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Pattern of Nodulation and Nitrogen Fixing Performance of Introduced Forage Legumes in Some Parts of North Gondar, Ethiopia

By Fekadu Shimekite

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ACRONYMS

ANOVA=Analysis of variance

AUCB=Addis Ababa University Bradyrhizobial isolates of cowpea (*Vigna unguiculata*)

AULB =Addis Ababa University Bradyrhizobial isolates of lablab (*Lablab purpureus*)

AUMR=Addis Ababa University rhizobial isolates of alfalfa (*Medicago sativa*)

AUVR=Addis Ababa University rhizobial isolates of common vetch (*Vicia dasycarpa*)

MgATP=Magnesium binding Adenosine triphosphate

Mo=Molybdenum

MoFe=Molybdenum-Iron

NDW= Nodule dry weight

NN=Nodule number

PCR=Polymerase Chain Reaction

SDW=Shoot dry weight

TN=Total nitrogen

ABSTRACT

Six exotic herbaceous forage legumes namely, ***Desmodium uncinatum*** (desmodium), ***Lablab purpureus*** (lablab), ***Medicago sativa*** (alfalfa), ***Trifolium repens*** (white clover), ***Vigna unguiculata*** (cowpea) and ***Vicia dasycarpa*** (common vetch) were induced for nodulation on Dembia and Wegera soils for which all except, desmodium and white clover legumes nodulated their host. From the nodulated legumes, nineteen isolates were isolated and characterized based on their morphological, cultural, and physiological properties. In the preliminary screening eleven isolates of alfalfa, three isolates of vetch were assigned as fast growing ***Sinorhizobium meliloti*** and ***R. leguminosarum*** var ***viceae*** respectively. The isolates of lablab (three) and cowpea (two) were assigned as slow growing ***Bradyrhizobium*** species. The vetch isolates AUVR11, AUVR13 from Wegera and AUVR14 (***R. leguminosarum*** var ***viceae)*** from Dembia and alfalfa isolates AUMR3 and AUMR10 from Wegera; and AUMR9 from Dembia (S***. meliloti***) were found to renodulate their respective hosts. The nodulating isolates were studied with respect to their relative effectiveness in nitrogen fixation. Inoculation of vetch by ***R. leguminosarum*** var ***viceae*** showed significant differences in percent effectiveness (30%-83%) on vetch, and (50-100%) on alfalfa. AUVR13 (vetch) and AUMR9 (alfalfa) were found to be the most efficient strains with percentage effectiveness of 83% and 100% respectively. Although the performance of the vetch isolates on two different soils showed that there was no any inter strain difference on each soil, Dembia soil responded better in SDW and TN than Holetta soil indicating that some soil-related factors such as pH affect symbiotic effectiveness. Generally, the fact that the number of infective and effective isolates of the introduced legumes in Dembia and Wegera weredas was very small may necessitate more exhaustive screening in different regions and the need for inoculation with effective exotic endosymbionts to fully realize the potential of Biological Nitrogen Fixation in forage production in animal husbandry.

Key words/Phrases: Introduced forage legumes, Nitrogen fixation, Nodulation, *Rhizobium leguminosarum* var *viceae*, *Sinorhizobium meliloti, Bradyrhizobium* spp

1 INTRODUCTION

Livestock contributes up to 80% of farmer's income and about 20% of agriculture gross domestic product in Ethiopia (Alemayehu Mengistu, 2002a). Traditional livestock production system solely depends upon poor pasturelands. Trees and shrubs mainly the legumes are part of the 200 main browse forage species for domestic and wild animals and represents 20-25% of the domestic ruminant intake of the silvopastoral area of Africa (Le-Houerou, 1987). Furthermore, in modern livestock production system, feed takes 60% of the total cost of the farm expenses (Thomas and Lascano, 1993). Thus, the incorporation of selected forage legumes into poor pastures was proved to be the best strategy to improve nitrogen nutrition and meet the protein demand of animals and humans reducing the high cost of the farm.

In Ethiopia development of exotic forage legumes have gradually become important mainly due to the supply of inadequate quantities and poor quality forages of the existing natural pastures with respect to energy and nitrogen. Since 1988, an extensive forage development program all over the country (Alemayehu Mengistu, 2002b) by national and regional agricultural offices and research institutions using various types of exotic forage seeds are incorporated into the livestock production system.

Some of these were *Macroptilium atropurpureum* (siratro), *Desmodium* and *Stylosanthes* spps, L*ablab purpureus* (lablab), *Vigna ungiculata* (cowpea), *Vicia dasycarpa* (vetch), *Medicago sativa* (alfalfa) and *Sesbania sesban* (sesbania). Most of these species and cultivars were imported from Australia, the Caribbean and Central America (tropics), and Mediterranean and West Asia (temperate) (Alemayehu Mengistu, 2002a).

The incorporation of forage legumes into pastureland is used to increase the herbage yield since they fix nitrogen with association of root nodule bacteria known as rhizobia. The Biological Nitrogen Fixation (BNF) through forage legume-*Rhizobium* partnership may provide up to 100% of the nitrogen needs of the plants from the atmosphere (Humphrey, 1980). The pattern and specificity of the association (nodulation) and the amount of nitrogen that can be fixed by the symbiosis (effectiveness) depends upon the type of legumes and rhizobia and the environmental

conditions (Vincent, 1970; Friedericks ***et al***., 1990 and Materon, 1991). It has been estimated that Alfalfa (***Medicago sativa***), White clover (***Trifolium repens***) fix nitrogen to the tune of 200-500kg/ha N, and that of ***Sesbania*** spp as high as 286 kg N/yr (Vallis, 1985; and Peoples and Crasswell, 1992). Skerman ***et al***. (1988) has reported that the use of cowpea and other leguminous green manure crops were found to incorporate 80.7-339.4 kg N/ha. The use of elite strains of rhizobia, therefore, highly improves the forage yield through fixation of large quantities of nitrogen (Hubbell, 1993).

In Ethiopia, the work on the nodulation status and nitrogen fixation potential of legumes is very scarce and has concentrated on highland pulses such as peas, beans, chickpeas, and lentils (Fassil Assefa, 1993). So far very few works have been reported on symbiotic effectiveness concerning forage legumes of Ethiopia that focused only on five African annual and two perennial clovers (Friedericks ***et al***., 1990 and Lupwayi ***et al***., 1997), and a few ***Acacia*** spp and ***Erythrina*** spp (Fassil Assefa, 1993 and Fassil Assefa and Kleiner, 1998).

Although exotic forage species have been extensively introduced in Ethiopia for experimental purpose (Thulin, 1989), and through fourth livestock development project, the performance of these species was found to be ambivalent in that exotic forage legumes such as common vetch, sesbania, treelucern and to a lesser extent alfalfa showed good performance, whereas desmodium, cowpea, lablab and trifolium species failed to persist in the farm (ILDP, 2005). The failure of these forage legumes might have come as a result of the absence of compatible and ineffective rhizobia due to the genotypic or edaphic factors. So far, studies on nodulation and nitrogen fixation of these legumes have not been reported. This study was, therefore, made to evaluate the status of compatible and effective rhizobia of the six introduced forage legumes cultivated in Dembia and Wegera, North Gondar.

2 OBJECTIVES

1. To assess the presence and diversity of rhizobia that form symbiosis with six exotic forage plants.
2. To isolate the efficient rhizobia in nitrogen fixation of theses forage plants.
3. To study their performance on two different soil types with contrasting pH

3 LITERATURE REVIEW

3.1 THE LEGUMINOSAE

The leguminosae is a family of flowering plants containing about 650 genera and 18,000 species (Giller and Wilson, 1991). It can be divided into three sub-families: ***Caesalpinioideae***, ***Mimosoideae*** and ***Papilionoideae***. Members of this family are known to form nodules in the roots to fix nitrogen in a symbiotic association with root nodule bacteria, generally known as rhizobia. From all the hitherto examined legumes, about 57% of the genera and 20% of the species are nodulated (de Faria ***et al***., 1989). The sub-family ***Caesalpinioideae***, which comprises about 1900 mainly woody and tropical species, only 23% of the examined species have nodules, followed by higher proportion of nodulation of 90% of examined species of the sub family ***Mimosoideae*** (2700 mainly woody species in all regions). The third, and largest sub-family, ***Papilionoideae***, contain 13,000 woody and herbaceous species with 97% of nodulating species.

Legumes have a number of advantages to agriculture. Cereal/legume shifting and use of legumes as green manure is very important in agriculture to prolong soil productivity. The legumes are used to increase the nitrogen content of the soil by symbiotic fixation and support the growth of crops. Planting fast perennial leguminous cover crops between rows of the main plantation crop are also used to protect the soil from erosion, protect the understorey vegetation from heavy rain and improve their water economy and photosynthetic efficiency. The use of cover crops has long been exploited in plantation crops such as coffee, tea, rubber, sisal, cacao, where the legumes provide light shed for moderation of soil temperatures, making conditions more favorable for microbiological activity towards slower mineralization and recycling of leaf litter (Dommergues, 1988). The accumulations of organic matter through mineralization increase infiltration beneath plants, and decrease soil loss by reducing surface flow during infrequent but intensive precipitation events (Dommergues, 1988). Many legumes provide valuable high heat value, fuel wood and charcoal, a source of 80% of the energy need in the tropics (Thulin, 1989). Wood for poles, pulp and craft uses are also important characteristics of many legumes. They are used as ornamentals, windbreaks, and fencerows. They also play an essential role in providing wood for construction, food, medicine, tannin, and gum Arabic for household and other uses. Since many legumes have high feeding value nutrients to ruminants, because of their lower contents of

structural fiber, higher protein contents and greater digestibility (Frame *et al*., 1998), they increase live weight of animals, milk production; and shorten first calving intervals (Thomas and Lascano, 1993).

3.2 FORAGE LEGUMES

Natural grazing land is the most important component of the traditional silvopastoral production system of intertropical Africa (Le-Houerou, 1987). Trees and shrubs mainly the legumes are part of the 200 main browse forage species for domestic and wild animals and represents 20-25% of the domestic ruminant intake of the silvopastoral area of Africa (Le-Houerou, 1987). The system covers also the montane (highland) silvopastoral together with the Afro–Alpine ecological zone that supports 120-150 million head of the intertropical African livestock population 40% of which belongs to Ethiopia (Le-Houerou, 1987). These montane silvopastoral systems play an essential role for both resident and transhumants herds as feed source and for cultivation of food crops to the farmers in the area. Owing to this fact, they are heavily exploited all year round resulting into infestation by plants of little forage value (Le-Houerou, 1987). The continuous loss of important forage cultivars and the ever increasing number of human population in the area therefore led to a dead end for the livestock industry in large parts of Africa, particularly in South-east and North Ethiopia (Le-Houerou, 1987). Therefore, development of forage/fodder of high nutritive value as supplementary feeds (20-25% of the total requirements) to the productive animals became crucial and very important (Alemayehu Mengistu, 2002a).

Forage legumes include herbs, trees and shrubs that are useful as forage/fodder (Alemayehu Mengistu, 1997). Farmers in Ethiopia developed the most important exotic tree and herbaceous legumes for animal production (Table1 and 2). The use of cultivated forage legumes was begun in the early 1970 in Chilalo Agricultural Development Project (CADU), Arsi and expanded to Wolayeta Agricultural Development Project (WADU), Sodo (Thulin, 1989). Its benefit was more realized when an international and national research centers and agricultural colleges put an effort to support high yielding dairy animals. In 1988 Fourth Livestock Development Project initiated the issue of animal feed security through the wise management of available lands for both crops and fodder production throughout the country (Alemayehu Mengistu, 2002a). Currently many

national and regional agricultural offices and research institutions are intensively incorporating various types of exotic multipurpose trees and herbaceous legumes in livestock production system.

