P and K significantly results in poor plant growth, yields and disease attraction, while those with higher availability of these nutrients records good crops development and production (Bationo *et al*., 2007; Williams *et al*., 2016; Zerihun Abebe, 2017).

This study shows that soil physico-chemical parameters have a direct effect on plant development and yields, and processes carried out by nitrogen fixing microorganisms such as rhizobia. This agrees with a study carried out by Zhong *et al.* (2019) who established that there is a direct and indirect effect of soil chemical properties on soil bacterial functions.

Understanding the direct and indirect effect of nutrients on plant development as well as microbial community can lead to maximization of C, and N_2 in the agricultural soils. Soil microorganisms such as; protozoa, bacteria (rhizobia), fungi, nematodes and actinomycetes play a vital and dynamic role in soil fertility (Chen *et al.,* 2019; Willy *et al*., 2019). Reduction of P, K, and N_2 in the soil can lead to unhealthy plants production and poor yields outcome as confirmed by (Thomas *et al*., 2015; De Castro Pires *et al*., 2018). They also added that microbes interact with the soil elements to augment the soil and boost crop yields. Soil microelements are necessary in the development of essential factors for the improvement of plants production, soil fertility, and a healthy ecosystem

(Pérez-Brandan *et al*., 2019). A study carried out by Kobraee *et al.* (2011) determined that application of Mn, Cu, P, and Zn markedly improved plants productivity.

5.1.2 Morpho-cultural characterization of rhizobia

The morphological characteristics of the 53 stored nodule isolates, resonates with the standard morphological characteristics of *Rhizobium* species by earlier studies that include; Beck *et al*. (1993); Somasegaran and Hoben (1994); Muthini *et al*. (2014); and Koskey *et al*. (2017). The presence of Gram negative rods isolated in the present study in addition to no absorbtion of congo red dye suggested that these isolates belonged to rhizobia due to the Gram negative endosymbionts results (Küçük *et al*., 2006; Boakye *et al*., 2016). The revealed fast growth among the isolates as indicated in all the groups except group C, group F, and group J, indicates that the isolates could probably fall under the genus *Rhizobium*. These suggestions are supported by Bergey's Manual of Systematic Bacteriology (Jordan, 1984) which has outlined similar observations for fast-growing rhizobia.

The colour change of YEMA-BTB from deep green to yellow is purely a characteristic of fast growing rhizobia (Jida and Assefa, 2011; Fernandes *et al*., 2012; Santos *et al*., 2016). All the isolates that were growing in YEMA with BTB showed yellow color on the plates (acid producers), this clearly indicated that all the isolates used were *Rhizobium* (Koskey *et al*., 2017; Diouf *et al*., 2019). In the current study, slow growth was observed in members that grouped under groups C, F and J even though they turned BTB to yellow. This unique trend could be closely associated with survival adaptation

of the isolates to stressful soil conditions, for example acidity above the optimum as revealed in some of the farms. Some rhizobia isolates are known to exhibit wide adaptation to the prevailing environmental conditions for their survival. The results of this study agree with Muthini *et al*. (2014) and Koskey *et al*., (2017) who in their studies morphologically characterized rhizobia nodulating plants from different agro-ecological zones.

The morpho-cultural characterized of the trapped native rhizobia isolates from Embu, Kitui and Tharaka Nithi Counties resonated with Elbanna *et al*. (2009); Ndungu *et al*. (2018) morphological and biochemical characterization. The rhizobia isolate obtained from the trapped experiment in family farms in Embu showed that groups VIII, IX, XI and XII are slow growing rhizobia, turning the YEMA-BTB media plates from green to yellow (Mwaangi *et al*., 2011). All the other groups were fast growing rhizobia. Furthermore, all the native rhizobia isolated from Kitui are fast growing rhizobia except isolates in groups VII, X, XI and XII, which are slow growing rhizobia (Agrawal *et al*., 2012). Interestingly, groups IV, VII, X, XI and XVI were slow growing rhizobia that turned BTB media from green to yellow (acid producers) from trapped cultured that were isolated from Tharaka Nithi farm soils (Koskey *et al.,* 2017).

