ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

SYMBIOTIC AND PHENOTYPIC CHARACTERIZATION OF COMMON BEAN (*PHASEOLUS VULGARIS*)- NODULATING RHIZOBIA FROM SOME AREAS OF SOUTHERN ETHIOPIA.

BY
ALEMAYEHU WORKALEMAHU

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Symbiotic and Phenotypic Characterization of Common Bean (*Phaseolus vulgaris*) Nodulating Rhizobia from Some Areas of Southern Ethiopia.

By

Alemayehu Workalemahu

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LIST OF SYMBOLS AND ABBREVIATIONS.

AUPR	Addis Ababa University *Phaseolus vulgaris* Rhizobia
AUER	Addis Ababa University rhizobia obtained from *Erythrina brucei*
AUAR	Addis Ababa University rhizobia obtained from *Acaciapolycantha*
AUMR	Addis Ababa University rhizobia obtained from *Milletict ferniginae*
BNF	Biological nitrogen fixation
FAO	Food and Agricultural Organization United Nation
N_2	Dinitrogen (Atmospheric nitrogen)
SNF	Symbiotic nitrogen fixation.
ha	Hectare
t	Tone
N	Nitrogen.
EARO	Ethiopian Agricultural Research Organization
M142	Mexican 142
RW	Red Woliata
YMA	Yeast Extract Mannitol Agar
W/V	Weight per volume
ppm	Parts per million
RCBD	Randomized complete block design
YMB	Yeast Extract Mannitol Broth
TY	Trypton Yeast Extract Agar Medium
PY	Peptone Yeast Extract Agar Medium
SD	Small dry
LM	Large mucoid
L\V	Large watery
YMA-BTB	Yeast Extract Mannitol Agar –0.5 % bromthymol blue medium
YMA-CR	Yeast Extract Mannitol Agar-Congo Red medium
TY-Ca	Trypton yeast extract agar lacking Ca
LB	Luria-Bertani medium
DAP	Days after planting
ca	About
IAR	Institute of Agricultural Research

Abstract

Eighteen isolates of common bean rhizobium from Konso (GI), Arbaminch (GII), Sodo (GUI), and Zeway-Awassa-Dilla (GIV) regions in Southern Ethiopia were isolated and characterized by their phenotypic diversity and symbiotic properties on Red Welaita (RW) and Mexican142 (Ml42). All isolates were infective on either of one or both *Phciseolus vulgaris* cultivars Red wolaita (RW) and Mexican 142 (Ml42) in glasshouse experiment. The cluster analysis grouped the isolates into two major diversity groups based on 80-100% similarity levels (Group I) and 39%-76%, similarity levels (Group II). Group I contained 78% of the isolates with two clusters la and lb with presumed similar characters of *Rhizobium legwninosarum/Rhizobium etli* group except AUPR10. Isolates such as AUPR8 from Group II displayed creamy colony appearance on Peptone Yeast Extract Agar medium (PY), tolerance to extreme pH, salt, and temperature and inability to utilize dulcitol and utilization of glycine that resembled with characters of *Rhizobium tropici.* Two isolates, AUPR9 (Group II) and AUPR10 (Group I), exhibited rough colony appearance on PY that resemble the characteristics of *Rhizobium gallicum.* There was significant difference in shoot dry, nodule fresh and dry weight on both cultivars ($P<0.05$). RW accumulated more shoot dry weight than M142 ($t=1.24$, $P<0.05$). There was also association between seed color of the cultivar cropped at site of bacterial isolation and isolates performance. In addition to authentication of bean isolates, three tree isolates were included for cross-inoculation to non-host *P.vulgaris* in that AUPR21 *(Erythrina brucei*) was effective on M142. Test on symbiotic properties of the five more effective isolates on RW cultivar on two soils of contrasting pH showed that all of them were equally effective in accumulating high diy matter and nitrogen on Gentameche soil. Kemogerbi soil poorly responded to inoculation by all the isolates. Soil factors such as high sum of cations and cation exchange capacity and low available phosphorus P (5.7 ppm) may have contributed to the difference of the two soils. We conclude that great variation exists among rhizobia spp. nodulating bean cultivars and selection of efficient symbiotic partners appears possible.

Key words: Common bean, Rhizobia, Southern Ethiopia, Symbiotic effectiveness.

1. INTRODUCTION

Biological nitrogen fixation (BNF) is the conversion of atmospheric nitrogen (N_2) to utilizable form of nitrogen (NII_3) by prokaiyotic microorganisms freely, associatively or in an endosymbiotic association with higher plants (Postgate, 1998). About 70 % of biologically fixed nitrogen comes from the symbiosis involving legumes and root nodulating bacteria (Peoples and Craswell, 1992). The most important nitrogen fixing legumes are cool season grain legumes and tropical grain legumes. These legumes can fix nitrogen to the tune of 50-250 Kg/ha/yr (Montanez, 2000) and improve soil fertility in agricultural systems. Thus, BNF is the main source of nitrogen in low input agricultural systems (Dobereiner *el cil.*, 1995; Dobereiner, 1997), and there are also opportunities to use it in intensive agriculture (Peterson and Russelle, 1991; Graham and Vance, 2003; Saghal and Johri, 2003).

Common bean *(Phciseohis vulgaris*) is one of the most important food crops (Broughton *et ai*, 2003). It is the cheaper source of proteins, vitamins and micronutrients and supplement to other staple foods in production areas (Graham and Vance, 2003; Broughton *et al.*, 2003). In the tropics and sub-tropics, 20% of the protein source for the population comes from common bean (Karanja and Wood, 1988). .

The crop originated from America and distributed to different parts of the world starting from the 16lh century (Gepts, 1990). It is grown widely in the tropics and sub-tropics, the most important of which are Latin America and Africa (Shellie-Dessert and Bliss, 1991). The area covered with the crop in production has been increasing due to its role in low input agricultural systems (Adams *et al.*, 1985; EARO, 2000) and as an export commodity crop (Ayel Haile, 1990; Broughton *et al.*, 2003).

It is one of the major crops in lowlands of Ethiopia, where more than 10 million people mainly depend on it (EARO, 2000). It is the major and inexpensive source of protein and cash crop to the farmers (Alelign Kefyalew, 1990; Asfaw Negasa and Abubakar Mussa, 1990; Tenaw Workayehu and Yeshi Chiche, 1990). It is also the main component of the cropping system because of its role in double cropping, ability of shade tolerance and early maturity (Shimelis W/Hawariate *et* *al.*, 1990; Tenaw Workayehu and Yeshi Chiche, 1990). In drought-prone areas, it is a risk aversion crop (Alelign Kefyalew, 1990; Shimelis W/Hawariate *et al.*, 1990).

The crop is expanding to areas with altitude between 2000-2200m *asl* where faba bean is not productive and areas consuming much amount of starchy foods like *enset* (Asfaw Negasa and Abubakar Mussa, 1990; Getachew Kasaye, 1990). In 1983/84 cropping seasons, a total of 37,146 ha of land was under bean production (Amare Abebe, 1987). This figure reached to 189,460 ha in 1996/97 that was 5-fold of the former (EARO, 2000). It has also increased its share in export from a total of 10% in 1973 to 86% of all exports in the pulses and oil seeds during 1989/1990 (Ayele Haile, 1990).

Even though the crop covers the large area, the average national yield per hectare is low (600kg/ha) comparing to other pulses such as faba bean (llOOkg/ha) and chickpea (760kg/ha). There is also a big difference between the national average and experimental yields (2500-3000kg/ha) (EARO, 2000). Though different factors may account to yield reduction, one of the most important factors is nitrogen deficiency of most Ethiopian soils (Desta Beyene and Angaw Tsigie, 1986) and its production by smallhold farmers with little accesses to external inputs (Amare Abebe, 1987; EARO, 2000).

Although the fertility problem can be partly solved through use of efficient rhizobial inoculant, the characteristics of indigenous soil rhizobial strains in different regions are not known (EARO, 2000; Desta Beyene *et al.*, 2004). This reflects the need for screening rhizobial isolates which are efficient and adaptable to different environmental conditions of the country. Use of these strains as inoculant, in turn, requires isolation, characterization and evaluation of their effectiveness in nitrogen fixation under different environmental conditions to fully realize biological nitrogen fixation.

Further more, common bean is characterized by its nodulation with a broad spectrum of endosymbionts. At least five named species of rhizobia can nodulate the crop and isolates from nodules of tree species (Martinez-Romero *et al.*, 1991; Segovia *et al.*, 1993; Hernandez-Lucas *et* *al.*, 1995; Amarger *et ah*, 1997). This characteristic of the crop can also be important in getting superior inoculant from strains of other legumes to improve the yield.

In Ethiopia, though some studies were undertaken on effectiveness of indigenous common bean-rhizobia from some sites, rigorous screening and the taxonomic relationship have not been well undertaken (EARO, 2000; Desta Beyene *et ah*, 2004). Hence, this study was initiated with the aim to isolate and characterize the phenotypic and symbiotic characteristics of rhizobia from some bean growing areas of Southern Ethiopia.

2. OBJECTIVES

2.1 General Objectives

- Symbiotic and phenotypic characterization of authenticated rhizobia from indigenous soiis.

2.2 Specific Objectives

- To isolate and authenticate bean-nodulating bacteria from the soils.
- To characterize the isolates in phenotypic characteristics.
- To evaluate symbiotic effectiveness of the isolates.
- To test cross- inoculation of three tree isolates on Ml42.

3. LITERATURE REVIEW

3.1. Nitrogen

Nitrogen is a major constituent of all proteins, nucleic acids and many metabolic intermediates involving in biological activities of all forms of life. It is the most important element that limits crop productivity since the nitrogen needs of most plants are second only to their photosynthetic requirements (Postgate, 1998; Broughton *et al*, 2003). Since most soils in tropics and subtropics are deficient in nitrogen (Hungria and Vargas, 2000), their nitrogen supply, management, and use efficiency are important for sustainable agricultural production (Graham and Vance, 2003).

3.2. Biological Nitrogen Fixation

Biological nitrogen fixation (BNF) is a fixation of atmospheric nitrogen into NH_3 by bacteria freely, associatively or in endosymbiotic association with leguminous or non-leguminous plants (Postgate, 1998). Peoples and Craswell (1992) estimated that 139-170 million tons of nitrogen is fixed through BNF annually, which is equivalent to the fertilizer nitrogen requirement worthy of US$ 7 to10 billion annually (Graham and Vance, 2003). It also needs significant amount of energy requirement for N-fertilizer synthesis and application that will contribute to environmental pollution (Graham and Vance, 2003; Crews and Peoples, 2004).

In order to feed the ever-increasing world population, there is a need to doubling crop productivity that in turn requires about 160 million tones of industrially fixed N_2, which is equivalent to annual biologically fixed N_2 (FAO, 2002). This shows how much industrially fixed nitrogen fertilizer will be required and the huge amount of energy for its production.

Uses of BNF offers an economically attractive and ecologically sound means of reducing external nitrogen input and improving the quality and quantity of internal resources (Graham and Vance, 2000). Though the role of BNF in developed countries is very limited, there are possibilities to increase its importance in food and forage production. Peterson and Russelle (1991) have calculated that the proper management of alfalfa (*Medicago saliva*) in rotation with corn in mid-western USA could reduce fertilizer nitrogen inputs by some 0.3 million kg y^{-1} without loss of production and give a net return of between $USA 50-90 million. It is also possible to use BNF in remediation of degraded lands and organic farming (Zaharan, 1999; Graham and Vance, 2000).

In terms of nitrogen fertilizer, Dobereiner *et al.* (1995) estimated that more than $ US 1.8 billion y^{-1} obtained from legume and associative nitrogen fixation in Brazil. Flood tolerant green manure species *(Sesbanici rostrota* and *Aeschynomene* species) enhanced the yield by 1.4 tones ha^{-1} as short season planting before rice (Becker *et al.*, 1995).

BNF is a viable, cost effective, alternative or complementary solution to industrially manufactured nitrogen fertilizers. Hence, expanded exploitation of BNF could reduce dependence on industrially produced fertilizer nitrogen that necessitates intensified studies on it. Since about 70% of biologically fixed N_2 comes from symbiotic nitrogen fixation (SNF) involving legumes and rhizobia (Peoples and Craswell, 1992), more attention is given for this symbiotic association to fully exploit the potential benefits of BNF technologies in agriculture and agroferestry.