Table1 Exotic Herbaceous Forage Legumes for Ethiopia

Species	Common Name	Altitude (masl)	Minimum rainfall (mm)
Macroptilium atropurpureum	Siratro	<2000	500
Macrotyloma axillare	Axillaris	<2400	500
Desmodium intortum	Greenleaf	<2400	800
Desmodium uncinatum	Silverleaf	<2400	700
Stylosanthes hamata	Verano	<2400	500
Stylosanthes scabra	Seca	<2400	500
Stylosanthes guianensis	Cook	<2400	600
Lablab purpureus	Lablab	<2400	400
Cassia rotundifolia	Wynn Cassia	<2400	400
Vigna unguiculata	Cow pea	<2400	350
Vicia dasycarpa	Common vetch	>2000	400
Medicago sativa	Alfalfa/Lucerne	>2000	350
Trifolium repens	White clover	>2000	500
Lotus pendiculatus	Maku Lotus	>2400	600

Table 2 Exotic Tree and Shrub Forage Legumes For Browse in Ethiopia

Species	Common Name	Altitude (masl)	Minimum Rainfall (mm)
Leucaena leucocephala	Leucaena	<2000	400
Sesbania sesban	Sesbania	<2400	600
Cajanus cajan	Pigeon pea	<2400	250
Chamaecytisus palmensis	Treelucern	<2000	350

Adapted from Alemayehu Mengistu *'Forage production in Ethiopia: A case study with Implications for Livestock Production*'

3.2.1 Characteristics of Forage Legumes Used for the Study

Desmodium

The genus ***Desmodium*** has some 300 species in tropical and temperate areas (Thulin, 1989). Several species were identified as good forage or cover crops. ***Desmodium uncinatum*** (Silverleaf) is the most widely utilized species in the tropical areas of Africa. It is a perennial, sprawling forage legume (Thulin, 1989). ***D. uncinatum*** is indigenous to Northern Argentina, Brazil and Venezuela. It uses specialized "Desmodium" group rhizobia (Skerman ***et al***., 1988).

Lablab

Lablab purpureus (lablab) is climbing or sub erect usually perennial legume (Thulin, 1989). It forms a vigorous, erect, trailing stems. Leaves are trifoliate; flowers are white, blue or purple. It has very large leaves; the small white flowers provide large, heavy pods that hold well on the plant (Humphrey, 1980; Skerman ***et al***., 1988). Lablab leaves supplement other forages well and its seed is an excellent human food (Alemayehu Mengistu, 2002a). Lablab has two important cultivars used particularly as forage: Rongai and Highworth. ***L purpureus*** cv Rongai was indigenous to the district of Rongai in Kenya whereas ***L. purpureus*** cv Highworth was introduced from India and improved in Australia (Humphrey, 1980).

Medicago

Medicago contains herbs and small shrubs with a number of annual and perennial species. The most important perennial species is ***Medicago sativa***. Common cultivars include Hunter river, Hairy Peruvian, Siriver, Paravivo, and Sequel (Thulin, 1989). Alfalfa produces good forage yields with more than 600mm of rainfall and is ideally suited to irrigation. It has high feed value and should be used as a supplement for crop residues and natural hay in a mixture of 30 percent alfalfa and 70 percent other roughages (Alemayehu Mengistu, 2002a). Moreover, its seed is used in making feed concentrates and its extracts have shown bactericidal properties. In addition to this, they are good sources of nectar for bees and useful in rotation with cereal crops to restore soil fertility.

Trifolium

This genus encompasses both annual and perennial herbs. Eventhough it is a large temperate genus containing many important fodder and bee plants, they are well represented in the Mediterranean region; but tropical Africa is recognized for its center of diversity (Thulin, 1989). In temperate region the two, most adapted trifolium species are ***T. repens*** (White clover) and ***T. subteranum*** (Red clover). ***T. repens*** is a versatile perennial clover, primarily for altitudes above 2400m (Alemayehu Mengistu, 2002a).

Vigna

There are nine species under the genus ***Vigna*** (Skerman ***et al***, 1988). The most known species as forage and food is ***Vigna unguiculata*** (L.) subsp. ***unguiculata*** (Cow pea). ***Vigna unguiculata*** is an annual legume suited to a wide range of environments. Cowpea grows from lowlands upto 2500m and is drought tolerant-maturing with anything more than 300mm annual rainfall, depending on cultivars (Skerman ***et al***, 1988). The mixed cowpea hay and stover makes excellent conserved forage for dairy animals (Alemayehu Mengistu, 2002a).

Vicia

There are two fodder species under the genus ***Vicia***; and they are ***Vicia dasycarpa*** (Common Vetch) and ***Vicia vilosa***. Common vetch is an annual herb that uses tendrils at the end of its leaves to climb up other plants. It grows well between 1500 and 3000m altitude. It produces much vegetative growth, a profusion of dark blue to purple flowers and pods with small round seeds (Thulin, 1989).

3.3 NITROGEN FIXATION

Nitrogen acquisition is one of the most important processes for plant growth and development (Zaharan, 1999). Globally the atmospheric gas contains about 10^{15} tonnes of nitrogen gas and the nitrogen cycle involves the transformation of some $3x10^{9}$ tonnes of nitrogen gas per year (Suba-Rao, 1977). Yet N availability can limit plant growth (Zaharan, 1999).

Nitrogen is incorporated to plant tissues in the form of ammonia, which is either supplied, by

artificial N fertilizer or using naturally available soil bacteria (Postgate, 1998). In nature nitrogen cannot be used directly by organisms, but it has to be combined with hydrogen to be incorporated into amino acids (Postgate, 1998). Thus, the reactions in which this triple bond is broken and N is combined with hydrogen require substantial inputs of energy, and only occur under highly specific conditions mainly in biological systems where the bacterial enzyme nitrogenase is expressed (Roughley, 1984; Postgate, 1998; and Zaharan, 1999).

According to Postgate (1998) 40 to 50% of the input of the world's nitrogen is globally supplied by nitrogen fixing bacteria. Other sources mainly industrial fertilizer contributes about 50 % (Peoples and Craswell, 1992; Postgate, 1998; and Zaharan, 1999).

Different groups of prokaryotes fix nitrogen either freely or in association with various legume plants. The free-living nitrogen fixers of the genera *Azosprilium* and *Azotobacter* contributes to the N balance in tropical cropping systems (Giller and Wilson, 1991). Other members of N_2 fixing genera of *Beijerinckia*, *Derixia* are associated with the rhizosphere of different grasses. Several species of truly microaerobic and aerobic N_2 fixing bacteria are also associated with plant roots such as *Herbasprillium*, *Aquasprillium*, *Pseudomonas*, *Klebsiella pneumoniae* and *Entrobacter* species.

The cyanobacteria (blue green algae) fix nitrogen freely or in associations with a variety of plants (Giller and Wilson, 1991). The others are the actinomycetes, mainly *Frankia* species, which form associations with actinorhizal plants such as alnus, casuarinas. Many of these host plants are woody perennials (Giller and Wilson, 1991). Rhizobia, the predominant group, which form symbiotic associations with legume plants contributes the greatest amount (70%) of biologically fixed nitrogen (Peoples and Crasswell, 1992).

3.4 BIOLOGICAL NITROGEN FIXATION

Symbiotic nitrogen fixation is a process in which root nodule bacteria are able to reduce atmospheric nitrogen into ammonia (Giller and Wilson, 1991). Root nodule bacteria (RNB), generally known as rhizobia, are microsymbionts that infect roots of some legumes and transform

atmospheric nitrogen into usable form by the plants. They are endosymbiotically associated with hairs of plant roots forming an excrescence known as nodule. RNB were isolated from a number of leguminous plants and pure cultures of the bacteria were shown to induce nodules when reinoculated onto the same host plants. The genus ***Rhizobium*** was originally classified into five groups based on the observation of host-range specificity. This led rapidly to the concept of 'cross-inoculation' group. A cross inoculation group is a group of host species supposedly nodulated by one set of rhizobial strains, and not by any rhizobial strains that could induce nodules of legumes not belonging to that particular cross-inoculation group (Fred ***et al.,*** 1932 sited in Giller and Wilson, 1991). Under this classification

R. leguminosarum nodulate ***Lathyrus***, ***Lens***, ***Pisum*** and ***Vicia***,

R. lupni the genus ***Lupinus***,

R. meliloti legumes of ***Meliloti***, ***Medicago*** and ***Trigonela*** species,

R. phaseoli make association with ***Phaseolus***

R. trifolii with many species of ***trifolium***.

As more RNB was isolated from several legumes, they were evaluated on the basis of biochemical and physiological characters. They were further classified into two genera as fast and slow growing ***Rhizobium*** and ***Bradyrhizobium*** respectively (Jordan, 1984).

The advent of molecular biology has strengthened rhizobial taxonomy through different tools. Sequence composition of 16S rRNA genes, finger-printing methods based on the use of PCR has been extensively used for characterizing rhizobia. Consequently, based on polyphasic approach 36 rhizobial species distributed among seven genera of rhizobia are recognized. These are ***Allorhizobium***, ***Azorhizobium***, ***Bradyrhizobium***, ***Mesorhizobium***, ***Methylobacterium***, ***Rhizobium*** and ***Sinorhizobium*** (Sahgal and Johri, 2003). According to this classification, forage legumes such as alfalfa (***M sativa***) are nodulated by fast growing rhizobia of ***S. meliloti***, Vetch (***V. dasycarpa***) and trifolium species are induced by ***R. leguminosarum*** (biovar ***viceae*** and ***trifolii***) respectively; whereas those forage legumes like desmodium, cowpea and lablab are all nodulated and fix nitrogen by a slow growing '***Bradyrhizobium***' spps generally known as the cowpea miscelany (Giller and Wilson, 1991).

3.5 EFFECTIVENESS OF NITROGEN FIXATION

Despite the large number of legume genera and species used for forage and green manure in the tropics and sub tropics (Peoples and Herridge, 1990), there are relatively few works on estimation of nitrogen fixation by herbaceous species because of methodological difficulties.

In an extensive review made by Peoples and Crasswell (1992) the amount of legume N derived from nitrogen fixation for cover crops and herbaceous forage legumes in mixed grass swards were estimated to be considerably high. The important forage legumes ***Centrosema pubescens*** fixed 150 kg N/ha, ***Desmodium intortum*** 183kg N/ha, ***Macroptilium atropurpureum*** 124kg N/ha, ***Stylosanthes*** spp. 84kgN/ha.

Quite high yields of nitrogen have also been recorded for perennial, temperate forage legumes grown under irrigated conditions. That is 250-500kg/ha N for Lucerne (***Medicago sativa***) and 200-500kg/ha N for white and red clovers (***Trifolium repens*** and ***T. pratense***) (Vallis, 1985). Similarly, large quantities of N from nitrogen fixation for tree and shrub legumes were estimated (Peoples and Crasswell, 1992). Accordingly, ***Leucena leucocephala*** from Malasia yields 182-231 kg N/ha; ***Sesbania*** spps yields 7-286 kg N/yr from different countries.