5.1.3 Authentication of native rhizobia isolates

Out of the 53 authenticated rhizobia isolates, forty-six isolates had characteristics of fast growing rhizobia and nodulated common bean, soyabean, cowpea, and green gram plant roots. The presence of nodules in all the legume plant roots indicated that the isolates

infected and induced nodulation and therefore, justified clearly that all the isolates were rhizobia. The infectivity (ability to induce nodules formation) and the effectiveness of the isolates inoculated to the plants varied. These results agree with Argawal, (2012); Lu *et al*. (2017), that there is a difference in symbiotic interaction between native rhizobia isolates strain with their host species. However, seven isolates did not induce any nodules formation, these findings agreed with Muthini *et al*. (2014); Karthik *et al*. (2017) that those isolates do not belong to the rhizobia species.

5.1.4 Genetic diversity of native rhizobia isolates (archived samples) and molecular characterization of the isolates from different regions in Kenya

The genetic diversity of the twenty-six selected native rhizobia isolates obtained from the nodulated plants of; common bean, soyabean, cowpea, and green gram plus the three commercial inocula used, were assessed and evaluated via molecular gene sequencing partially targeting and aiming for 16S rRNA gene (16S rDNA) region. Inside the genome of the rhizobia, the DNA is extremely and highly preserved, therefore making it possible to distinguish and express the different populations and strains within the nodulated plant roots bacteria (Alves. 2009; Gray *et al*., 2016 and Karthik *et al*., 2017).

Due to the evolutionary affiliation and connection of the twenty-nine isolates obtained, the rhizobia isolates were assembled and clustered into two phenotypic clusters and two sub-clusters. The diverse clusters all contained bacteria from Genus *Rhizobium.* This showed high variation and disparity among the rhizobia isolates from the studied areas. It has been speculated that the genetic variation might be due to the different varities of

legume crops used and the soil biological conditions as articulated by Fening *et al*. (2004); Gentzbittel *et al*. (2015); Koskey *et al.* (2017).

The keys findings of these study indicate that there is a varying nature in the tendency in which plants uptake (P, Ca, Cu, N and Zn) and nodulate with rhizobia isolate. This feature helps crops to thrive successfully, where others cannot survive due to extreme environmental factors (biotic, edaphic and climatic factors) that significantly affect the process of BNF (Zahran, 2009). Despite the ecological variation and distance among the study areas of the revived stored isolates; Embu, Tharaka Nithi and Kisumu Counties, the study revealed little genetic difference of the native rhizobia populations in those agro-ecological zones. The findings from the 16S rDNA gene sequences of the revived glycerol stocks cultures suggest that there are different species of rhizobia, this sp. belong to *Rhizobium* genus. According to Morgan *et al*. (2005) and Saif *et al.* (2017) this might be correlated to the soil parameters for example; pH, P, acidic soils, and total soil organic matter content.

In addition, some rhizobia isolates that were clustered with similar morphological characteristics were not closely clustered genetically based on 16S rRNA gene sequence of the bacterial genome. This correlates with a previous study carried out in a semi-arid region in Ghana that showed the absence of correlation in genotypic and phenotypic features of *Vigna unguiculata* nodulating *Bradyrhizobium* sp. (Fening *et al.*, 2004). Similar findings in India by Rai *et al.* (2012) reported on native rhizobia nodulating *Cicer arietinum* (chickpea). The findings of the study were supported by Lu *et al.*

(2017), who reported that genetic diversity and alterations can cause profound changes in phenotypic and genetic (genotypic) characterization of the 16S rDNA gene in bacteria (rhizobia) which can lead to unclear identification and clustering. Similar results were obtained by Young *et al.* (2001) supporting the findings of the present study.

Similar rhizobia species obtained in this research have been isolated previously from root nodules of cowpea plants in South America in Brazil (Leite *et al*., 2017). Also, similar findings were presented by Kawaka *et al.* (2014) on common bean in Western Kenya. Furthermore, similar comparable and related soil microbe's were obtained in lower Eastern Kenya in selected small-holder farms via PCR-Box fingerprinting (Ntambo *et al.*, 2017). Based on the symbiotic efficiency results in this study, all the isolates obtained from the nodulated plants are rhizobia and can fix nitrogen. This agreed with results obtained by De Castro Pires *et al.* (2018) who studied the biodiversity of native rhizobia isolates that nodulate with *Vigna unguiculata* from the mining place of iron in Nova Lima (South Eastern Brazil) which affect and destroy the DNA (16S rRNA gene) of the bacteria. Similar studies of native rhizobia populations by Zhong *et al*. (2019) showed that the rhizobia sp. in China are closely related to rhizobia sp. in Northern America and Japan. These findings also agree with Ismail *et al.* (2013). They reported that rhizobial communities in legume root plants are diverse in different agro-ecological zones in Egypt were closely related, based on the information obtained from molecular identification. The genetic sequencing and cluster W analysis of native rhizobia groups, the study sites and the source of the rhizobia isolates does not show any significant connection and relationship with the sequenced isolates as observed by Da