3.3. Legume-Rhizobia Symbiotic Nitrogen Fixation (SNF).

Nitrogen fixation, in general, is the process by which atmospheric N_2 is converted into NH_3. The net reaction is:

$$N_2 + 8\wedge + 8e^{-} + 16ATP + 16H20 \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$$

SNF by legume-rhizobia is performed by the coordinated activities between the symbionts (Broughton *et al.*, 2003). It takes place in root nodules with a complex enzyme system called nitrogenase that reduces N_2 into reduced form, NH_3. The nodules provide enosymbiotic bacteria with microenvironment suitable for nitrogenase activity (Marschner, 1995). The enzyme consists of two components, dinitrogenase reductase (Fe-protein) and dinitrogenase (FeMo-protein) (Thourneley, 1992).

The enzyme in nodules is maintained in the strict anaerobic microenvironment by the special protein, leghaemoglobin. This protein provides sufficient oxygen required to generate sufficient respiratory energy to support the modified rhizobia (bacteroids) in nodules and protects nitrogenase from molecular oxygen (Marschner, 1995).

3.4. Legumes

Legumes are categorized into one of the largest group of flowering plants, family leguminaseae. It contains a large and diverse type of plants ranging from huge, long-lived forest trees to tiny, annual herbaceous plants (Somasegran and Hoben, 1994). There are about 18,000 described species of legumes (Thulin, 1983). They are subdivided into three subfamilies, the Cesalpinioideae, the Mimosoideae, and the Papilionoideae (Postgate, 1998).

Legumes are important components of food, forage, and agro forestry cropping systems. More than 50% of crops grown in, for example, Africa, India and Latin America are either intercropped or rotated with nitrogen fixing plants (Broughton et al., 2003). Legumes are also important in human and animal diets. They provide 25-35% of the worldwide protein intake (Graham and Vance, 2000; Broughton *et* «/.2003). Grain legumes are rich in protein especially amino acids, complementary to that of cereals and hence play a major role in animal and human diets. It is also rich in vitamins and microelements and has energy content close to that of cereals (National Academy of Science, 1979; Messina, 1999).

Legumes such as dry beans and soybeans are important source of oil and unique phytochemicals that seem to promote health (Anderson *et al.*, 1999). Besides, their medicinal and food values, legumes are also used in synthesis of dyes, flavors and other products (National Academy of Science, 1979; Graham and Vance, 2003).

Although there are 600 species of legumes recorded in Ethiopia (Thulin, 1983), a few groups such as peas, lentil, chickpea, common bean, faba bean and cowpea are largely grown as grain legumes for human consumption. Most of the works on nitrogen fixation have been conducted on faba bean followed by chickpea (Tekalign Mamo and Asgelil Debabe, 1994). Regarding tree legumes, only a hand Rill of studies was conducted (Fassil Assefa, 1993; Million Yohannes, 2002; Shasho Megersa, 2002; Yonas Yohannes, 2005). Generally work on BNF of legumes in Ethiopia has been limited to cool season legumes such as faba bean, field pea and lentils and a few tree species such as *Acacia* and *Erythrina* species. Although almost more than half of Ethiopian land mass covers the lowlands (Alemayehu Mengistu, 2003), there is little information on tropical leguminous crops such as common bean, soybean *(Giycin max)* and Groundnut *(Arachis hypogaea)* that are widely grown in these areas.

3.4.1. Common bean

Common bean is an important food crop originated in America and exported to different parts of the world starting the 16th century (Gepts, 1990). Afier its dissemination, it is grown extensively in five major continental areas: Africa, North and Central America, South America, Eastern Asia, and Western and Southern-eastern Europe (Adams *et al.*, 1985).

From a total production of 23 million metric tones of common bean, 7 million metric tones are produced in Latin America and Africa (Broughton *et al.*, 2003). Latin America was the leading bean producer, with approximately 30% of the world's production of diy bean in the 1980s whereas Africa produces approximately 10% of world dry bean production (Shellie-Dessert and Bliss, 1991). Brazil and Mexico produce 78% of the dry bean in the region followed by Argentina, Chile, Guatemala and Colombia in 1980s (Shellie- Dessert and Bliss, 1991).

In dietary use, common bean is the first standing crop among legumes conveys up to half of grain legumes consumed worldwide (Broughton *et al.*, 2003). It is the cheaper source of proteins and supplements other staple foods in production areas (Graham and Vance, 2003). It is also rich in carbohydrates, vitamins and minerals, especially high in iron and zinc.

Bean production in Latin America, for example, has increased by 3% per year over the decades (Broughton *et al.*, 2003). This suggests per capita consumption has also increased. The estimated per capita consumption in Latin America during 1980s was 13.3 kg/year. During the same period, the mean per capita consumption of dry bean in Africa was higher about 31.4kg (Sheilie-Dessert and bliss, 1991). This amount has reached to 24 kg per year in Americas and 40-60kg per year in Africans (Broughton *etal.,* 2003).

Common bean is grown as an intercropped or involve in crop rotation (Woolley *et al.,* 1991). This is due to its various growth habits, early maturity, possibility of double cropping and the role in soil fertility due to its symbiotic association with rhizobia (Broughton *et al*, 2003). Since common bean is widely cultivated, their N-fixing ability can play an important role in improving soil fertility in low input agricultural systems.

Despite its importance worldwide, the average yield of the crop is very low when compared with the experimental average yield (Broughton *et al.,* 2003). Although the potential average yield could reach 6000 to 8000kg ha^{-1} depending on the varieties (Sheilie-Dessert and Bliss, 1991), the average national yield is below this amount almost in all common bean producing countries (Karanja and Wood, 1988). Since common bean is widely grown in tropics and sub- tropics, the main reason for yield reduction is believed to be poor soil fertility particularly nitrogen since they are produced by resource poor fanners (Amijee and Giller, 1998; Hungria and Vargas. 2000). Under such conditions, the ability of the crop to fix atmospheric nitrogen is likely to be an important and cheaper option especially if high nitrogen fixing combination of rhizobia and bean genotypes could be identified.

While common bean has often been regarded as weak in nitrogen fixation which is about 50kg N ha^{-1} (Montanze, 2000). Surprisingly, large rates of nitrogen can be obtained under appropriate conditions that showed maximum rates of nitrogen fixation equivalent to 64-121kg N ha^{-1} per growth cycle (Adames *et al.,* 1985). Nipe-Nolt and Pineda (1988) also indicated that some strains of rhizobia have produced bean yields as high as those obtained from application of 180kg N ha^{-1} was applied (2.2t ha^{-1}inoculated and 2.4t ha^{-1}N fertilized). Results from some cultivars have also indicated high rate of nitrogen fixation to the tune of 165kg N ha^{-1} (Table1) (Montanez, 2000).

Table1: Nitrogen fixing potential of cultivars and breeding lines of common beans.

Country	Common Bean tested	Total N fixed (Kg ha^{-1})	Selected Cultivars
Australia (Seibersdorf)	29	25-165	Riz 44,Bat322
Brazil (Goiania)	17	4-12	Honuras35 Corioca
Chile	7	10-50	Red Mexican INIA Don Timotco
Colombia (CIAT)	9	20-35	A268
Guatemala	10	92-125	ICTA San Martin ICTA Panamos ICTA Qucnackchc
Mexico	18	0-70	Azufrado Negro Colima Negro Poblano
Peru Summer Winter	20	12-59	Summer Cabalero,Caraota,Blanc Winter Bayo Normal, Canari OG-62-2-6, Bavo-G759

Adopted from Montanez, 2000.

Considering its importance in area coverage, diet, economy and broad range of environments and cropping systems, it can be one of a good candidate grain legume for BNF technologies by resolving its poor fixing ability. This requires studies on screening bacteria from their well-adapted indigenous habitats, factors affecting the symbiotic characteristics and selection of bean genotypes with high N-fixation (Hungria and Vargas, 2000; Rengel, 2002).

The environmental factors that affect N-fixation are temperature, moisture, acidity and several chemical components of the soil such as nitrogen, phosphorus, calcium and molybdenum content and the interaction of those factors (Zaharan, 1999; Hungaria and Vargas, 2000; Rengel, 2002).

The factors should be considered during selection and use of the bean genotypes and rhizobial strains for better nitrogen fixation.

It is also possible to develop bean cultivars that obtain most of their nitrogen from fixation without sacrificing actual and potential yield levels. There is substantial genotype variability in nitrogen fixation in common beans (Table 1). The high value for N- fixation were observed on adapted cultivars and breeding lines (Hardarson *et al*, 1993; Jebara *et al.*, 2001). These can be used either directly as cultivars or production of inbreeding programme to enhance nitrogen fixation.

Selection of rhizobia that can tolerate different factors and compete with indigenous rhizobia is also crucial (Lupwaye and Mkandowire, 1996; Rengel, 2002). To enhance nitrogen fixation, rhizobia with high nitrogen fixation and competitive ability in the soils could be screened from different environmental conditions (Anyango *et al.*, 1995; Mhamdi *et al.*, 1999; Montanez, 2000; Bouhmouch *et al.*, 2001).

Strains with high tolerance to soil acidity and high temperature were obtained through screening (Anyango *et al.*, 1995; Pinto *et al.*, 1998). Osmotolerant strains with interesting potential of effectiveness were identified from Moroccan soils (Bouhmouch *et al.*, 2001). Similarly, in Tunisia, indigenous rhizobia with higher performance than CIAT 899, recommended as inoculant to Africa, were obtained (Mhamdi *et al.*, 1999). Hungria *et al.* (2000) also found temperature and soil acidity tolerant strains with good result in field experiments. These results are indicative of benefits which could be achieved by rigorous screening and characterization of large number of isolates from natural population and thus for selecting competitive and adapted inoculant strains.

3.4.1.1. Common bean in Ethiopia

Common bean was introduced to Ethiopia in the 16th C. It is well distributed in most part of Ethiopia. It is a major crop that about 10 million people mainly depend for food (EARO, 2000). Central Ethiopia is the highest bean producer followed by Sidamo, Harrarghe and Wollega in dry bean production (Amare Abebe, 1987). The suitable areas for bean production are outlined in Table 2.

Table 2: Suitable areas for common beans production.

Altitude	Rainfall	Humidity	Temperature
●1400-2000m *asl* for fed conditions ●≥700 m *asl* for irrigation	●Well-distributed rain fall off 350-550 mm for over 70-90 days depending on altitude.	●Not above 75%	●Mean maximum ‹30-32^{0}c ●Mean minimum ›10-12^{0}c

Adapted from Amare Abebe, 1987.

Common bean is the major and inexpensive source of protein and cash crop to farmers (Aleligne Kefyalew, 1990; Asfaw Negasa and Abubakar Mussa, 1990; Tenaw Workayehu and Yeshi Chiche, 1990). The 22% protein content and amino acid composition (high in lysine) complement cereals and other staple foods in the diet (Asfaw Negasa and Abubakar Mussa, 1990; Getachew Kassaye, 1990). Early maturity and double cropping make common bean the first food in the growing seasons. Its high iron content can also play an important role in the diet of Ethiopian farmers (Broughton *et al.*, 2003). The fresh leaves, straw and pods are also used as high protein supplement to grass fodders (EARO, 2000). Common bean currently is extended to different regions where starchy foods are consumed and faba bean failed to be productive to supplement protein to farmers' diet (Asfaw Negasa and Abubakar Mussa, 1990; Getachew Kassaye, 1990).

In addition to its role in the diet, in some regions it is the first or the second important cash crop (Asfaw Negasa and Abubakar Mussa, 1990). It is mainly produced for export in the rift valley whereas in Harrarghe and Southern regions grown for both food and cash (Habtamu Assefa *et al.*, 2003). A climbing type of bean with large seeds is preferred in Wollega regions where people consume it as green beans (Asfaw Negasa and Abubakar Mussa, 1990).

Common bean is also the main component of cropping systems in the regions (Tenaw and Yeshi, 1990; Habtamu Assefa *et al.*, 2003). Double cropping with lowland pulses in eastern and southern zones enable the farmers to produce two- three crops in a year (Shimelis W/Hawariate *et al.* 1990; Tenaw Workayehu and Yeshi Chiche, 1990). Besides, its shade tolerance and early maturity make the crop to be intercropped with sorghum, maize, chat and coffee (Broughton *et al.*, 2003). One study showed that upto 85 % of all sorghum in the eastern highlands is in intercropped systems with bean (Shimelis W/Hawariate *et ah*, 1990). In drought-prone areas, it is risk aversion crop due to its early maturity and drought resistance (Aleligne Kefyalew, 1990; Shimelis W/Hawariate *et al*, 1990).

Its wide range of growth habits has enabled the crop to fit diverse situations (Woolley *et al.*, 1991). Prostrate bush types achieve rapid growth cover; compete well with weeds and save labor for other farm operations (EARO, 2000). Traditionally, small farmers grow bean on poor soils or lands that have been depleted of essential nutrients after long years of planting with cereals. It also intercropped or used in crop rotation due to the belief that bean can renew the poor soils (Amare Abebe, 1987; Mitiku Haile, 1990). Scientifically, it is due to symbiotic association with root nodulating bacteria. Hence, this ability of the crops has significant impact in enhancing soil fertility that increases the yield of the companion and subsequent crops in the low input agricultural systems of Ethiopia.