3.6 FACTORS AFFECTING SYMBIOSIS

The nitrogen fixation efficiency of legume-rhizobia symbioses is affected by three sets of variables: the plant genotype, the rhizobial genotype, and the environment. The later includes a range of abiotic and biotic factors such as temperature, rainfall, drainage, soil pH, soil nutrient status (including N), competition between plants, pests, diseases, and the cutting/grazing regime. Typical environmental stresses by the legume nodules and their symbiotic partner (rhizobia) may include salinity, soil nitrate, temperature, and soil acidity (Zahran, 1999).

Increasing salt concentrations may have detrimental effects on soil microbial populations because of direct toxicity (Marschner, 1995). The legume-***Rhizobium*** symbiosis and nodule formation on legumes are more sensitive to salt stress. Salt stress inhibits the initial steps of ***Rhizobium*** legume symbiosis. Successful ***Rhizobium*** legume symbiosis under salt stress requires the selection of salt

tolerant rhizobia from those indigenous saline soils (Zahran, 1991). According to Hussain ***et al.*** (2002) inoculation of R***hizobium*** to saline soil improves the dry matter, nitrogen fixing abilities, nodule number, and dry weight of ***Trifolium alexandrium***. Temperature also affects root hair infection, bacteroid differentiation, nodule structure, and the functioning of the legume root nodule (Roughley, 1984).

Soil acidity is a significant problem facing agriculture in many areas of the world and limit legume productivity (Bordelea and Prevosl, 1994). Most leguminous plants require a neutral or slightly acidic soil for growth, especially when they depend on symbiotic nitrogen fixation (Bordelea and Prevosol, 1994). Legumes and their rhizobia exhibit varied responses to acidity. Some species like alfalfa are extremely sensitive to low soil pH. Soil acidity constrains symbiotic nitrogen fixation limiting ***Rhizobium*** survival and persistence in soils and reducing nodulation (Brockwell ***et al.***, 1991). ***Rhizobium*** with a higher tolerance to acidity has been identified (Graham ***et al***, 1982).

4 MATERIALS AND METHODS

4.1 STUDY SITES

The study sites are Dembia and Wegera, which are located in the Northwestern part of the country, Gondar (Map). Dembia is located at 20 ^{o} 24' N and 37^{o} 18'E, and elevation of 1821 m. a. s. l. at Koladeba town with a temperature range between 13.8-28.7 ^{0}C. The rainfall pattern is of monomodal type with an annual rainfall of 1021 mm (MoA, 2000). Its soil type is Nitosol. Wegera is situated at 12^{o}46'N and 37^{o}37'E and altitude of 2640 m. a. s. l. at Ambagiorgis town, with mean annual temperature of 14 ^{o}C and annual rainfall of about 1100 mm. Its soil type is Vertisol. Both sites are characterized by mixed agriculture system in which major food crops such as teff, wheat, beans, peas, lentils, and chickpeas are cultivated. Cattle, sheep, and goats are dominant in the mixed farming system.

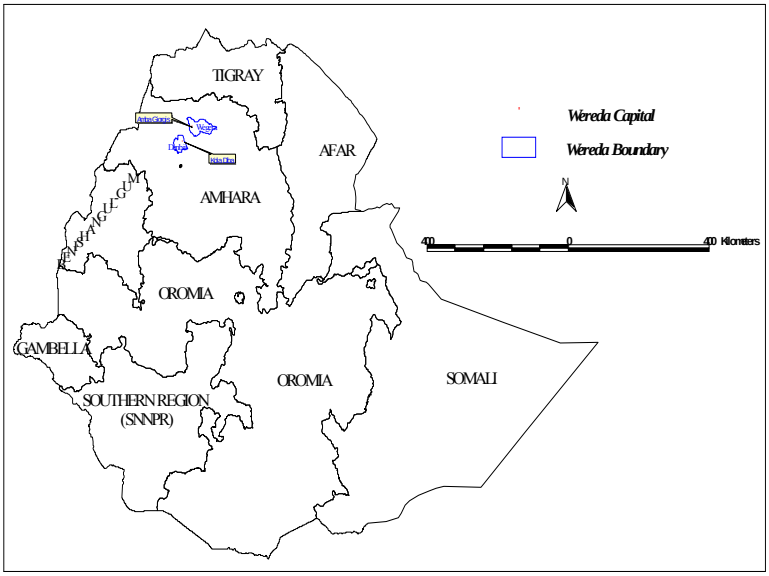

Figure 1. Location Map of Sampling Sites

The major exotic forage legumes that are used as animal feed in both sites include *Chamaecytisus palmensis*, *Sesbania sesban*, *Vicia dasycarpa*, *Medicago sativa*, *Desmodium intortum* and *D. uncinatum*. Other forage legumes like, *Trifolium repens, Lablab purpureus*, *Vigna unguiculata*, *Macroptilium atropurpureum*, and *Stylosanthes scarba* were also introduced. For the last ten years, 24.19 million tree forage legume seedlings and 5279qt of forage seeds that covers an area

of 15,837 ha of land were distributed in the region of which 60% of them were vetch, alfalfa, desmodium, cowpea, and lablab (ILDP, 2005).

4.2. SOME FORAGE LEGUMES CULTIVATED IN THE FIELD

Figure 2 *Desmodium uncinatum*

Figure 3 *Lablab purpureus* cv. Rongai

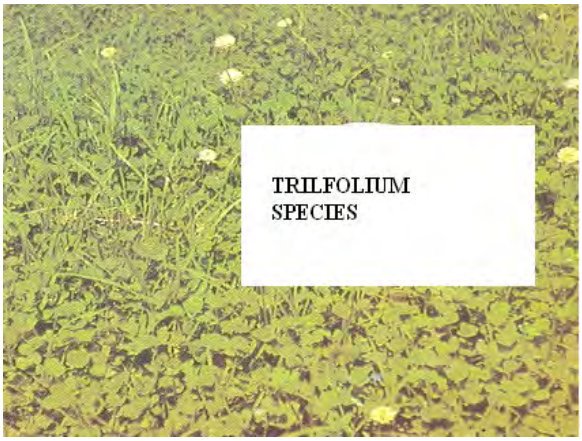

Figure 4 *Trifolium repens*

Figure 5 *Vicia dasycarpa*

4.3 SOIL COLLECTION

Farmers' fields were selected with past-history of mixed cultivation of forage and food legumes. Five soil samples from different corners of the sampling plots were taken from the depth of 15-30

cm of surface. The samples were pooled, mixed, and kept in surface sterilized plastic bags. They were air dried at room temperature and transported to Addis Ababa University, Biology Department for further study. Similarly, soil samples were also collected from Holetta for studying the rhizobial efficiency on lower pH.

4.4 SEED COLLECTION

For this study forage seeds were acquired from the genetic bank of International Livestock Research Institute (ILRI). The accessions were: *Desmodium uncinatum 6765*, *Lablab purpureus 147, Medicago sativa 6984, Trifolium repens, Vicia dasycarpa 6213* and *Vigna unguiculata 9334.*

4.5 INDUCTION OF NODULATION

Nodulation was induced by 'plant trap' method as described by Vincent (1970). Two kilograms of field soil from each sample was thoroughly mixed and sieved through 5 mm mesh sieve and prepared for potting. They were filled in 3 kg capacity plastic pots that had been surface sterilized (using 70% alcohol). Similar sized seeds of each forage legume were surface sterilized by 95% ethanol for 5 seconds and with acidified mercuric chloride (0.2% w/v in 5 ml HCl) for 3-5 minutes, and washed thoroughly with five changes of sterile water. 20-25 seeds aseptically transferred to water agar and incubated at $28\pm2\ ^{o}$C until 1-1.5 mm of the radicle and hayla were emerged. Four germinated seeds were then transplanted on their respective pots, which were thinned to three after a week. The pots were kept in the green house and watered at filled capacity with distilled water every day for two months.

4.6 ISOLATION OF ROOT NODULE BACTERIA

Sixty days after planting seedlings were carefully pulled out to remove soil particles. Nodules were then detached from the roots and their shape, color, and number were recorded. Five to ten pink and healthy nodules were selected, surface sterilized as before (Vincent, 1970). The sterile nodules were then crushed aseptically with sterile glass rod and the milky fluid were streaked on YEMA plates and incubated at $28\pm 2\ ^{o}$C for 5 to 7 days. Typical colonies from each plate were picked and repeatedly sub cultured on the same media to obtain pure culture. Pure colonies were

then preserved at 4^{o} C on YEMA slants containing 0.3% (w/v) $CaCO_3$ (Vincent, 1970) for further study.

Yeast Extract Mannitol Broth (YEMB) (g/l)

K_2HPO_4 ----------0.5

$MgSO_4.7H_2O$----------0.2

NaCl----------0.1

Yeast extract----------0.5

Mannitol----------10

Yeast Extract Mannitol Agar Medium (YEMA) (g/l)

YMB plus

Agar----------15g

4.7 CHARACTERIZATION OF ROOT NODULE BACTERIA

All isolates were characterized based on cultural and physiological features. All experiments were undertaken with inoculations with approximately 10^4 cells ml^{-1} using 3.3µl calibrated inoculating loop after growing the isolates on Yeast-extract-Manito Broth (YMB), adjusted to pH 6.8-7.2 on Gyratory shaker at 125 rev min^{-1}, at room temperature. All assays except were performed in triplicates (Lupwayi and Haque, 1994), and test plates were incubated at 28 ^{o}C, for 5-7 days.

4.7.1 Cultural and Morphological Characterization of Root Nodule Bacteria

Colony morphology and acid/base reaction were evaluated on Yeast-extract-Manitol agar medium (YEMA) containing, 25 µgml^{-1} each of Congo red and bromothymol blue (BTB) (Vincent, 1970; Ahmed ***et al***, 1984; Lupwayi and Haque, 1994). Colony size, diameter, and appearance were also characterized on YEMA according to Ahmed ***et al***, (1984).

Isolates were inoculated to check their growth on peptone glucose agar to which rhizobia do not grow well or change the color of the media as described by Lupwayi and Haque (1994). PGA

Medium contains: Glucose (5g), Peptone (10g), Agar (15g) and Bromocresol purple (1% in alcohol) (10g).

4.7.2 Growth Rate Determination

Growth of the isolates was determined as described by Somasegaran and Hoben (1994). Bacterial growth was assessed on YEM broth incubated in a Gyratory shaker at 125 rev. min^{-1}, by measuring the optical density at 540 nm every 6 hrs. Viable counts were also taken every 6 hrs on YEMA by colony-spread plate Method, and incubated for 3-5 days. Doubling time was calculated from the logarithmic phase according to White, (1995).

g= t/y, Where g=generation time;

t=time elapsed;

y= the number of generation

4.7.3 Physiological Characterization of Root Nodule Bacteria

All isolates were checked for their growth at different nutritional and environmental conditions. The characters were: tolerance to NaCl, pH, temperature, antibiotics, and growth on different carbon sources. One loopful of each tested isolates ($\approx 10^4$ cells/ml) rhizobia were inoculated in triplicate on YEMA containing various concentrations of each content of tested material and incubated at 28$\pm$ 2^oC for 5-10 days according to Somasegaran and Hoben (1994) and Lupwayi and Haque (1994) unless stated otherwise.