Silva Júnior *et al*. (2018). Rejii *et al.* (2014) revealed and established similar findings that there are no associations and correlation between the genetic variations of leguminous nodulating plants bacteria and the environmental distribution in Tunisia. It also concluded that native rhizobia diversity cannot be subject to the original study sites based on genetic diversity. Also, Torres *et al.* (2018) made similar conclusions in their findings.

5.1.5 Symbiotic efficiency of rhizobia isolates

The difference in nodules number plant^{-1}, in the current study, can be linked to varied characteristics of the indigenous rhizobia isolated. Rhizobia have been shown to have an influence on nodule establishment in legume crops (Ferguson *et al*., 2013). The ability of common bean plants inoculated with isolates IsAMR2, IsAMR18, IsAMR12 and IsAMR14 (in sterile and non-sterile soil) to induce the development of more nodules and growth performance, as compared to that inoculated with other isolates could be linked to the symbiotic association among these rhizobia and the legume plants. Muthini *et al*. (2014), demonstrated that the relationship between rhizobia and common beans is crucial in nodule development. The author further emphasized that nodule development becomes more effective when the rhizobia isolates are well adapted to the environmental conditions of the agro-ecological zones. The ability of isolates IsAMR2 and IsAMR18 to enhance more nodule formation than the rest of the tested isolates suggests that the isolates were well adapted to the existing environmental conditions (temperature between 26 °C to 30 °C and low soil pH) as compared to the other isolates tested (Okazaki *et al*., 2016).

This study revealed that forty-six of the total revived stock isolates (archived samples) used were able to enhance and induce the development of nodules up to a certain level. Generally, native rhizobia isolates are varied diversely at species and strain levels (Lindstrom *et al*., 2010). Different studies have shown that the common bean plants is able to nodulate when infected with diverse indigenous *Rhizobium* sp. The ability of the twenty-six rhizobia isolates to induce the formation of nodules than the commercial strain isolates (CIAT889, USDA110, and USDA3456) suggests that the isolates could be used as bio-inocula in the productivity of legume crops and to improve soil fertility (Oliveira *et al*., 2017). The un-inoculated plants revealed no (zero) formation of nodules in sterile soils, demonstrating that aseptic conditions were met in the experimental setup and maintenance of the plants in the greenhouse.

The plant dry matter parameters showed that the effectiveness of the inoculated rhizobia isolates varied. These findings are supported by Elbanna *et al*. (2009) who demonstrated that there was a disparity in legumes effectiveness among indigenous rhizobia strains linked with particular host species. Significant differences in nodule dry weights as observed in this study have also been reported by other researchers like Gicharu *et al*. (2013); Koskey *et al*. (2017). In their study, the researchers reported significant differences in nodule dry weight between three cultivated bush bean and climbing bean grown in Kenya, that were treated and inoculated with different native rhizobia isolates. The dry nodule weight and the number of nodules positively correlated with the dry shoot weight and the symbiotic effectiveness of the isolates, which is supported by Minalku *et al.* (2009) and Agrawal *et al*. (2012).