Due to its importance of the crop in low input cropping systems, the area covered with common bean has been increasing (Table3). In the 1983/84 cropping seasons a total of 37,146 ha land was under bean production (Amare Abebe, 1987). Recently, it covered 189,460 ha in 1996/97 that was 5-fold of the former (EARO, 2000).

Table 3: Area, production and productivity of common bean in the 1994/95-1997/98 cropping season.

Year	Season	Area (ha)	Production (ton)	Yield (kg/ha)
1994/95	*Meher*	68,750	36,363	529
1995/96	*Meher*	101,170	78,361	775
	Belg	54,360	30,743	566
1996/97	*Meher*	112,810	947,640	839
	Belg	76,650	448,720	585

Adopted from EARO, 2000.

Ethiopia has also been earning significant amount of money from export of common bean (Tabled). However, almost all export production comes from small farms, mostly from Rift Valley (Habtamu Assefa *et al*, 2003). In 1973, 10% of Ethiopia's total earnings came from common bean. This figure reached to about 86% of all exports in the pulses and oil seeds by 1989/90 (Ayele Haile, 1990). In 1997/98 Ethiopia earned about $US 16,128,665 (EARO, 2000). This shows the place and importance of common bean export in the country's national economic development.

Table 4: Total amount of dry bean and snap bean exported to the international markets and value obtained during 1992/93 to 1997/98.

Year	Amount (ton)	Value ($US)
White pea beans		
1992/93	1843	737200
1994/95	26670	-
1995/96	30274	12715248
1996/97	32054	13207607
1997/98	32755	13265613
Snap beans		
1996/97	2286.1	1657444
1997/98	3096.0	2863052

Adopted from EARO, 2000.

Despite its increased need for domestic use and export, the yield is found to be low when compared to that of other legume crops. There is also a big difference between national average (600kg/ha) and experimental yields in Ethiopia (2500-3000) (EARO, 2000). Even though, different factors attributed to yield reduction, nitrogen deficiency is the most important of Ethiopian soils (Desta Beyene and Angaw Tsigie, 1986).

Poor nitrogen fixation coupled with unavailability of nitrogen fertilizer by resource poor farmers growing common bean may further aggravate its low productivity. Further more, characteristic of the indigenous soil rhizobial strains, which are one of a cheaper possible solution to increase productivity, in different agro ecological zones is not known (EARO, 2000; Desta Beyene *et al*., 2004).

Although some studies showed the presence of indigenous rhizobia nodulating common bean, no rigorous screening and characterization of the isolates were undertaken (Desta Beyene *et al*., 2004). Increasing the rate of inoculum per seed was shown by Amare Abebe (1982) to improve nitrogen fixation. Mitiku Haile (1990) also showed the need for inoculation with rhizobia to improve the yield. The work by Mitiku Haile (1994) clearly showed all tested varieties responded to inoculation. One study showed that the importance of *Rhizobium* inoculant and use of starter nitrogen to improve the yield (Shibru Daba and Mitiku Haile, 2000). Hence, these results are indicative of the need of highly effective strains adaptable to local environments and tolerant to adverse soil conditions to improve productivity.

The large area coverage and importance of the crop in this country emphasize the need to study and understand the genetic resource of bacteria that nodulate bean and attain maximum utilization of the crop by taking advantage of biological nitrogen fixation. This necessitates the screening of indigenous rhizobial population.

3.5. Root Nodulating Bacteria

Rhizobium is generally a name given for symbiotic bacteria capable of infecting and inducing N_2- fixing nodules on leguminous plants. All rhizobia are root nodulating bacterium and are gram negative, aerobic without end spores, normally rod shaped cell and motile (Jordan, 1984).

Classification of these bacteria formerly based on their growth rate generally into fast growing and slow growing groups .The slow growing root nodulate bacteria categorized into *Bradyrhizobium* and fast growing as *Rhizobium* (Jordan, 1984). Another approach was by grouping these bacteria into different cross inoculation groups based on the host they can nodulate (Postgate, 1998). Recently, due to the invention of molecular techniques along with phenotypic characteristics, the taxonomy of root nodulating bacteria has been revised and several new species are named. There are about 36 species of rhizobia that are divided in to seven genera from a-proteobacteria namely *Allorhizobium, Azorhizobium, Bradyrhizobium, Rhizobium, Mesorhizobium, Sinorhizobium* and *Methylobacterium* (Sahgal and John, 2003). Additionally *Burkholderia caribemis, Rolstonia (ahvanensis* and *Burkholderia phymatum* from p-proteobacteria were also identified (Chen *et al.*, 2003).

3.5.1 Rhizobia Nodulating *Phaseolus vulgaris*

The genetic diversity of rhizobia strains that can nodulate *P.vulgaris* has been extensively studied for the last two decades. These rhizobia were initially assigned to *Rhizobium leguminosarum* bv. phaseoli on the basis of their host specificity, separate from *R. leguminosarum* bv. viciae and *R. leguminosciriim* bv. trifoli, symbiont of peas (*Pisum spp.,Viciae* spp.) and clover (*Trifolium* spp.), respectively (Jordan, 1984). *R. leguminosarum* bv. phaseoli was recognized to be taxonomically heterogeneous as evidenced by multilocus enzyme electrophoresis (Martinez-Romero *et a!.*, 1991;Eardly *et a!.*, 1995) and the 16S rRNA genes sequencing (Segovia *et ah*, 1993; Hernandez-Lucas *et al.*, 1995).

R. leguminosarum bv. phaseoli from Mexico and South America was first divided into *R. leguminosarum* bv. phaseoli type I and type II. *R. tropici* types A and B were first proposed for type II strains carrying a single *nifH* gene copy. *R. tropici* types A and B are distinguished by their DNA-DNA hybridization values, a number of phenotypic characteristics such as their growth on LB and PY-Ca and the presence of a specific megaplasimid (Martinez-Romero *et al.*, 1991). *Rhizobium etli* was then proposed for *R. leguminosarum* bv. phaseoli type I strains which contain multiple copies of the nitrogenase reductase gene (*nifH*) on their symbiotic plasmids (Segovia *et al.*, 1993).

More recently, two additional groups of *Rhizobium* strains nodulating bean plants were characterized in Europian soils and proposed as two new species, *Rhizobium gallicum* and *Rhizobium gicirdinii* (Amarger *et al.*, 1997). The crop can nodulate with indigenous rhizobia where no history of inoculation. Some studies were showed indigenous rhizobia from some African countries could nodulate the crop (Anyango *et al.*, 1995; Mhamdi *et ah*, 1999; Bouhmouch *et ah*, 2001). Similar results were also observed in Ethiopian soils (Amare Abebe, 1982; Mitiku Haile, 1990; Desta Beyene *et al.*, 2004).

Phaseolus vulgaris, due to its promiscuity, can be nodulated by bacteria that nodulate other legumes such as alfalfa *(R. meliloti)*, soybean *(R. fredii)*(Hernandez-Lucas *et ah*, 1995). Bacteria that nodulate, for example, *Leucaena* spp, *Clitoria ternatea*, *Dalea leporinci* and *Acacia* spp are able to nodulate *P. vulgaris* (Hernandez-Lucas *et al.*, 1995). This ability of the crop has a practical implication to increase the rhizobial reservoir for effective inoculant selection.

4. MATERIALS AND METHODS

4.1. Soil Sampling Sites

Soil samples were collected from some common bean producing areas of southern Ethiopia indicated in Table 5 and the major reference regions indicated on figure 1.

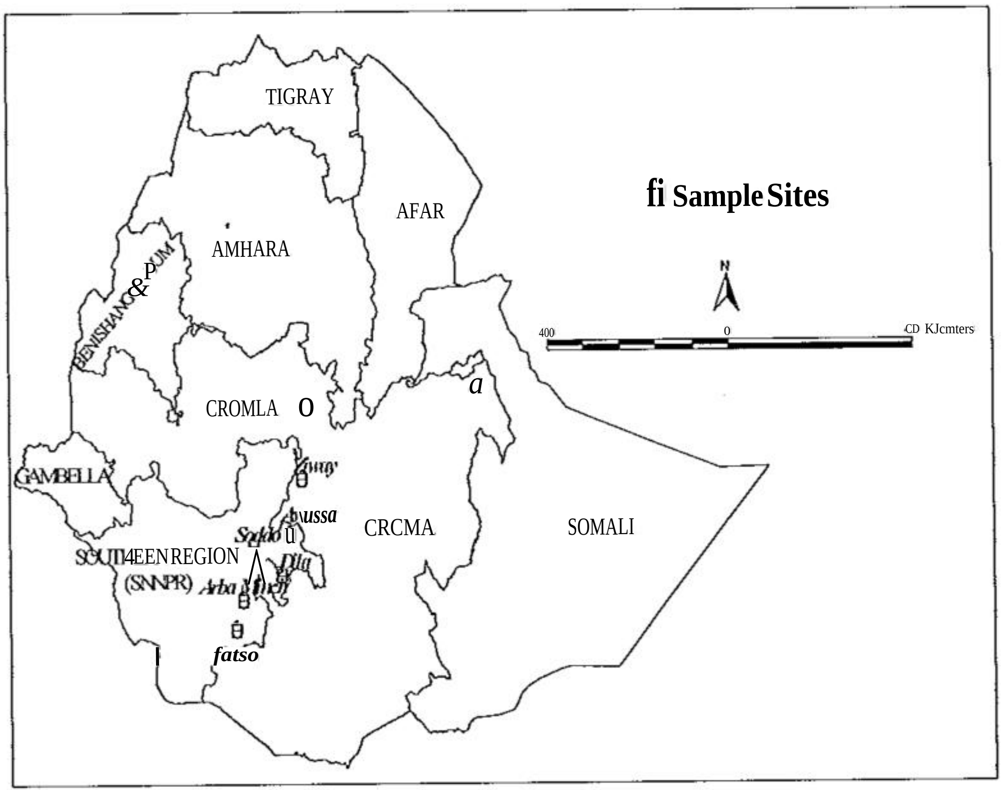


Figure 1. Location Map of Sampling Sites

4.2. Soil Sampling

Soil samples were collected from farmers' field in which common bean has been grown with no history of inoculation with rhizobia. Samples were randomly taken using sterilized polyethylene bags from a depth of 0-30 cm from the surface of several spots of each sampling site. They were sub-sampled and transported to Addis Ababa University Applied Microbiology Laboratory for nodulation test and pot experiments. Soil chemical analysis was selectively undertaken in National Soil Laboratory for Kemogerbi and Getameche sites for symbiotic effectiveness traits. The chemical characteristic of the soils is tabulated (Table 9).

4.3. Seed Samples

The seeds of cultivars, Mexican 142 (Ml42) with white seeds and Red Wolaita (RW) with red seeds of *Phaseolus vulgaris* were provided by Melkasa Agricultural Research Center.

4.4. Nodule Induction

Induction of nodules was made using plant trap method according to Vincent (1970). Plastic pots with 3 kg capacity were surface sterilized with 95% ethanol and filled with each of the homogenized and sieved soil samples using 2 mm mesh size. Seeds of *P. vulgaris* were briefly surface sterilized with 95% ethanol and 0.1% acidified mercuric chloride (5ml cone. MCI with 1L distilled water) lor 4 min. They were repeatedly washed with sterilized distilled water and allowed to germinate on sterile water agar plates (7.5g of agar in 1L water) for 2 days. Four pre-germinated seeds were transplanted to each pot, which were, then, thinned down to 3 after a week. The pots were watered at full capacity every three days for 45 days.

4.5. Rhizobium Isolation

Forty-five days after planting (DAP), the plants were gently uprooted from the pots and several times immersed in a container containing water to remove soil particles. Nodules were collected from roots and surface sterilized as before and transferred into sterilized Petri dishes to be crushed with flamed glass rod. The extract from nodules was streaked on to Yeast Extract Mannitol Agar-Congo red (YMA-CR) plates with pH adjusted to 6.8 by using IN HC1 or IN NaOH and incubated at 28^{0}C ± 2^{0}C. YMA-CR contains (Vincent, 1970):

	[gL^{-1}]
Mannitol	10
K_2HPO_4	0.5
MgS04.7H20	0.2
NaCl	0.1
Yeast extract	0.1
Congo red	0.025
Agar	15

After 5 days, single colonies were taken and re-streaked on YMA plates for purity. The pure isolates were then preserved at 4^{0}C on YMA slants containing 0.3 % (W/V) CaCCE (Vincent, 1970).

