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Symbiotic and phenotypic characterization of rhizobia nodulating common bean (*Phaseolus vulgaris* L.) from Eastern Ethiopia

By

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LIST OF SYMBOLS AND ABBREVIATONS

BNF	Biological Nitrogen Fixation
CEC	Cation Exchangeable Capacity
Con-	Negative control with out inoculum and nitrogen fertilizer
Con+	Positive control with nitrogen fertilizer
CRD	Complete Random Design
E	Effective
EC	Electrical Conductivity
g/liter	Gram per liter
ha	Hectare
hrs	Hours
IAR	Intrinsic Antibiotic Resistance
I	Ineffective
Kg	Kilogram
LB	Luria-Bertani medium
masl	Meter above sea level
mg	Milligram
MGT	Mean Generation Time
mM	mill molar
mm/annum	millimeter per annum
MT	Metric tone
min	minute
N	Nitrogen
N_2	Nitrogen molecule
NSPVR	National Soil *Phaseolus vulgaris Rhizobium*
P	Phosphorus
PGA	Peptone-Glucose Agar
ppm	Part per million

PY	Peptone-Yeast extract medium
PY-Ca	Peptone lacking Calcium medium
SDW	Shoot Dry Weight
SE	Symbiotic effectiveness
TCP	Tricalcium phosphate
TY	Tryptone -Yeast extract medium
ug/ml	Microgram per milliliter
YEMA	Yeast extract Mannitol Agar
YEMB	Yeast extract Mannitol Broth
UPGMA	Unpaired Group Method Analysis
VE	Very Effective
V/V	Volume per volume
YEMA-BTB	Yeast extract Mannitol Agar-0.5% bromothymol blue medium
YEMA-CR	Yeast extract Mannitol Agar-0.25% Congo red medium.

ABSTRACT

Common bean (Phaseolus vulgaris L.) is the most widely distributed and has the broadest range of genetic resources. In Ethiopia, the yield of common bean is extremely low mainly due to nitrogen deficiency and also little information is available regarding diversity of rhizobia nodulating common bean. Hence, this study initiated to know their phenotypic diversity and maximize the symbiotic nitrogen fixation with common bean in Eastern Ethiopia in particular, 72 isolates of bean rhizobia were isolated from three zones, where common bean is commonly cultivated. The efficiency of infectivity and effectiveness of isolates was examined using the Awash Melka variety. Results revealed that 62 isolates were able to form nodules on the roots of the common bean variety and 10 isolates failed to nodulate the host. The tested isolates showed two different ranges of growth rates: 61 isolates as fast growing and isolate NSPVR-62 from Goro Gutu woreda as slow growing. The numerical analysis of the phenotypic characteristics of 62 isolates formed two major diversity groups based on 75% level of relative similarity. The phenotypic characterization and the numerical analysis indicated that 57 isolates as *Rhizobium leguminosarum*-like or *Rhizobium etli*-like, 4 isolates as *Rhizobium gallicum*-like and 1 isolate as *Bradyrhizobium*-like rhizobia were isolated from Eastern Ethiopia soils nodulating common bean. On the basis of preliminary screening of symbiotic effectiveness on sand culture from 62 isolates, 89% of them are effective and very effective in terms of symbiotic effectiveness. Based on SDW yield, strains NSPVR-2 from Eastern Shewa Zone and NSPVR-26, NSPVR-29, and NSPVR-31 from Western Hararghe Zone were selected to study symbiotic effectiveness on Ayenew and Awash Melka varieties on Melkassa and Babille soils. From the selected isolates, isolate NSPVR-31 showed different effectiveness of nitrogen fixation on Awash Melka and Ayenew varieties. While isolate NSPVR-29 showed significantly ($p<0.01$) higher SDW than uninoculated plants on both Ayenew and Awash Melka varieties. Therefore, isolate NSPVR-29 could be used as an inoculum on Babille soil on both tested varieties. Nevertheless, on Melkassa soil on Ayenew and Awash Melka varieties showed that non-statistical difference of SDW, plant nitrogen contents, nodule number, and weight among all treatments. This is due to Melkassa soil has sufficient number of effective rhizobia nodulating common bean. Isolates NSPVR-8 and NSPVR-11 both from Bost woreda showed TCP solubilizer under preliminary screening of phosphorus solubilization and effective nitrogen fixers. We conclude that significant variation exists among rhizobial isolates nodulating common bean in Eastern Ethiopia and can be possible to select superior rhizobia that is in terms of symbiotic effectiveness, competitiveness ability and tolerate to adverse conditions.

Key Words/Phrases: Common bean, Rhizobia, Eastern Ethiopia, Phenotypic, Symbiotic effectiveness, TCP solubilizer.

1. INTRODUCTION

The genus *phaseolus* comprises over 30 species (Debouck 1991). Of which, the common bean (*Phaseolus vulgaris* L.) is the most widely distributed and has the broadest range of genetic resources (Singh 1999). It is the third most important legume crop grown worldwide, superseded only by soyabean (*Glycine max* L.) and peanut (*Arachis hypogea* L.). In developing countries, most beans are growing to feed the local population, as an important source of dietary protein.

Common bean is widely cultivated in many parts of the tropics and sub-tropics and throughout the temperate regions (Westphal 1974). It is mostly used as food crop throughout the world with a center of diversity in Mesoamerica (Middle America), and in the Andean region of South America in Northwestern Argentina, in Ecuador and in Northern Peru (Gepts 1998). Worldwide production of common bean exceeds 23 million metric tone (MT), of which 7 million MT are produced in Latin America and Africa (Broughton *et al.*, 2003).

Common bean is a warm-season crop. However, high temperatures (>30^0C) can cause flower blasting, which reduces yield (Fageria *et al.*, 1997). The crop is adapted to an altitude ranging from sea level to nearly 3000 meter above sea level (masl) (CIAR, 1988).

The common bean requires moderate amounts of water (300-600mm/am) and cultivated under different cropping systems from the highly mechanized, irrigated, and intensive production of monocropped bush beans to complex associations of indeterminate or climbing beans with maize, other cereals, sugarcane, coffee, or plantarum (Schoonhoven and Vouset, 1991). Beans are consume as mature grain, as immature seed, as well as a vegetable that are both leaves and pods (Broughton *et al.*, 2003).

In Ethiopia, Common bean is widely cultivated in areas with attitude between 1400-2000, as a sole crop or inter cropped with sorghum, maize and other. The Rift valley contributes to 48% out of 163,688ha and 55% of 1,384,216quintals production of the country (Teshale Assefa *et al.*, 2006). The Hararghe highland is one of the major common bean producing areas in the

country (Wortmann and Allen, 1994). It is estimated to cover 11,696.4 ha of land with a production of 1 ton/ha (CACC, 2001). It is becoming important as short duration crop because of the recurrent late onset and early termination of rainfall in these areas.

The yield is extremely low due to low soil fertility, smallholder farming and limited access to external inputs (Amare Abebe 1987; EARO, 2000). One of the most important factor of soil fertility is nitrogen deficiency of most Ethiopian soils (Desta Beyene and Angaw Tsigie, 1986). Studies carried out by the National Soil Survey Project (NSSP 1990) and by the Alemaya University of Agriculture (Mitiku Haile 1990) clearly revealed the need for inoculation with rhizobia to improve the yield of common bean in Ethiopia. Recently, Asfaw Hailemariam and Angaw Tsigie (2006) reported research on *Rhizobium leguminosarum* revealed 10-50% yield increase on faba bean, field pea and lentil , and appropriate *Rhizobium sp.* Strain for chick pea gave up to 38% yield increase. Therefore, biological nitrogen fixation should be more exploited to increase nitrogen for common bean cultivation in Africa.

Most of the studies showed that some rhizobial strains recommended as inoculum for common bean production were not satisfactory for nodulation and nitrogen fixation (EARO, 2000). Nevertheless, 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*., 2000). These reflect the need for screening rhizobial isolates which are efficient and adaptable to different soils of the county. Use of these strains as inoculant, in turn, requires isolation, characterization, and evaluation of their effectiveness in nitrogen fixation under different soil conditions to fully realize biological nitrogen fixation

This is not only limited by the nitrogen-fixing potential of the rhizobia symbiosis but also by the edapho-climatic conditions that effect on the establishment of the symbiosis (Sessitsch *et al.*, 2002; Howieson and Balland, 2004). However, there are soils where common bean crops have been effectively established that contain strains well adapted to specific environmental conditions. This can be isolated and evaluated for use as common bean inoculants.

Furthermore, common bean is known to be a relatively permissive host, nodulating effectively with many rhizobial species (Michiels *et al*., 1998). At least six rhizobia *Rhizobium etli* (Segovia *et al*., 1993), *Rhizobium tropici* (Martinez-Romero *et al*., 1991), *Rhizobium leguminosarum* biovar. *Phaseoli* (Jordan 1984), *Rhizobium giardinii* and *Rhizobium gallicum* (Amarger *et al*., 1997) and *Bradyrhizobium* (Hungria *et al.*, 1993), nodulate this promiscuous legume (Michiels *et al*., 1998).

From different regions in the world, there are many reports as well on genetic uniformity and on large biodiversity of rhizobial strains nodulating common bean (Martinez-Romero 2003). In Ethiopia, through some studies were undertaken on effectiveness of indigenous common bean rhizobia from some part of central and southern Ethiopia, rigorous screening and the taxonomic relationship have not been well undertaken (EARO, 2000; Desta Beyene et al., 2004; Alemayehu Workalemahu, 2006). Hence, this study was initiated with the aim to isolate rhizobia nodulating common bean and characterize phenotypically and its symbiotic performance from some bean growing area of Eastern Ethiopia.

2. OBJECTIVES

2.1. General objective

- To evaluate symbiotic and phenotypic characterization of common bean-nodulating rhizobia from eastern Ethiopia

2.2. Specific objective

- To isolate and authenticate common bean-nodulating bacteria from nodules
- To examine phenotypic characterization of the isolates
- To check cross-inoculation test on *Leucaena leucocephala*
- To evaluate symbiotic effectiveness of the selected isolates on different varieties of common bean

3. LITERATURE REVIEW

3.1. Importance of legume plants

Leguminous crops (pulses) are important as animal feed they provide not only high quality protein but also a variety of nutrients such as vitamins, minerals, and other nutrients (Burris and Roberts, 1993). They fix nitrogen in an endosymbiotic nitrogen fixation with root nodule bacteria known as rhizobia. They fulfill most of their N requirements through this process. They occupy 12-15% of the earth's arable land and account for a third of human dietary protein needs and for up to 2/3 of subsistence livelihood (Graham and Vance, 2003).

A number of studies have indicated that cultivation of N_2-fixing legumes benefits the yield of the succeeding crops (Senaraine and Hardarson, 1988). The residual nitrogen benefits from legumes are usually evident in the available soil mineral nitrogen pool. Asefa Taa and Kedir Nefo,(2006) indicated that the double cropping of bread wheat following field pea have been increased total farm output compared to either fallow practice or bread wheat following barley. Generally, the inclusion of grain legumes in cropping sequences increases soil nitrate, grain yield, total nitrogen accumulation and nitrogen use efficiency of a subsequent cereal crop with inorganic fertilizer applied (Badaruddin and Meyer, 1994).

Introducing more legumes into farming systems can itself help to reduce soil erosion losses. They also provide a good maintenance of soil cover, in pasture lands by contributing to nitrogen through nitrogen fixation and quality litter for decomposition (Robbins *et al.*, 1989). Leguminous plants that fix nitrogen may well grow on soils that are poor in available nitrogen, reducing the amendments with expensive nitrogen fertilizers (Burris and Roberts, 1993).

3.2. Distribution of common bean in Ethiopia

Common bean (*Phaseolus vulgaris* L.) is grown in Ethiopia primarily as a crop for small-scale production with little inputs (Wortman and Allen, 1994). Although it is distributed in many parts of the country, most of the production is concentrated mainly in east and the central Rift Valley representing semi-arid areas, the south and south-west representing mid altitude and the west representing the sub-humid climate (Habtu Assefa 1994). Suitable production areas in Ethiopia are between 1200-2000 masl mean maximum and mean minimum temperature of less than 30^0C and greater than 10^0C respectively, the seasonal rainfall of 350-700mm are suited (Ohlander 1977).

It can be grown on light sandy soils to heavy clay soils provided that they are well drained since it is sensitive to water logging (IAR 1979). Despite its high economic importance, the average yield of bean obtained by farmers in Ethiopia is extremely low (800-900Kg/ha) (CSA 2006) as compared to 2500 kg/ha in Egypt, which is the highest yield in the world (Dev and Gupta, 1997). Recent report indicated that the area devoted to common bean production in 2005/6 cropping season in the country was 163,688ha, which is mainly grown as cash crop by farmers (CSA 2006).

3.3. Biological nitrogen fixation

One of the driving forces behind agricultural sustainability is effective management of N in the environment. Nitrogen is present in various form, primarily as dinitrogen gas (N_2), organic nitrogen (in plants, animals, microbial biomass and soil organic matter), and ammonium (NH_4^+) and nitrate (NO_3^-). Soil inorganic nitrogen pools are usually small, generally just a few mg N kg^{-1} in natural ecosystems and rarely exceeding 100 mg N/kg in the plow layer of recently fertilized agricultural soils (David *et al.*, 2005).

Application of fertilizer N increased approximately 10-fold to 90 million Mt between 1950 and 1995 with significant energy consumption for N fertilizer synthesis and application (Frink *et al.,* 1999). However, in developing countries the high cost of nitrogen fertilizer, the energy requirements for production, and the suboptimal transportation capacities limit its use especially on small farms (Vance 1997). Consequently, farming practices can make use of the more economically viable and environmentally prudent biological nitrogen fixation (BNF), in which agriculture and the environment will benefit (Peoples *et al.,* 1995).

Roughly, half of the 23 million metric tons of nitrogen consumed as human food sources (grains and livestock) comes from biological nitrogen fixation by prokaryotes (Socolow 1999). Out of this, rhizobia in root nodules are estimated to carry out between 50-70% of the world's biological nitrogen fixation (Burris and Roberts, 1993) and the estimated annual biological fixation of atmospheric nitrogen varies between $100x10^6$ and $180x10^6$ Mt per year (Philips 1980; Burris and Roberts, 1993). Tropical grain legumes such as common bean fix nitrogen to the tune of 25 to 210 kg N ha^{-1} (Dakora and Keya, 1997).

3.3.1. Biochemistry of BNF

Biologically available nitrogen, also called fixed nitrogen, is essential for life. All known nitrogen-fixing organisms (diazatrophs) are prokaryotes, and the ability to fix nitrogen is widely distributed across both the bacterial and archaeal domains (Raymond *et al.,* 2004). In the legume-rhizobia interaction, symbiotic nitrogen fixation takes in a symbiosome, an organelle inside the root nodules (Hadri and Bisseling, 1998). The nitrogenase complex catalyzes biological nitrogen fixation. The nitrogenase complex consists of two metalloproteins, highly conserved in sequence and structure throughout nitrogen-fixing bacteria (Dean and Jacobsen, 1992). The protein containing the site of substrate reduction is nitrogenase molybdenum-iron (MoFe) protein, also known as dinitrogenase or component-I. While, the obligate electron donor to MoFe protein is nitrogenase iron protein (Fe protein), also known as dinitrogenase reductase or component-II (Dean and Jacobsen, 1992). Nitrogenase catalyzes the conversion of N_2 to NH_4^+, as represented by:

$N_2+10H^++8e^-+nMgATP \longrightarrow 2NH_4^-+H_2+nMgADP+nP_i$ (n=16)

As can be seen from this reaction to the nitrogen assimilation reactions, nitrogen fixation is very expensive in biological energy equivalents, requiring large amounts of both reducing power and high-energy phosphate (Adenosine triphosphate, ATP). The capacity for nitrogen fixation in these organisms relies solely upon the nitrogenase enzyme system, which, at 16 ATPs hydrolyzed per one mole of N_2 fixed, carries out one of the most metabolically expensive processes in biology (Simpson and Burris, 1984). Moreover, obligate proton reduction occurs during nitrogenase catalysis, with a minimum of one mole of (hydrogen molecule) H_2 produced per mol of N_2 reduced (Simpson and Burris, 1984). The proportion of electrons allocated to proton reduction increases under conditions of limiting electron flux, further increasing the consumption of MgATP (Burgess and Lowe, 1996).