Most of the highly nodulated plants had higher dry shoot weight when compared to other plants with less nodulation. These findings are in line with Rejii *et al.* (2014) studies. Who worked on nodulating roots rhizobacteria on *Lotus* species in semi-arid soil of Tunisia, they observed that inoculation of plants with root nodulating bacteria enhanced the vegetative growth and dry matter. This demonstrates that the treatment and inoculation of common bean plants with native rhizobia isolates improves shoot growth. The rhizobia isolates had significant differences in shoot dry weight of the plants due to the significantly variable ability in fixing nitrogen. Shoot dry weight is one of the methods used to assess and evaluate the symbiotic efficiency of indigenous rhizobia isolates (Sharma and Kumawat, 2011). This results corresponds well with Delic *et al*. (2010). Statistically, there was a significant rise in the dry shoot weight of the inoculated legume plants as compared to the un-treated (un-inoculated) control plants. This study's findings agreed with the findings by Agrawal *et al*. (2012); and Muthini *et al*. (2014) who reported a significant rise in shoot dry weight of *lentil* plant and common bean plants when inoculated with *Bradyrhizobium* sp. and *Rhizobium leguminosarum* var. *viciae*. This study's findings can be related to the potential of some native rhizobia isolates' capability to produce plant-growth-promoting hormones (PGPH) as well as fixing nitrogen (Agrawal *et al*., 2012).

The significant difference in the dry root weight of the inoculated plants indicates the different abilities of the inoculated native rhizobia strains to enhance root growth. These findings are contrary to Stajkovic *et al.* (2011) who established no significant difference in root dry weight when plants were inoculated with different native rhizobia. However,

plants that were not inoculated with rhizobia registered root dry weight higher than plants inoculated with IsAMR16 *Rhizobium* sp. This unique observation can be related to alteration in microbial processes involving IsAMR16 that mediate nitrogen cycling and root development (Vargas *et al.,* 2017). Some native rhizobia have been shown to not only be effective in symbiosis but also have the ability to enhance plant development due to their adjustment to the soil environmental conditons (Shamseldin *et al*., 2007).

5.1.6 Effectiveness of native rhizobia isolates in non-sterilized soil in nodulation

The improved growth parameters of green gram, cowpea, common bean and soyabean when grown in non-sterile soils inoculated with native rhizobia isolates has been reported in other studies. For instance, in a study carried out by Guiñazu *et al.* (2010), the authors' related increase in plant growth and yields with inoculation of rhizobia isolates. Elsewhere, Mburu *et al*. (2016); Koskey *et al.* (2017), in a field study carried out in Eastern Kenya showed that the effect and interaction of rhizobia and other biotic and abiotic soil elements significantly increased nodule number per plant, relative growth rate and yield of the plants. Green gram and common bean plants, mainly draw nutrients from a native pool of soil and rhizobia helps in fixing N_2. Legumes depend on their symbionts for a large part of their nitrogen requirements for growth and increased dry matter production. Since rhizobia are commonly found inhabiting the common rhizosphere and colonizing the roots of legume plants in the agricultural soil, they possibly worked in synergy with the introduced native rhizobia to enhance water and nutrient (phosphorus, calcium and nitrogen) transport and also increases growth and

yield of the studied plants (Gopalakrishnan *et al*., 2015). In recent years, the effect of inoculation with native rhizobia isolates have been reported to further increase and enhance the growth and productivity of some plants including; green gram and cowpea (Aryal *et al.,* 2007; Meghavansi *et al.,* 2008; Abd-Alla *et al.,* 2014). The rhizobia that recorded higher common bean, cowpea, soyabean, and green gram growth parameters compared to their counterparts can be said to be important symbionts associated with plant roots that improve N_2-fixation, plant growth and induce nodulation.

The association of rhizobial strains with the roots of the studied plants may have improved the soil health and nitrogen fixation, thus further enhancing higher growth parameters (Thomas *et al*., 2015). In a related study, the tripartite association of root nodule bacteria (*Bradyrhizobium japonicum*) and soyabean host to find its effect on the promotion of growth and yield of plants (Meena *et al*., 2018). It was reported that the bacteria exerted positive effects and a direct correlation on plant growth by improving P and N_2 availability. The findings of the current study support suggestions that the rhizobia inoculation may be of crucial importance within sustainable, low-input agricultural cropping systems to maintain soil fertility and legume crops' health (Muthini *et al*., 2014; Sureshbabu *et al*., 2016; Koskey *et al*., 2017).