4.6. Authentication and Effectivity of the Isolates

Each isolate was authenticated as root nodulating bacteria by re-inoculating them on to the host. Three-kilogram capacity pots were filled with acid washed and heat sterilized (121^{0}C, 15Ib/in) river sand. As a starter, 20 ppm of nitrogen was given for all pots before planting (Gibson, 1980). Four surface sterilized and pre-germinated seeds as before were transferred into the pots. Each seedling was inoculated with 1 ml of each isolate with an inoculum size of 109cells/ml (Somasegaran and Hoben, 1994).

After a week, the seedlings were reduced into three per pot. As controls two treatments, one without inoculation and nitrogen addition (TN0), and un-inoculated but with 0.05% (W/V) KNO_3 per week (TN+). This experiment was made in triplicates and the plants were grown under glasshouse condition with mean minimum temperature of 18±3^{0}C and mean maximum temperature of 29±2^{0}C. The pots were arranged in Randomized Complete Block Design (RCBD) and fertilized with quarter strength Broughton and Dilworth N-free medium (Somasegaran and Hoben, 1994).

Broughton and Dilworth Medium

	Stock solution	g/L	V (nil) of stock solution for 10L of medium
A	CaCl2.2H20	294.1	5
B	KH_2PO_4	136.1	5
C	Fe-citrate MgSo4.7H20 K_2S04 MnS04.H20	6.7 123.3 87.0 0.338	5
D	H_2BO_3 ZnS04.7H20 C«S04.5H20 COS04.7H20 NaMo02.H20	0.247 0.288 0.1 0.056 0.048	5

Taken from Lupwayi and Haque, 1994

Forty-five DAP, the plants were uprooted and data on nodule fresh and dry weight, and shoot dry weight of the plants were taken after drying at 70^{0}C for 2 days in oven (Lupwayi and Haque, 1994). The isolates with relatively good performance with regard to shoot dry matter accumulation in RW were selected to evaluate the symbiotic effectiveness for the following experiment

4.7. Methods of Statistical Analysis

The results were compared with the negative and positive controls by analysis of variance and correlation coefficient to see the correlation between the shoot dry matter with nodule fresh and dry weight. t-test was also used to compare the mean shoot dry matter between the cultivars. Statistical analysis was done using SPSS vrl3.

4.7. Designation of the Isolates

All isolates have same letter of designation but they differ in number that represent place of isolation (Table 5). AUPR refers Addis Ababa University *Phaseohis vulgaris* rhizobia.

4.8. Characterization of the Isolates

All isolates were characterized by their morphological, biochemical and physiological features. All inoculations were standardized by growing the isolates in shaker at 125 rev /min at room temperature with an inoculum size of approximately 104cells/10pl unless other wise specified.

All tests were carried out in triplicates on Tryptone Yeast Extract medium (TY) except tests for morphological, acid-alkaline production, and growth on 2% (W/V) urea, which were made on YMA medium. In addition, Peptone Yeast Extract Agar medium (PY) was used for further morphological characteristics of bean isolates.

TY medium (g/ L)		**PY mediuin(g/L)**	
Tryptone	5g	Peptone	5g
Yeast extract	3g	Yeast extract	3g
$CaCl_2$.H20	0.87g	$CaCl_2$	lg
Agar	15g	Agar	15g

PH adjusted to 6.8-7.2

Each plate was spot inoculated eight times with 10pl(104 cells) of the isolate (Amarger *et al.*, 1997). Except cultures used to determine minimum and maximum growth temperature, all inoculated plates were incubated at 28± 2^{0}C. Monitoring was made after 5 days. Result for growth tests was determined qualitatively presented as '+'= growth and '-' = no growth.

4.8.1. Morphological characteristics

Loop full of test isolates was inoculated by streak plating into YMA and PY medium and incubated at 28±2^{0}C. They were checked after 5 days. Colony diameter and morphology were recorded as SD (small dry), LM (large mucoid) or LW (large watery) according to Ahmed *et al.* (1984). Their growth on PY also determined and the colonies were characterized as gummy, creamy or rough colony appearance (Martinez-Romero *et al.*, 1991; Silva *et al.*, 2003).

4.8.2. Acid and base Production

Acid or alkaline production of the isolates was detected on YMA containing 0.5% Bromthymol blue (YMA-BTB). The isolates were grown in 10ml of YMB to approximately 10 ^{6}cells/ml and loop lull of suspension was streaked on to YMA-BTB. The color change (yellow) was observed after three days for fast growing (Jordan, 1984).

4.8.3. Growth rate determination

Each isolate was streaked on YMA plates and single colony was transferred into test tubes containing 10ml Yeast Extract mannitol broth (YMB) which were then incubated on a rotary shaker (125 rev/min) at room temperature for 48 hours. One ml of cell suspension (ca 10 ^{6}cells) were transferred into 250ml Erlenmeyer flasks containing 100ml of YMB and incubated on rotary shaker (125 rev /min) at 28^{0}C. Turbidity was measured every 6 hours at 540 mn. The experiment was conducted in triplicates. Mean generation times were then determined from logarithmic growth according to Martinez-Romero *et al.* (1991).

4.8.4. Biochemical and physiological characteristics

4.8.4.1. Salt tolerance

Salt tolerance of the isolates was assessed by growing them on TY agar medium containing 0.5,1, 1.5 and 2% (W/V) NaCl. Growth was evaluated qualitatively after 5 days (Amarger *et al.*, 1997).

4.8.4.2. Temperature tolerance

Growth of isolates was detected at 4,10,30,35,40 and 45°C on YMA medium after 5 days (Jordan, 1984). Their growth also detected at 37 and 40°C on TY agar medium (Hungria *et al.*, 2000).

4.8.4.3. pll tolerance

Tolerance of isolates to pH was tested on TY medium adjusted to pH of 4, 4.5, 5, 8.5 and 9(Amarger *et al.*, 1997).

4.8.4.4. Growth on TY lacking Calcium (TY-Ca)

This was determined on TY medium by excluding Calcium (Amarger *et al.*, 1997).

4.8.4.5. Melanin Production

Test for melanin production of isolates was made on TY medium containing tyrosine at 1.2 mg/ml, and $CUSO_4$ at 40pg/ml as described by Rodrigue- z Navorro *et al.*, 1996 cited in Hungria *et al.*, (2000). Production of melanin was detected by the formation of black pigment.

4.8.4.6. Growth on 2%Urea

The ability of isolates to grow on YMA medium containing 2% (W/V) urea was undertaken according to Andrade *et al.* (2002).

4.8.4.7. Growth on Luria -Bertani (LB) medium

Isolates were inoculated on LB medium to check growth as described by Amarger *et al.* (1997). The constituents of LB medium g/L:

Tryptone	10
Yeast extract	5
NaCl	5
Agar	15g

The pH of the medium was adjusted to 7.4

4.8.4.8. Carbohydrate utilization

The different carbon sources were added as described by Amarger *et al.* (1997) at a final concentration of 1g/L to a basal medium containing (g/ L): K_2HPO_4, 1; KH_2P04, 1; FeCf $6H_2O$, 0.01; MgS04.7H20, 0.2; $CaCl_2$, 0.1; $(NH_4)_2$ S04, 1; and 15g of agar. The following filter sterilized (0.22pm millipore) sole carbon sources were added after autoclaving: L- arabinose, D-fructose, D-galactose, D-glucose, lactose, maltose, D- mannose, raffinose, L- rhamnose, D-sorbitol, xylose, dulcitol, inositol, citrate, tartarate, cellobiose, glycerol, anoditol and gluconate.

4.8.4.9. Nitrogen Source utilization

Filter sterilized L- tryptophan, L- tyrosine and glycine were used as a sole nitrogen source for isolates by adding a final concentration of 0.5g L^{-1} to the above basal medium from which ammonium sulfate had been omitted and to which mannitol had been added at a concentration of IgL^{-1} (Amargere*tal.*, 1997).

4.8.4.10. Phosphate solubilizing ability

This was determined by inoculating the isolates on Pikovskaya Agar medium (PA) containing (g/L): Glucose (10), Tricalcium phosphate (5), Ammonium sulphate (0.5), Yeast extract (0.5), Magnesium sulphate heptahydrate (0.1), Sodium chloride (0.2), Manganese sulphate (0.002), Ferrous sulphate (0.002) and Agar (15). The pH of the medium was adjusted to 7.00. This ability was detected checked based on growth and the presence of clear zone around the colonies(Nautiyal, 1999).

4.9. Antibiotic resistance

It was determined on TY medium containing the following filter sterilized (0.22 pm millipore) antibiotics (pg/ml): Naldixic acid (40 and 60), Streptomycin (3 and 10), Kanamycin (10 and 15), Erythromycin (5 and 10), Chloramphenicol (5 and 10), Rifampicin (5 and 10), Ampicillin (5 and 10)(Amarger *et al.*, 1997). Kanamycin and Ampicillin were dissolved in water; Streptomycin, Erythromycin and Chloramphenicol were dissolved in ethanol, Rifampicin in methanol and Naldixic acid in 1M NaOH.

4.10. Numerical Analysis

Traits were coded ‘2’ for growth and ‘1Tor no growth. Sixty phenotypic traits of the 18 isolates were used. A computer cluster analysis of the 60 phenotypic traits was carried out using similarity coefficient and a dendrogram was constructed by the unweighted pair group method with average (UPGMA) clustering method using NTSYS-pc version 2.1.

4.11. Cross-inoculation test

Three tree isolates from *Milletia ferruginae* (AUMR1) and *Erythrina brucei* (AUER21) isolated from nodules collected from Arat-kilo campus, and *Acacia polycantha* (AUAR31) provided by Applied Microbiology Laboratory were used for this test. Approximately 109 cells/ml of each isolate was used to inoculate non-host *Phaseolus vulgaris* (M142). Methods of isolation, incubation, inoculation and glasshouse conditions were similar as used before.

4.12. Symbiotic effectiveness of selected isolates on pot trial

For this experiment, soil samples collected from farmers' field in Gentameche (near Arbaniich) and Kemogerbi (near Ziway) were used. Their major chemical characteristics are shown in Table 6. The selected effective inoculants for this study were AUPR1, AUPR2, AUPR5, AUPR7 and AUPR13. Thoroughly mixed and sieved with 2 mm mesh was filled in surface sterilized 3-kilogram capacity pots. To optimize nitrogen fixation, the two soils were fertilized with the following nutrients mg pot^{-1} according to Somasegran and Hoben (1994): 661.25 KH_2PO_4, 505.25 K_2SO_4, 66.825 $MgS04.7H20$, 61.875 $ZnS042H20$, and 2.4375$(NH_4)_6$ $Mo7024.H20$. Four surface sterilized and pre-germinated RW cultivates seeds were transferred into the pots, which were thinned down to three after seven days. Each seed was inoculated with 1 ml of YMB grown (109 cells) test isolates. The experiment was conducted in triplicates in glasshouse mean minimum and mean maximum temperatures of 19±2°C and 30±1°C, respectively. Two controls, without inoculation and N-addition and uninoculated with 0.05% (W/V) KNO_3 week^{-1}.The pots were arranged in RCBD.

45 DAP, the plants were uprooted and their effectiveness was evaluated on the basis of nodule fresh and dry weight, and shoot dry weights after drying at 70°C for 2 days in oven. The results were compared with the controls.

4.12.1. Total nitrogen determination

Kjeldhal method was used as National Soil Laboratory Manual procedure to determine plant total nitrogen(Sahlemedhin Sertsu and Taye Bekele, 2000). Dried and finely grounded 0.3g of the plant sample was transferred into a digestion tube (Digestion system 20 1015 Digester). Two point five ml of the digestion mixture (Sulfuric acid (1L cone.)- Selenium (3.5g) and Salicylic acid (7.2g)) was added to it. The mixture was carefully swirled and allowed to stand for 2 hours. The tubes were then heated to about 100^0C for 2 hours in heating blocks. The tubes were removed from the heating blocks and cooled down to room temperature and three 1ml 30% (V/V) H_2O_2 solution was successively added and mixed thoroughly. The tubes were placed again in preheated block and heated to 300^0C until the digest turned to colorless or light yellow.

The tubes were removed from the blocks and cooled down to room temperature. Forty-eight point three (48.3) ml of distilled water was added to the digest and mixed thoroughly, and allowed to stand overnight. The digest was then mixed and filtered on a flask. Seventy-five ml 40% NaOH was then poured to each tube containing the digest and mixed gently, and distillated (Kjeltec system 1002 Distilling Unit). The distillate was received in 20 ml of boric acid containing two drops of the indicator solution until about 80 ml of the distillate had been collected. The distillate was then titrated with 0.1N H_2SO_4 until the receiver flask solution changes from green to a pink endpoint. A blank tube was also titrated similarly as the tubes with samples. Finally, the percentage of total nitrogen was calculated as:

$$\% N = (a-b)/S \times NX0.014 \times 100$$

Where,
a= ml of H_2SO_4 required for titration of sample
b= ml of H_2SO_4 required for titration of blank
S= sample weight in grams
N= Normality of H_2SO_4 (0.IN)
0.014= meq weight of nitrogen in g.