At least 25% and as much as 60% of the energy consumed in nitrogen fixation is wasted in the production of H_2 (Arp 1992). Some strains of *Bradyrhizobium japonicum* are known to be able to oxidize the H_2 produced during the nitrogenase reaction because of the presence of uptake hydrogenase (Hup) system. As a result, much of the energy consumed in the production of H_2 is recovered. Due to the presence of this system, *B. japonicum* can increase soybean yield by as much as 17% (Evans *et al.*, 1987.). However, this characteristic is very rare among strains used to inoculate other legumes such as common bean, alfalfa, or peas (Maier and Triplett, 1996).

Nitrogen fixation is regulated at the transcriptional level in response to environmental oxygen and ammonium levels. Because the nitrogenase components are oxygen labile, it is advantageous for bacteria to repress transcription when oxygen levels are high. It is also advantageous to repress the expression of the metabolically expensive nitrogenase system when the cellular level of fixed nitrogen is sufficiently high. The degree to which each stimulus affects transcription is characteristic of the particular diazotroph.

3.3.2. Signaling in symbiotic association of *Rhizobium* and legume plant

Formation of symbiotically effective root nodules involves signaling between host and microsymbioint. Numerous changes occur in host and bacterial gene expression during infection, nodule development, and function, with approximately 100 host legume and rhizobial genes involved (Vance 2002). The initial recognition between compatible partners is crucial for the successful development of a symbiotic nodule and it seems logical that surface interactions between the two partners may be involved in this complicated recognition process (Long and Ehrhardt, 1989). That is through specific binding of particular polysaccharide structures present on the bacterial cell surface to host plant lectins (Bohlool *et al.*, 1974).

The chemical mediators involved in the molecular dialogue include flavonoids, Nod factors, surface polysaccharides, and extracellular proteins (Perret *et al*., 2000). Legumes excrete a number of secondary metabolites released into their rhizosphere. Flavonoids and/or isoflavonoids are two such metabolite released from the root of the legume host that induce transcription of nodulation genes on compatible rhizobia, leading to the formation of lipochitooligosaccharide molecules that, in turn, signal the host plant to begin nodule formation (Long 1996). Flavonoids concentrations in the rhizosphere increase in response to compatible rhizobia (Recourt *et al.*, 1992; Schmidt *et al.*, 1994; Zuanazzi *et al.*, 1998). Moreover, rhizobia use these plant products for their own ends. This is the first specifity interaction in the symbiosis.

Some flavonoids induce the expression of nodulation (*nod*, *noe*, and *nol*) and related genes (Broughton and Perret, 1999); interact with rhizobial NodD proteins that serve as both environmental sensors and activators of transcription. A second set of signals is synthesized when NodD-flavonoid complexes activate transcription from *nod* boxes. Rhizobia and the Nod factors they secrete (Cohn *et al.*, 1998; Downie 1998) stimulate the reorientation of root hair cell wall growth (Dart 1977), resulting in curled root hairs. Most of the genes immediately downstream of these promoters are involved in the synthesis of lipo-oligosaccharidic Nod factors that provoke deformation of root hairs and allow rhizobia to enter the root through infection threads. This is the second level of host specifity. The nature of fatty acid and the

substitutions, such as acetyl, carbamoyl, methyl, sulphate, and sugar groups of Nod factor seems to determine host specificity (Perret *et al.*, 2000).

3.3.3. Biological Nitrogen Fixation in *Phaseolus vulgaris* L.

Common bean is considered a poor nitrogen fixing pulse in comparison with other grain legumes (La Rue and Patterson, 1981; Hardarson 1993). As an example faba bean (*Vicia faba*), lupin (*Lupinus spp*.) and pigeon peas (*Cajanus cajan*) have been found to be very efficient; soybean (*Glycine max*), groundnut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata)* to be average; and common bean (*Phaseolus vulgaris*) and pea (*Pisum sativum*) rather poor in fixing atmospheric nitrogen (Hardarson 1993).

Moreover, sparse nodulation or a lack of response to inoculation in field experiments has been frequently reported worldwide, raising doubts about the benefits of inoculation (Graham 1981; Buttery *et. al*., 1987). This fact could be related to the promiscuity observed in common bean (Hernandez-Lucas *et al.*, 1995; Michiels *et al.*, 1998) or to other limiting nodulation factors, like the high rate of nitrogen- fertilizer used for intensive agriculture (Diouf *et al.*, 1999).

Furthermore, when native bacteria exist in the fields they often out-compete the inoculant strains that only occupy a small proportion of nodules as observed in some areas of Latin America (Aguilar *et al.*, 2001; Burgos *et al.*, 1999; Graham 1981). This is also indicated in Eastern and Southern Africa due to the presence of large populations of indigenous, compatible rhizobia, which are effective in nitrogen fixation (Ssali 1988), lack of available phosphorus in the soil or other limiting environmental factors (Anyango *et al.*, 1995). On the contrary, field trials have indicated that it is possible to increase bean (*Phaseolus vulgaris* L) yields by inoculation with selected strains of *Rhizobium leguminosarum* biovar *Phaseoli* in many location in Eastern Africa (Keya *et al.*, 1982), and also bean inoculation with *R. tropici* in Brazil (Hungria *et al.*, 2000).

In case of Ethiopia, studies clearly revealed that there is a need for inoculation of common bean with rhizobia to improve the yield of common beans in Ethiopia (Mitiku Haile 1990). Amare Abebe (1987) conducted a study using two strains of inoculants and indicated that

Phaseolus vulgaris L fixed 12-20Kg/ha, of nitrogen compared to uninoculated control. Alemayehu Workalemahu (2006) on his report symbiotic properties of the effective isolates showed different response depending on soil factors such as cations, cation exchange capacity, and available phosphorus.

3.4. Taxonomy of rhizobia nodulating *Phaseolus vulgaris* L.

According to Jordan (1984), rhizobia were classified into two genera, *Rhizobium* and *Bradyrhizobium*. The early classification of rhizobia was maintained on the ability of infection and nodulation of particular legume species (Somasegaran and Hoben, 1985). Two different types of symbiotic plasmid among rhizobial isolates were obtained from common bean nodules as reported by Martinez-Romero *et al*., (1985).

Symbiotic plasmids of type I strains have multiple copies of *nifH* gene, a narrow nodulation host range (Quinto *et al.*, 1982; Martinez-Romero *et al.*, 1985). On the other hand, the symbiotic plasmids of type II strains have a single copy of *nifH* gene, nodulate *Leucaena spp*. (Martinez-Romero *et al.*, 1988; Brom, *et al.*, 1988).

Type II strains were proposed a new species named *R. tropici,* which revealed further subdivision into two similar but distinct subgroups using multilocus enzyme electrophoresis (MLEE) (Martinez-Romero *et al.*, 1991). *Rhizobium tropici*, further classified into two subgroups IIA and IIB accommodated by the relatively low level of DNA-DNA homology (36%) and differences in phenotypic and genotypic characteristics (Martinez-Romero 1996). However, there are also *R. tropici* strains with intermediate characteristics, which do not fall into either type A or type B (Hungria *et al.*, 2000; Martinez-Romero 1996). Segovia *et al.*, (1993) reported that *R. leguminosarum* bv. *phaseoli* type I strains could be reclassified on the basis of a sequence analysis of 16S ribosomal RNA, as a new species, named *R. etli*.

In addition, Amarger *et al.*, (1997) proposed two new species of *Rhizobium* that nodulate *Phaseolus vulgaris* in France based on the results of DNA–DNA hybridization experiments, restriction analysis of the amplified fragments of 16S rDNA and sequence of 16S rDNA. The

proposed names for these two new species were *R. gallicum* and *R. giardinii*, each with two biovars based on the symbiotic and molecular characteristics.

Rhizobium gallicum bv. *gallicum* nodulating *Phaseolus spp*., *L. leucocephala*, *Macroptillium atropurpureum* and *Onobrychis vicifolii* had only one single copy of the nitrogenase *nifH* gene. *Rhizobium gallicum* bv. *Phaseoli* nodulating *Phaseolus spp*., and *Macroptillium atropurpureum*, failed to nodulate *L. leucocephala* had three copies from the *nifH* gene. *Rhizobium giardinii* bv. *giardinii* is able to nodulate the same hosts as *R. gallicum* bv. *gallicum*, failed to hybridize with *nifH* gene. *Rhizobium giardinii* bv. *phaseoli* is weakly efficient in nitrogen fixation with *Phaseolus vulgaris.* They can also nodulate *Macroptillium atropurpureum* after delaying in nodulation for at least one month, and also characterized by three copies of *nifH* gene.

In Ethiopia, Mitiku Haile (1990) revealed that Ethiopian soils have indigenous rhizobia nodulating common bean during the survey of nodulation status of the crop. Amare Abebe (1982) found the presence of bean-nodulating rhizobia from Ethiopian soils. Alemayehu Workalemahu (2006) found the presence of *R. leguminosarum* bv. Phaseoli, or *R. etli*, *R. tropici*- and *R. gallicum*- like strains were characterized phenotypically isolated from South Ethiopia. Desta Beyene *et al.*, (2004) indicated that *R. leguminosarum* has acquired the determinants for nodulation of bean from a low number of introduced bean-nodulating rhizobia.

3.5. Factors affecting nodulation and nitrogen fixation of rhizobia

Environmental factors influence all aspects of nodulation and symbiotic N_2 fixation. They affect rhizobial survival and diversity in soil; and growth, nodulation and nitrogen fixation of the host. Lack of response to inoculation can be attributed to great sensitivity of the symbiosis to environmental stresses, such as high temperature, soil dryness, and low soil fertility (Graham 1981; Hungria *et al.*, 1997).

Salinity of soil

Nearly 10% of the world's land surface can be classified as endangered by salinity. Most of such areas are found in the tropical and Mediterranean regions (Surange *et al.*, 1997). Zahran (1999) has reported variability in salt tolerance among legume crops. Legumes such as *Phaseolus vulgaris*, *Vicia faba* and *Glycin max* are more salt tolerant than *Pisum sativum* (Cordovilla *et al.*, 1995). Moreover, the legume plants are more sensitive to salt or osmotic stress than the rhizobia (Elshinnawi *et al.*, 1989; Zahran and Sprent, 1986). Amarger *et al.*, (1997) noted that tolerance to salinity, acidity and alkality is more strain- specific than species-specific. Graham and parker, (1964) showed that strains of fast growing acid-producing rhizobia such as *R. etli* are generally more salt tolerant than slow growing alkali producing strains.

There are also significant positive correlation between salt tolerance and adaptation of rhizobial strains in alkaline condition (Kulkarni *et al.*, 2000; Abdelaal Shamseldin, 2005). *Rhizobium* nodulating *Phaseolus vulgaris* isolated from Morocco were able to resist a sodium chloride concentration up to 4% NaCl (680 mM NaCl) in liquid culture (Priefer *et al.*, 2001). There are some evidence that rhizobia strains isolated from alkaline soils are rather tolerant to high temperature, pH, and salt stress (Surange *et al.*, 1997).

High salt concentration was reported to decrease nodulation and amounts of N_2 fixed (Bekki *et al.*, 1987). The amount of nitrogen fixed per unit weight of nodules was also found to decline with salt stress (Miller and Wood, 1996). Cordovilla *et al.*, (1999) found a reduction in nodule number and nodule weight in fababean by factor 45% and 59% respectively, at 0.45% NaCl. Therefore, the success of *Rhizobium*-legume symbiosis under salt stress requires a good selection for both salt tolerant rhizobial strains (Zahran 1991) and host plants (Saadallah *et al.*, 2001).

Soil acidity

One of the main environmental factors that disrupt the symbiotic association between *Phaseolus* and *Rhizobium* is soil acidity (Graham 1981). The low pH of soil affects all stages of the legume-*Rhizobium* symbiosis, including strain survival in the soil, root hair infection, nodule initiation, and nitrogen fixation (Graham *et al.*, 1982). The early steps during pre-infection are the more acid sensitive events as a result of negative influence of the low pH on the bacterial attachment to roots as reported by Caetano Anolle and Favelukes, (1986). In addition, the failure of nodulation was reported in legumes especially in the acid soil below pH 5 due to the inability of *Rhizobium* strain to survive under these conditions (Graham *et al.*, 1982).

Poor nodulation of bean roots was commonly observed in the tropics due to soil acidity (Ssahi 1981). Soil acidity also causes Aluminium and Manganese toxicity on the host, rhizobia and symbiosis (Franco and Munns, 1982). Phosphorus (P) availability is often reduced with increasing soil acidity due to high P fixation (Sanchez and Vehara, 1980) which is the characteristics of some soils in Eastern Africa (Le Mare 1984). Management of soil acidity for nitrogen fixation depends the selection of acid-tolerant legume cultivars and compatible rhizobia (Howieson *et al.*, 1995).

Soil acidity is affecting the diversity of rhizobia. In Kenya, the dominant types of *Phaseolus*-nodulating rhizobia differ between an acidic soil and a high-pH soil, with *Rhizobium tropici* dominating in the acidic soil (Anyango *et al.*, 1995.). It is tempting to assume that *R. tropici* might generally be better adapted to acidic soils than other species of *Phaseolus*-nodulating rhizobia. Because *R. tropici* is the most acid-tolerant *Rhizobium* species described to date (Graham *et al.*, 1994.), a potential to replace other less tolerant bean rhizobia.

Since, pH values between 5.5 and 6.5 have been reported to be optimum for bean growth (Munns and Fox, 1979), identification of both acid-tolerant host and rhizobial endosymbionts are priority areas to increase bean production is prone areas. Moreover, amendment of acid

soils with lime provides nutrients (Ca and Mg) and creates better conditions for growth of bacterial cells in the short time by altering soil pH and increasing the availability of phosphorus and molybdenum(Andrade *et al.*, 2002).

Soil temperature

Elevated temperatures in tropical soils are major problems restricting the response of inoculation with introduced or indigenous rhizobial strains (Michiels *et al.*, 1994). Temperature affects several stages of symbiosis such as root hair infection, bacteroid differentiation, nodule structure, and functioning (Roughley and Dart, 1970). High soil temperature is associated with delaying or restricting nodulation in the subsurface region (Graham 1992).

Nodule functioning in common bean is optimal between 25 and 30 °C, but is hampered by root temperatures between 30 and 33 °C (Piha and Munns, 1987). Michiels *et al.* (1994) found that the acetylene reduction activity of common bean plants was strongly diminished at 35 °C when plants were inoculated by heat-sensitive or heat tolerant strains. Nevertheless, Surange *et al.* (1997) isolated highly temperature (50°C) tolerant strains of *Rhizobium* nodulating leguminous trees from tropical soils. The remedy is administration of inoculum in deeper soils and application of surface mulch to reduce soil temperature (Roughley 1980).

Soil nutrients

Nodulation and N_2-fixation by many legumes are limited by deficiencies in soil nutrients such as N, P, and micronutrients (Giller and Wilson, 1991; Sanginga *et al.*, 1995). Studies in Zambia (Sakala 1984) found that inoculation in combination with a starter dose of nitrogen increased yield of common bean by 73%. Chemical fertilization in doses normally recommended for agricultural fields also diminish the genetic diversity of rhizobia in bean nodules (Caballero-Mellado and Martinez-Romero, 1999). Although mineral nitrogen in the soil affects the process of nodulation, it may be promoted by relatively low levels of available nitrate or ammonia. However, higher concentrations of nitrogen always depress nodulation (Eaglesham

1989). The usually recommended rates of 40-60Kg N/ha suppresses nitrogen fixation (Graham 1981). Application of nitrogen for *Phaseolus vulgaris* L. was found to suppress nodulation but resulted in yield increase ion a vertisol at Alemaya (Mitiku Haile, 1990). Danso *et al.* (1990) found that soybean N_2 fixation is inhibited at higher N levels (83 mg of N kg of soil) which subsequently reduced production.

The inhibitory effect of nitrate on N_2 fixation has been attributed to a direct competition between nitrate reductase and nitrogenase for reducing power (Straub *et al.*, 1997) or to the hypothesis that nitrite as intermediate of nitrate reductase inhibits the function of nitrogenase or leghaemoglobin (Becana and Sprent, 1987). Gates and Miller, (1979) observed that nodulation in soyabean is affected by unbalanced nutritional conditions of nitrogen (N), phosphorus (P) and sulfur(S). Amare Abebe (1987) indicated that the application of P at the rate of 100Kg/ha have improved nitrogen fixation.