The low soyabean, greem gram, cowpea, and common bean dry matter parameters recorded in the current study in the plants grown in non-sterile soils can be associated with antagonistic effects between the soil microorganisms, microelements and the rhizobia inocula. Related suggestions were reported by Lima *et al.* (2017) where they

sought to characterize and evaluate the antagonistic effect of action bacteria on rhizobia from a semi-arid region. The variation in recorded low growth parameters of the study crops can be linked to the inocula used. It has been revealed in the past studies that the antagonistic activity is largely determined by the strain of rhizobia isolates used (Vargas *et al*., 2017). For instance, non-acid *Rhizobium* strains sp. have been found to be less susceptible to antagonistic activities of *Actinomycetes* than acid strains (Volpiano *et al*., 2019). Previously, antagonistic mycorrhizal fungus *Glomus* on *Bradyrhizobium* sp. was reported for legume plants when one of the microsymbionts had colonized the roots system before to the other (Hindumathi and Reddy 2012; Nadeem *et al*., 2014).

The development of the rhizosphere in natural soils is a dynamic process that incorporates physical, chemical and biological modification at the root-soil surface (Espeleta *et al.,* 2017). The qualitative and quantitative changes of microbial populations in the rhizosphere ecology might have influenced the required components for the plants growth. Generally, the ability of some native rhizobia inocula in the current study to enhance growth parameters higher than the commercial inoculant has been documented in past studies (Gary *et al*., 2016; Kyei-Boahen *et al*., 2017; Gouda *et al*., 2018).

5.1.7 Effects of cross-inoculation with native rhizobia isolates on growth parameter of different host legume plants

The significant difference in nodule numbers of common beans, cowpeas and soyabeans

is an indication of how soil alteration and cross-inoculation can dictate the impact of native rhizobia strains on the development of plants. Different rhizobia isolated from different plants have a specific impact on the growth of other plants (Ferguson *et al*., 2013). Native rhizobia strains impact on plant roots nodulation is dependent on the ability of the isolates to adapt and survive to the prevailing environmental and biological conditions (Alves. 2009; Ferguson *et al*., 2013).

Isolates IsM, IsG2, IsD, IsB, and IsF which showed the highest nodule numbers in cross-inoculation between common bean and cowpea plants, were well suited for the change in original host plants, as compared to the commercial inoculant (CIAT899) plants, as has been previously reported by Kyei-Boahen *et al*. (2017). On the contrary, rhizobia isolates IsA, IsE, IsL, and IsG, which showed the lowest or no nodulation numbers in cowpea plants were not well adapted to the change in original host plants conditions and their infection potential is not suitable (Nadeem *et al.,* 2014). The capacity of rhizobia inoculated to enhance and induce nodulation independently or in symbiotic interaction might have been altered due to the change of legume plants (Thilakarathna *et al*., 2017). Therefore, the effect of the original host plants can be linked to the difference in nodulation potential in these plants. Contrary to this, the native rhizobia candidates that registered highest nodule numbers in cowpea plants could be well adapted to working individually leading to the high nodulation and plants production improvement (Leite *et al*., 2017).

The nodulation ability of the non-original host species of the revived rhizobia isolates obtained from soyabean (SB-126), were tested by inoculating on common bean plants. Isolates IsAGR10, IsAGR12, IsAGR81 and IsAGR13 induced more nodulations compared to the commercial inoculant CIAT899 this was previously reported by Muthini *et al*. (2014); and Kyei-Boahen *et al*. (2017).

Isolates IsA, IsB, IsC, and IsD which showed the highest nodule numbers in cross-inoculation between common bean and green gram plants, were well suited for the change in original host plants, as compared to the commercial inoculant USDA110, and the other rhizobia isolates (Wertz *et al*., 2007). Furthermore, rhizobia isolates IsG, Isk, IsI, and IsE, which showed the lowest nodulation numbers in green gram plants were not well adapted to the change in original host plants conditions and their infection potential became unsuitable for nodulating with different plants (Larimer *et al.,* 2014). The non-effectiveness of commercial inoculants under field conditions has been linked to the inability of the inoculants to adjust to their new environments and the new plants introduced and to stay abreast of the expanding root system (Lesueur *et al.,* 2016). Therefore, this suggests that bio-inoculants are more likely to be active and effective if they are developed and produced from isolates found in the area or zone in which they are to be used.