5. RESULT

5.1. Authentication of isolates

All isolates obtained from different geographical locations, ranged from low lands (1210m *asl)* to a highland (2200m *asl)* and soil with contrasting pH (5.5-8.7) (Table5), induced nodule formation on either RW or M142 or both cultivars of *Phaseolus vulgaris* (Table 8).

Table 5: Soil sampling sites of authenticated isolates.

No	Name of the site	Altitude (m asl)	Soil pH	Code	Seed color of the cultivar	Group	Major Reference Site
1	Gaho	1840	6.6	AUPR2	Red	GI	Konso Area
2	Mechela	1720	6.7	AUPR5	If	GI	u
3	Tishalc	1210	6.9	AUPR6	If	GI	‘‘
4	Gato	1295	8.7	AUPR7	II	GI	u
5	Gentanieche	2200	5.5	AUPR1	II	GII	Arba Minch area
6	Pura	1230	6.7	AUPR8	ND	GII	*a*
7	Marka	1310	7.0	AUPR9	White	GII	*tc*
8	Ambokesha	1780	5.8	AUPR10	White	GII	*‘s*
9	Selam Ber	1380	6.5	AUPR3	Red	GUI	Sodo Area
10	Gogara	1300	6.6	AUPR11	White	GUI	u
11	Wachigo Esho	1730	5.6	AUPR12	//	GUI	it
12	Shoya	1860	5.7	AUPR13	Red	GUI	it
13	Gedeba	1800	6.8	AUPR14	II	Gill	ct
14	Awergama (Siraro)	1700	6.4	AUPR15	White	Gin	tt
15	Kemo Gerbi	1630	7.9	AUPR4	II	GIV	Zevvay-Awassa-Dilla Area
16	Leku	1850	6.8	AUPR16	Red	GIV	ct
17	Tugaweransa	1760	6.7	AUPR17	Red	GIV	u
18	Adello	1900	6.1	AUPR18	White	GUI	(t

ND=Not determined.

5.2. Characteristics of the Isolates

5.2.1. Morphological and Cultural characteristics

All isolates displayed large mucoid (LM) colonies with 2-4 mm, except AUPR 8 characterized by large watery (LW) appearance on YMA medium (Table6). AUPR18 was found to exhibit small dry (SD) colonies with the maximum of 1mm diameter after 5 days of growth on the same medium. All except AUPR9 and AUPR10, and AUPR8 showed smooth and gummy whereas the latter displayed rough and creamy colonies grown on PY medium, respectively.

5.2.2. Growth on YMA-BTB and mean generation time (MGT).

All isolates turned the YMA-BTB medium into yellow after 3 days of incubation. Isolates also displayed different growth rate with fastest doubling time of 1.2 hr by AUPR8 and the majority between 2-3 hr, except AUPR18 exhibiting doubling time of 4hr. Based on the results, all isolates were fast growing rhizobia.

Table 6: Morphological and cultural characteristics of Isolates.

Characteristics	ΛD CL C4 1	ΛD CM S	ΛD O■ Qi m	ΛD O S	ΛU CM Pi 5	ΛO CM Oi VO	ΛUPR7	ΛD CU Pi 00	ΛU PM Pi Os	ΛD Cu Pi 1O	ΛD CU Pi 11	ΛD Cu 2 N	ΛU Cu Pi r"i	ΛD Cu Pi 14	ΛD CM Pi 1 w	ΛD OH 2 VO	ΛD PM 2 t■	ΛU PM 2 oo
Morphological characteristics																		
*> Colony size (mm)	2.4	3.5	2.5	2.3	2.5	2	2	4	2.3	2.4	2	2	2	3	2.6	2.2	2.2	1
❖ Colony morphology	LM	LM	LM	LM	LM	LM	LM	LW	LM	LM	LM	LM	LM	LM	LM	LM	LM	SD
Growth rate (hr)	2	1.5	2	2.08	1.94	2.14	3.81	1.22	1.90	2.0	3.0	2.74	3.0	2.33	2.0	2.70	2.50	4.0

Abb: LM =large mucoid; LW = large watery; SD = small dry;

5.2.3. Physiological and Biochemical Characteristics

Biochemical and physiological characteristics of isolates was depicted in Table 7. Isolates that grew well on all tested NaCl concentrations were AUPR8 and AUPR9. AUPR18 grew on all except at 2% (w/v) NaCl. Almost all isolates were found to grow at pH 5.0-8.5, with the exception that of AUPR12 that failed to grow at high pH (8.5). Although all isolates but AUPR8 did not grow at pH 4.0, 2/3 of the isolates were found to grow at pH 4.5. All except AUPR12 were found to grow at 37 ^{0}C but failed to grow at 40^{0}C except AUPR8 on TY medium. Although all grew at 15 and 35^{0}C, all but AUPR8 did not grow at 10 and 40^{0}C on YMA medium. This indicates a narrow range of tolerance at higher temperature on TY medium.

All isolates did not grow on LB, TY-Ca and 2% urea except AUPR8 and AUPR9.This indicates the two isolates have distinct features. Melanin production was common feature of most isolates, except AUPR3, AUPR8 and AUPR9 whereas phosphate solubilization did not exist in all isolates. All isolates grew on almost all carbohydrates, except AUPR4 and AUPR8, which failed to grow on fructose and dulcitol, respectively. Although L- tryptophan and L-tyrosine were used as nitrogen source for almost all isolates, glycine was not utilized by all isolates except AUPR8. Thus, AUPR8 had unique characters.

5.2.4. Antibiotics resistance

The inhibitory concentration of similar antibiotics was evident in Erythromycin and Rifampicin in that only 22% of isolates were inhibited at Erythromycin (5) than 78% of which at Erythromycin (10) (Table 7). Similarly, about 40% of isolates were inhibited by Rifampicin (5) whereas about 90% were inhibited by Rifampicin (10). Such a difference was not clearly seen between Kanamycin (10) and Kanamycin (15). Low concentration of Streptomycin (3) did not also show significant difference of inhibition compared to that of Streptomycin (10) as the former antibiotics.

AUPR12, the most sensitive to five antibiotics followed by AUPR8 and AUPR13 inhibited by 4 and 3 antibiotics with at least one of the tested concentrations. AUPR7 was found to resist every concentration of all tested antibiotics followed by AUPR3 inhibited by only Rifampicin (10). AUPR9 and AUPR16 were inhibited by Erythromycin (10) and Streptomycin (10), and Erythromycin (10) and Rifampicin (10), respectively. Most isolates were found to be inhibited by Rifampicin (10) followed by Erythromycin (10).

Ampicillin, Chloramphenicol and Naldixic acid were not found to inhibit almost all isolates with exception of AUPR12, and AUPR8. Almost half of the isolates were inhibited by Kanamycin (10, 15), Streptomycin (10) and Rifampicin (5). This mean that these antibiotics could differentiate isolates into two large groups.

Table 7: Physiological and biochemical characteristics of the isolates.

Characteristics	AUPR 1	AUPR 2	AUPR 3	AUPR 4	AUPR 5	AUPR 6	AUPR 7	AUPR 8	AUPR 9	AUPR 10	AUPR 11	AUPR 12	AUPR 13	AUPR 14	AUPR 15	AUPR 16	AUPR 17	AUPR 18
Salt tolerance																		
❖ 0.5% (W/v) NaCl	4	4	+	+	4	4	4	4	4	4	4	4	4	4	4	4	4	4
❖ 1% (W/v) NaCl	-	-	-	-	-	-	-	4	4	-	-	-	-	-	-	-	-	4
❖ 1.5% (w/v) NaCl	-	-	-	-	-	-	-	4	4	-	-	-	-	-	-	-	-	4
❖ 2% (W/v) NaCl	-	-	-	-	-	-	-	4	4	-	-	-	-	-	-	-	-	-
Temperature tolerance																		
TY	4	4	+	4	4	4	4	4	4	4	4	-	4	4	4	4	4	4
❖ 37°C	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-
❖ 40°C																		
YMA	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-
❖ io°C	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-
* 40°C																		
PH tolerance																		
❖ 4	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-
❖ 4.5	4	4	-	-	4	4	4	4	-	-	4	-	4	4	4	4	-	4
❖ 5	+	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
❖ 8.5	4	+	4	4	4	4	4	4	4	4	4	-	4	4	4	4	4	4
❖ 9	-	-	-	-	-	-	-	4	4	4	-	-	-	-	-	-	-	-
Antibiotics Resistance(mg/ml)																		
Naldixic acid																		
❖ 40	4	4	4	4	4	4	4	-	4	4	4	4	4	4	4	4	4	4
❖ 60	4	4	4	4	4	4	4	-	4	4	4	4	4	4	4	4	4	4
Streptomycin																		
❖ 3	4	4	4	4	4	4	4	4	4	4	-	4	4	4	4	4	4	4
❖ 10	-	4	4	4	4	+	4	-	-	-	-	-	4	4	4	4	-	-

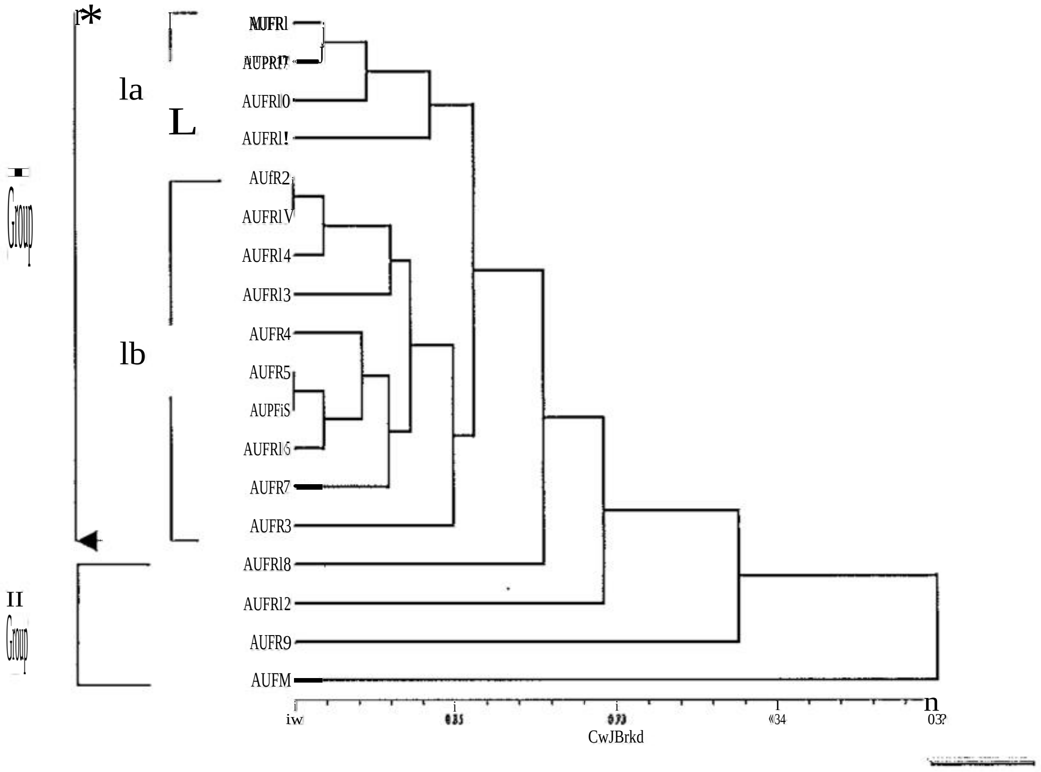
Characteristics	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Kanamycin																		
❖ 10	+	-	+	+	+	+	+	-	+	+	+	-	-	-	-	+	+	+
❖ 15	+	-	+	+	+	+	+	-	+	+	+	-	-	-	-	+	+	+
Erythromycin																		
❖ 5	-	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	-	+
❖ 10	-	-	+	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-
Rifampcin																		
❖ 5	-	+	+	-	-	-	+	+	+	-	+	+	-	+	+	+	-	+
❖ 10	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
Chloramphenicol																		
❖ 5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
❖ 10	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Ampicillin																		
❖ 5	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
❖ 10	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Characteristics	1 AUPR	2 AUPR	3 AUPR	4 AUPR	5 AUPR	6 AUPR	7 AUPR	8 AUPR	9 AUPR	10 AUPR	11 AUPR	12 AUPR	13 AUPR	4 AUPR	15 AUPR	16 AUPR	17 AUPR	18 AUPR
Growth on Dulcitol	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Nitorgen utilization																		
Glycine	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
L- tryptophan	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Tyrosine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth on LB	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Growth on TY-Ca	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Phosphate solubilizing ability	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth on 2% urea	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Melanin production	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+

5.3. Numerical analysis

The result of cluster analysis performed on the 18 isolates for 60 phenotypic traits is shown in Fig. 2. The result placed the isolates into two major phena at 39% similarity level. Phenon I contains only AUPR8 that displayed unique characteristics on most traits and Phenon II contains all the rest isolates. In phenon II, there are 4 major sub-phena in which they were separated at 57, 70 and 76% level similarity. The first 3 sub-phena of phenon II, each contains a single isolates AUPR9, AUPR12, and AUPR18, respectively.