Drought stress

Establishment and activity of the legume-*Rhizobium* symbiosis have been found to be extremely sensitive to drought stress. In a two year field experiment with Soyabean, Sinclair *et al.*, (1987) found that nodule number and dry weight decrease after a severe drought. Compared to host plants, rhizobial strains are quite resistant to soil desiccation and can survive in water films surrounding soil particles (Williams and De Mallorca, 1984). Fast-growing rhizobia, however, are sensitive to soil dehydration as compared to slow-growing strains (Sprent 1971). An evidence indicated that the decline of soyabean nitrogen fixation under drought was associated with a decline in photosynthesis (Huang *et al.*, 1975). Zahran *et al.*, (1994) showed that exposing rhizobia to osmotic stress bring about alteration of bacterial membrane lipopolysaccharides, which are involved in the *Rhizobium*-host plant recognition process.

4. MATERIAL AND METHODS

4.1. Sampling site

Nodules were collected in August 2006 at 50% flowering stage of the plants from Eastern Ethiopia covering some parts of Eastern Shewa (17 isolates), Eastern Hararhge (19 isolates) and Western Hararghe (26 isolates) from as many sampling sites where common bean has bean grown for a long time with no history of inoculation with rhizobia. The areas are indicated in table 1. in addition, location of sampling site is shown in figure 1.

4.2. Nodule collection and isolation of rhizobia

More than 70 isolates were randomly obtained from well-developed pinkish nodules on plant samples collected from Eastern part of Ethiopia. The collected nodules in the field were kept in closed vials with silica gel at room temperature until the isolation of rhizobia was undertaken at the National Soil Research Center, Soil Microbiology Laboratory. The undamaged nodules were sterilized by 95% ethanol for 10sec and transferred to a 3% solution of sodium hypochlorite for 3min. Then, the nodules were rinsed five times by sterilized water and crushed in normal saline solution (0.85% NaCl) as indicated in Somasegaran and Hoben (1994). The suspension was streaked on yeast extract mannitol agar (YEMA). The purity of cultures was checked by repeatedly streaking the bacteria on YEM agar medium (Jordan 1984).

The component of YEMA medium

Mannitol----------10g

K_2HPO_4----------0.5g

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

NaCl----------0.1g

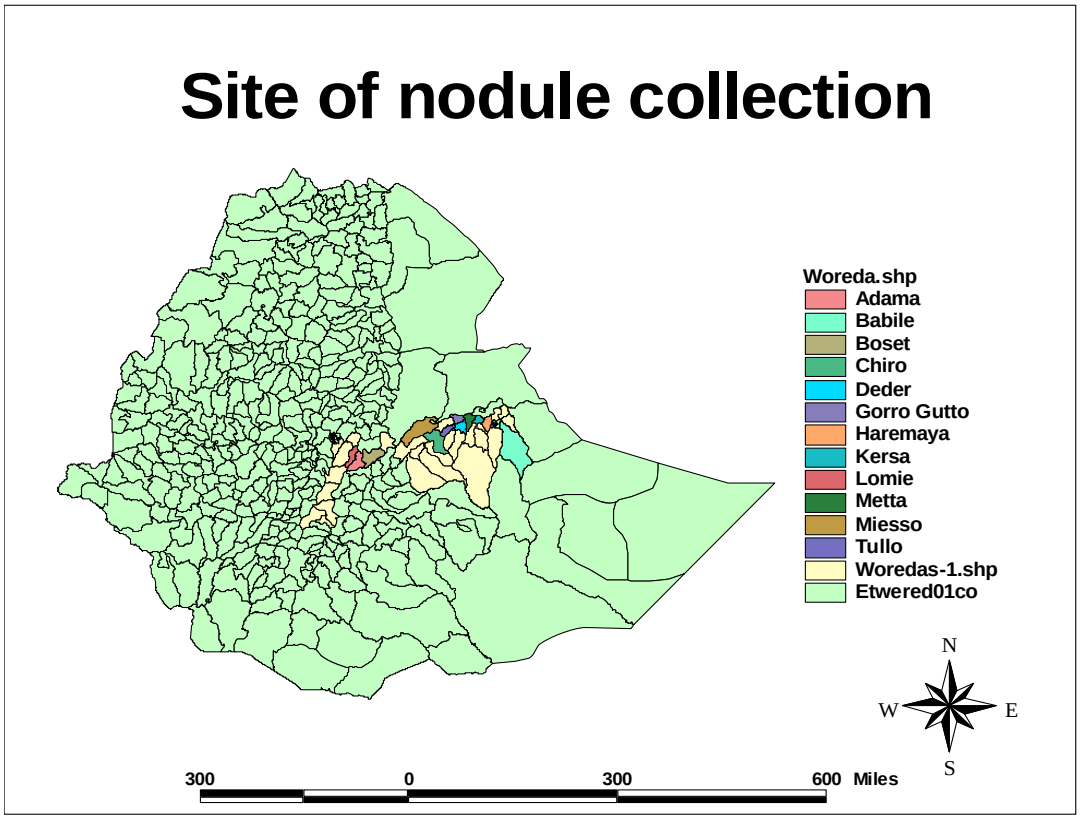
Yeast extract------1g

Congo red---------0.025ml

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

Distilled water—1000ml

Taken from Somasegaran and Hoben (1994)



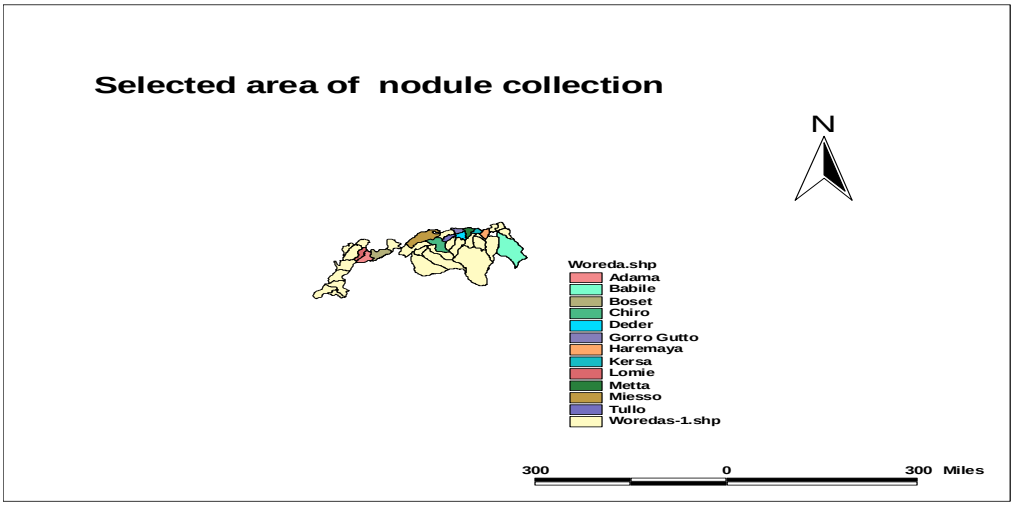


Figure 1. Location map of collected site

Table 1. The area (woreda and Kebele) of nodules collection

Isolates	Woreda	Kebele	Major reference site	Zone
NSPVR 1	Lome	Tede	Adama area	Eastern shewa
NSPVR2	Adama	Adama	"	“
NSPVR3	Adama	Adulala Ate Aroerti	"	“
NSPVR4	Adama	Awash Melka	"	“
NSPVR5	Adama	Melkasa	"	“
NSPVR6	Bost	Kilo Degaga	Boffa area	“
NSPVR7	Bost	Kelechacule and Duga	"	“
NSPVR8	Bost	Sarareda	"	“
NSPVR9	Bost	Arareso Bero	"	“
NSPVR10	Bost	Arareso Bero	"	“
NSPVR11	Bost	Kelechacule and Duga	"	“
NSPVR12	Bost	Kelechacule and Duga	"	“
NSPVR13	Nazereth	Kurfana Soloke	Adama-Meisso area	“
NSPVR14	Nazereth	Debisa	"	“
NSPVR15	Nazereth	Guraja Minmama	"	“
NSPVR16	Bost	Dengura Teyo	"	“
NSPVR17	Deyma	Asebo	"	“
NSPVR18	Mieaso	Mieso	Meisso-Chiro area	Western Hararghe
NSPVR19	Mieaso	Gulfa	"	“
NSPVR20	Chiro	Chiro Kela	Chiro-Tulo area	“
NSPVR21	Chiro	Keliso	"	“
NSPVR22	Chiro	Keliso	"	“
NSPVR23	Chiro	Alberekti	"	“
NSPVR24	Chiro	Akachebsa	"	“
NSPVR25	Chiro	Fanan Dimo	"	“
NSPVR26	Chiro	Shola	"	“
NSPVR27	Chiro	Efabas	"	“
NSPVR28	Ana Tulo	Debeso	"	“
NSPVR29	Chefe	Ourgi Dimtu	"	“
NSPVR30	Tulo	Terkam Feta	"	“
NSPVR31	Tulo	Terkam Feta	"	“
NSPVR32	Tulo	Lubu Dekem	"	“
NSPVR33	Tulo	Ouda Nega	"	“
NSPVR34	Tulo	Efabas	"	“
NSPVR35	Tulo	Afan Zic	"	“
NSPVR36	Tulo	Eimde Missoma	"	“
NSPVR37	Tulo	Edo Bas	"	“
NSPVR38	Alemaya	Fendisha	Alemaya area	Eastern Hararghe
NSPVR39	Alemaya	Awedai	"	“
NSPVR40	Alemaya	Gendeja	"	‘’
NSPVR41	Alemaya	Efabate	"	“
NSPVR42	Alemaya	Tineka	"	“
NSPVR43	Eide Teyara	Sibelu	"	“
NSPVR44	Kombolcha	Erer Guda	"	“
NSPVR45	Babile	Erer Guda	Babille area	“
NSPVR46	Alemaya	Adala Keke	Alemaya area	“
NSPVR47	Alemaya	Ware	"	“
NSPVR48	Kersa	Golawachu	Kersa a rea	“
NSPVR49	Kersa	Metekoma	"	“
NSPVR50	Kersa	Gale Mirga	"	“
NSPVR51	Kersa	Lange	Lange-Hirna area	“
NSPVR52	Kersa	Ofanse	"	“
NSPVR53	Meta	Kulebi	"	“
NSPVR54	Meta	Chelnko	"	“
NSPVR55	Meta	Chelenko Hara	"	“
NSPVR56	Meta	Chelenko Hara	"	“
NSPVR57	Deder	Welta Geba	"	Western Hararghe
NSPVR58	Deder	Kobo	"	“
NSPVR59	Goro Gutu	Wedai	"	“
NSPVR60	Goro Gutu	Sherbe	"	“
NSPVR61	Goro Gutu	Abti Hati	"	“
NSPVR62	Goro Gutu	Edo Jalella	"	“

4.3. Seed Samples

The seeds of variety, Awash Melka with white seeds (export variety) and Ayenew variety (highly produced in Hararghe areas for food purpose) were provided by Melkassa Agricultural Research Center.

4.4. Gram staining test

All isolates were tested in gram stain for rapid means of identification of gram-positive contaminants as indicated in Lupwayi and Haque, (1994).

4.5. Growth on Peptone-glucose medium

All isolates were streaked on yeast-mannitol agar plus 25ppm congo red (YEMA-CR), and glucose-peptone agar plus 25ppm bromocresol purple (Somasegaran and Hoben, 1994).

4.6. Designation of isolates

All isolates were designated as NSPVR (National Soil *Phaseolus vulgaris Rhizobium*), with different in numbers for each strains as indicated in table 1.

4.7. Authentication of rhizobia and preliminary screening of symbiotic effectiveness on sand culture

All the purified isolates were screened for infectivity and effectivity using 5Kg capacity pots containing 3Kg of sterilized and nitrogen-free sand. Plastic pots were surface sterilized with 95% ethanol whereas the river sand was sterilized with concentrated sulphuric acid (H_2SO_4) and then again using autoclave as indicated in Lupwayi and Haque, (1994).

Five surface sterilized and pre-germinated seeds (Awash Melkassa variety) were transferred into the pots, which were latter (after a week of planting) thinned to three seedlings per pot.

Each seedling was inoculated with 1ml of each isolate with an inoculum size of 10^9cell/ml. As controls, each block contains two pots negative control with no chemical fertilizer (no KNO_3) and inoculum (Con-) and positive control with chemical fertilizer (Con+) (0.05% KNO_3). All pots were fertilized with quarter strength of Broughton and Dilworth N-free medium described in Somasegaran and Hoben (1994). The experiment was statistically laid out with three replications using a Complete Random design in the National Soil Research Center's green house.

The plants were harvested eight weeks after inoculation and the following measurements were made: number and dry weight of nodules, above ground dry weight and percentage nitrogen of the host plants. A strain was arbitrarily rated very effective (VE) when the dry matter yield of the associated host was higher than the total mean of all strains plus the standard deviation (more than 0.83); effective (E) when its yield was between that of the mean $\pm$ the standard deviation (between 0.83-0.52) and ineffective (I) when its yield was smaller than the mean minus the standard deviation (less than 0.52) described in Lalande *et al* ., (1990).

Table 2. N-free Nutrient Solution (Broughton and Dilworth, 1970)

Stock Solution	**Chemical**	**g/liter**
1	$CaCl_2 \cdot 2H_2O$	294.1
2	KH_2PO_4	136.1
3	$FeC_6H_5O_7 \cdot 3H_2O$	6.7
	$MgSO_4 \cdot 7H_2O$	123.3
	K_2SO_4	87.0
	$MnSO_4 \cdot H_2O$	0.338
4	H_3BO_3	0.247
	$ZnSO_4 \cdot 7H_2O$	0.288
	$CuSO_4 \cdot 5H_2O$	0.100
	$CoSO_4 \cdot 7H_2O$	0.056
	$Na_2MoO_2 \cdot 2H_2O$	0.048

Taken from Somasegaran and Hoben (1994)

4.8. Colony morphology

Loop full of test isolates was inoculated by streaking plating into YEMA and PY medium and incubated at 28±2°C. They were checked after 5 days. Colony diameter and morphology were recorded as small dry, large mucoid and large watery according to Ahmed *et al.*, (1984)
In addition, peptone-glucose(PY) agar were used for determination of smooth gummy, creamy or rough appearance as indicated in Martinez-Romero *et al.*, (1991).

The component of PY-medium
Peptone----------5g
Yeast extract--------3g
$CaCl_2$----------1g
Agar----------15g
Distilled water—1000ml
PH adjusted to 6.8-7.0
Adopted from Silva *et al.*, (2003)

4.9. Generation time

Each isolate was streaked on YMA plates and single colony was transferred into test tubes containing 10ml Yeast Extract Mannitol Broth (YEMB), which were then inoculated, on a rotary shaker (125rev/min) at room temperature for 48hrs. One ml of cell suspension were transferred into 250ml Erlenmeyer flasks containing 100ml of YEMB and incubated on rotary shaker (125rev/min) at 28°C. Turbidity was measured every 6hrs at 540nm using spectrophotometer. Mean generation time or doubling time was calculated from the logarithmic phase (White, 1995).

4.10. Acid-Base production test

The ability of isolates to produce acid or alkaline in the medium was evaluated on YEMA containing bromothymol blue (BTB) (0.125%) as indicator. 48hrs old culture for each isolates was incubated on the medium for 3-7 days and observed for a color change as indicated in Alberton, *et al*., (2005).

4.11. Phosphorus solubilization efficiency

Rhizobial isolates were tested for synthetic tricalcium phosphate solubilization following the method of Alikhani *et al*., (2006).

The ingredients of the Basal Sperber agar medium (gm/liter) were as follows:

Glucose----------10.0

Yeast extract--------0.5

$CaCl_2$----------0.1

$MgSO_4.7H_2O$-------0.25

$Ca_3 (PO_4)_2$----------2.5

Agar----------15.0

All isolates were tested with three replications. Inoculated plates were incubated at 28 ± 2^oC and the diameter of clear zone (halo) as well as the diameter of colony was measured.

4.12. Biochemical and physiological tests

All tests were carried out in triplicate. Before incubation, isolates were grown on YEMB to 10^9cells/ml. When test plates were used, inoculation was performed with 30ul of these cultures. The results were scored after 5 days of incubation at 28 ± 2^oC unless stated otherwise (Somasegaran and Hoben 1994).

Temperature tolerance

To verify the tolerance to high temperatures a loop of each bacterium was streaked on triplicate plates containing TY agar medium and allowed to grow at 37 and 40^{0}C as indicated Hungria *et al.*, (2000). While, growth of isolates was also detected at 4, 10, 15, 20 and 45^{o}C on YEMA medium (Jordan, 1984).

Salt tolerance

It was determined on TY agar medium plates containing different concentration of salt (0%, 0.5%, 1%, 1.5%, and 2%... up to 10% NaCl) as described by Bernal and Graham, (2001).

pH tolerance

Tolerance to extreme pH was tested on TY agar medium set at 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, and 10.5 pH values as indicated by Bernal and Graham, (2001).