The native rhizobia isolates had significant differences in shoot dry weight of common bean, cowpea, and green gram and hence significantly variable ability in fixing nitrogen. This study findings can be related to the potential of some rhizobia isolate’s ability to

produce plant-growth promoting hormones (PGPH) as well as fixing nitrogen (Agrawal *et al*., 2012). Production of shoot yields is a complex process and has been associated with the plant genotype (Zhong *et al*., 2019). The crops genotype significantly modifies the performance of *Rhizobium* strains. A highly active and most effective strain in one species, might be rated as moderately efficient in another type of species (Mendonça *et al*., 2017). Shoot dry weight is one of the methods used to determine the effectiveness of rhizobia isolates (Sharma and Kumawat, 2011). Thus, the interaction among plant varieties and *Rhizobium* strains inoculum in the current study indicates the need for assessing specific strains for individual legume crops varieties for specific farming practices. On the contrary, rhizobia isolates IsA, IsE, IsM, IsG2, IsAGR1, IsAGR10, IsAGR12, IsAGR13, IsAGR26, and IsAGR81, which showed highest shoot dry weight from non-original host plants can be used as bio-inoculants for legume plants production and soil fertility. This study's findings demonstrate that the inoculation of the plants with native rhizobia from non-original host plants can increase and improve the crop dry matter.

The nodule dry weight of common bean, cowpea, and green gram positively correlated with their nodule numbers. This demonstrates that the inoculation of the plants with native rhizobia isolates in the soils improves nodule dry matter (Vargas *et al.*, 2017). However, some of the inoculants caused high nodule numbers in green gram but this did not translate to a higher nodule dry weight. This could be associated to the presence of some rhizobia isolates that are parasitic (deleterious) to the crops and do not fix nitrogen effectively for the plants (Volpiano *et al*., 2019). This suggestion supports that rhizobia

effectiveness cannot be sufficiently determined by nodule dry weight and nodule number (Jida and Assefa. 2011). They further articulated that in the rhizobia legume association, rhizobia may infect the host plant but not be effective in nitrogen fixation.

This study also evaluated the interaction and effect of native rhizobia, hence cross-inoculated with different legume crops (cowpea, common bean and green gram) for growth parameters under sterilized and non-sterilized soil conditions. The significant difference in nodule numbers and dry matter of plants reveals the specificity of the studied rhizobia isolates to influence growth parameters in the crops. However, different rhizobia sp. have a specific impact on the growth of legume crops (Alves. 2009; Ferguson *et al.,* 2013). Rhizobia affect plant development by the production and release of secondary metabolites (Singh *et al*., 2019). The metabolites are critical in the facilitation of uptake of minerals from the plants root environment. In the current study, rhizobia that enhanced higher growth parameters due to the cross-inoculation with the different legume plants measured above grown in sterilized and non-sterilized soil might be well adapted for the aforementioned functions.

The common bean, cowpea, and green gram dry matter parameters effectiveness of the inoculated native rhizobia varied. These findings are supported by a study carried out by Elbanna *et al*. (2009) who demonstrated that there was a disparity in the effectiveness of indigenous rhizobia strains linked with particular host species. The significance of the findings in the current study is that the effectiveness of an individual *Rhizobium* sp. isolates varies with their ability to act independently and while interacting with soil

biota with the non-original host plants. This explanation supports suggestions that agricultural management practices may influence the effectiveness of BNF by the indigenous rhizobia bacteria (Schmidt *et al*., 2017). Garg *et al.* (2016) postulated in their studies that nodulation efficiency among rhizobia isolates inducing nodulation in legume plants was highly determined by the ability to adapt and adjust to the prevailing soil environments and non-original host plants. The change of original host plants of the isolates IsA, IsP, IsM, IsG2, IsB, IsAGR10, IsAGR13, IsAGR26, and IsAGR81 when inoculated with different legume plants performed better than the commercial isolates CIAT899 and USDA3456. Hence, this isolates can be used as bio-fertilizer for further legume plants production, soil fertility and soil stability.

5.2 Conclusions

i. A total of 311 nodules rhizobia isolates were obtained, from Tharaka Nithi, Embu and Kitui Counties farms. Based on morphology, the isolates were placed into 42 groups. In addition, a total of 53 isolates from the revived stored cultures were grouped morphologically into 11 groups. Out of the 53 isolates, 26 outcompeted the commercial inoculum and were sequenced, and identified as rhizobia species based on 16S rRNA gene.