4.7.3.1. pH Tolerance

The ability of isolates to grow in acid and alkaline media was tested by inoculating strains into Keyser defined agar plates as modified by CIAT (Lupwayi and Haque, 1994) in which the pH had been adjusted to 4.0, 5.0, 8.0, and 9.0 respectively with sterile 0.1N HCl or NaOH solutions. Growth was evaluated qualitatively as (+) for growth and (-) for no growth. Keyser medium contained: -

A. Micronutrient stock solution (g/l)

$MnCl_2.4\ H_2O$---------- 0.504

$ZnSO_4.7H_2O$ ----------0.227

$CuCl_2.2H_2O$ ----------0.034

$NaMO_4.2H_2O$ ---------- 0.008

B. Phosphate Stock solution (g/l)

KH_2PO_4 ----------1.36

C. Vitamin stock solution (g/100ml)

Thiamin HCl ---------- 0.400

d-pantothenic acid (Ca)---------- 0.400

Biotin ---------- 0.0001

Medium (per lt)

Glycerol---------- 5ml

K_2SO_4 ---------- 0.131g

Na glutamate----------0.220g

$MgSO_4.7H_2O$----------0.074g

$CaCl_2.\ 2H_2O$ ----------0.007g

Fe-EDTA----------0.035g

Solution A----------0.5ml

Solution C ----------1.0ml

Solution B ----------1.0ml

Agar ----------20g

4.7.3.2 Salt Tolerance

Isolates were inoculated on YEMA plates containing 0.01 (control) 0.5, 1, 2, 3, 4, 5 % (w/v) NaCl. Growth was compared with control plates and scored as above (Lupwayi and Haque, 1994).

4.7.3.3 Temperature Tolerance

The ability of isolates to grow at different temperature gradients was monitored by inoculating them on solid YEMA medium and incubating at 5, 10, 35, and 45 ^{o} C, respectively. They were first left for two hours at room temperature to assist them to establish on the media followed by incubation to 5-10 days. Tolerance was evaluated qualitatively as (+) for growth and (-) for absence of growth.

4.7.3.4 Carbohydrate Utilization

Isolates were checked for their ability to utilize the different carbohydrate sources (Somasegaran and Hoben, 1994). The test carbohydrates were prepared in 10% (w/v) solutions in water. Nineteen carbohydrates were used as carbon source replacing mannitol and the yeast extract was reduced to 0.05 g/l according to Somasegaran and Hoben (1994). All heat labile carbohydrates were filter sterilized using disposable membrane filter of 0.22μm sizes and added to the basal medium after sterilization when the medium temperature was reduced to 50 ^{o}C, but heat stable carbohydrates were autoclaved together with the medium. The heat labile carbohydrates used were arabinose, rhaminose, xylose, gluconate, mannose, maltose, raffinose, tartarate, citrate, sorbitol, adonitol, inositol, D-galactose, cellobiose and glycerol while the heat stable carbohydrates used were lactose, glucose, sucrose and fructose. Each YEMA medium containing the above carbohydrates was used to streak the test isolates then they were allowed to stay for 2 hours at room temperature and incubated for a maximum of 10 days at 28 ± 2^{o} C. Data were recorded qualitatively as above.

4.7.3.5 Intrinsic Antibiotic Resistance (IAR)

Isolates were inoculated on YEMA media containing different concentrations of seven antibiotics to realize their intrinsic antibiotic resistance (IAR) following the method described by Somasegaran and Hoben (1994). Antibiotics were filter sterilized using 0.22 μm millipore filters and stored in one-use aliquots at 0 ^{o}C. One loopful ($\approx10^{4}$cells/μl) of broth containing the tested isolate was transferred to YEMA plates supplemented with each antibiotic. The plates were

prepared in duplicate and incubated at 28 $\pm$2^{o}C for 5-10 days and growth was scored by visual inspection as above.

Antibiotics

Group I (inhibitors of Protein Synthesis)

Antibiotics	final conc. (µg/ml)
Erythromycin (Ery)----------	5, 10
Streptomycin (Str)----------	3, 5

Group II (Inhibitors of DNA and RNA synthesis)

Nalidixic acid (Nal)----------	5, 10
Rifampicin (Rif)----------	3, 5

Group III (Inhibitors of protein synthesis and cell wall membrane permeability)

Kanamycin (Kan)----------	3, 5
Chloramphinicol (Chl)----------	5,10
Ampicilin (Amp)----------	3,10

4.8 AUTHENTICATION AND SYMBIOTIC EFFECTIVENESS OF ISOLATES

Authentication and effectivity of isolates were undertaken in the greenhouse using the methods described by Lupwayi and Haque (1994). Small and large seeded legumes were grown in test tubes and plastic pots respectively.

4.8.1 Authentication and Symbiotic Effectiveness (sand pot experiment)

Large seeded forage legumes of common vetch, lablab, and cowpea were surface-sterilized and germinated in a water agar and incubated at 28$\pm$2^{o}C as before until the hayla and radicle reached 1-1.5cm. For authentication, four seedlings were transferred to pots that contained acid washed (1N HCl) sand, which were later thinned down to three. Each strain was grown on YEMB to 10^{9} cells/ml and inoculated into each seedling. The experiment was statistically laid out with three replications as Randomized Complete Block Design (RCBD). Each block contained two pots, negative control (T_0) and positive control (T_N) with uninoculated seedlings. The positive control was supplied with N weekly at a rate of 70mg/lt applied as 0.05% KNO_3 (w/v) solution. Plants were also fertilized with nitrogen-free nutrient solution (Norris and Date, 1976 sited in Lupwayi

and Haque, 1994) once in a week and they were grown in the greenhouse with 12-day/night photoperiod. Forty-five days after planting, nodule numbers (NN), nodule (NDW) and shoot dry weight (SDW) were recorded.

N-free Nutrient Solution for Forage Legumes

Solution	Reagents	Concentrations (g/l)	Vol. (ml) of stock solution per liter of medium
A	KCl	29.8	2.5
B	K_2HPO_4	69.8	2.5
C	$MgSO_4.7H_2O$	98.6	2.5
D	Micronutrients		0.5
	$CuSO_4.7H_2O$	0.078	
	$ZnSO_4.2H_2O$	0.22	
	$MnSO_4.4H_2O$	2.03	
	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.01	
	H_3BO_3	1.43	
E	Ferric citrate	1.795	1.0
F	$CaSO_4.2H_2O$	-	0.344g

4.8.2 Authentication and Symbiotic Effectiveness (test tube experiment)

Small seeded plants of alfalfa were grown in 20x200mm test tubes dispensed with 15mm aliquots of N-free nutrient solution agar and autoclaved at 121 ^oC for 15 minutes (Lupwayi and Haque, 1994). The seeds were surface-sterilized and germinated as before (Vincent, 1970) and one seedling to each test tube was aseptically transferred and allowed to grow for 5-7 days at room temperature. The seedlings were then inoculated with 1ml ($\approx 10^9$ cells) of their respective strains and replenished with quarter strength of the N free nutrient solution and sterile water up to eighty days. Test tubes were arranged in duplicates as RCBD and placed into the glasshouse using a wooden rack. Two uninoculated seedlings (T_O and T_N) were also used as controls.

After 80 days of growth, the whole plants were removed from the tubes. The nodulation capabilities of the isolates were assessed by visual inspection and effectivity of the homologous isolates was assessed on the bases of nodule number, nodule color and nodule mass and whole plant dry weights after drying at 70 ^{o}C for 48 hours.

4.9 SYMBIOTIC EFFECTIVENESS OF VETCH ON SOIL CULTURE

The *in situ* symbiotic effectiveness of the vetch isolates was tested on Dembia and Holetta soils. They were filled into 3kg capacity pots, and were once fertilized with the following N free fertilizers (mg/pot) according to the suggestion of Somasegaran and Hoben (1994):

KH_2PO_4	468
KCl	404.2
$MgSO_4.7H_2O$	53.3
$ZnSO_4.7H_2O$	49.5
$(NH_4)_6 MO_7O_{24}. H_2O$	1.95

The soil in each pot was mixed thoroughly to ensure uniform distribution of nutrients. Urea ($CO(NH_2)_2$) (219 mg/pot) was used as nitrogen source for N-control pots. 25% of N was applied at planting and the remaining 75% at 3 weeks interval. Seeds of vetch were germinated as before. The tested strains AUVR11, AUVR13, and AUVR14 were cultured into 250 Erlenmeyer flasks for 48 hours. 1ml of each culture (10^9cells/ml) was inoculated into each seedling. Pots were labeled and assigned in three block numbers and randomized in the greenhouse. All pots were prepared in triplicates and supplied with tap water to field capacity every day. Fourty-five days after planting (DAP), plants were harvested and dry weight of nodule; shoot and nodule number were recorded with two uninoculated controls (UNI+ and UNI-).

4.10 TOTAL NITROGEN DETERMINATION

Total nitrogen content of the forage legume samples was analyzed by using modified kjeldahl procedure described by Sahelemedhin Sertsu and Taye Bekele (2000) using the following chemicals:

H_3BO_3	20g
Bromocresol green	0.5g
Methyl red	0.1g
40% NaOH	400g
Conc. H_2SO_4	1lt
Metallic selenium	3.5g
Salicylic acid	7.2g
0.1N H_2SO_4	2.82ml
95% ethanol	100ml

The dried plant samples were first finely ground using pistil and mortar followed by sieving with 0.5mm sieve size of which 0.3g of the ground samples were subjected Kjeldahl procedure for determination of total nitrogen in the sample. Macrokjeldahl procedure for determination of total nitrogen involves three processes: digestion, distillation and titration. 2.5 ml of the digestion mixture composed of 1lt sulfuric acid, 3.5g selenium powder and 7.2g of salicylic acid/100ml H_2SO_4-selenium mixture were added to each macrokjeldahl tube and swirled carefully so as to moisten the plant material and let them stand for 2hrs. The digestion tubes were placed on a heating block and heated at 100^o C for 2hrs. After 2hrs, the tubes from the block were removed and allowed to cool; then three-1ml hydrogen peroxide were added and mixed thoroughly. The digestion tube was again heated at 300 ^{o} C on a preheated block until the digest turns to colorless or light yellow. After removing the tube from the block and cooling to room temperature, 48.3ml of distilled water was added, mixed and left overnight. In the next day the mixtures were again mixed, filtered on 100 ml volumetric flask, bring to volume with distilled water and made ready for further analysis.

The acid digest was transferred quantitatively to the macro-kjeldahl flasks. 20 ml of boric acid solution was measured into a receiver Erlenmeyer flask corresponding to the number of samples. 2 drops of mixed indicator solution composed of 0.5g bromocresol green, 0.1g methyl red dissolved in 100ml of 95% ethanol were added and placed under the condenser. After adding 75ml of 40% NaOH solution to the digestion flasks containing the digests, it was fitted to the corresponding holder to start distillation. When the distillation was completed, i.e. when about

80ml of distillate has been collected to boric acid, the flask was removed and the digestion process for another samples continued.