Carbon and nitrogen sources utilization

To test for carbohydrate utilization, the different carbon sources; L-arabinose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, raffinose, L-rhamnose, D-sorbitol, trehalose, xylose, dulcitol, glycerol, starch, citrate, tartarate, lactate, and glucuonic acid were added at a final concentration of 1g/liter to a basal medium containing (per liter) 1g of K_2HPO_4, 1g of KH_2PO_4, 0.01g of $FeCl_3.6H_2O$, 0.2g of $MgSO_4.7H_2O$, 0.1g of $CaCl_2$, 1g of $(NH_4)_2SO_4$ and 15g of agar.

Different amino acids utilization; L-arginine, L-alanine, L-isoleucine, L-aspargine, L-glutamine, L-leucine, L-lysine, L-phenylalanine, Glycine, L-valine, L-tryptophan and L-tyrosine When substrates were tested as nitrogen sources, they were added at a concentration of 0.5g/liter to a similar basal medium from which ammonium sulfate had been omitted and to

which mannitol had been added at a concentration of 1g/liter. Substrates were sterilized by filteration with pore size of 0.22um as indicated in Amarger *et al.*, (1997).

Intrinsic antibiotic resistance

Resistance to low concentrations of antibiotics was determined by preparing the fresh solutions of filtered sterilized (0.22um) antibiotics in YMA medium to give the following concentrations (ug ml^{-1}): Kanamycin sulfate, 5 and 15; novobiocine, 0.5 and 1.5; streptomycin sulfate, 2.5 and 10; spectinomycin, 2.5 and 5; naldixic acid, 40 and 60; and tetracycline 0.1 and 0.2 as described by Amarger *et al.*, (1997).

Kanamycin was dissolved in water; Streptomycin was dissolved in ethanol and Naldixic acid was dissolved in 1M NaOH as indicated in Somasegaran and Hoben (1994).

Growth on LB and PY-Ca media

All the isolates were tested for growth on Luria broth (LB) and Peptone yeast extract minus Calcium (PY-Ca) media described in Martinez-Romero *et al.*, (1991).

The ingredients of the Luria-Bertani medium (LB) agar medium were as follows:

Tryptone	10g
Yeast extract	5g
NaCl	5g
Agar	15g

Distilled water-1000ml

Growth on YEMA with 2%Urea

All isolates were tested on yeast extract mannitol agar (YEMA) containing 2% urea described in Andrade *et al.*, (2002).

Detection of Melanin production

Synthesis of melanin was evaluated in TY agar medium supplemented with 1.2mg/ml of L-tyrosine and 40ug/ml of Copper sulphate ($CuSO_4$) as indicated Hungria *et al*., (2000). Production of melanin was detected by the formation of black pigment.

4.13. Screening for host range test

Cross-inoculation ability of rhizobia strains was tested on *Leucaena leucocephala* under greenhouse in pouches experiment as indicated in Martinez-Romero *et al*., (1991). Prior to germination, seeds of *Leucaena leucocephala* were treated for 20 min in concentrated sulphuric acid (98%) and rinsed successively with water. The germination was carried out in Petri dish containing filter paper and water. The well-germinated seeds were inoculated with 1ml of rhizobial cultures containing 10^9cells/ml. The experiment was laid out in three replication with negative control with out inoculation. All pouches were fertilized with quarter strength of Broughton and Dilworth N-free medium.

4.14. Soil for pot experiment

Soils samples for pot experiment were taken from Melkassa and Babille Agricultural Research Center to estimate density of indigenous rhizobia and for symbiotic competency studies.

Soil nitrogen analysis

Modified kjeldahl method was used to determine soil total nitrogen following the manual produced by Sahlemedhin Sertsu and Taye Bekele, (2000).

Soil available phosphorus analysis

Olsen method was used to determine soil available phosphorus following the manual produced by Sahlemedhin Sertsu and Taye Bekele, (2000).

Cation Exchange Capacity (CEC) analysis

The ammonium acetate method was used to determine CEC following the manual produced by Sahlemedhin Sertsu and Taye Bekele, (2000).

Soil organic carbon analysis

Soil organic carbon analysis was determined following the manual produced by Sahlemedhin Sertsu and Taye Bekele, (2000).

4.14.1. Enumeration of rhizobia

The numbers of indigenous rhizobia present in the Melkassa and Babille soils, which could nodulate *Phaseolus vulgaris*, were estimated by the most-probable-number, plant infection technique as indicated by Somasegaran and Hoben (1994). Seeds were surface sterilized with 95% of ethanol and in 3% (v/v) solution of sodium hypochlorite. The seeds were successively rinsed in sterilized distilled water, allowed to germinate, and stored at 28+ 2oC on sterilized petri plates containing moistured filter paper. One healthy well-grown seedling with similar size and radicle length were transferred into growth pouches aseptically. After 5-7 days old, the pouches were reorganized on the rack.

Two varieties (Ayenew and Awash Melka) and two soils from Babille and Melkassa with four combinations, Ayenew with Babille; Ayenew with Melkassa; Awash Melka with Babille and Awash Melka with Melkassa were tested for estimation of numbers of indigenous rhizobia. For each combination, tenfold soil dilutions (10^{-1}-10^{-10}) and one control pouch following each group of inoculated pouch were used with four replicate pouches. After three weeks, nodulation was assessed and the number of rhizobia was calculated from the following formula:

m=Likely number from the MPN table for the lower dilution of the series
d=Lowest dilution (first unit used in the tabulation)

v=Volume of aliquot applied to plant

The MPN per gram of inoculant is:

$$X=\frac{m \times d}{v}$$

4.14.2 Symbiotic effectiveness test in soil

Symbiotic effectiveness of selected strains NSPVR-2, NSPVR-26, NSPVR-29, and NSPVR-31 was evaluated in pot experiment with two genotypes (Awash melka and Ayenew) on two different soils was conducted under greenhouse condition of the National Soil Research Center. Babille and Melkassa soils were air-dried, sieved (<2mm) and thoroughly mixed. All pots were fertilized with N (12.5 mg/kg soil), P (20.0mg as P_2O_5/kg soil) and K (10 mg K_2O/kg soil) in the form of urea, triple superphosphate and potassium chloride, respectively and Zinc, Molybdenum and Iron added at the rate of 5mg/kg soil in the form of $ZnSO_4$, $NaMoO_4$ and $FeSO_4$ and watered as recommended when needed (Somasegaran and Hoben 1994).

Seeds of common bean were surface sterilized using sodium hypochlorite and then rinsed several times in sterile water prior to sowing. Five seeds were sown in each pot and thinned to three plants one week after planting. Each seed was inoculated with 1ml of YMB grown 10^9cells/ml test isolates. The experiment was conducted in triplicates in green house mean minimum and mean maximum temperatures of 19 ± 2^oC and 30 ± 1^oC, respectively. Two controls, without inoculation and N-addition, and uninoculated with 0.05% (W/V) KNO_3 $week^{-1}$. The pots were arranged in CRD.

Eight week after planting, the plants were uprooted and their effectiveness was evaluated on the basis of nodule dry weight, nodule number and shoot dry weights after drying at 70^oC for 2days in oven. Then the result was compared with the controls.

4.15. Total nitrogen determination

Modified kjeldahl method was used to determine plant total nitrogen following the manual produced by Sahlemedhin Sertsu and Taye Bekele, (2000). First 0.2gm of ground samples were measured for analysis. A mixture 10 gm of K_2S0_4; 2gm of $CuS0_4$. $5H_20$ and 0.2gm of selenium were prepared as catalyst and 0.5gm of the mixture was added into ground sample. Successively 7ml of sulfuric-salicylic acid mixture were added and allowed to react for 30min. Then 0.5gm of $Na_2S_20_3$. $5H_20$ were added and shaked and allowed to react for 5 minutes. For blank 0.5 gm salt mixture and 7ml of sulfuric-salicylic mixture was prepared. Then after, digestion was undertaken with temperature of 380^0C for 3 hours till the color is clear. The digested sample was distilled by adding 40ml of 40% NaOH and received its nitrogen with in 20ml boric acid containing flask. The distillation was complete when its volume of distillate became 110ml. Finally, the distillates were titrated by 0.1N H_2SO_4, record the reading of the burette, and then calculated the nitrogen percentage by the following formula:

$$\text{Nitrogen (\%)} = \frac{(V_1\text{-}V_2) \times N. \times 0.014 \times 100}{S}$$

Where:

V_1 = m1 of titrant used for the sample

V_2 = m1 of titrant used for the blank

N = Normality of the acid

S = Weight of the plant material, g

4.16. Numerical analysis

A computer cluster analysis of phenotypic variables was carried out using a similarity coefficient and a dendrogram was constructed by the unweighed pair group method with the average (UPGMA) clustering method using NTSYS-pc version 2.1.

4.17. Data Analysis

Comparison between treatments was analyzed by one-way ANOVA (Turkey's HSD tests) using GMP-5 software.

5. RESULT

5.1. Preliminary test of Purity

Seventy-two strains of root nodule bacteria were isolated from sampling sites in Eastern Shewa (17 isolates), Western Hararghe (19 isolates) and Eastern Hararghe (26 isolates) region. All isolates were gram negative and rod shaped bacteria with little or no absorption of congored grown on YEM-CR medium. Out of the 72 bacteria isolates, only ten isolates grew on peptone-glucose medium supplemented with 25ppm bromocresol purple.

5.2. Authentication of rhizobia

All isolates were reinoculated into the host on acid-treated, sterilized river sand to evaluate their ability to form nodules. Consequently, 62 of the tested isolates formed nodules on the host; and hence were authenticated as root nodule bacteria.

5.3. Characteristics of the isolates

5.3.1. Morphology and Cultural characteristics

The diversity of the isolates was verified using colony diameter and colony morphology as large mucoid, large watery and small dry on YEMA medium (Table 4). 61 isolates (95%) of the tested isolates formed colonies diameter greater than 2.5mm; whereas isolate NSPVR-62 from Goro Gutu woreda formed colony diameter with 1.5mm. 77% of isolates displayed large mucoid and 20% characterized by large watery colony morphology. The remaining isolate, NSPVR-62 from Goro Gutu woreda was found to exhibit small dry colony morphology.

All except NSPVR-18, NSPVR-31, NSPVR-32, and NSPVR-57 showed smooth and gummy whereas the latter displayed rough colonies grown on PY-medium, respectively (Table 3).

5.3.2. Growth on YEMA-BTB and mean generation time (MGT)

Almost all isolates except isolate NSPVR-62 from Goro Gutu woreda produced acid on YEMA-BTB medium and did change the YEMA-BTB medium in to yellow. However, isolate NSPVR-62 from Goro Gutu woreda produced alkaline on YEMA-BTB medium and changed the medium color into blue.

On the basis of their generation time, isolates displayed different doubling times ranging from 0.6-6hrs (Table 3). Among the isolates, 66% showed the fastest growth with doubling time less than 1.9hr and NSPVR-62 isolate from Goro Gutu woreda showed slow growing isolate with doubling time 6hr. The doubling time of 32% of the isolates were displayed between 2.5 and 4.3hr categorized as fast growing isolates.

5.4. Growth characteristics of isolates on other media

From the 62 tested isolates, 79 % were able to grow on LB-medium and 83% grew on TY-Ca medium. Eight isolates, NSPVR-2, NSPVR-3, and NSPVR-13 from Eastern Shewa; NSPVR-27, NSPVR-29, NSPVR-59, and NSPVR-60 from Western Hararghe; and NSPVR-48 from Eastern Hararghe were unable to grow on both LB and TY-Ca media. Seven isolates, NSPVR-12 from Bost woreda; and NSPVR-17, NSPVR-18, NSPVR-19, NSPVR-31, NSPVR-32, NSPVR-57 from Western Hararghe were able to grow on PY-Ca medium but not on LB-medium. All isolates grew on YEMA containing 2% Urea medium. Similarly, the data indicated that 68% of the tested isolates produced dark color on TY medium supplemented with tyrosine and $CuSO_4$ (Table 3).

Table 3. Growth characteristics of rhizobia nodulating common bean.

Tested isolates	LB-Medium	TY-Ca Medium	Melanin Production	YMA 2% Urea	Phosphorus solubilization ratio (x)	Mean generation time (hrs)	Colony morphology	Colony diameter (mm)	Colony texture on PY-medium
NSPVR 1	+	+	+	+	0.1	3.40	LM	5.0	gummy, smooth
NSPVR2	-	-	-	+	-	0.75	LM	4.5	gummy, smooth
NSPVR3	-	-	-	+	-	3.33	LM	3.0	gummy, smooth
NSPVR4	+	+	-	+	-	1.20	LW	6.0	gummy, smooth
NSPVR5	+	+	+	+	-	0.80	LM	5.5	gummy, smooth
NSPVR6	+	+	+	+	-	0.97	LM	5.5	gummy, smooth
NSPVR7	+	+	-	+	-	1.60	LM	5.5	gummy, smooth
NSPVR8	+	+	+	+	1.22	0.93	LM	4.0	gummy, smooth
NSPVR9	+	+	+	+	-	0.60	LM	5.0	gummy, smooth
NSPVR10	+	+	-	+	-	3.23	LM	4.0	gummy, smooth
NSPVR11	+	+	+	+	1.31	1.05	LM	4.0	gummy, smooth
NSPVR12	-	+	+	+	-	0.92	LM	2.5	gummy, smooth
NSPVR13	-	-	-	+	0.45	3.75	LM	2.5	gummy, smooth
NSPVR14	-	-	+	+	-	0.98	LM	2.5	gummy, smooth
NSPVR15	+	+	+	+	-	4.20	LW	6.0	gummy, smooth
NSPVR16	+	+	+	+	-	3.00	LW	6.0	gummy, smooth
NSPVR17	-	+	-	+	-	1.50	LM	4.5	gummy, smooth
NSPVR18	-	+	-	+	-	1.20	LM	4.0	rough
NSPVR19	-	+	+	+	-	1.33	LM	2.5	gummy, smooth
NSPVR20	+	+	+	+	0.57	1.00	LM	5.0	gummy, smooth
NSPVR21	+	+	+	+	-	0.70	LM	5.0	gummy, smooth
NSPVR22	+	+	-	+	-	1.07	LM	5.0	gummy, smooth
NSPVR23	+	+	+	+	0.32	3.08	LM	5.5	gummy, smooth
NSPVR24	+	+	-	+	0.12	3.00	LM	3.5	gummy, smooth
NSPVR25	+	+	+	+	0.12	3.30	LM	4.0	gummy, smooth
NSPVR26	+	+	-	+	-	4.17	LM	4.0	gummy, smooth
NSPVR27	-	-	+	+	-	3.68	LM	3.5	gummy, smooth
NSPVR28	+	+	-	+	-	4.20	LM	2.5	gummy, smooth
NSPVR29	-	-	+	+	0.56	3.83	LM	4.0	gummy, smooth
NSPVR30	+	+	+	+	-	0.98	LW	6.0	gummy, smooth
NSPVR31	-	+	+	+	-	3.17	LM	2.0	rough
NSPVR32	-	+	+	+	0.46	1.28	LM	4.0	rough
NSPVR33	+	+	-	+	0.36	4.25	LM	5.5	gummy, smooth
NSPVR34	+	+	+	+	-	0.93	LW	6.0	gummy, smooth
NSPVR35	+	+	+	+	0.55	1.35	LM	2.5	gummy, smooth
NSPVR36	+	+	+	+	0.11	0.67	LW	6.0	gummy, smooth