The fourth sub-phenon contains the rest 14(78%) isolates. This sub-phenon formed two main clusters at 83% similarity level. Cluster I contains most isolates of the sub-phenon with many sub-clustering. In this cluster, 100% similarity between AUPR5 and AUPR6, and AUPR2 and AUPR15 was observed from different sub-clusters. Generally, even though most of the isolates are similar at 83 % level, this analysis showed the diversity of strains among the isolates.



Levels of similarity

Fig.2. Dendrogram highlighting the phenotypic similarities among the isolates.

5.4. Relative effectiveness of isolates

Sand culture study of nodulating cultivars showed variation in mean shoot dry matter, nodule fresh and dry weight among the treatments at p<0.05. As summarized in Table 8, in RW cultivar five isolates did not show significant difference with TN+ in mean shoot dry matter accumulation at p=0.05 by Tukey's test. AUPR 2 accumulated the highest dry matter (2.16 g/plant which was 72.73 % of TN+) followed by AUPR1 (2.01 g/plant which was 67.68% of TN+). The least performance by AUPR9 (0.28g/plant) and AUPR12 (0.31 g /plant). In this cultivar, there was also correlation between nodule fresh(i=0.745) and dry weighty=0.732), and shoot dry weight.

In M142, four isolates were not significantly different with TN+ in mean shoot dry matter at p=0.05 by Tukey's test (Table 8). AUPR15 was the most effective isolate in mean shoot dry matter (ca 2.06 g/plant which was about 71% of TN+) followed by AUPR11 (1.85 g/plant which was 63.67 % of TN+). The least effective isolate was AUPR3 (0.25 g/plant) and AUPR7 (0.37 g/plant). Correlation was also observed between nodule fresh (r=0.614) and dry weight(r=0.837), and shoot dry weight.

The effectiveness of isolates was associated with the seed color of the cultivars cropped in the place where they were isolated (Table 5). Almost no isolate performed well on both cultivars. The RW cultivar performed well and was stronger with good stand and dry matter accumulation than M142 in sand experiment (t=1.24, p< 0.05).

Table 8: Symbiotic characteristics of the cultivars and isolates on sand experiment (g /plant).

Red Wolaita					Mexican 142			
Teatment	Number of nodules	Nodule fresh weight	Nodule dry weight	Shoot dry weight	Nodule number	Nodule fresh weight	Nodule dry weight	Shoot dry weight
TN+	ND	ND	ND	2.97±0.50a	ND	ND	ND	2.91±0.64a
AUPR1	154±7a	1.22±0.06ab	0.29±0.01ab	2.01±0.27abc	ND	ND	ND	ND
AUPR2	128±4abc	0.89±0.01b	0.2±0.03bcd	2.16±0.20ab	35±7d	0.27±0.04fg	0.06±0.01de	0.46±0.19cd
AUPR3	80±14cde	0.98±0.13b	0.21±0.03bcd	1.19±0.60bcde	17±7d	0.11±0.02g	0.04±0.03e	0.25±0.07d
AUPR5	92±11cde	1.24±0.33ab	0.25±0.04ab	1.60±0.10abcde	ND	ND	ND	ND
AUPR6	95±21cd	0.94±0.1b	0.22±0.02bc	0.64±0.50cde	ND	ND	ND	ND
AUPR7	149±5ab	1.67±0.09a	0.31±0.04ab	1.87±0.40abcd	48±7bcd	0.26±0.04fg	0.06±0.03de	0.37±0.21d
AUPR8	ND	ND	ND	ND	46±6cd	0.37±0.03efg	0.09±0.01cde	0.55±0.28cd
AUPR9	11±4f	0.04±0.01c	0.03±0.01e	0.28±0.14e	ND	ND	ND	ND
AUPR10	ND	ND	ND	ND	25±3d	0.39±0.06edefg	0.11±0.03cde	0.56±0.31cd
AUPR11	ND	ND	ND	ND	88±11a	1.09±0.16a	0.27±0.03b	1.85±0.40abc
AUPR12	5±2f	0.04±0.01c	0.03±0.01e	0.31±0.16e	71±10abc	0.91±0.07ab	0.19±0.04bc	1.14±0.24bcd
AUPR13	76±2de	1.54±0.13a	0.37±0.06a	1.64±0.33abcde	33±4d	0.73±0.04bcd	0.61±0.01a	0.89±0.24bcd
AUPR14	97±18cd	0.95±0.07b	0.22±0.04bc	1.48±0.33bcde	ND	ND	ND	ND
AUPR15	45±14cf	0.4±0.04c	0.09±0.01cde	0.52±0.33de	78±11ab	0.99±0.03ab	0.24±0.03b	2.06±0.41ab
AUPR17	101±22bcd	0.93±0.04b	0.2±0.04bcd	1.25±0.42bcde	98±10a	0.76±0.23abc	0.19±0.04bc	1.54±0.34abcd
AUPR18	25±7f	0.37±0.04c	0.08±0.03de	0.63±0.24cde	86±6a	0.60±0.03cdef	0.16±0.03bcd	1.44±0.62bcd
AUER21	ND	ND	ND	ND	95±7a	0.64±0.03cde	0.24±0.03b	1.53±0.23abcd

ND= Not determined.

Numbers in the same column followed by the same letter do not differ significantly at p=0.05 by Tukey’s test.

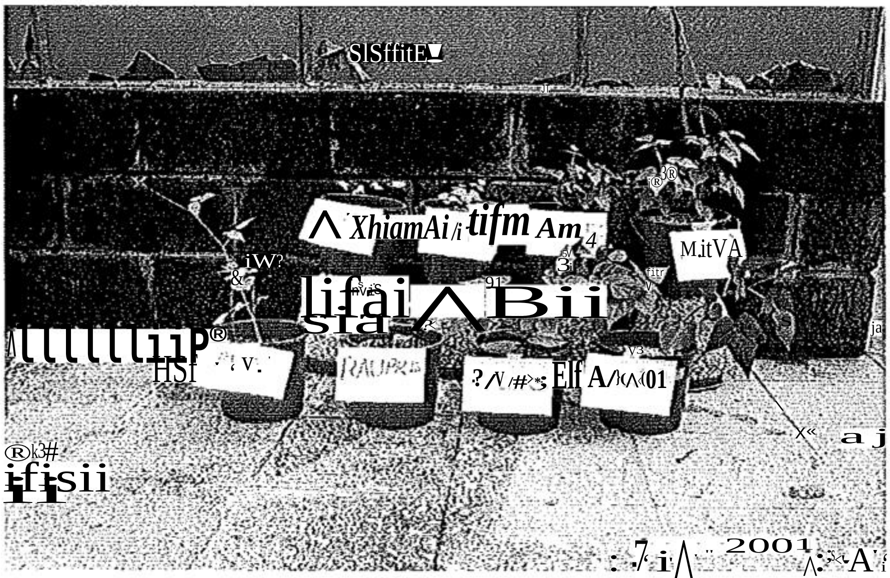


Fig.3. Performance of some isolates. The upper and the lower pots represent growth of M142 and RW Respectively. PVE represent (AUER21) on M142.

5. 5. Cross-inoculation result

Isolates AUER21 (*Erythrina brucei*) and AUAR31 (*Acacia polycantha)* were infective on the non-host *Phaseolus vulgaris* M142. AUER21 induced pinkish nodules and accumulated good dry matter comparable to several bean isolates (Table 8).

5.6. Effectiveness of the isolates on soil trial in pots

The chemical analysis of the two selected soils is indicated in Table 9. There was variation in the performance of isolates between the two soils (Fig.4, 5 and 6). Their performance was good on soil brought from Gentameche than brought from Kemogerbi in shoot dry weight ($t=8.10$, $p<0.05$) and total nitrogen ($t=6.64$, $p<0.05$). This implies soil brought from Kemogerbi inhibited or reduced the inocula activities whereas soil brought from Gentameche was suitable for both symbionts that was reflected by good plant vigor (Fig.4). Similarly, the performance of the selected isolates on soil brought from Gentameche showed similar trend with that of sand experiment.

Table 9: Chemical characteristics of the soil samples.

Parameters	Soil from Kcmogerbi	Soil from Gentameche
PH	7.90	5.50
EC ds/m	0.18	0.10
Na C mol (+)/kg	0.80	0.02
K C mol (+)/kg	2.50	0.74
Ca C mol (+)/kg	36.08	6.09
Mg C mol (+)/kg	1.98	2.47
Sum	40.95	9.27
CEC C mol (+)/kg	52.80	24.60
Bas.Sa. %	78.00	38.00
TN %	0.127	0.25
O.C %	1.46	3.14
C/N	11.00	13.00
Av.P ppm	5.74	56.50
HC03 Me/I00g	0.30	0.14

EC: Electrical conductivity, CEC: Cation exchange capacity, TN: Total nitrogen, O.C :Organic carbon, C/N: Carbon to nitrogen ratio, Av.P: Available phosphorus, Bas. Sa.: Base saturation.

There was significant difference in nodule fresh weight, shoot dry weight and total nitrogen in soil brought fromGentameche at $p<0.05$. Even though there was no significant difference in nodule number, AUPR2 was significantly different in nodule fresh weight with AUPR7 by Tukey’s test at $p=0.05$. All isolates, except AUPR1 were inferior to TN+ in shoot dry weight while all isolates, except TNO had no significant difference in total nitrogen with TN+. In this study, shoot diy weight and total nitrogen was also highly correlated ($r=0.947$, $p< 0.01$). Generally, the result showed the relative positive effect of inoculation, size of inoculum and the competitiveness of AUPR2, which was the second to AUPR1 in most parameters.

On soil brought from Kemogerbi, significant difference in nodule fresh weight, shoot dry weight and total nitrogen at $p<0.05$. In almost all parameters, TN+ was superior by Tukey's test at $p=0.05$. Even though there were no significant difference between TNO and the inocula in almost all parameters, AUPR2 showed significant difference in nodule fresh weight with AUPR5 and AUPR7 and not significantly different in nodule fresh and dry weight with TN+. Besides, this isolate was also the second highest in nodule number and fresh weight, shoot dry weight and total nitrogen. This result showed the effect of nitrogen on nodulation, the relative good performance by AUPR2 and the role of adapted indigenous rhizobia.

Table 10: Symbiotic performance of the isolates on Kemogerbi and Gentameche soils (g/ plant unless otherwise specified)

Treatements	Kemogerbi					Gentameche				
	Nodule Number	Nodule fresh weight (mg)	Nodule dry weight (mg)	Shoot dry weight	Total nitrogen 〈%)	Nodule Number	Nodule fresh weight	Nodule dry weight	Shoot dry weight	Total nitrogen(%)
TN+	56±5a	166±64a	45±28a	2.49∧0.01a	3.05±0.24a	S$±40a	0.3S±0.11abc	0.08±0.03a	2.92±0.12a	3.32±0.3a
AUPR1	\S±5bc	33±10bc	25±5ab	0.25±0.2b	U.8±0.2b	S8±15a	0.60±0.04ab	0.14±0.02a	2.04±0.34ab	2.73±0.3a
AUPR2	23±7b	94±13ab	21±10ab	0.43±0.2Sb	1.67±0.4b	101±44a	0.63±0.2a	0.14±0.04a	2.02±0.22b	2.73±0.22ab
AUPR5	5±2c	4±2c	1±0.Sb	0.09±0.011b	1.21±0.0b	70±10a	0.45±0.0Sabc	0.13±0.02a	2.01±0.18b	2.64±0.22ab
AUPR7	14±6bc	7±0.9c	*6±0.6b*	0.17±0.09b	1.12±0.7b	63±32a	0.26±0.126c	0.19±0.06a	1.84db0.72b	2.64±0.51ab
AUPR13	16±3bc	36±17bc	20±9ab	0.2S±0.2b	1.13±0.2b	137±72a	0.53±0.07abc	0.16±0.02a	1.98±0.11b	2.76±0.2ab
TNo	14±5bc	27±15bc	9±4b	0.32±0.22b	1.64± 0.2b	57±4a	0.32±0.05bc	0.22±0.18a	1.S7±0.06b	2.55±0.05b

Numbers in the same column followed by the same letter do not differ significantly at p=0.05 by Tukey’s HSD test at p=0.05.