Titration was then performed using 0.1N H_2SO_4 until the color of the distillate turns from green to pink at the end and the utilized sulfuric acid for titration was recorded volumetrically. Finally, the total nitrogen contents of the samples were determined by using the following formula after correcting for the blank.

$$\%N = \frac{(a-b)}{s} X\ N\ X 0.014 X\ 100 X\ mcf$$

Where
a=ml of H_2SO_4 required for titration of sample
b=ml of H_2SO_4 required for titration of blank
s= air dried sample weight in grams
N=Normality of H_2SO_4 (0.1N)
0.014=meq weight of nitrogen in g
mcf= moisture correction factor

4.11 DATA ANALYSIS

Phenotypic variability was analyzed using a computer cluster analysis applying UPGMA method (NT-SYS version 2.1) software. One-way analysis of variance of data was also undertaken using the (SPSS 11.5 version) statistical program. Mean separation was calculated using the Turkey's values when the F-test was significant at P=0.05.

RESULTS

5.1 SOIL CHEMICAL ANALYSIS

The soil chemical characters are shown in table 3. All the three soil types contained high N contents (Desta Beyene and Angaw Tsigie, 1989) and phosphorus except Holetta soil, which is very deficient in P content according to Ngeborg (1986). All the three soil types have high cation exchange capacity and low electric conductivity (Giller and Wilson, 1990). The two soils used for evaluation of symbiotic effectiveness of vetch isolates have slightly acidic pH (Dembia, 6.8) and very acidic pH (Holetta, 4.8), respectively.

Table 3 Soil Chemical Characteristics

Parameter	Wegera	Dembia	Holetta
pH H_2O 1:2.5	7.2	6.8	4.8
EC, ds/m^{2} [1]	0.060	0.183	0.059
Na, Meq/100gm soil [2]	0.200	0.410	2.60
K, Meq/100gm soil [3]	0.370	2.990	1.81
Ca, Meq/100gm soil [4]	27.940	21.860	6.24
Mg, Meq/100gm soil [5]	9.140	3.540	8.72
CEC, Meq/100 [6]	50.60	46.60	30.0
T.N. % [7]	0.129	0.249	0.166
O.C. % [8]	1.556	2.713	1.549
Av.P. Ol (ppm) [9]	21.44	69.70	6.44

1. Electric conductivity of the soil
2. Total sodium content of the soil
3. Total potassium of the soil
4. Total calcium of the soil
5. Total magnesium of the soil
6. Cation exchange capacity of the soil
7. Total nitrogen content of the soil in percent
8. Organic carbon content of the soil in percent
9. Available phosphorus

5.2 PATTERN OF NODULATION

Among the six tested forage legumes *Medicago sativa* (alfalfa*)*, *Vigna unguiculata* (cowpea), *Lablab purpureus* (lablab) and *Vicia dasycarpa* (vetch) induced nodulation on either or both of Dembia and Wegera soils (Table 4). Nodulation of ***M. sativa*** and ***V. dasycarpa*** occurred on both Dembia and Wegera soils whereas, nodulation on ***L. purpureus*** and ***V. unguiculata*** species was found only on Dembia soil. Two species of *Desmodium uncinatum* (silverleaf) and *Trifolium repens* (white clover) failed to form nodules on their respective host

Table 4 Pattern of Nodulation of Tested Forage Legumes

No	Host Plant species	Dembia		Wegera	
		Nod	NN	Nod	NN
1	*Medicago sativa*	+	10	+	19
2	*Vigna unguiculata*	+	10	-	-
3	*Desmodium uncinatum*	-	-	-	-
4	*Lablab purpureus*	+	10	-	-
5	*Trifolium repens*	-	-	-	-
6	*Vicia dasycarpa*	+	10	+	7

(+)= Nodulated, (-) = Non nodulated, Nod= nodulation, NN= nodule number

5.3 CULTURAL AND MORPHOLOGICAL CHARACTERISTICS OF ISOLATES

The colony characteristics showed that most isolates from ***M. sativa*** and two isolates from ***V. dasycarpa*** displayed large mucoid (LM) colonies, whereas AUMR7 (***M. sativa***) and AUVR14 (***V. dasycarpa***) showed large watery (LW) colony types. Isolates from ***V. unguiculata*** and ***L. purpureus*** showed small and dry (SD) colony types (Table 5). All strains did not absorb Congo red from YEMA media except AUMR1 and AUMR12 (***M. sativa***), AULB15, AULB17 (***L. purpureus***) and AUCB19 (***V. unguiculata***) that appeared to be bright red as a result of taking Congo red. Almost all isolates of alfalfa, vetch and one isolate from lablab (AULB16) changed the color of BTB-YEMA from green to yellow. However, AUMR3 (***M. sativa***), and AULB15 and

AULB17 (*L. purpureus*) and AUCB18 and AUCB19 (*V. unguiculata*) changed the medium to blue (Table 5). No isolate grew on peptone glucose agar media (PGA) containing Bromocresol purple indicator.

Table 5 Morphological and Growth characteristics of isolates

Strain	Type of Colony	GT (h)	Colony size (mm)	Presumptive test		
				YMA-CR	YMA-BTB	PGA-BCP
AUMR1	LM	2.1	2	Red	Yellow	-
AUMR2	LM	2.6	4	Colorless	Yellow	-
AUMR3	LM	2.2	1	Colorless	Blue	-
AUMR4	LM	2.5	2	Colorless	Yellow	-
AUMR5	LM	1.8	2	Colorless	Yellow	-
AUMR6	LM	3.2	3	Colorless	Yellow	-
AUMR7	LW	2.9	4	Colorless	Yellow	-
AUMR8	LM	3.5	3	Colorless	Yellow	-
AUMR9	LM	3.5	4	Colorless	Yellow	-
AUMR10	LM	2.9	2	Colorless	Yellow	-
AUMR12	LM	2.1	3	Red	Yellow	-
AUVR11	LM	2.4	5	Colorless	Yellow	-
AUVR13	LM	1.9	6	Colorless	Yellow	-
AUVR14	LW	2.1	5	Colorless	Yellow	-
AULB15	SD	7.9	<1	Red	Blue	-
AULB16	SD	8.2	<1	Colorless	Yellow	-
AULB17	SD	13.9	<1	Red	Blue	-
AUCB18	SD	5.8	<1	Colorless	Blue	-
AUCB19	SD	1.9	<1	Red	Blue	-

(-) = No growth, GT=Generation time, LM=Large mucoid, LW=Large watery, SD=Small dry, h=hours

All isolates from alfalfa and vetch displayed fast growth with colony diameter of about 2-6mm, and generation times of 1.8-3.5hrs, except AUMR3 that attained a colony size of 1mm with little exoploysaccharide production. The other isolates from lablab and cowpea attained colony size diameters <1mm and generation times 5.8-13.9hrs except, AUCB19 (Cowpea) that displayed faster growth with doubling time of 1.9h (Table 5).

5.4 PHYSIOLOGICAL CHARACTERISTICS Of ISOLATES

5.4.1 pH Tolerance

All isolates grew on YEMA media between pH 5 and pH 9 except, AUMR12 (***M. sativa***) that failed to grow on both pH values (Table 6). None of the isolates could show any growth at pH 4.

5.4.2 Salt Tolerance

All isolates grew at 1-4% NaCl except AUVR11, AUVR13 (vetch) and AUMR12 (alfalfa) that failed to grow on YEMA containing 4% NaCl (Table6). Many isolates (eight) from alfalfa, except AUMR4, AUMR9, and AUMR12; and three isolates from lablab and cowpea, AULB17, AUCB18 and AUCB19 were found to be resistant to 5% NaCl. No vetch isolate was found to grow at this concentration.

5.4.3 Temperature Tolerance

With regard to temperature, four alfalfa isolates (AUMR5, AUMR6, AUMR7 and AUMR8) managed to grow at 5^{o}C whereas all isolates, except AUVR11, AUVR14 (vetch isolates) and AULB15 (lablab) showed good growth at 10^{o}C. However, seven isolates of alfalfa (AUMR1, AUMR3, AUMR4, AUMR5, AUMR8, AUMR9 and AUMR10), all vetch and four lablab and cowpea (AULB15, AULB17, AUCB18 and AUCB19) isolates showed good growth on YEMA medium at 40 ^{o}C, A slight growth was also displayed by AUMR4 (***M. sativa***) and AULB16 (***Lablab purpureus***) at an incubation temperature of 45 ^{o}C (Table 6).

Table 6 Tolerance of isolates to pH, salinity and temperatures

Parameters/	pH			NaCl				**Temperature**			
Strains	4	5	9	5%	4%	2%	1%	5 °C	10 °C	40 °C	45 °C
AUMR1	-	+	+	+	+	+	+	-	+	+	-
AUMR2	-	+	+	+	+	+	+	-	+	-	-
AUMR3	-	+	+	+	+	+	+	-	+	+	-
AUMR4	-	+	+	-	+	+	+	-	+	+	±
AUMR5	-	+	+	+	+	+	+	+	+	+	-
AUMR6	-	+	+	+	+	+	+	+	+	-	-
AUMR7	-	+	+	+	+	+	+	+	+	-	-
AUMR8	-	+	+	+	+	+	+	+	+	+	-
AUMR9	-	+	+	-	+	+	+	-	+	+	-
AUMR10	-	+	+	+	+	+	+	-	+	+	-
AUMR12	-	-	-	-	-	+	+	-	+	-	-
AUVR11	-	+	+	-	-	+	+	-	-	+	-
AUVR13	-	+	+	-	-	+	+	-	+	+	-
AUVR14	-	+	+	-	+	+	+	-	-	+	
AULB15	-	+	+	-	+	+	+	-	-	+	-
AULB16	-	+	+	-	+	+	+	-	+	-	±
AULB17	-	+	+	+	+	+	+	-	+	+	-
AUCB18	-	+	+	+	+	+	+	-	+	+	-
AUCB19	-	+	+	+	+	+	+	-	+	+	-

(+) =Growth, (-) = No growth (±) = slight growth

5.4.4 Carbon Utilization

Nearly all rhizobial isolates utilized almost all carbon sources except isolate AUMR10 that showed no growth response to arabinose, galactose and inositol and isolate AUVR11 that did not show any growth on gluconate and tartarate. No strain utilized the carbohydrate metabolism intermediate, citrate.

Table 7 Carbohydrate Utilization of the Four forage Isolates

Carbohydrates	Strains																		
	AUMR											AUVR			AULB		AUCB		
	1	2	3	4	5	6	7	8	9	10	12	11	13	14	15	16	17	18	19
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gluconate	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Tartarate	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Citrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhaminose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adonitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inositol	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(+)=Growth and (-)=No growth

5.4.5 Intrinsic Antibiotic Resistance (IAR)

Isolates exposed to seven types of antibiotics with 14 different concentrations in µg/ml showed wide variations in tolerance (Table 8). No isolate from all legumes could show any growth on Rif (5), but almost all isolates grew on Amp (3) and Amp (5). Generally, tolerant strains were isolated from alfalfa represented by the most tolerant isolates of AUMR1, AUMR2, and AUMR12, that resisted many of the tested antibiotics; whereas the most sensitive isolates were AULB15 and AULB17, (lablab) and AULB18 and AULB 19 (cowpea), followed by vetch isolates. However, isolate AULB16 (lablab) was found to be as resistant to many concentrations of antibiotics as several of the isolates from alfalfa strains (Table 8).