NSPVR37	+	+	+	+	0.14	0.90	LM	5.5	gummy, smooth
NSPVR38	+	+	+	+	0.60	0.85	LM	3.0	gummy, smooth
NSPVR39	+	+	-	+	0.45	0.73	LW	6.0	Gummy, smooth
NSPVR40	+	+	+	+	-	0.95	LM	4.5	Gummy, smooth
NSPVR41	+	+	+	+	0.34	0.98	LM	5.0	Gummy, smooth
NSPVR42	+	+	+	+	0.60	1.35	LW	6.0	gummy, smooth
NSPVR43	+	+	-	+	0.12	1.50	LM	5.5	gummy, smooth
NSPVR44	+	+	-	+	-	0.85	LW	6.0	gummy, smooth
NSPVR45	+	+	+	+	-	0.87	LM	4.0	gummy, smooth
NSPVR46	+	+	+	+	0.14	1.78	LW	6.0	gummy, smooth
NSPVR47	+	+	-	+	-	1.20	LM	5.0	gummy, smooth
NSPVR48	-	-	+	+	-	0.95	LM	3.5	gummy, smooth
NSPVR49	+	+	+	+	-	3.12	LM	5.5	gummy, smooth
NSPVR50	+	+	+	+	0.16	0.90	LW	6.5	gummy, smooth
NSPVR51	+	+	+	+	-	1.33	LM	5.0	gummy, smooth
NSPVR52	+	+	+	+	0.45	3.00	LM	5.0	gummy, smooth
NSPVR53	+	+	-	+	0.39	0.47	LW	6.5	gummy, smooth
NSPVR54	+	+	+	+	-	0.87	LM	4.5	gummy, smooth
NSPVR55	+	+	-	+	0.18	1.17	LM	5.5	gummy, smooth
NSPVR56	+	+	+	+	-	1.20	LM	4.0	gummy, smooth
NSPVR57	-	+	+	+	-	3.62	LM	4.0	rough
NSPVR58	+	+	+	+	-	3.00	LM	5.5	gummy, smooth
NSPVR59	-	-	+	+	0.32	0.95	LM	3.0	gummy, smooth
NSPVR60	-	-	+	+	0.23	2.57	LM	3.5	gummy, smooth
NSPVR61	+	+	-	+	-	1.38	LM	3.0	gummy, smooth
NSPVR62	+	+	+	+	-	6.00	SD	1.5	gummy, smooth
% of isolates tolerated/ grown.	77%	83%	68%	100%					

Key: (+): Presence of Growth; (-): Absence of Growth; x-diameter of halo zone /the diameter of colony

SD-Small dry
LM-Large mucoid
LW-Large watery

5. Biochemical and physiological test

Salt tolerance

The isolates showed differences to grow on YEMA adjusted to different NaCl concentration (Figure 2). All isolates grew at 0.5%NaCl concentration, whereas three of the isolates, NSPVR-4, NSPVR-24, and NSPVR-58 from Adama, Chiro, and Deder woreda respectively were found to be highly tolerant up to 10% NaCl. Similarly, 71%, 52% and 5% of the isolates tolerated salt concentration of 3.5%, 8%, and 9% respectively. The most sensitive isolates less than 1%NaCl constituted 16% of all the tested isolates. These were NSPVR-2, NSPVR-3, NSPVR-12, NSPVR-13, NSPVR-14, NSPVR-17, NSPVR-18, and NSPVR-19, from East Shewa zone, NSPVR-27, NSPVR-29, and NSPVR-62 from Western Ethiopia Zone and NSPVR-48 from Kersa woreda of all the isolates tested.

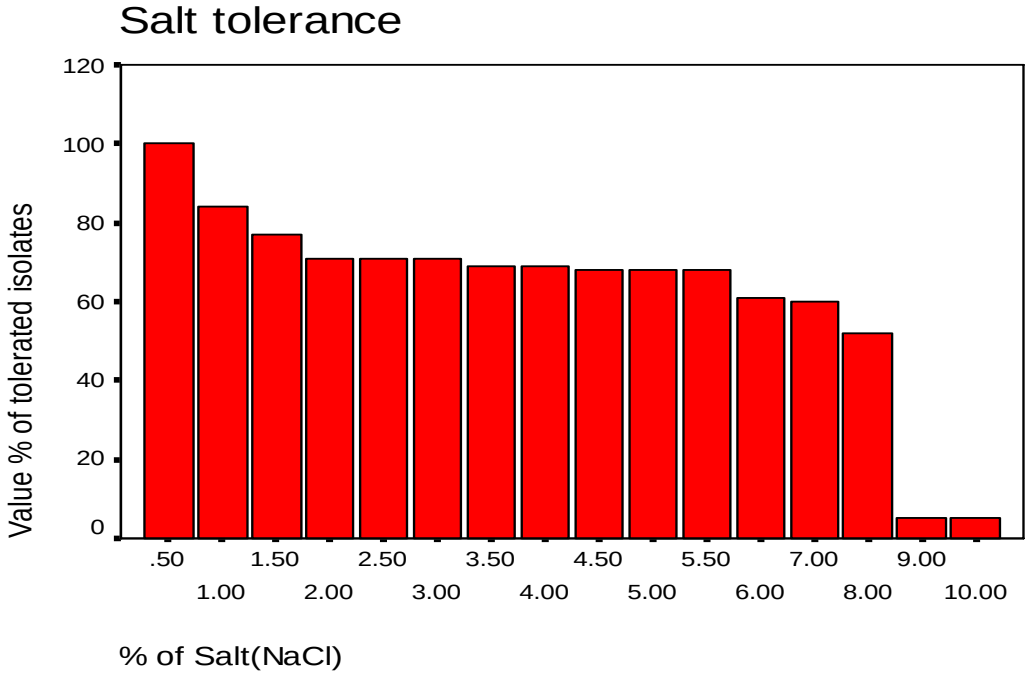


Figure 2.Tolerance of rhizobia nodulating common bean at different concentration of salts.

Temperature tolerance

Almost all tested isolates grew between 15^0C and 30^0C incubation, whereas 55% of isolates were able to survive at 37^0C. Isolates NSPVR-4 from Adama woreda and NSPVR-24, NSPVR-25, and NSPVR-27 from west Hararghe managed to survive at 45^0C. Likewise, 39% of the isolates were found to tolerate and grow at a temperature of 5^0C (Figure 3).

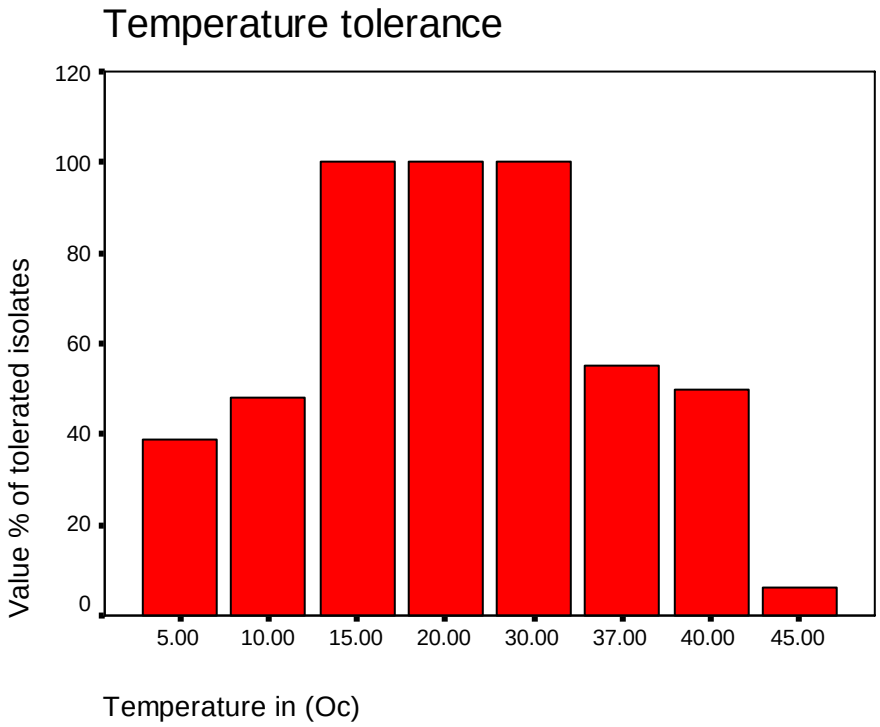


Figure 3. Tolerance of rhizobia nodulating common bean at different range of temperatures.

pH tolerance

Resistance to grow at different pH was found to vary amongst the test isolates. All tested isolates (100%) grew in between slightly acid pH 5.5 and alkaline pH 8.5 interval. 24% of isolates were adapted to grow at pH 4.5, whereas 71% of the isolates managed to grow at pH 10.00. None of isolates grew at pH-4.0. However, isolates that tolerate pH 4.5 like NSPVR-12, NSPVR-14, NSPVR-17 from East Shewa and NSPVR-18, NSPVR-29, NSPVR-57, and NSPVR-61 from West Hararghe were found to be sensitive to alkaline pH greater than 8.5(Figure 4).

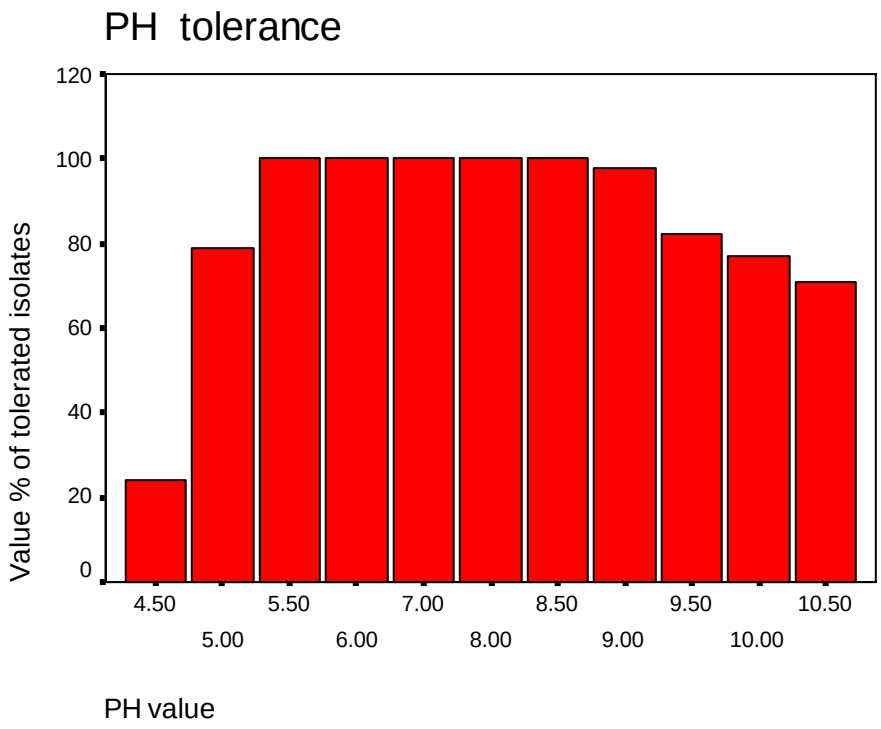


Figure 4. Tolerance of rhizobia nodulating common bean at different pH range.

Amino acid utilization

Almost all of the isolates were able to catabolize a large variety of nitrogen substrates (Table 4). All isolates were utilized L-tyrosine, L-aspargine, and L-arginine, and more than 90% of isolates grew on L-tryptophan, L-phenylalanine, L-lysine, L-leucine, L-glutamine. 89% of them grew on L-valine, L-leucine, L-lysine, and alanine. Likewise, glycine was found to be utilized by 87% of tested isolates.

Carbohydrate utilization

Almost all of the rhizobia were able to catabilize a large variety of carbon substrates (Table 4). All isolates grew on D-glucose, D-fructose, xylose, malate, eructose, and 90% of isolates were grown on lactose, D-glucoinic acid, raffinose, mannose, cellobiose, galactose, L-rhaminose, sorbitol, glycerol and trehalose. More than 80% of the isolates were found to utilize starch.

With regard to the remaining carbohydrates such as tartarate, citrate, L-arabinose, and dulcitol were displayed diversity of tested isolates. As tartarate, citrate, and dulcitol were metabolized by 69%, 61%, and 53% respectively; only 37% and 13% managed to utilize L-arabinose and dextrin respectively.

Rhizobial isolates NSPVR-3 from Adama woreda; NSPVR-26 from Chiro woreda; NSPVR-48 and NSPVR-56 from Eastern Hararghe; and NSPVR-62 from Goro Gutu woreda utilized 95% of the tested carbohydrates whereas, NSPVR-17 from Deyima woreda; NSPVR-23 and NSPVR-27 from Chiro woreda; NSPVR-35 from Tulo woreda; and NSPVR-45 from Babille woreda were able to utilize less than 65% of the tested carbohydrates.

Table 4. Comparative analysis of rhizobia isolates for utilization of different carbohydrates and amino acid

Amino acid	**% of isolates utilized the tested amino acid**	**Carbohydrate**	**% of isolates utilized the tested carbohydrates**
L-tyrosine, L-aspargine, L-arginene	100%	D-glucose, D-fructose, xylose, Eructose, Malate	100%
L-tryptophan, L-phenylalanine, L-lysine, L-leucine, L-glutamine	98-90%	Lactose, D-glucoinic acid, Raffinose, Mannose, lactate, trehalose, glycerol, sorbitol, L-rhaminose, galactose, cellobiose	98-90%
Glycine, L-valine, L-isoleucine, L-alanine	85-89%	Starch Citrate, tartarate Dulcitol L-arabinose Dextrin	82% 60-70% 53% 37% 13%

Intrinsic antibiotic resistance

The different isolates displayed a large spectrum of antibiotic resistance on the different types and concentration of antibiotics (Figure 5). Almost all isolates exhibited resistant to Novobiocin and Spectinomycin of all tested concentration and types of antibiotics except NSPVR-27 and NSPVR-60, and NSPVR-27 and NSPVR-46 that were inhibited by both antibiotic, respectively followed by high concentration of Oxytetracycline and lower concentration of Naldixic acid. Hence, almost 50% of the isolates were found to be tolerate to different concentrations of Streptomycin followed by almost 40% of the isolates were sensitive for Kanamycin.

Twenty five percent of the isolates were found to be tolerate to all tested antibiotics and 25% of the isolates were found to be tolerate to almost 80% of the tested antibiotic. Isolate NSPVR-27 from Chiro woreda was observed to be the most sensitive strain capable of growing on only Streptomycin followed by NSPVR-30 and NSPVR-32 from Tulo woreda; NSPVR-46 from Alemaya woreda; and NSPVR-57 from Deder woreda. Streptomycin and Kanamycin were found to be the most potent antibiotics that allow the growth of a few isolates.

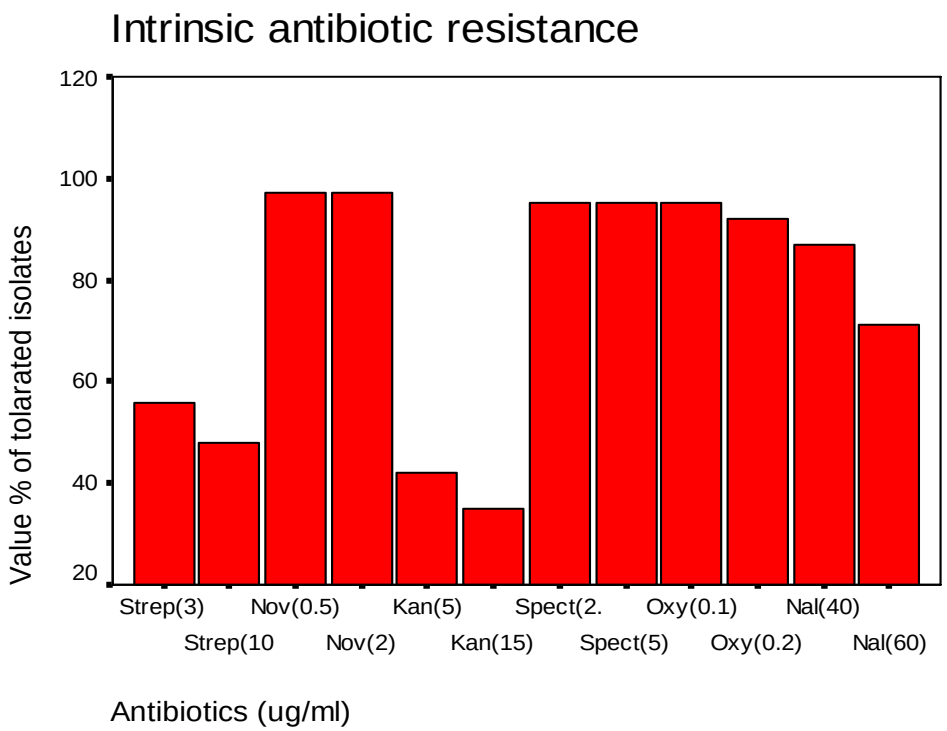


Figure 5. Tolerance of rhizobia nodulating common bean of different antibiotics

5.6. Cross inoculation test

All isolates were tested for cross-inoculation on *Leucaena leucocephala* under greenhouse condition on pouch experiment. None of the isolates were able to nodulate the tested plant.

5.7. Phosphorus solubization in the Solid Sperber medium

From the 62 isolates, 42% were able to solubilize tricalcium phosphate (Table 3). Of which, only NSPVR-8 and NSPVR-11 both from Bost woreda were able to produce the ratio diameter of halo zone to colony diameter of 1.22 and 1.31, respectively. The remaining 26 isolates phosphorus solubilizing microorganisms were produced the ratio value between 0.1 and 0.6.