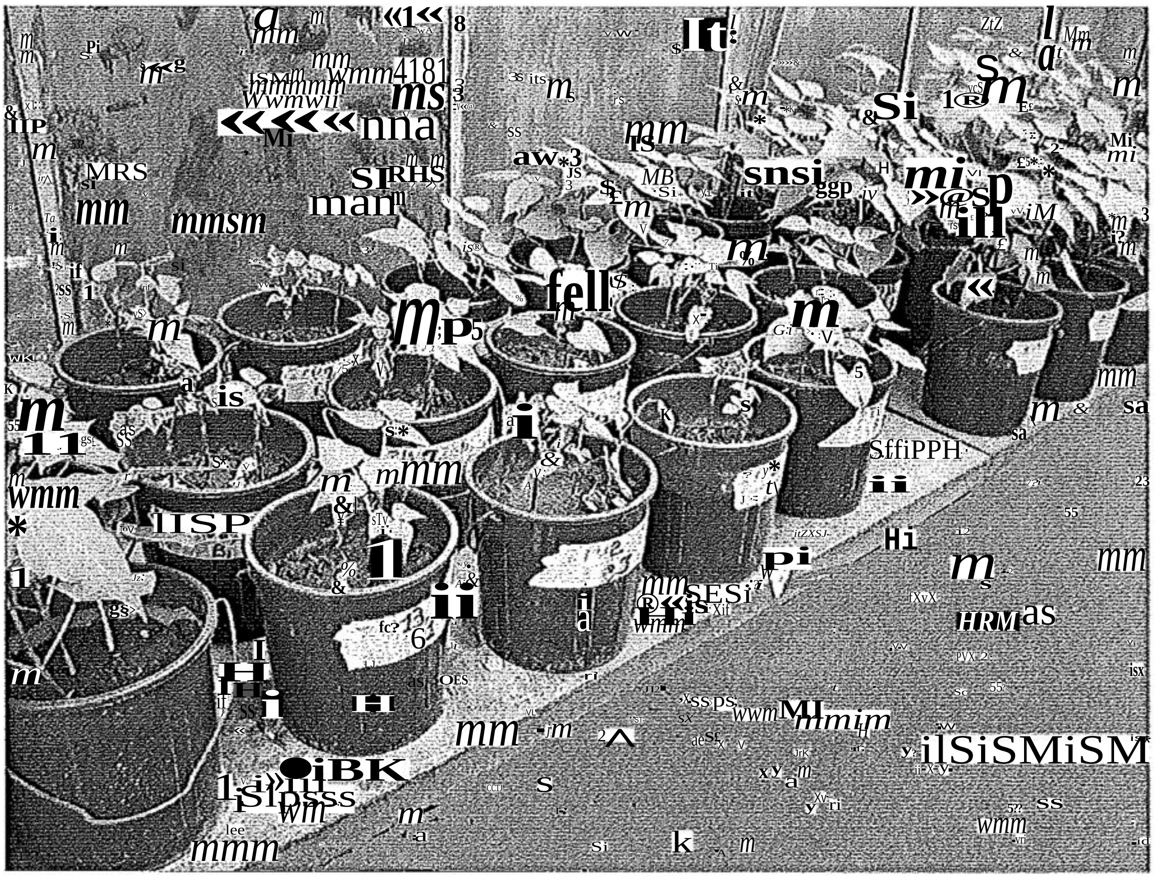


Fig. 4. Greenhouse experiment on the two soils. Growth on the left and right side were on soils brought from Gentameche and Kemogerbi, respectively.

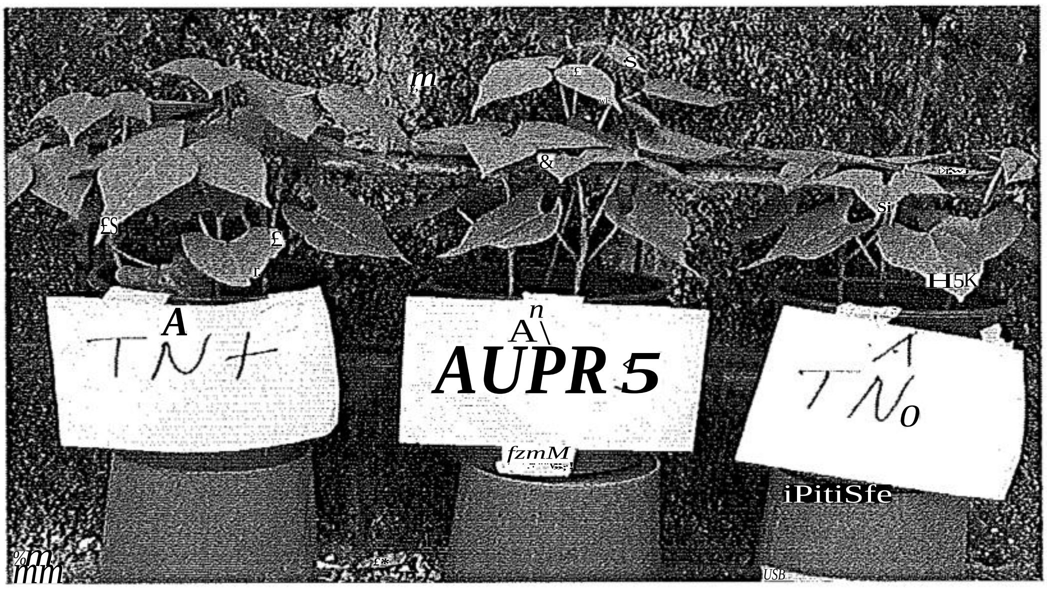


Fig 5. Performance of TN+, AUPR5 and TNO on brought from Gentamecha.

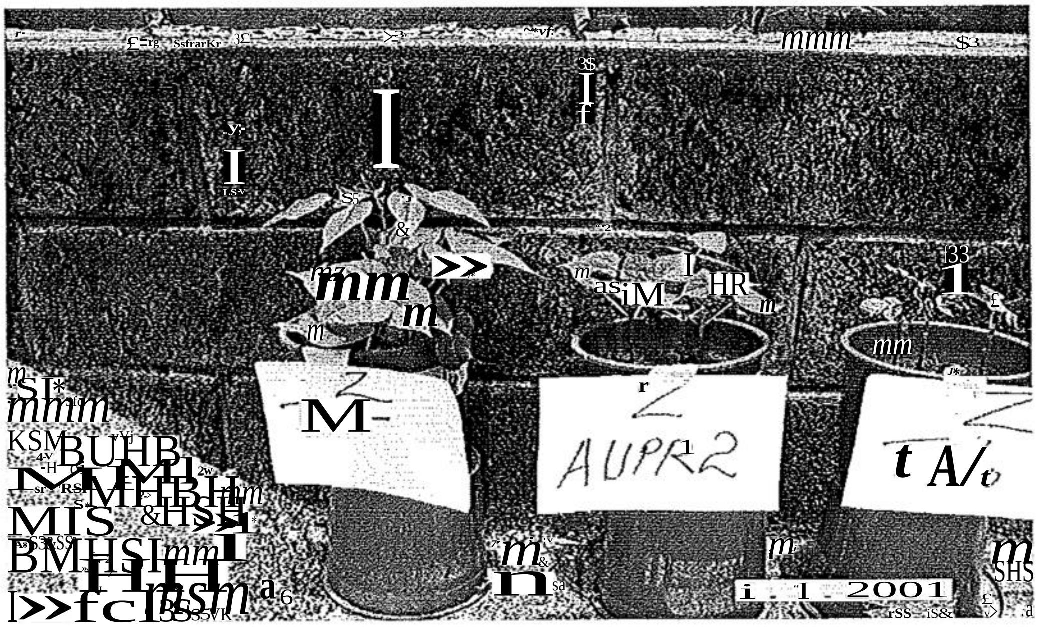


Fig. 6.Performance of TN+, AUPR2 and TNO on soil brough from Kemogerbi.

6. DISCUSSION

In this study we examined the presence and effectiveness of rhizobia isolated from 18 bean-growing areas. All isolates that represent wide geographical locations and soil pH (5.5-8.7) were able to induced nodulation on *P.vulgaris*. Mitiku Haile (1990) revealed that nodulation by indigenous rhizobia during the survey of nodulation status of the crop. Similarly, Amare Abebe (1982) and Desta Beyene *et al*. (2004) found the presence of bean-nodulating rhizobia from Ethiopian soils. These results indicate that effective strains of rhizobia, adaptable to local environments can be obtained through surveys.

According to the classification of Rhizobiaceae in Bergey's Manual (Jordan, 1984), our isolates fall in to fast growing rhizobia based on their generation time, acid production and large growth with production of copious exopolysaccharide at optimum temperature range (25-30^{0}C) and pH of the medium (6-7). Fast growing bacteria nodulating common bean were also identified by several workers (Amarger *et al*., 1997; Aguilar *et al*., 1998; Diouf *et al*, 2000; Andrade *et al*, 2002; Desta Beyene *et al*, 2004).

Cultural and morphological characteristics of most isolates were similar. Most isolates displayed LM colonies except LW and SD colonies by AUPR8, and AUPR18, respectively. Morphological characteristics of bean isolates on PY medium differentiated them into respective type groups. AUPR8 found to be creamy colonies, which is a characteristic of *R. tropici* strains (Martinez-Romero *et al*., 1991). AUPR9 and AUPR10 found to be rough colonies, which is a characteristic of *R. gallicum* (Silva *et al*., 2003). All the rest isolates were found to be smooth gummy colonies, which could be *R. leguminosarum* or *R. etli* (Martinez-Romero *et al*., 1991; Silva *et al*., 2003).

Isolates generally were found to be salt sensitive. All except AUPR8, AUPR9 and AUPR18 were unable to grow above 0.5% (w/v) NaCl. Amarger *et al*. (1997) found no bean isolates were tolerant at 1% (W/V) NaCl except *R. giardinii* and *It. tropici* strains. Molecular tools have also identified six osmotolerant strains that could grow at 2% (w/v) NaCl as *R. tropici* in Morocco (Bouhmouch *et al*., 2001). Similarly, Boncompagni *et al.* (1999) reported reference strains of *R. leguminosarum* bv. phaseoli and *R. etli* could not tolerate at 100mM NaCl whereas *R. tropici* is inhibited at 200mM NaCl. Bouhmouch *et al.,* 2001 also found isolates from saline soils were more tolerant than others. These results revealed that in addition to their genotype, the tolerant isolates were naturally selected by saline soils

All isolates but AUPR8 were unable to grow at low (10^{0}C) and high (40^{0}C) temperature. Thus, our result suggests that most isolates were sensitive at 10^{0}C (YMA) and 40°C on TY and YMA media. Pinto *et al.* (1998) and Raposeiras *et al.* (2002) indicated *R. tropici* strains revealed less alteration in phenotypes than *R. leguminosarum* bv. phaseoli strains after their exposure to thermal shock at 45^{0}C. Hungria *et al.* (2000) also found *R. tropici* was tolerant at 40°C on TY medium.

All isolates could grow at pH 5 and 8.5 except AUPR12 that failed to grow at 8.5. In contrast to high pH, all isolates except AUPR8 were unable to grow at low pH (4). This suggests that most isolates are sensitive to lower pH and tolerant to higher pH. Similar explanation was made by Jordan (1984), fast- and slow-growing rhizobia were more tolerant at alkaline and lower pH, respectively. However, some fast growing strains such as *R. tropici* and *Mesorhizobiwn loti* could grow at a pH of 4 (Cooper 1982; Cunningham and Munns, 1984; Graham 1992; Gao *et al.*, 1994). Amarger *et al.,* 1997 found that all strains of *R. tropici* were able to grow at pH of 4. However, only some strains of *R.giardinii* and *R.leguminosarum* bv. phaseoli. These showed tolerance to pH is not only related to their growth rate but also to the type of strains and probably their adaptation to acidic soils.

There was difference in antibiotics sensitivity among isolates. Rhizobia showed wide variability in resistance to antibiotics (Young and Chao, 1989; Amarger *et al.*, 1997). Because of this, antibiotics resistance could be used as supplementary diagnostic character to show diversity among strains (Josey *et al.,* 1979; Amarger *et al.*, 1997). Therefore, difference in growth in different antibiotics in our result indicated that the diversity in strains among isolates.