Table 8 Intrinsic Antibiotic Resistance (IAR)

Isolates/ Antibiotics (µg/ml)		Strains																		
		AUMR											AUVR			AULB(CB)				
		1	2	3	4	5	6	7	8	9	10	12	11	13	14	15	16	17	18	19
Rif	3	+	+	-	+	-	+	+	+	+	-	+	-	+	-	+	+	-	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ery	5	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-	-	-	-
	10	+	+	-	-	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-
Kan	3	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-
	5	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-
Str	3	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	5	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Nal	5	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-	-
	10	+	+	+	+	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-
Chl	5	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	-	-
	10	+	+	+	+	-	+	+	+	+	-	+	+	-	+	-	+	-	-	-
Amp	3	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+
	10	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+

Rif= Rifampicin, Ery=Erythromycin, Kan=Kanamicin, Str=Streptomycin, Nal= Nalidixic acid, Chl= Chloramphinicol, Amp= Ampiciline

5.4.6 Numerical Analysis

The use of Euclidean distance in taxonomy has resulted in clustering the nineteen isolates based on their physiological characteristics. At 68% of similarity, the phenotypic characteristics of these strains were clustered into 4 groups. Group 1 was the largest group formed by 81% of the isolates of alfalfa (AUMR1, AUMR2, AUMR3, AUMR4, AUMR6, AUMR7, AUMR8, AUMR9 and AUMR10) and 33% of vetch (AUVR14). Group 2 was the second that contained 80% of lablab and cowpea (AULB15, AULB17, AUCB18 and AUCB19) and 9% of alfalfa (AUMR5) isolates. Group 3 contained AUMR12 and AULB16; and group 4 separated the two isolates of vetch (AUVR11 and AUVR13) (fig9). When a high Euclidean distance of about 82% was established as a base, the nineteen strains of the four forage plants formed 12 distinct groups. The majority of isolates from alfalfa (73%) formed the first 4 groups, but the remaining 27% separated into three clusters formed by AUMR5, AUMR10 and AUMR12 respectively. And 80% of the isolates from lablab and cowpea were clustered in one group. Isolates of vetch were differentiated between three separated clusters. In general, the homogeneity of all isolates was rather clearly discriminated at 68% than 82% at of similarity.

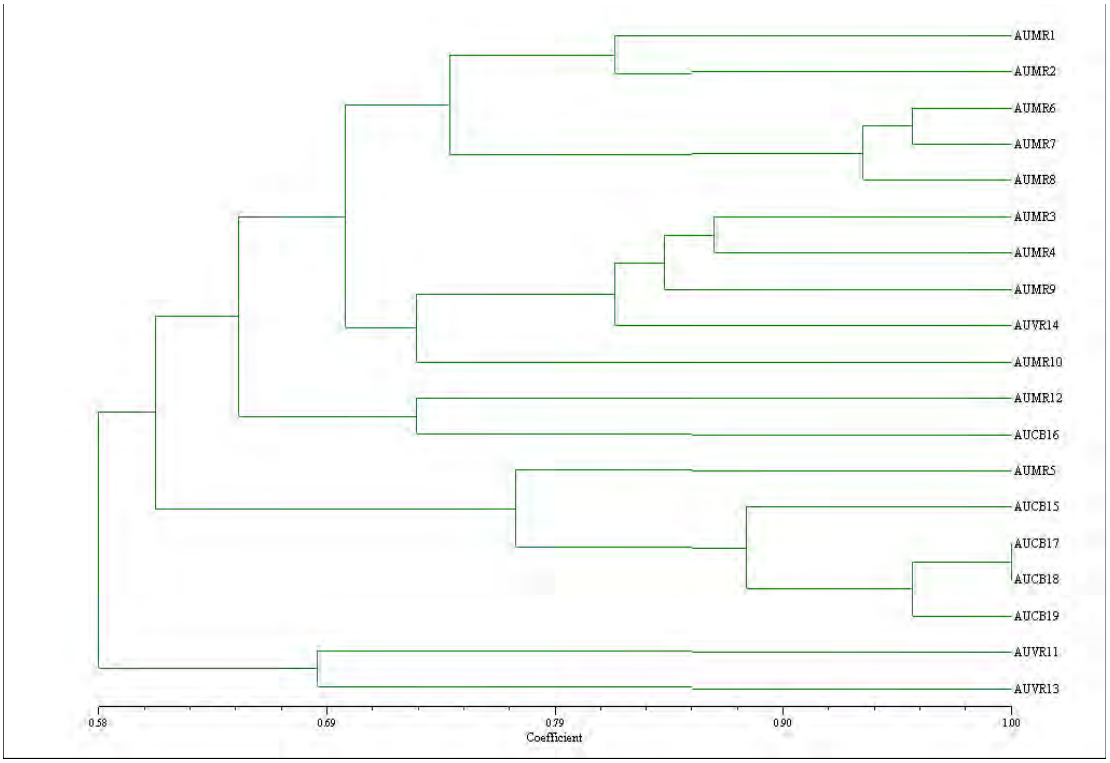

Figure 6 Dendogram, the two genera showing their tolerance to different stresses and utilization of carbohydrates

Generally, the physiological examination with respect to high salt percentage, tolerance to extreme temperatures, to high and low pH and to high concentrations of various antibiotics clearly revealed that there is diversity among the four plant isolates (Fig 6, Table 6, 7 and 8).

5.5 AUTHENTICATION OF ROOT NODULE BACTERIA OF THE FORAGE LEGUMES

Nineteen strains of rhizobial bacteria isolated from the nodules of the four forage legumes were reinoculated into their respective hosts for authentication (Table 9).

Table 9 Study sites and isolates of the nodulating forage legumes

No	Origin of Isolates	Host plants	Isolates	Authenticity
1	Wegera	*Medicago sativa*	AUMR1	-
	Wegera	*Medicago sativa*	AUMR2	-
	Wegera	*Medicago sativa*	AUMR4	-
	Wegera	*Medicago sativa*	AUMR5	-
	Wegera	*Medicago sativa*	AUMR12	-
2	Wegera	*Medicago sativa*	AUMR3	+
	Dembia	*Medicago sativa*	AUMR9	+
	Wegera	*Medicago sativa*	AUMR10	+
3	Dembia	*Medicago sativa*	AUMR6	-
	Dembia	*Medicago sativa*	AUMR7	-
	Dembia	*Medicago sativa*	AUMR8	-
4	Wegera	*V. dasycarpa*	AUVR11	+
	Wegera	*V. dasycarpa*	AUVR13	+
5	Dembia	*V. dasycarpa*	AUVR14	+
6	Dembia	*L purpureus*	AULB15	-
	Dembia	*L purpureus*	AULB16	-
	Dembia	*L. purpureus*	AULB17	-
7	Dembia	*V. unguiculata*	AUCB18	-
	Dembia	*V. unguiculata*	AUCB19	-

(+)= Authenticated, (-) = Not authenticated

A few of *Medicago sativa* and *Vicia dasycarpa* isolates were authenticated as true root nodule bacteria. The authenticated isolates from ***M. sativa*** were AUMR3 and AUMR10 (Wegera); and AUMR9 (Dembia) whereas AUVR11 and AUVR13 (Wegera) and AUVR14 (Dembia) were found to renodulate *Vicia dasycarpa* (Table 9). 73% of isolates from alfalfa and all isolates from lablab and cowpea (AULB15, AULB16, AULB17, AUCB18 and AUCB19) did not form any nodule upon authentication. Consequently, the color of leaf blades of all these plants found to be deep yellow (fig 7) with spots and scorching followed by foliage. Plant growth was poor and most of them were dead.

Figure 7 *Lablab purpureus* inoculated by AUCB16 that failed to renodulate

5.6 PRELIMINARY SCREENING FOR SYMBIOTIC EFFECTIVENESS OF VETCH AND ALFALFA

Based upon the relative shoot dry matter accumulation of inoculated plants with Nitrogen-fertilized control plants (Date, 1993 cited in Purchino, 2000), the symbiotic efficiency of isolates on V. *dasycarpa* was found to be very effective (83%) with AUVR13, and effective (50%) and ineffective (30%) with isolates AUVR11 and AUVR14, respectively (Table 10). The best isolate, AUVR13 (Wegera) gave 64 and 90 % ($P<0.05$) SDW yield advantage over the lowest strain

AUVR14 and the N free treatment, respectively (Table 10). This plant showed good growth performance with deep green shoots as compared to others (fig 8).

Similarly, from the alfalfa isolates, AUMR9 displayed a very effective rate (100%); whereas AUMR3, and AUMR 10 showed effective (64%), and (50%) symbiosis, respectively. Similar variations were observed between the three isolates with regard to NN and NDW.

Table 10 Evaluation of effectiveness of vetch and alfalfa on sand and test tube experiment

TREATMENT	SDW (mg/pl)	NN/pl	NDW (mg/pl)	% EFFECTIVENESS	RATE
AUVR11 (vetch)	209[b]	64[a]	33[a]	48	3
AUVR13 (vetch)	347[a]	69[a]	38[ab]	83	1
AUVR14 (vetch)	125[c]	37[b]	22[b]	30	4
UNI+(vetch)	421[a]	-	-	-	-
UNI- (vetch)	34[d]	-	-	-	-
AUMR3 (alfalfa)	29[b]	13[a]	4.7[a]	64	2
AUMR9(alfalfa)	46[a]	17[a]	5.6[a]	100	1
AUMR10(alfalfa)	21[c]	14[a]	5.6[a]	50	3
UNI+(alfalfa)	46[a]	-	-	-	-
UNI- (alfalfa)	6[d]	-	-	-	-

Numbers in the same column followed by different letters are significantly different at 5% level (Tukey HSD);
Letters in the columns (a, b, c) are rank of the means
1=Very effective (80-100%); 2=Effective (50-80%); 3=Lowly effective (35-50%); 4=ineffective (<35%) (Date, 1993), NN= Nodule number, NDW=Nodule dry weight**,** SDW=Shoot dry weight