5.8. Numerical analysis

About 92 parameters were considered for the cluster analysis using UPGMA method (Figure 6). Two big clusters were formed on the basis of 75% level of relative similarity. Cluster-I included 37 isolates with four separated sub-clusters in which they were separated at 82%, 83%, 85%, and 89% level of relative similarity.

Cluster-II contains 25 isolates and displayed two sub-clusters at 80% level of relative similarity. Of which NSPVR-62 isolate from Goro Gutu woreda that displayed unique characteristics and separated from cluster-II at 83% level of relative similarity, and isolate NSPVR- 27 from Chiro woreda from cluster-II separated at 84% level of relative similarity.

NSPVR-31, NSPVR-32, and NSPVR-18, NSPVR-57 all from Western Hararghe separated from cluster-I at 83% level of relative similarity and cluster-II at 78% level of relative similarity respectively. Generally, even though most of the isolates are similar at 80% level, this cluster analysis indicated that there is phenotypic diversity of rhizobia that nodulate common bean in Eastern Ethiopia.

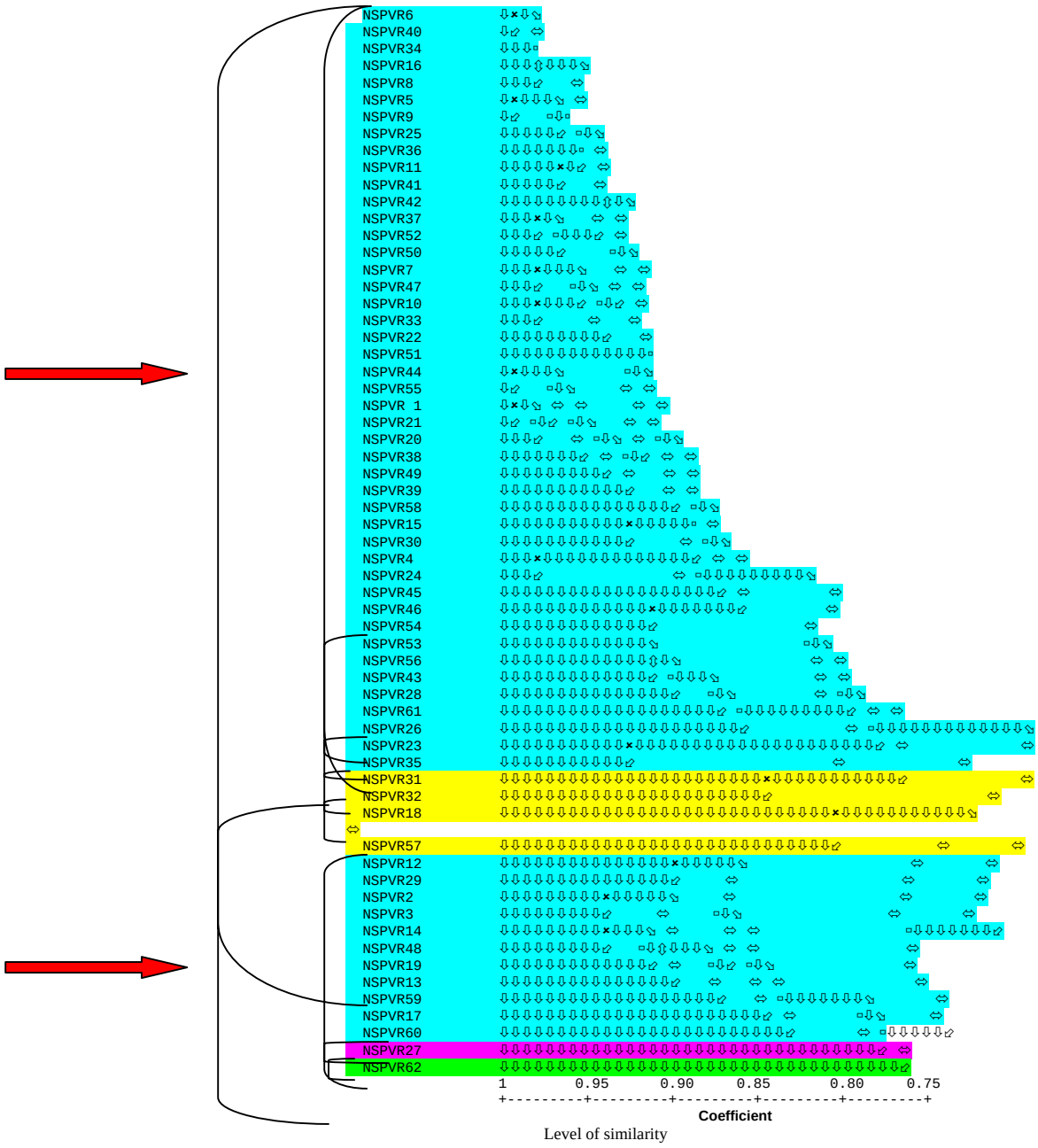


Figure 6. Dendrogram highlighting the phenotypic similarities among the isolates.

5.9. Enumeration of rhizobia

The plant infection tests were under taken to evaluate the most probable number (MPN) density of indigenous population of rhizobia at Melkassa and Babille soils on Ayenew and Awash Melka varieties of common bean. This experiment indicated that number of indigenous rhizobia greatly varied between the two soils. Consequently, Melkassa soil was found to contain 10^8 rhizobia/gm of soil; whereas Babille soil harbored only 10^3 rhizobia/gm of soil estimated from both common bean varieties (Table 5).

Table 5. Number of indigenous rhizobia from Melkassa and Babille soils.

Varieties	**Number of rhizobia in(number of cell/ gm of soil)**	
	Melkassa Soil	**Babille Soil**
Ayenew	$1.7x10^8$	$1.7x10^3$
Awash Melka	$5.9x10^8$	$1x10^3$

5.10. Soil chemical and physical properties

As Table 6. indicated Melkassa and Babille soils had similar soil chemical properties. These are: total nitrogen, organic carbon, C/N ratio, available phosphorus, and base saturation. Likewise, Melkassa and Babille soils had neutral pH. Nevertheless, Melkassa soil had higher electron conductivity (EC) and CEC than Babille soil. With regard to soil physical property, Melkassa and Babille soils had loamy and sandy loam soil respectively.

Table 6. Chemical and physical analysis of selected soils.

Parameters	Babille	Melkassa
PH	6.8	7.6
EC (ds/m)	0.044	0.117
Total nitrogen (%)	0.07	0.08
Organic carbon (%)	0.73	0.85
C/N	10	11
Available phosphorus(ppm)	11.5	13.48
Na Cmol (+)/kg	0.02	0.28
K Cmol (+)/kg	0.36	2.8
Ca Cmol (+)/kg	5.44	15.32
Mg Cmol (+)/kg	1.69	2.8
CEC Cmol (+)/kg	8.0	23.2
Base saturation (%)	94	91
Texture class	Sandy loam	Loamy

5.11. Preliminary screening of symbiotic effectiveness on sand culture

Sand culture study of nodulating Awash Melka variety showed variation in shoot dry weight, nodule number, nodule weight and plant total nitrogen contents among the treatments at $p<0.01$. Based on the relative plant dry matter accumulation of inoculated plants with nitrogen-fertilized control (Lalande *et al*., 1990), 10% (6 isolates) were as very effective, 79% (49 isolates) effective, and 11% (7 isolates) were ineffective. With regard to site of collection, all isolates from Eastern Shewa found to be effective, while 20% of Western Hararghe isolates; NSPVR-22, NSPVR-25, NSPVR-27, and NSPVR-28 from Chiro woreda and NSPVR-30 from Tulo woreda and 10% of Eastern Hararghe isolates; NSPVR-41 and NSPVR-48 from Alemaya and Kersa woreda were poorly effective (Table 7).

Four isolates NSPVR-2, from Adama woreda; NSPVR-26, from Chiro woreda; NSPVR-29, from Tulo woreda and NSPVR-31 from Tulo woreda together with the nitrogen fertilized plants displayed the highest shoot dry matter of the other isolates. Of which, NSPVR-29 accumulated the highest shoot dry weight (1.13gm/plant) followed by NSPVR-26 (1.1gm/plant), NSPVR-2 (1.09gm/plant) and NSPVR-31 (1.09gm/plant). The least performance was recorded by NSPVR-22 (0.39gm/plant) from Chiro woreda and NSPVR-41 (0.40gm/plant) from Alemaya woreda.

Isolates NSPVR-12 from Bost woreda; and NSPVR-37 from Tulo woreda showed significantly ($p<0.01$) higher plant nitrogen contents than uninoculated plants and exhibited statistically similar plants nitrogen contents with nitrogen fertilized. The plant nitrogen contents recorded ranged from 1.40%N/gm of plant sample for isolate NSPVR-38 to 2.63% N/gm of plant sample for isolate NSPVR-12.

Three isolates NSPVR-12, from Bost woreda; NSPVR-17, from Deyma woreda and NSPVR-37, from Tulo woreda showed the highest nodule number and nodule dry weight than other isolates tested. (Table 7). The nodule number recorded from 21/plant for isolate NSPVR-61 to 136/plant for isolate NSPVR-37. Similarly, nodule dry weight recorded from 0.041gm/plant for isolate NSPVR-36 to 0.215gm/plant for isolate NSPVR-37.

Table 7. Nodulation data of collected isolates of common bean rhizobia with variety Awash Melka

Isolates	Nodule number	Nodule weight	Shoot dry weight	Plant total nitrogen (%N/gm of plant sample)	SE(%)	Effectiveness
Control(-)	0x	0s	0.398±0.091b	1.43±0.036c-d	39	
Control(+)	0x	0s	0.98±0.115ab	2.28±0.071a-d	100	
NSPVR 1	91±8.5d-l	0.096±0.005c-r	0.706±0.293ab	1.66±0.164 a-d	72	E
NSPVR2	124±12.5a-e	0.099±0.023c-r	1.09±0.086a	1.86±0.127 a-d	111	VE
NSPVR3	67±16.5g-u	0.082±0.007e-r	0.72±0.156ab	1.8±0.355 a-d	73	E
NSPVR4	109±8.5a-g	0.12±0.013b-j	0.51±0.165ab	2.32±0.33 a-d	52	E
NSPVR5	31±17.1t-x	0.044±0.008q-s	0.543±0.275ab	2.01±0.011 a-d	55	E
NSPVR6	112±5a-f	0.127±0.009b-i	0.73ab	2.13±0.202 a-d	74	E
NSPVR7	37±14.73s-x	0.054±0.001k-s	0.59±0.23ab	1.88±0.08 a-d	60	E
NSPVR8	107±8.6a-h	0.131±0.005b-g	0.5±0.07ab	2.32±+0.4 a-d	51	E
NSPVR9	77±5f-s	0.084±0.017e-r	0.633±0.265ab	2.11±0.12 a-d	54	E
NSPVR10	23±6.5v-x	0.048±0.015n-s	0.533±0.123ab	1.64±0.13b-d	54	E
NSPVR11	47±28.6m-w	0.05±0.009n-s	0.68±0.311ab	1.92±0.176 a-d	69	E
NSPVR12	150±8.9a	0.149±0.013b-d	0.643±0.051ab	2.63±0.842a	66	E
NSPVR13	54±3.6k-w	0.065±0.005i-r	0.887±0.171ab	2.39±0.28a-c	90	VE
NSPVR14	34±2.6s-x	0.053±0.007j-s	0.543±0.102ab	2.12±0.058 a-d	55	E
NSPVR15	70±3.2f-t	0.061±0.042l-s	0.765±0.375ab	2.26±0.207 a-d	78	E
NSPVR16	95±5c-k	0.072±0.006g-r	0.67±0.141ab	1.85±0.11 a-d	68	E
NSPVR17	149±8.6a	0.169±0.007a,b	0.68±0.215ab	1.97±0.07 a-d	69	E
NSPVR18	88±27d-n	0.111±0.01b-n	0.636±0.058ab	1.95±0.089 a-d	66	E
NSPVR19	40±3q-x	0.051±0.013m-s	0.79±0.072ab	2.14±0.226 a-d	81	E
NSPVR20	90±8.7d-m	0.11±0.006b-o	0.677±0.068ab	1.46±0.051 a-d	69	E
NSPVR21	66±13.6g-u	0.144±0.077b-e	0.82±0.07ab	1.71±0.22 a-d	84	VE
NSPVR22	128±6a-d	0.124±0.007b-i	0.33ab	1.9±0.01 a-d	34	I
NSPVR23	108±7a-h	0.093±0.007d-r	0.623±0.092ab	1.84±0.14 a-d	64	E
NSPVR24	36±3.5s-x	0.116±0.007b-l	0.6±0.32ab	1.89±0.145 a-d	61	E
NSPVR25	92±10.6d-l	0.09±0.005d-r	0.48±0.028ab	1.82±0.037 a-d	49	I
NSPVR26	93±4.5c-k	0.117±0.009b-k	1.13±0.100a	1.62±0.256 b-d	115	VE
NSPVR27	141±9.1a,b	0.115±0.008b-l	0.487±0.074ab	1.98±0.028 a-d	50	I
NSPVR28	44±10.3o-w	0.071±0.005g-r	0.477±0.236ab	2.18±0.144 a-d	49	I
NSPVR29	111±34.8a-f	0.105±0.005c-q	1.11±0.095a	1.71±0.011 a-d	113	VE
NSPVR30	105±14.2b-i	0.112±0.01b-n	0.5±0.099ab	1.89±0.023 a-d	51	I
NSPVR31	50±17l-w	0.108±0.008b-p	1.09±0.046a	2±0.33 a-d	111	VE
NSPVR32	64±5.3i-v	0.102±0.004c-r	0.743±0.268ab	1.79±0.168 a-d	76	E
NSPVR33	37±2.1r-x	0.081±0.01f-r	0.643±0.229ab	1.81±0.045 a-d	66	E
NSPVR34	80±10f-r	0.11±0.008b-o	0.55±0.12ab	1.64±0.213b-d	56	E
NSPVR35	45±5n-w	0.106±0.006b-q	0.643±0.287ab	1.74±0.046 a-d	66	E
NSPVR36	43±1.5p-w	0.041±0.008r,s	0.75±0.02ab	1.65±0.204b-d	76	E
NSPVR37	136±9a-c	0.215±0.017a	0.71±0.385ab	2.43±0.682a,b	72	E

NSPVR38	83±8.5e-q	0.079±0.01g-r	0.567±0.153ab	1.4±0.07d	58	E
NSPVR39	89±7.1q-m	0.115±0.01b-l	0.68±0.182ab	1.78±0.276 a-d	69	E
NSPVR40	84±11.9e-p	0.09±0.01d-r	0.743±0.12ab	1.85±0.447 a-d	76	E
NSPVR41	70±6.5f-t	0.097±0.005c-r	0.4±0.187ab	1.72±0.141 a-d	41	I
NSPVR42	87±5.7d-o	0.143±0.011b-f	0.61±0.255ab	1.89±0.41 a-d	62	E
NSPVR43	26±26.9u-x	0.06±0.037j-s	0.633±0.233ab	1.52±0.136b-d	65	E
NSPVR44	66±4g-u	0.064±0.001i-r	0.657±0.222ab	1.64±0.203b-d	67	E
NSPVR45	61±24.7j-w	0.08±0.013d-r	0.623±0.166b	1.92±0.208 a-d	64	E
NSPVR46	63±6.1i-w	0.08±0.003g-r	0.523±0.175ab	2.34±0.203a-d	53	E
NSPVR47	31±2.5t-x	0.045±0.009o-s	0.577±0.04ab	1.8±0.055 a-d	59	E
NSPVR48	68±10g-u	0.074±0.023g-r	0.477±0.086ab	1.81±0.38 a-d	49	I
NSPVR49	29±13.4t-x	0.088±0.013d-r	0.677±0.04ab	1.68±0.053 a-d	69	E
NSPVR50	67±6.5g-u	0.114±0.007b-m	0.633±0.29ab	1.59±0.31b-d	65	E
NSPVR51	65±18.5h-v	0.081±0.004e-r	0.61±0.377ab	1.72±0.361 a-d	62	E
NSPVR52	100±33b-j	0.118±0.013b-j	0.71±0.324ab	1.82±0.02 a-d	72	E
NSPVR53	60±8.9j-w	0.088±0.025d-r	0.533±0.142ab	2.07±0.133 a-d	54	E
NSPVR54	68±5g-u	0.111±0.038b-o	0.663±0.41ab	1.67±0.415 a-d	65	E
NSPVR55	37±13.7s-x	0.074±0.02g-r	0.613±0.098ab	1.82±0.286 a-d	63	E
NSPVR56	60±8.7j-w	0.158±0.02a-c	0.657±0.125ab	1.62±0.357b-d	67	E
NSPVR57	68±2g-u	0.112±0.005b-n	0.735±0.375ab	1.96±0.79 a-d	75	E
NSPVR58	83±16.5e-q	0.114±0.008b-m	0.633±0.035ab	2.1±0.1 a-d	66	E
NSPVR59	88±10.4d-n	0.088±0.004d-r	0.757±0.14ab	2.16±0.672 a-d	77	E
NSPVR60	31±3.2j-x	0.05±0.011n-s	0.63ab	2.03±0.306 a-d	64	E
NSPVR61	21±3.2w-x	0.067±0.057h-r	0.53±0.099ab	2.32±0.216 a-d	54	E
NSPVR62	90±9.5d-m	0.129±0.022b-h	0.697±0.251ab	2.03±0.306 a-d	71	E

Key: Levels not connected by same letter are significantly different ($p<0.01$).