   Most of the sequenced native isolates were closely related to *Rhizobium etlt*, *Rhizobium* sp. and *Rhizobium leguminosarum* sp. when BLAST in the NCBI database. In addition, soil physico-chemical parameters also play a key role in legume plants health development and food production.

ii. Native rhizobia isolated from different study sites had a significant difference at $p \leq 0.05$ symbiotic efficiency as compared to the commercial inocula. Variation in soil physico-chemical properties influenced the number of nodules and shoot dry weight with P, pH, N, C, Ca, and TOC was having the highest impact.

The performance of the native rhizobia isolates across the tested legume (common bean and soyabean) plants in sterile soils demonstrated their adaptability and symbiotic potential in inducing nodulation and nitrogen fixation (NF).

iii. The effectiveness of native rhizobia isolates in non-sterilized soils was confirmed as some of the isolates out-competed the commercial inoculants. However, their diversity differed significantly (at $p \leq 0.05$) in growth parameters of the plants in non-sterile soils. The study demonstrated that native rhizobia isolates can form a beneficial symbiosis with common bean, cowpea, green gran and soyabean as they are able to favourably compete with the indigenous microflora and establish a relationship with the legume plants tested.

iv. Different native rhizobia isolates had a noteworthy effect, and influence on the growth parameters of cowpea, green gram, and common bean on cross-inoculation. Several native rhizobia isolates produced better or similar results to that of the commercial inocula in cross-inoculation. These determine the

presence of highly effective native rhizobia in the studied revived native rhizobia isolates.

5.3 Recommendations

i. Molecular methods should be carried out to identify and check the presence of other rhizobia species from the trapped nodule isolates, using other different primers such as CTO189f - Pf1053r and BAMO143f - BAMO1315r and molecular vast sequences.

ii. Native rhizobia isolate with the highest symbiotic efficiency of above the commercial inocula of 100.00 % (IsAMR2, IsAMR3, IsAMR6, IsAMR12, IsAMR14, IsAMR18, IsAMR18, IsAMR22, IsAMR25, IsAMR27, IsAS14, IsAGR1 and IsAGR81) should be subjected to further screening to test for their potential to fix Nitrogen using other legumes of economic importance. These are the potential rhizobia candidates for soil fertility and production of an effective common bean and soyabean plants inocula for lower-income farmers in Kenya and sub-Saharan African.

iii. The revived rhizobia isolates (IsAS11, IsAMR2, IsAMR12, IsAMR14, IsAGR10, IsAGR12, IsAGR5, IsAS02, IsAS10 and IsAS14) that outcompeted the commercial inoculants in non-sterile soils should be used as biofertilizers for common bean, cowpea, green gram, and soyabean production by

smallholder farmers as an alternative and cheaper sustainable agricultural practice.

iv. Notably, some of the native isolates IsA, IsE, IsG and IsL in Table 4.16 are known only to nodules with their trapped host plants. These findings highlight the need for continuous investigation in cross-inoculation with legume-rhizobia microsymbionts from non-original host plants of economic significance. Further research focusing on unearthing the vast diversity of cross-inoculation of indigenous rhizobia with their potential to improve legume plants growth from other parts of the country that has not been studied should be investigated.

Field trials for the native rhizobia isolate (IsB, IsD, IsF, IsG2, IsM, IsAGR10, IsAGR12 and IsAGR81) with the highest symbiotic efficiency (SyE) between 101.01 % - 128.69 % and highest nodulation number should be carried out to establish their competitiveness in various soils and different regions in Kenya.

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APPENDICES

Appendix I: Preparation of Yeast Extract Mannitol Agar (YEMA)

	Reagents/Chemical for Rhizobia media	Quantity/Amont
1	Mannitol	10.0g
2	K_2HPO_4	0.5g
3	$MgSO_4.7H_2O$	0.2g
4	NaCl	0.1g
5	Yeast Extract	0.5g
6	Congo red dye/Bromothymel Blue	0.025g
7	Bacteriological Agar	16.0g
8	Distilled H_2O	1.0L

Appendix II: Preparation of Yeast Extract Mannitol Broth (YEMB)

	Reagents/Chemical for Nutrient Broth	**Quantity/Amont**
1	Mannitol	5.0g
2	K_2HPO_4	0.25g
3	$MgSO_4.7H_2O$	0.1g
4	NaCl	0.5g
5	Yeast Extract	0.25g
6	Distilled H_2O	0.5L