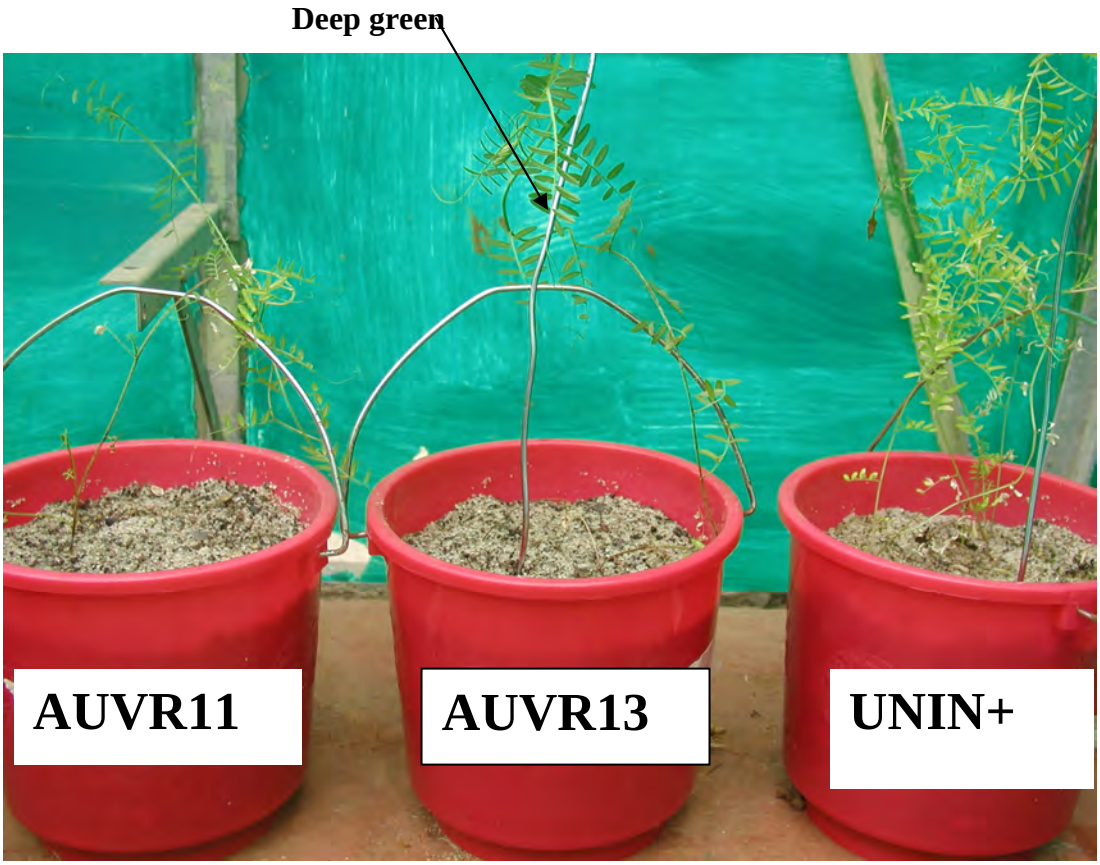

Figure 8 *Vicia dasycarpa* inoculated by AUVR11, AUVR13, and positive control

5.7 SYMBIOTIC EFFECTIVENESS OF VETCH ON SOIL CULTURE

Symbiotic effectiveness of the three isolates from vetch together with two controls (N-fertilized and uninoculated, UNI+ non-N-fertilized and uninoculated, UNI-) was tested on two different soil types with contrasting pH (Dembia 6.8 and Holetta 4.8). Although there was significant difference in nodule number and nodule dry weight of plants inoculated with AUV13 on Dembia soil (at $\alpha < 0.05$ (Table 11), there was no difference in shoot dry weight, and nitrogen content amongst the rhizobial treatments in both soils. The shoot dry matter of N-fertilized plants on Holetta soil was slightly higher than all the other treatments (Table 11).

The rhizobial treatments accumulated 74% (AUVR11), 86% (AUVR14), and 122% (AUVR13) of SDW of the N-fertilized on Dembia soil, whereas the same inoculants accumulated 42%, 42%, and 47% of SDW on Holetta soil, respectively. This means that the soil factors in Dembia soil enabled the isolates to increase this parameter 2-3 times higher than Holetta soil. There was no such difference between the N-fertilized and rhizobia-inoculated treatments with respect to total nitrogen content, although Dembia plants accumulated one and half times more nitrogen than Holetta soil.

Table 11 Evaluation of symbiotic effectiveness of vetch upon inoculation with their homologous endosymbionts on soil culture

TREATMENT	NN/pl		NDW (mg/pl)		SDW (mg/pl)		TN (%)	
	Dembia	Holetta	Dembia	Holetta	Dembia	Holetta	Dembia	Holetta
AUVR11	12±1[b]	16±4 [a]	3.7±0.5[b]	4.0±1.0 [a]	147.9±48.0[ab]	77.7±11.6[b]	2.5±0.1[a]	*1.5±0.4[a]*
AUVR13	22±6[a]	19±10 [a]	5.8±0.6[a]	9.9±6.7 [a]	244.5±62.2[a]	87.3±24.9[b]	2.7±0.4[a]	*1.6±0.3[a]*
AUVR14	12±1[b]	19±13 [a]	3.8±0.4[b]	8.1±4.9 [a]	172±23.8[a]	78.1±9.3[b]	2.5±0.9[a]	*ND*
UNI+	-	-	-	-	200.6±49.1[a]	183.8±41.5[a]	2.6±0.8[a]	*1.7±0.2[a]*
UNI-	-	-	-	-	108.7±7.8[b]	36.9±2.7[c]	2.1±0.3[a]	*1.3±0.3[a]*

Numbers in the same column followed by different letters are significantly different at 5% level (Tukey HSD)

Numbers are means and Standard deviation of three replicates (3 plants in each pot)

Letters in the columns (a, b, c) are rank of the means; ND=Not determined, NN= Nodule number, NDW=Nodule dry weight**,** SDW=Shoot dry weight, TN=Total nitrogen

6. DISCUSSION

In this study of the pattern of nodulation of six introduced (exotic) forage legumes to Ethiopia showed that only four species (*Medicago sativa, Vigna unguiculata, Lablab purpureus* and *Vicia dasycarpa*) were found to induce nodulation on Dembia and Wogera soils, in North Gondar. The two cultivated forage species; *T. repens* and *D. uncinatum* failed to nodulate indicating that their specific rhizobia were either eliminated due to shifting of grazing lands for food crop production or, if present, incompatible to the introduced cultivars. According to O'Hara *et al* (2002) it is very unlikely that all agricultural soils contain bacteria capable of effectively nodulating some legumes and absence of nodulation or ineffective symbiosis is quite common for the situation where new legumes are being introduced to new lands.

Furthermore, other factors such as specific endosymbiont-host cultivar interaction may limit *Rhizobium* legume recognition, which is the primary step of successful nodulation and nitrogen fixation (Saubert and Scheffler, 1967; Lupwayi *et al*., 1997; O'Hara *et al*., 2002). These authors indicated that clovers formed no effective associations with rhizobia of European origin and vice versa and called this relationship "reciprocal incompatibility". Consequently, inoculation of exotic forage species with compatible *Rhizobium* is found necessary; and it was recommended that *Rhizobium* inoculation is definitely needed for exotic species such as *Desmodium intortum* and *Desmodium uncinatum*. (Desta Beyene, 1984).

From the nodulating hosts, 19 isolates of root nodule bacteria were isolated and characterized. All isolates from alfalfa displayed growth rate of (1.8h-3.5h), and attained 2-4mm in colony diameter, except AUMR3 (1mm) within 3-5 days of incubation. They all showed yellow color on BTB-YEMA medium indicating the production of acid. Similarly, the growth rate of vetch isolates ranged between 2-2.4h, but larger colony diameter of about 5-6mm with copious production of exopolysaccharides (EPS), and production of acid under the same conditions. On the contrary, the growth rate of almost all isolates of lablab and cowpea ranged between 5.8-13.9h, with less than 1mm colony diameter, and changed BTB-YEMA medium to blue indicating alkali production within 7-10 days.

Therefore, isolates of alfalfa and vetch showed typical properties of fast growing ***Rhizobium*** while that of lablab and cowpea showed the characteristics of slow growing ***Bradyrhizobium*** according to the classification of the family ***Rhizobiaceae*** (Jordan, 1984). Hence, based on criterion of cross-nodulation host pattern alfalfa and vetch isolates could be categorized into ***Sinorhizobium meliloti***, ***Rhizobium leguminosarum*** var ***viceae***, respectively. Similarly, isolates from both lablab and cowpea fall into the ***Bradyrhizobium*** spp (Cowpea miscellany) (Jordan, 1984).

Although fast/slow growth rate, large/small colony, and acid/base reactions are the basis of differentiating into fast growing ***Rhizobium*** and slow-growing ***Bradyrhizobium***, isolates AUMR3 (alfalfa) (***Sinorhizobium***), and AUCB19 (cowpea) (***Bradyrhizobium***) attained smaller diameter (1mm) and displayed fast growth respectively. Such anomalies were reported by several workers (Trinick ***et al***, 1983; Dakora and Vincent, 1984; Hernandez and Focht, 1984; Odee ***et al***., 1997).

Generally, the physiological examination with respect to resistance to salt, temperatures, pH and various antibiotics clearly revealed that there is diversity among the different isolates within their respective genera. All isolates were found to grow at 1-2% NaCl. However, most isolates from ***S. meliloti***, (alfalfa) and ***Bradyrhizobium*** (Cowpea, and lablab) species were found to grow at 4-5% salt concentrations. The vetch isolates, ***Rhizobium leguminosarum*** var ***viceae*** were sensitive to these concentrations as opposed to the report of Graham and Parker (1964) and Odee ***et al***. (1997). All fast and slow growing isolates were also found to grow well on YEMA media at pH 5 and 9 except AUMR12. But no growth was observed on the same media at pH 4. Although Jordan (1984) reported that slow-growing strains appear to be more tolerant to low pH than fast-growing strains, such a difference was not observed in the present study. In the present work, the ability of isolates to grow at 5 ^{o}C was limited mainly to alfalfa isolates (***S. meliloti***); although Graham and Parker (1964) reported that ***R. leguminosarum*** and ***Sinorhizobium meliloti*** were tolerant to 4^{o}C. The majority of isolates from all groups including vetch isolates managed to grow at 40 ^{o}C despite the fact that growth temperature of most rhizobial (***Rhizobium/Sinorhizobium***) strains varied between 10^{o}C and 37^{o}C (Lindstorm and Lehtomaki, 1988; Graham, 1992).

Regarding the intrinsic antibiotics resistance, ***S. meliloti*** (alfalfa) strains tolerated many of the tested antibiotics (43%-100%) and concentrations (29%-86%) followed by ***R. leguminosarum*** var ***viceae*** (29%-71%) of the antibiotics and (21%-43%) of their concentrations. Four bradyrhizobial isolates of lablab/cowpea were found to be very sensitive in their IAR pattern as opposed to the reports of Jordan (1984) and Madrzak ***et al.*** (1995) that fast-growing strains are more sensitive than slow-growing rhizobia.

More than two third of the isolates of ***S. meliloti*** and ***R. leguminosarum*** var ***viceae*** but all strains of ***Bradyrhizobium*** species utilized all carbon sources except, citrate. This report is different from Stowers (1985) and van Rossum ***et al***. (1995) who reported that fast growing rhizobia could metabolize a wider range of carbon substrates than slow growing rhizobia. There was a slight difference between the two fast growing species, ***R. leguminosarum*** var ***viceae*** (vetch) and ***S. meliloti*** (alfalfa) with respect to carbon utilization. One of the ***R. leguminosarum*** var ***viceae*** strains, AUVR11, did not utilize gluconate and tartarate whereas AUMR10 (M. sativa) failed to catabolize arabinose, inositol and D-galactose.

Although about nineteen rhizobial strains were isolated from nodules of the four forage plants, preliminary screening for effective strains showed that only six of them (AUMR3, AUMR9, AUMR10, AUVR11, AUMR13 and AUVR14) were found to authenticate on sand and test tube experiments. According to Odee ***et al***. (1997) who isolated over 480 rhizobia from root nodules of woody legume and herbaceous trap host species in soils collected from 12 different Kenyan sites, all of them could not be authenticated on their homologous hosts, viz. ***Acacia tortilis, Acacia nubica*** and ***Acacia polyacantha***.

Furthermore, fast growing ’rhizobia-like ‘bacteria frequently occurred in cowpea nodules (Dakora and Vincent, 1984). It was experimentally found that whenever fast forms are supplied in mixed form with Bradyrhizobia, the former was found to cohabit using the entry of the sole. They also collated that non-invasive forms can genuinely be found within the nodule of this host as well as others. These bacteria may not exactly be rhizobia. Because these and several fast growing isolates of alfalfa seem to be ‘rhizobia-like’ strains similar to the report of Fassil Assefa

(1993) who found the same from species of *Erythrina*, *Milettia* and *Tephrosia*. In this study, isolates failed to renodulate their homologues hosts on authentication test.