Symbiotic effectiveness(SE) %=shoot dry weight(SDW) of inoculated plant/ SDW of positive control

Mean of Shoot dry weight is 0.675

Standard deviation of Shoot dry weight is 0.155

VE- SDW greater that 0.83

E- SDW between 0.83 and 0.52

I- SDW below 0.52

5.12. Symbiotic effectiveness of selected isolates on soil

The relative evaluation of symbiotic effectiveness of the four selected isolates NSPVR-2, from Adama woreda; NSPVR-26, from Chiro woreda; NSPVR-29, from Tulo woreda and NSPVR-31 from Tulo woreda with two Awash Melka and Ayenew varieties on Melkassa soil did not show significant difference of nodule number, nodule weight, and shoot dry weight (SDW) among treatments (Table 8 and Table 9). However, Awash Melka variety on Melkassa soil showed a statistically significant difference of plant total nitrogen contents among treatments (Table 8). The nitrogen-fertilized, NSPVR-29, and NSPVR-2 plants displayed significantly higher plant nitrogen contents than the uninoculated, NSPVR-31, and NSPVR-26 inoculated plants.

Generally selected isolates accumulated 1.78- 1.96 gm/plant shoot weight on Awash Melka variety and 2.64-3.07 gm/plant on Ayenew variety on Melkassa soil. Selected isolates increased SDW accumulation 12-24%for Awash Melka variety and 9-27% for Ayenew variety on Melkassa soil compared with SDW obtained from uninoculated and unfertilized plants.

On Babille soil on Awash Melka and Ayenew varieties, the four selected isolates showed statistically significant difference with regard to nodule number, nodule weight, Shoot dry matter, and total plant nitrogen (Table 10 and Table 11).

On Babille soil on Awash Melka variety (Table 10), isolate NSPVR-31 showed significantly higher nodule number and nodule weight than the uninoculated plants. Nevertheless, isolate NSPVR-31 did not indicate significant difference of SDW compared with all treatments including uninoculated plants. Isolate NSPVR-29 showed higher nodule weight than nitrogen-fertilized and uninoculated plants. Moreover, NSPVR-29 isolate showed significantly higher total plant nitrogen contents than the other all treatments. With regard to shoot dry weight, NSPVR-29 showed statistically equal with NSPVR-26, NSPVR-31, and NSPVR-2 including nitrogen-fertilized plants.

Regarding nitrogen fertilized plants showed statistically higher SDW than uninoculated plants. However, both nitrogen-fertilized and uninoculated plants showed statistically lower nodule weight than inoculated plants.

On Babille soil on Ayenew variety (Table 11), showed significant variation of nodule number, nodule weight, shoot dry weight, and plant nitrogen contents by the application of inoculum and nitrogen fertilizer. NSPVR-29 showed significantly higher nodule weight and SDW than uninoculated plants. Whereas, NSPVR-31 showed statistically lower SDW and plant nitrogen contents than nitrogen-fertilized plants. Moreover, NSPVR-31 showed statistically similar SDW and plant nitrogen contents with uninoculated plants. The remaining NSPVR-2 and NSPVR-26 showed statistically similar SDW with nitrogen-fertilized and NSPVR-29 plants but they are not statistically different from uninoculated plants. Nitrogen-fertilized plants showed statistically lower nodule number than inoculated and uninoculated plants whereas, uninoculated plants showed statistically lower nodule weight, SDW and plant nitrogen contents than inoculated and nitrogen-fertilized plants.

Generally selected isolates accumulated 0.90-1.14 gm/plant shoot weight on Awash Melka variety and 1.2-1.87 gm/plant on Ayenew variety on Babille soil. Selected isolates increased SDW accumulation 28-62% for Awash Melka variety and 0-56% for Ayenew variety on Babille soil compared with SDW obtained from their uninoculated plants.

Table 8. Nodulation data of selected effective isolates of common bean rhizobia with variety Awash Melka on Melkassa soil.

Isolates	Nodule number/plant	Nodule weight (gm/plant)	Shoot dry weight (gm/plant)	Plant total nitrogen (%N/gm of plant sample)
AW/ NSPVR-31/Mel.	105.7±21.6a	0.163±0.051a	1.78±0.19a	2.12±0.036b
A W/ NSPVR-29/Mel.	159.3±24.9a	0.257±0.151a	1.96±0.34a	2.27±0.185ab
AW/ NSPVR-2/Mel.	136.3±18.9a	0.207±0.093a	1.92±0.14a	2.2±0.118ab
AW/ NSPVR-26/Mel.	130.7±17.6a	0.267±0.138a	1.83±0.58a	2.08±0.047b
AW/con+/M.el	91±26.9a	0.153±0.045a	1.89±0.38a	2.49±0.179a
AW/con-/Mel	100.3±18a	0.09±0.055a	1.58±0.23a	1.72±0.011c

Key: Levels not connected by same letter are significantly different ($p<0.01$).

Table 9. Nodulation data of selected effective isolates of common bean rhizobia with variety Ayenew on Melkassa soil.

Isolates	Nodule number/plant	Nodule weight (gm/plant)	Shoot dry weight (gm/plant)	Plant total nitrogen (%N/gm of plant sample)
AY/ NSPVR-31/Mel	138.7±74.1a	0.22±0.111a	2.65±0.5a	2.19±0.03a
AY/ NSPVR-29/Mel	97±17.3a	0.197±0.032a	2.75±0.13a	2.04±0.153a
AY/ NSPVR-2/Mel	89.3±5.03a	0.147±0.032a	2.64±0.22a	2.31±0.08a
AY/ NSPVR-26/Mel	106±27a	0.23±0.072a	3.07±0.32a	2.02±0.187a
AY/ con+/Mel	77±20.9a	0.167±0.047a	2.87±0.17a	2.32±0.121a
AY/ con-/Mel	108±3.5a	0.193±0.015a	2.42±0.13a	2.23±0.072a

Key: Levels not connected by same letter are significantly different ($p<0.01$).

Table 10. Nodulation data of selected effective isolates of common bean rhizobia with variety Awash Melka on Babille soil.

Isolates	Nodule number/plant	Nodule weight (gm/plant)	Shoot dry weight (gm/plant)	Plant total nitrogen (%N/gm of plant sample)
AW/ NSPVR-31/Bab	282.3±74a	0.27±0.0462a	0.9±0.1ab	2.26±0.065cd
AW/ NSPVR-29/Bab	115±15b	0.19±0.025ab	1.11±0.51ab	2.89±0.01a
AW/ NSPVR-2/Bab	198.3±67.1ab	0.22±0.060ab	1.14±0.2ab	2.48±0.15bc
AW/ NSPVR-26/Bab	117.3±16.5b	0.21±0.091ab	0.97±0.37ab	2.41±0.069bcd
AW/ con+/Bab	101.3±11.7b	0.11±0.015b	1.7±0.3a	2.49±0.081b
AW/ con-/Bab	86.3±5.5b	0.12±0.030b	0.51±0.15b	2.24±0.017d

Key: Levels not connected by same letter are significantly different ($p<0.01$).

Table 11. Nodulation data of selected effective isolates of common bean rhizobia with variety Ayenew on Babille soil.

Isolates	Nodule number/plant	Nodule weight (gm/plant)	Shoot dry weight (gm/plant)	Plant total nitrogen (%N/gm of plant sample)
AY/ NSPVR-31/ Bab	14.4±11.12b	0.15±0.01ab	0.78±0.22b	1.65±0.146b
AY/ NSPVR-29/ Bab	54.2±8.19a	0.19±0.01a	1.87±0.32a	2.06±0.261ab
AY/ NSPVR-2/ Bab	13.0±7.53b	0.13±0.036ab	1.69±0.28ab	2.03±0.116ab
AY/ NSPVR-26/ Bab	19.3±11.13b	0.097±0.035ab	1.55±0.2ab	1.94±0.128ab
AY/con+/ Bab	8.5±4.91b	0.117±0.032ab	1.81±0.55a	2.31±0.092a
AY/ con-/ Bab	38.2±12.05ab	0.09±0.043b	0.78±0.39b	1.63±0.241b

Key: Levels not connected by same letter are significantly different ($p<0.01$).
Aw-Awash Melka variety.
Ay-Ayenew variety.
Mel-Melkassa soil.
Bab-Babille soil.
Con+-Positive control with nitrogen fertilizer (nitrogen-fertilized plants).
Con- -Negative control without inoculum and nitrogen fertilizer (uninoculated plants).

6. DISCUSSION

6.1. Authentication of rhizobia

In this study, nodules were collected from as many sites of common bean growing areas of Eastern Shewa (17 isolates), Eastern Hararghe (19 isolates), and Western Hararghe (26 isolates). Of which, 62 isolates renodulated the host plant, gram negative and rod shaped bacteria with little or no absorption of congored grown on YEM-CR medium characterized as Rhizobiaceae according to Jordan (1984). Similarly, Alemayuhu Workalemahu (2006) revealed all collected rhizobia from common bean nodules found to be authenticated as root nodule bacteria. Desta Beyene *et al*., (2004); Mitiku Haile (1990) and Amare Abebe (1982); revealed the presence of indigenous bean-nodulating rhizobia from Ethiopian soils.

Ten of the isolates failed to nodulate the parent host and grew on peptone-glucose medium (PGA) that is used to preliminary screening of root nodulation bacteria from the contaminant (Lupwayi and Haque 1994). Similarly, Zerihun Belay (2006) identified one isolate from faba bean nodule that failed to be authenticated as root nodule bacteria. Some works also showed a failure of nodulation of rhizobia isolated from nodules upon reinoculation of *Rhizobium leguminosarum* on the host. This suggests that there are rhizobia that either lost their nodulation capacity due to a loss of plasmids (Segovia *et al*., 1991) and some contaminated bacteria that penetrate the nodule (Johnston and Beringer, 1976).

6.2. Characteristics of the isolates

6.2.1. Morphology and Cultural characteristics

Cultural and morphological characteristics of isolates showed three colony morphology. 77% and 20% of isolates displayed large mucoid and large watery respectively. Isolate NSPVR-62 from Goro Gutu woreda was found to exhibited small dry colony morphology (Table 3). Similarly, Alemayehu Workalemahu (2006) revealed almost all isolates of rhizobia from common bean have shown large mucoid colony morphology and two isolates displayed large watery and small dry colony morphology.

Morphological characteristics of bean isolates on PY-medium differentiated them into respective type groups (Table 3). All isolates except NSPVR-18, NSPVR-31, NSPVR-32, and NSPVR-57 from Western Hararghe showed smooth and gummy grown on PY-medium. Smooth and gummy colonies of common bean rhizobia could be *Rhizobium leguminosarum* or *Rhizobium etli* (Martinez-Romero *et al.*, 1991; Silva *et al.*, 2003). Isolates NSPVR-18, NSPVR-31, NSPVR-32, and NSPVR-57 from Western Hararghe showed rough grown on PY-medium, which is a characteristic of *Rhizobium gallicum* (Martinez-Romero *et al.*, 1991; Silva *et al.*, 2003). Similarly, Alemayehu Workalemahu (2006) reported almost all common bean rhizobia of Southern Ethiopia isolates displayed smooth gummy colony except AUPR-9 and AUPR-10 isolates from Wellayita Sodo areas displayed rough colony morphology whereas AUPR-8 isolate from Arbaminch areas displayed creamy colony morphology on PY-medium.

6.2.2. Growth on YEMA-BTB and mean generation time (MGT)

According to the classification of Rhizobiaceae in Bergey's Manual (Jordan 1984), almost all isolates categorized in to fast growing rhizobia based on their generation time, acid production and large growth with production of copious exopolysaccharid and colony diameter greater than 2mm except isolate NSPVR-62 from Goro Gutu woreda at optimum temperature (25-30^{0}C) and pH of the medium (6-7) (Table 3). Isolate NSPVR-62 from Goro Gutu woreda formed colony diameter of 1.5mm, alkaline production and

with generation time of 6hr categorized as slow growing *Rhizobium* known as *Bradyrhizobium* according to Jordan (1984). Fast growing bacteria nodulating common bean were also identified by several workers (Alemayehu Workhalemahu 2006; Amarger *et al.*, 1997; Aguilar *et al.*, 1998; Desta Beyene *et al.*, 2004). Odee *et al.*, (1997) revealed slow and fast growing common bean rhizobia recorded from Kenyan soils. Similarly, Hungria *et al.*, (1993) reported that *Bradyrhizobium*-like bacteria was isolated from Latin American soils that nodulate common bean.

6.3. Growth characteristics of isolates on other media

Most strains (68%) were found to be melanin producers. Andrade *et al.*, (2002) found that melanin production was observed in *R. tropici* strains. On the other hand, melanin production was observed in *R. leguminosarum* bv. *phaseoli* (Cubo *et al.*, 1988). Alemayehu Workalemahu (2006) found that melanin production was observed in most of South Ethiopia isolates of common bean.

From the 62 tested isolates, 79 % were able to grow on LB-medium and 83% grew on TY-Ca medium (Table 3). Similarly, some isolates were not able to grow on LB and TY-Ca media, which is in agreement with (Martinez-Romero *et al.*, 1991; Andrade *et al.*, 2002) that are the characteristics of all rhizobia nodulating common bean except *R. tropici*. According to Martinez-Romero *et al.*, (1991), isolates that are able to grow on these media were characterized as *R. tropici*. Alemayehu Workalemahu 2006 found that except two isolates, all bean isolates are unable to grow on both LB and TY-Ca media. Nevertheless, Grange and Hungria, (2004) showed other isolates of common bean like *R. etli* strains are also able to grow on LB medium.

Seven isolates namely, NSPVR-12 from Bost woreda, NSPVR-17, NSPVR-18, NSPVR-19, NSPVR-31, NSPVR-32, and NSPVR-57 isolated from Western Hararghe Zone were showed the presence and absence of growth on PY-Ca and LB-media respectively. According to Amargar *et al.*, (1997) these are the properties of *Rhizobium gallicum*.

All isolates grew on YEMA containing 2% Urea medium. Alemayehu Workalemahu (2006) revealed that isolates of common bean rhizobia from Southern Ethiopia showed similar pattern of growth on LB, TY-Ca, and YEMA containing 2% Urea media.

6.4. Biochemical and physiological test

Salt tolerance

More than 68% of the isolates continuously tolerated up to 4%NaCl (Figure 2). *R. Phaseoli* is one of the most halotolerant rhizobia and several isolates have been reported to grow at high salt concentrations (4%-5%) (Hungaria *et al.*, 2000).Similarly, two isolates of common bean from Southern Ethiopia tolerated higher concentration of salt reported by Alemayehu Workalemahu (2006).

Most of fast growing isolates (74%) could tolerate more than 2% NaCl while slow growing isolate NSPVR-62 from Goro Gutu woreda was highly sensitive to salt at less than 1% NaCl (Figure 2). Maaoui and Baghdalik, 2002 reported that fast growing strains are generally more tolerant to salt than slow growing strains and Zahran (1997), showed that fast growing *Rhizobium*, in general, grew well at NaCl concentration between 3-5%.

All isolates that were found to tolerate up to 8%NaCl were also indicated highly resistant to alkaline pH at 10.5. This result agreed with the observation of Abdelaal Shamsedin and Werner, (2004). However, the legume-*Rhizobium* symbioses and nodule formation are more sensitive to salt or osmotic stress than are the free- living rhizobia (Delgado *et al*, 1994; Zahran, 1999), and therefore rhizobial strains that are tolerance to high salinity levels in laboratory may not be effective in nitrogen fixation.