We found that generally no difference in carbohydrates utilization among isolates. Amarger *et al.* (1997) found most carbohydrates could be utilized by all tested rhizobia. However, most of bean nodulating bacteria could grow on dulcitol except *R. giardinii* and all strains of *R. tropici*. Similar finding was also shown by Andrade *et al.* (2002) that the *R. tropici* strains were unable to grow on dulcitol. Since similar observation was made in our study, this carbohydrate test was used as one of the traits for tentative classification of isolates. All isolates except AUPR8 were unable to grow on glycine. Amarger *et al.* (1997) and Andrade *et al.* (2002) also found most of bean isolates could grow on L- tryptophan and L- tyrosine whereas glycine was utilized only by strains of *R. tropici.*

All isolates but AUPR8 and AUPR9 could not grow on LB, TY- Ca and 2% urea media. Amarger *et al.* (1997) and Andrade *et al.* (2002) that most bean isolates were unable to grow on LB and TY- Ca media except *R. tropici* strains. Similarly, Martinez-Romero *et al.* (1991) found except *R. leguminosarum,* all *R. tropici* strains were able to grow on LB and TY-Ca media. In our result except AUPR8 and AUPR9, all bean isolates were unable to grow that seems this character was correlated with salt tolerance.

In the present study, no isolate was found to exhibit the ability to solubilize phosphate. On the other hand, melanin production was common feature of all isolates except AUPR3, AUPR8 and AUPR9. Andrade *et al*., (2002) found that melanin production was observed in *R. tropici* strains. Similarly, melanin production was observed in *R. leguminosarum* bv. phaseoli (Cubo *et al.*, 1988).

Even though bean rhizobia are diverse and hence difficult to assign to their respective species without genetic study, it is possible to use standard phenotypic features to tentatively group these bacteria into their respective species types. Phenotypic features such as growth rate and colony morphology as described by Jordan (1984) and other phenotypic features that differentiate bean nodulating rhizobia as described by Martinez-Romero *et al.* (1991), Amarger *et al.* (1997) and Silva *et al.* (2003) were used to make preliminary classification of the isolates.

Based on morphological, biochemical and physiological characteristics, the isolates can be tentatively placed into three groups. AUPR8 was classified as *R. tropici*- like strain mainly by its growth on glycine and failur to grow on dulcitol, AUPR9 and AUPR10 as *R. gallicium-like* strains because of their rough appearance on PY medium and the rest isolates into *R.*

leguminosarum- or *R. etli*-like strains because of the lack of clear demarcation in phenotypic features between them. In pervious study, except a single *R. etli* strain, most bean rhizobia were classified as *R. leguminosarum* by multilocus enzyme electrophoresis. However, based on 16S rRNA most resembled *R. etli*. It is inconclusive that those strains were *R. leguminosarum* or *R. etli* (Desta Beyene *et al*., 2004).

Our result is different to this result by tentative placement of isolates into *R. gallicum*- and *R. tropici*-like strains. Since the sites of bacterial isolation in this study, and Desta Beyene and his colligues were different, the bacterial diversity seems also different. Our result is most likely in agreement with their generalization that it is unlikely that Ethiopian soils extensively colonized by rhizobia of American and Europe origin. Because in some African countries, rhizobia of American and Europe origin were common and abundant (Anyango *et al*., 1995; Mhamdi *et al*., 1999; Diouf *et al*., 2000). Therefore, to have a complete picture of bacteria that nodulate common bean, the genetic study that covers most of bean growing regions would be important.

Generally the result of cluster analysis clearly revealed the presence of strain diversity among the isolates and the possibility to screen different strains from wide bean growing areas. The analysis grouped the isolates into two major diversity groups based on 80-100% similarity levels (Group I) and 39%-78%, similarity levels (Group II). Group I contained 78% of the isolates with two clusters la and lb with presumed similar characters of *Rhizobium leguminosarum/R. etli* group except AUPR10. Isolates such as AUPR8 from Group II displayed characters resemble with *Rhizobium tropici*. Two isolates, AUPR9 (Group II) and AUPR10 (Group I), exhibited rough colony appearance on PY that resemble the characteristics of *Rhizobium gallicum*. The place of AUPR8 is in agreement with its placement to different species type. However, the result and placement of AUPR10 that has 92% similarity with other isolates rather than AUPR9 is conflicting. This might be due to use of a single trait to place them in to *R. gallicum*-like strains and most traits were strain specific (Amarger *et al*., 1997). Even though the trait was used in the previous works and it was similar with genetic classification, this work should be supported by other phenotypic and genotypic studies for the case in Ethiopia.

The effectiveness of isolates from wide range of geographical locations and pH ranges showed the potential benefit of the crop from BNF through screening more effective indigenous rhizobia. The presence of relatively more effective isolates comparable to TN+ in all geographical locations such as lowlands (AUPR7 and AUPR11), intermediate altitude (AUPR2 and AUPR15) and highland (AUPR1) together with a wide area distribution of the crop in our country are interesting. It indicates the possibility of obtaining effective rhizobia adaptable to different bean-growing areas. The role of these isolates becomes more important when we consider the crop production by poor farmers and N-deficiency of most Ethiopian soils (Desta Beyene and Angaw Tsigie, 1986). Since bean is the basic component of most cropping systems in Ethiopia, inoculant would not only improve bean yield but also other crops in the cropping systems. These in turn improve the farmers' life and ultimately the country's economy by improving the average national yield. This suggests that the importance of using effective rhizobia that can improve bean production in Ethiopia.

Even though no much work has been done regarding effectiveness in Ethiopia, the benefit that can be achieved through isolation of indigenous rhizobia was revealed in some African countries. *R. gallicum*-like isolates that could perform better than the CIAT 899 that is recommended as inoculant for bean were obtained in Tunisia (Mhamdi *et ah,* 1999). Isolates those possessed tolerance to environmental stress such as acidity and salt were identified (Anyango *et ah*, 1995; Bouhmouch *et al.,* 2001). Interestingly in Moroccoo, effective isolates with salt tolerance could produce 69-72% diy matter accumulated by the plant supplied with mineral nitrogen (Bouhmouch *et al.,* 2001). Thus, our results reflected the presence of effective bean rhizobia in Ethiopian soils with the possibility of selecting interesting strains able to nodulate the host abundantly and effectively.

In sand pot experiment, difference in dty matter accumulation between the cultivars was observed. The RW cultivars showed good plant vigor visually. This is supported by higher mean shoot dry matter accumulation in RW. Similarly, Hardarson *et al.* (1993) found the presence of difference in nitrogen fixation among cultivars of common bean that was reflected by different nitrogen accumulation by using ^{15}N-isotope dilution method. The study conducted in Tunisia also showed such a difference that whatever the rhizobia, NAG310 genotype was about 50% of shoot growth of other cultivars (Jebara *et al.,* 2001). In Ethiopia, genotypes of common bean also showed difference in nodulation status that the EXRICO 23 nodulated less than others on intercrop and sole systems. On the other hand, the Black Bessie produced the highest number of effective nodules when inoculated on both systems (Mitiku Haile, 1994).

Further more, in our study there was interaction between the strain performance and the seed color of cultivars cropped at the site of bacterial isolation. This could be one of the reasons for the difference in shoot dry matter between the two cultivars since most isolates were obtained from red seed type regions. Similar to this observation, in terms of their effectiveness a strong interaction between the bean cultivar and the native rhizobia population was observed. Most native rhizobia were more effective than the reference strains of *R. leguminosarum* bv. phaseoli, *R. elli,* and *R. tropici* (Rodriguez-Navarro *et al.,* 2000). Native rhizobia isolated from Tunisian soils induced more nodular tissue than CIAT899 (Jebara *et al.,* 2001). One study in Kenya also showed that the presence of such Rhizobium and cultivar interaction in that not all strains were able to nodulate all the three tested cultivars (Karanja and Wood, 1988). Aguilar *et al.* (1998) found some accessions of *P. vulgaris* were able to restrict nodulation by some rhizobial strains. This result suggested that selection of cultivars type and site of bacterial isolation for specific purpose to screen out most efficient inoculant strains would be important.

P. vulgaris is a promiscuous host that rhizobia that nodulate other legumes could induce symbiotic characteristics. In our study, AUER21 and AUAR31 were infective. AUER21 was effective that induced 53 % of the diy matter accumulation by TN+. Though Fassil Assefa (1993) found that isolates from *Acacia* and *Erythrina* species were unable to nodulate *P. vulgaris,* generally, it is not uncommon to find nodulation of this crop by isolates from arboreal legumes since these legumes were inhabitant of the area of bean origin (Hernandez-Lucas *et al.,* 1995; Lopez-Lara *et al.,* 1995). Their co-existence in this area could limit a particular specificity between the host and microsymbiont (Aguilar *et al.,* 1998). Findings of Diouf *et al.* (2000) can be additional evidence for this generalization that isolates from *P.vulgaris* induced nodulation on *Acacia seyal, Acacia sengal, Luecenia leucocephala* and *Faidherbia albidia.*

The result indicated the possibility of isolating effective rhizobia from tree legumes particularly from indigenous one. The promiscuous character of *P. vulgaris* has some practical importance. If such tree strains can prove competitive with specific strains in field, they can be used as additional reservoir from where superior inoculant can be selected in order to improve the yield of the crop (Fassil Assefa, 1993).

Soil type affected the isolates performance. The trend of isolates performance on soil brought from Gentameche was similar with isolates performance in sand experiment. The result of soil chemical analysis indicated that this soil was about four fold in sum of cations; two fold in CEC and base saturatition of soil brought from Gentameche. However, comparing to Gentameche it was low in nitrogen content and alkaline in pH.

The fact that the soil brought from Kemogerbi infected the tested plant with few nodules without inoculation on the TN+ and TNO controls indicated that indigenous rhizobia existed in the soil. The activity of almost all isolates was inhibited due to separate or combination effect of CEC, base saturation, sum of cations, phosphorus deficiency, or alkaline pH on this soil. Salt accumulation and available phosphorus, and capable of nodulation suggests that poor nitrogen fixation by Kemogerbi soil may be due to mineral content, especially phosphorus that may have limited symbiotic characteristics. Such a phenomenon of low nitrogen fixation and poor plant growth in faba bean have been improved by phosphorus fertilization in phosphorus deficient acidic soils in various parts of the country (Amanuel Gorfu *et al*, 2000; Ayneabeba Adarnu *et al*, 2001, Assefa Keneni, 2002)

In soil brought from Gentameche, suitable soil factors including very high nitrogen (Desta Beyene and Angaw Tsigie, 1986) contributed for the high performance for shoot dry weight and total nitrogen in all treatments. High nodule number and significant difference in nodule fresh weight revealed that the very high nitrogen of the soil did not inhibit nodulation and still the difference in performance among the rhizobial strains. Similar observation also made by Yonas Yohannes (2005) that high nitrogen content of the soil did not inhibit nodulation in *Acacia* species. This experiment generally revealed the positive effect of inoculation. All treatments except TNO were not significantly different in total nitrogen. The difference was due to inoculation over the background rhizobia that make AUPR1 and the rest treatments were superior over TNO. In addition to that AUPR2 were next to AUPR1 (which includes background rhizobia and 109 cells) almost in all parameters.

7. CONCLUSIONS AND RECOMMENDATIONS

From result of phenotypic characterization, we concluded that the presence of *R. leguminosarum* bv. phaseoli, or *R. etli*, *R. tropici*- and *R. gallicum*-*like* strains in the tested soils. Since in this study only 18 sites were included and phenotypic features were used, we recommend further genetic and phenotypic characterization that covers most bean growing areas to know taxonomic groups in Ethiopia.

From the result of sand experiment, we concluded that isolates from common bean nodules were infective with variation in their effectiveness. Some of the isolates showed good performance close to TN+. This shows the possibility of obtaining effective isolates from Ethiopian soils that could be used as inoculant. Thus, we recommend rigorous screening and characterization of isolates from soils. In addition, commercial inoculants that are recommended for bean should be included to compare the effectiveness of indigenous rhizobia to recommend as inoculant.

Difference in performance on nitrogen fixation between cultivars was observed. In addition to that the symbiotic preference between the isolates and cultivars was also recognized. Based on the result, we recommend that selection of cultivars type and site of isolation for bacteria for specific purpose to screen out most efficient inoculant strains would be important.

From the result of potted soils, we concluded that generally soil factors affected the symbiotic characteristics of the tested effective isolates. Based on the result, we recommend that before using inoculants in fields, soil factors that affect symbiosis should be studied. AUPR2 showed good performance on sand and both soils so that further competitive studies both in greenhouses and field trials would be important since a success in greenhouse experiment may not necessarily assure a success in the field.

Among the tree isolates, isolate from *Erythrina brucei* and *Acacia polyccmiha* were infective. However, only the *Erythrina brucei* isolate was effective. This result revealed the possibility of obtaining effective isolates from tree legumes. Therefore, we recommend that test for infectivity and effectivity of isolates from diverse tree legumes would be important to increase the rhizobial inoculant reservoir for common bean in Ethiopia.

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