Martins *et al*. (1997) also reported that some Bradyrhizobia formed pseudonodules with soybean. But this did not contribute significantly to the nitrogen content of plant tissue; however, signal exchange between plant and rhizobia is occurring to some degree. Genes required for nodulation can be located in rhizobia on a plasmid (Hynes and Macgregor, 1990). These plasmids can be lost spontaneously at high frequency and can undergo frequent rearrangements, resulting in a loss of symbiotic capacity. From such reports, it is tempting to assume that the rhizobia-like isolates may be either rhizobia that lost their plasmids, or some related free cohabiting bacteria that may stimulate nitrogen fixation. However, it must be experimentally substantiated before these rhizobia like isolates are identified as simple soil contaminants or genetically defective rhizobia.

Based upon the relative shoot dry matter accumulation of inoculated plants with Nitrogen-fertilized control plants (Date, 1993 cited in Purchino, 2000), the symbiotic efficiency of isolates on V. *dasycarpa* was found to be very effective (83%) with AUVR13 (Wegera isolate), and effective (50%) and ineffective (30%) with isolates AUVR11 (Wegera) and AUVR14 (Dembia), respectively (Table 10). The best isolate, AUVR13 (Wogera) gave 64 and 90 % ($P<0.05$) SDW yield advantage over the lowest strain AUVR14 (Dembia) and the N free treatment, respectively. As a result, AUVR11 and AUVR13 authenticated significantly large number of nodules than AUVR14 (Table 10).

From the authenticated root nodule bacteria from alfalfa, AUMR9 (Dembia) was found to be as effective (100%) as the N-fertilized control, where as isolate AUMR3 (Wegera) and AUMR 10 (Wegera) were effective with symbiotic efficiency of 64% and 50% respectively. This represents 25% of the population in the tested soils. Generally, the work showed that Wegera was found to contain very efficient strains of *R. leguminosarum* var *viceae* on vetch; whereas the Dembia soil contains the highly effective isolate on alfalfa despite the fact that both are known pulse (beans, field peas and lentils) growing regions. Variation in symbiotic effectiveness from population of *R. leguminosarum* var *viceae* of faba bean from Australian soils (Slattery and Pearce, 2002) and

Rhizobium meliloti (15% effectiveness*)* of alfalfa from the Middle East (Materon, 1991) was well recorded.

Although there was no interstrain significant difference on SDW on both Dembia and Holetta soils, the rhizobial treatments accumulated 74% (AUVR11), 86% (AUV14), and 122% (AUVR13) of SDW of the N-fertilized on Dembia soil, whereas the same inoculants accumulated 42%, 42%, and 47% of SDW on Holetta soil. The same pattern was found with respect to total nitrogen content in that rhizobial treatments accumulated 1.5 times more nitrogen in Dembia than the Holetta soil. This means that some soil factors enabled the isolates and the host to respond to more growth and nitrogen fixation.

Even though availability of nitrogen source in the soil was known to limit the symbiotic relationship between the symbionts (Dowling and Broughton, 1986), in the present study it is unlikely to conclude that nodulation thereby nitrogen fixation was affected by the availability of high N contents of the soil since all the three strains induced sufficient number of nodules against very high (>0.2%) and high nitrogen (0.15-0.2%) contents of Dembia and Holetta soils, respectively (Desta Beyene and Angaw Tsigie, 1989).

Vetch plants harvested from both soils contained 1-2% N that seems much lower than the maximum (1-5% N) that can be accumulated according to Carlsson (2005). However, Dembia plants accumulated higher N content (>2%) than Holetta soil (<2%) indicating that nitrogen recovery of the plants was from both inoculation and the soil.

Although the two soils did not significantly differ in their nitrogen content, Holetta soil contains 10 times less available phosphorus than Dembia soil which is very low to support plant growth, which is a characteristic of more than 70% of vertisols in Ethiopia (Birhanu Debele, 1993). Given that Holetta soil is acidic it is likely that its deficiency in P may be due to acidity-related P fixation (Tekalegn Mamo and Haque, 1987). Low nitrogen fixation and poor plant growth in vetch-related faba bean due to phosphorus deficiency have been reported in Ethiopia (Desta Beyene and Angaw Tsigie, 1989; Ayneabeba Adamu *et al,* 2001). Although there were general

reductions of shoot dry weight and nitrogen content on Holetta soil, the isolates managed to improve production compared to the uninoculated and unfertilized control plants.

Generally, in this study the plant *Rhizobium* relationship is classified into three categories. First, those rhizobial isolates that effectively fixing the host plant from which they have been isolated; second, rhizobial isolates that failed to renodulate; and third those forage plants, which did not bait the bacteria during isolation from the tested soil samples. Failure of the majority of the introduced legumes in terms of nodulation and effective nitrogen fixation in North Gondar draws special attention.

Given that several indigenous species of medics (7 species), 1 species of lablab, and about 11 species of desmodium are found in Ethiopia including Gondar, (Thulin, 1989); and are reported to be nodulating elsewhere (deFaria *et al*, 1989), compatible indigenous rhizobia may be present in the soil. It is, therefore, tempting to assume that if more screening is undertaken from the indigenous population of medics, cowpea, lablab, and desmodium from Gondar and elsewhere, it is likely that compatible infective and effective rhizobia can be obtained to fully realize the potential of Biological Nitrogen Fixation in forage production in animal husbandry.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1. CONCLUSIONS

From the present study the following conclusions could be drawn.

- *Desmodium uncinatum* and *Trifolium repens* could not get trapped by the background soils as a result cultivar differences in which the two exotic forage legumes typically did not find appropriate genotypes that form symbiotic association with them.

- *Lablab purpureus* and *Vigna unguiculata* were unable to renodulate due to either they had been nodulated by other distant-related cross-nodulating rhizobia that is common among slow growing rhizobia. This, however, has to be substantiated by yet another extensive screening works.

- The authentication and effective nodulation of almost 30% of the isolates from alfalfa indicated that infective endosymbionts for this legume are abundant in both Dembia and Wogera soils, but effectiveness is limited to a few isolates

- The isolation and authenticity results of vetch plant confirmed that abundant rhizobial strains of this species existed in the background soil. However their efficiency varied in that only 50% from Wogera were found to be highly effective. It is, therefore possible to conclude that the arable soils of Northwestern Gondar contain large and adapted population of ***R. leguminosarum*** var ***viceae*** species including the ineffective strains that equally compete for nodulation.

- The fact that large number of ineffective alfalfa (***Rhizobium meliloti)*** and vetch (***R. leguminosarum*** var ***viceae)*** isolates from Wogera and Dembia often pause a considerable barrier to effective strains affecting the exploitation of annual vetch and perennial *Medicago* species for livestock production.

- Requirement of inoculation and introduction of compatible rhizobia either from the indigenous soils or from abroad is very important for those forage legumes such as *Desmodium uncinatum* and *Trifolium repens*.

- Physiological characteristics of all authenticated strains indicated that there is great diversity among species and strains in terms of tolerance to tropical temperature, alkaline and acidic soil are important characters for inoculant's selection. Even in acid soil like Holeta they performed much better than the negative control that their tolerance to tropical soil acidity made them good candidates for inoculant production.

7.2. RECOMMEENDATIONS

Based on the above conclusions the following recommendations are forwarded.

- For better understanding of the performance of the introduced forage species, it is imperative to undertake more extensive screening of their rhizobia association where they had been introduced.

- Forage agronomists and development agents must always take the rhizobiology of forage legumes into consideration, which is one of the most neglected part of research in forage agronomy and agroforestry; not the least, whenever they plan to introduce any exotic forage species to the country.

- The respective institutions should show relentless effort to improve forage production through the incorporation of appropriate rhizobial inoculants to those imported forage legumes and try to examine their potential, adaptation and competence to indigenous rhizobia.

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APPENDICES

Appendix 1-ANOVA, effect of ***Rhizobium*** treatment on variability of shoot dry weight, Nodule number, nodule dry weight in ***V. dasycarpa*** on Sand Experiment

Parameter	Source of Variation	d.f.	Sum of square	Mean square	F-value	p-value
Shoot Dry Weight	Between treatments Within treatments Total	4 10 14	300,785.5 14495.7 315281.2	75,196.4 1449.6	51.9	<0.05
Nodule Number	Between treatments Within treatments Total	2 6 8	4184.2 2268.7 6452.889	2092.1 378.1	5.5	<0.05
Nodule Dry Weight	Between treatments Within treatments Total	2 6 8	365.3 308.2 673.5	182.6 51.4	3.6	<0.05

Appendix 2 ANOVA, effect of ***Rhizobium*** treatment on variability of Nodule number, shoot dry weight in ***M. sativa***

Parameter	Source of Variation	d.f.	Sum of square	Mean square	F-value	p-value
Shoot Dry Weight	Between treatments Within treatments Total	4 5 9	2321.0 795.8 3116.8	580.3 159.2	3.65	<0.05
Nodule Number	Between treatments Within treatments Total	2 3 5	25.3 4.0 29.3	12.7 1.3	9.8	<0.05
Nodule Dry weight	Between treatments Within treatments Total	2 3 5	21.1 4.1 25.2	10.5 1.4	7.5	<0.05

Appendix 3 ANOVA, effect of *Rhizobium* treatment on variability of Nodule number, nodule dry weight, shoot dry weight, total nitrogen in ***V. dasycarpa*** (Pot Experiment, Dembia)

Parameter	Source of Variation	d.f.	Sum of square	Mean square	F-value	p-value
Shoot Dry Weight	Between treatments	4	16254.3	4063.6	1.06	<0.05
	Within treatments	25	38377.2	3837.7		
	Total	29	54631.5			
Nodule Dry Weight	Between treatments	2	8.7	4.3	14.3	<0.05
	Within treatments	4	1.6	0.3		
	Total	6	10.3			
Nodule Number	Between treatments	2	193.6	96.8	8.9	<0.05
	Within treatments	4	65.3	10.9		
	Total	6	258.9			
Shoot total N	Between treatments	4	0.672	0.168	.136	<0.05
	Within treatments	10	12.326	1.233		
	Total	14	12.998			

Appendix 4 ANOVA, effect of *Rhizobium* treatment on variability of Nodule number, nodule dry weight, shoot dry weight, total nitrogen in *V. dasycarpa* (Pot Experiment, Holeta)

Parameter	Source of Variation	d.f.	Sum of square	Mean square	F-value	p-value
Shoot Dry Weight	Between treatments	4	26174.4	6543.6	9.9	<0.05
	Within treatments	24	6581.4	658.1		
	Total	28	32755.9			
Nodule Dry Weight	Between treatments	2	54.2	27.1	1.2	<0.05
	Within treatments	6	139.4	23.2		
	Total	8	193.6			
Nodule Number	Between treatments	2	18.0	9.0	.09	<0.05
	Within treatments	6	572.0	95.3		
	Total	8	590.0			
Shoot total N	Between treatments	3	5.7	1.4	3.3	<0.05
	Within treatments	8	4.3	0.4		
	Total	11	10.0			

Declaration

I, the under signed, declare that this thesis is my original work. It has never been submitted in any institution and that all sources of materials used for thesis have been dully acknowledged.

Name: Fekadu Shimekite

Place: Addis Ababa University

Signature_____

Date_____

This thesis has been submitted for examination with our approval as

Advisor: Fassil Assefa, Ph.D

Signature_____

Date_____

Advisor: Alemayehu Mengistu

Signature_____

Date_____