Temperature tolerance

In this study 55% of the isolates were able to grow at 37^0C and 6% survived to a temperature of 45^0C (Figure 3). Kucuk *et al.*, (2006) isolated common bean rhizobia from Turkey soil capable of resisting an incubation temperature of 42^0C. Similarly, Hungria *et al.*, (2000) also found common bean rhizobia were tolerated at 40^0C on TY medium. Similar response of *Rhizobium tropici* to incubation of high temperature was recorded to tolerate at 40^0C on different media (Martinez-Romero *et al.*, 1991).

pH tolerance

In this experiment, small number of the isolates (24%) was able to grow at pH- 4.5. Of which, NSPVR-62 slow growing isolate from Goro Gutu woreda was able to tolerate at pH-4.5 (Figure 4). The fact that different strains of the same species may vary widely in their pH tolerance has been demonstrated previously (Glenn and Dilworth, 1994). The result also agrees with Jordan (1984) shown that fast growing strains appear to be more sensitive to low pH than slow growing strains. Similarly, almost all fast growing isolates of Southern Ethiopia common bean rhizobia failed to tolerate pH lower than 5 (Alemayehu Workalemahu 2006).

Whereas, 77% of isolates were able to grow at pH-10, which is similar to other common bean isolates from Turkey soil (Kucuk *et al.*, 2006) and all Southern Ethiopia isolates of common bean rhizobia grew on medium with pH value 8.5 (Alemayehu Workalemahu 2006).

None of the isolates was able to grow at pH-4.0. One isolate from Southern Ethiopia finally characterized as *R. tropici*-like isolate tolerated pH-4.0 (Alemayehu Workalemahu 2006) and some fast growing strains are able to grow at a pH as low as 4.0(Jordan, 1984).

Amino acids utilization

More than 90% of the tested isolates were able to catabilise L-tryptophan, L-phenylalanine, L-lysine, L-leucine, L-glutamine L-tyrosine, L-aspargine, and L-arginine (Table 4). Sessitsch *et al.*, (1997) reported most of the nitrogen sources were utilized by rhizobia nodulating common bean. Similarly, Alemayehu Workalemahu (2006) reported all the tested isolates of common bean from south Ethiopia utilized L-tryptophan and L-tyrosine.

13% of the tested isolates were unable to utilize glycine agreed with glycine were utilized by some rhizobia isolated from common bean (Amarger *et al.*, 1997; Alemayehu Workalemahu 2006). Moreover, glycine rarely utilized by some species of rhizobia as reported by Jordan (1984).

Carbohydrates utilization

All isolates grew on D-glucose, D-fructose, xylose, malate, eructose, and 90% of isolates were grown on lactose, D-glucoinic acid, raffinose, mannose, cellobiose, galactose, L-rhaminose, sorbitol, glycerol and trehalose (Table 4). Similarly, Alemayehu Workalemahu (2006); Stowers (1985); Amarger *et al.*, (1997); Hungria *et al.*, (2000) found that most carbohydrates could be utilized by all tested rhizobia. The catabolism of monosaccharides and disaccharides are widespread in fast growing rhizobia including *Rhizobium leguminosarum* var *Phaseoli* (Jordan 1984).

Nevertheless, 13% of the isolates in my study were able to utilize dextrin as a carbon source, which is in agreement with other works indicating that dextrin is rarely utilized by *Rhizobium* (Jordon, 1984). 53% of isolates were able to utilize dulcitol and 61% of isolates utilized citrates, which is similar to other *Rhizobium* bacteria (Jordan 1984). In addition, isolates unable to utilized dulcitol are categorized in *R. giardinii*, *R. gallicum*, and *R. tropici* in contrast with isolates categorized under *R. leguminosarum*, and *R. etli* as indicated in Amarger *et al.*, (1997).

Intrinsic antibiotic resistance

Resistance patterns of the isolates to six antibiotics were studied to provide phenotypic data on common bean isolates to determine the diversity among the isolates (Figure 5). The concentrations used to characterize in this experiment were in the range of those used by Amarger *et al.*, (1977), who used a similar approach to characterize rhizobial populations isolated from common bean. Almost all tested isolates grew on media supplemented with all concentration of Oxytetracycline, Spectinomycin, and Novobiocine. More than 50% of isolates sensitive for Kanamycin and Streptomycin in this study agrees with the results reported previously by Amarger *et al.*, (1997); Kucuk *et al.*, (2006); Alemayehu Workalemahu (2006) this is the characteristics of *R. leguminosarum* bv *Phaseoli*-like bacteria .

More than 71% of tested isolates were resistant to high-level Naldixic acid (60mg/ml). According to Amarger *et al*., (1997), indicated isolates with tolerance to Naldixic acid are the properties of bean-nodulating rhizobia particularly *R. etli*. Therefore, difference in growth in different antibiotics in our result indicated that the diversity in strains among isolates.

6.5. Cross inoculation test

In the present study, none of the isolates was found to exhibit the ability to nodulate *Leucaena leucocephala.* According to Martinez-Romero *et al.*, (1991), isolates that are able to infect *Leucaena leucocephala* are characterized as *R. tropici.*

6.6. Phosphorus solubization in the solid Sperber medium

From the 62 common bean rhizobial isolates, 26 were able to solubilize tricalcium phosphate in sperber solid medium (Table 3). In fact, a large number of *Rhizobium* strains are able to solubize insoluble phosphorus compounds but this ability is variable among the strains (Halder and Chakrabartla, 1993). Out of these 26 isolates NSPVR-8 and NSPVR-11 isolated from Bost woreda produced large halo zones and ratio of halo diameter/colony diameter>1.2, and it is important for preliminary selection of phosphorus solubilizing microorganism (Alikhani *et al.*, 2006).

6.7. Numerical analysis

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 tentatively group these bacteria into their respective species types (Figure 7). Phenotype 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); Grange and Hungria, 2004; Amarger *et al.*, (1997) and Silva *et al.*, (2003) were used to make preliminary classification of isolates.

Based on morphological, biochemical and physiological characteristics, the isolates of common bean rhizobia from Eastern Ethiopia and cluster analysis supported that diversity of rhizobia are found in soils of Eastern Ethiopia that nodulate common bean. All isolates except NSPVR-62 from Goro Gutu woreda and NSPVR-18, NSPVR-31, NSPVR-32, and NSPVR-57 all from Western Hararghe Zone were classified as *Rhizobium leguminosarum*-like or *Rhizobium etli*-like strains because of the lack of clear demarcation in phenotypic features between them. *Rhizobium leguminosarum*-like or *Rhizobium etli*-like mainly characterized by its most of them grew on dulcitol, tolerance to Naldixic acid, sensitive to Streptomycin and Kanamycin, melanin production, and grew on LB-medium; their smooth and gummy colony on PY-medium; growth on urea

20% on YEMA medium; failed to nodulate *Leucaena leucocephala* and failed to tolerate at pH-4.0 (Grange and Hungria, 2004; Martinez-Romero *et al.*, 1991; Cubo *et al.*, 1988; Silva *et al.*, 2003) and supported by numerical analysis.

Similarly, the previous study, except a single *Rhizobium etli* strain, most bean rhizobia were classified as *Rhizobium leguminosarum* by multilocus enzyme electrophoresis that covered central Ethiopia and some parts of North Ethiopia and East Ethiopia from Amaresa woreda (Desta Beyene *et al.*, 2004). Diouf *et al*., (2000) reported that *R. etli* are the dominant species in common bean nodules in some African soils particularly Senegal and Gambia.

In Eastern Ethiopia, Common bean is widely cultivated as intercropping system with sorghum, maize and other (Wortmann and Allen, 1994). This is also supported that domination of *R. etli*-like in Eastern Ethiopia due to *R. etli* have found as maize endophytes in the traditional agricultural fields where common bean and maize are intercropped in Mexico (Gutierrez-Zamora and Martinez-Romero, 2001)

Isolates NSPVR-31 and NSPVR-32 from cluster-I and NSPVR-18, and NSPVR-57 from cluster-II all from Western Hararghe Zone were tentatively classified as *R. gallicum* mainly characterized by its rough colony morphology on PY-medium, presence of growth on PY-Ca medium, absence of growth on LB-medium, unable to nodulate *Leucaena leucocephala* and unable to utilize dulcitol (Silva *et al.*, 2003; Amarger *et al.*, 1997). Alemayehu Workalemahu (2006) reported that two isolates *R. gallicum*-like isolated from Wollayita Sodo woreda soil that nodulate common bean characterized using phenotypic parameters. Similarly, *Rhizobium gallicum* were found to nodulate beans in Europe (Amarger *et al.*, 1997), and in Africa from Tunisia (Mhamdi *et al.*, 1999) and in Latin America from Mexico (Sessitsch *et al.*, 1997).

Isolate NSPVR-62 from Goro Gutu woreda was classified as *Bradyrhizobium*-like isolate. *Bradyrhizobium*-like isolate mainly characterized by its slow growing generation

time, colony diameter less than 2mm, highly sensitive for NaCl more than 1%, resistant to acidic pH, highly sensitive for alkaline pH and alkaline producer on YEMA-BTB medium (Jordan, 1984). Moreover, numerical analysis displayed isolate NSPVR-62 had unique characteristics from other tested isolates.

Isolate NSPVR-27 from Chiro woreda was displayed highly sensitive acid and alkaline pH, salt concentration more than 0.5% NaCl, high and low temperatures, most of tested antibiotics and unable to utilize most of tested carbohydrates and amino acids compared to other tested isolates. Moreover, NSPVR-27 categorized under ineffective strain. On the other hand, isolate NSPVR-37 from Tulo woreda was shown highly tolerant for wide temperature range from 5^0C up to 45^0C, high salt concentration up to 7%NaCl, wide pH range from 5-10.5, all antibiotic tested except kanamycin and able to utilize almost all carbohydrates and amino acids sources. With regard to symbiotic effectiveness, NSPVR-37 was categorized as effective nitrogen fixer.

6.8. Enumeration of rhizobia

Both Melkassa and Babille soils had almost equal level of soil fertility in terms of soil chemical and except CEC and EC (Table 6). In addition to this, both soils had neutral pH values. Nevertheless Melkassa and Babille soils had loamy and sand loamy soil texture. Alemayehu Workalemahu (2006) found that pH may have contributed to P-deficiency and hence affected negatively *Rhizobium* presence in some of the tested areas of Southern Ethiopia.

However, the plant infection tests to evaluate the most probable number (MPN) density of indigenous population of rhizobia at Melkassa soil was found to contain 10^8 rhizobia/gm of soil; whereas Babille soil harbored only 10^3 rhizobia/gm of soil estimated from both common bean varieties (Table 5). This is may be due to Melkassa found in highly common bean producing area in the rift valley (Teshale Assefa *et al.*, 2006); in Babille woreda, on the other hand farmers mainly cultivated peanut intercropped with maize, sorghum, and chat but not common bean (Tamado Tana, 1994). Therefore, the

absence of the homologues host in cropping system or failure to grow the host for quite a long time from cropping system in Babille woreda leads to very low number of endosymbiote for that specific host (Date 1982; Loutfi *et al.*, 1980).

6.9. Preliminary screening of symbiotic effectiveness on sand culture

Based on shoot dry matter accumulation of inoculated plants over the uninoculated control plants, 89% of the isolates were categorized into effective and very effective groups (Table 7). Similarly, Alemayehu Workalemahu (2006) reported that more effective isolates were obtained from wide range of geographical locations and pH ranges of South Ethiopia soils. Thus, result 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.

The highest scores of effectiveness of symbiotic nitrogen fixation were displayed by isolates NSPVR-2 isolated from East Shewa Zone; NSPVR-26, NSPVR-29, and NSPVR-31 from West Hararghe Zone. Four isolates inoculated plants were shown significantly ($p<0.01$) higher SDW than uninoculated plants (negative control). This result agreed with observation of Alemayehu Workalemahu (2006) revealed inoculation of rhizobia isolated from Southern Ethiopia on common bean improved total plant nitrogen contents, shoot dry weight and nodulation of the plants. The benefits that can be achieved through isolation of indigenous rhizobia were 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 al.*, 1999). Interestingly in Morocco, effective isolates with salt tolerance could produce 69-72% dry matter accumulated by the plant supplied with mineral nitrogen (Bouhmouch *et al.*, 2001).

Based on preliminary selection of phosphorus solubilizing microorganisms, isolates NSPVR-8 and NSPVR-11 isolated from Bost woreda were grouped under better inorganic phosphorus solubilizer rhizobia. Moreover, NSPVR-8 and NSPVR-11 are characterized as effective nitrogen fixing rhizobia.

6.10. Symbiotic effectiveness of selected isolates in soil

Soil type affected the isolates performance. Babille soil on both Ayenew and Awash Melka varieties showed significant variation of shoot dry weight by the application of inoculant and nitrogen fertilizer. That is may be due to Mitiku Haile (1990) on his survey status of BNF on common bean inoculation and production indicated that the nodulation status of common beans in different agroecological area of Eastern Ethiopia especially Babille area is very limited.

Babille soil on both varieties (Table 10 and Table 11)showed significantly ($p<0.01$) higher shoot dry weight for nitrogen fertilized and inoculated plants than uninoculated plants except NSPVR-31 that showed poor shoot dry weight similar to uninoculated plants on Ayenew variety (Table 11), suggested that the Babille soil did not have effective nitrogen fixing rhizobia on common bean. Lopez-Bellido and Fuentes (1986) showed that clearly increases in lupin yield due to N- fertilizer application occurred only on soils with out initial effective *Bradyrhizobium* population.

Nitrogen-fertilized plants of Ayenew and Awash Melka varieties on Babille soil displayed low nodule number and nodule weight but increase shoot dry weight plant nitrogen contents that clearly indicated by Tamado Tana and Chemeda Fininsa (2006) the application of nitrogen fertilizer generally increased grain yield of common bean but the nodulation was decreased with increased rate of nitrogen in Eastern Ethiopia. Similarly, Desta Beyene and Angaw Tsigie, (1986) reported that very high nitrogen contributed for the high performance for shoot dry weight and total nitrogen. Nevertheless, some mineral N is needed to achieve maximum yield and to improve capable of fixing large amounts of atmospheric N_2 (Tsai *et al.*, 1993; Ayneabeba Adamu *et al.*, 2001; Diouf *et al.*, 1999). This is possible to conclude that in Babille soil response to inoculation can revert poor yields and depletion of soil N content and it is important especially in developing countries where the gap between actual and potential crop yield is large.

On Babille soil on Awash Melka variety all inoculant plants had similar performance with nitrogen-fertilized plants in terms of shoot dry weight yield whereas, on Ayenew variety NSPVR-31 showed statistically lower shoot dry weight than nitrogen-fertilized plants (Table 11). This result indicated that variation of nitrogen fixation potential of isolate NSPVR-31 between Ayenew and Awash Melka varieties. Similarly, Alemayehu Workalemahu (2006) reported that no isolates performed well on both tested varieties on the basis of shoot dry weight. Montealegre and Kipe-Nolt (1994); Handarson *et al.*, (1993); Manrique *et al.*, (1993) indicated that specific interaction between *Rhizobium* strain with bean genotype was highly significant. This is due to some accessions of common bean were able to restricted nodulation by some rhizobial strains (Aguilar *et al.*, 1998; Redden *et al*., 1990). Similarly, wild common bean accessation of Mesoamerican origin has been restricted nodulation by the *R. tropici* strain CIAR 899 and performed better than any other isolates (Kipe-Nolt *et al*, 1992). This observation indicated that interaction between the bean varieties and the some tested isolates. Nevertheless, NSPVR-29 showed statistically higher shoot dry weight on both Ayenew and Awash Melka varieties than uninoculated plants (Table 10 and Table 11). Therefore, NSPVR-29 performed well on Ayenew and Awash Melka varieties. This result suggested that NSPVR-29 isolate is most efficient inoculum for both Ayenew and Awash Melka varieties.

On Melkassa soil on Ayenew and Awash Melka varieties, the application of inoculum and nitrogen-fertilizer did not indicate difference on nodule number, nodule weight, shoot dry weight and plant nitrogen contents (Table 8 and Table 9) may be due to the Melkassa soil it self had high number of effective indigenous rhizobia (Table 5) that is why the application of nitrogen and inoculum did not revert the yield (Singleton and Tavares 1986). In addition, that may be due to the inoculum strains tested were not substantially better than the indigenous strains (Larson *et al.*, 1989).

However, Plant nitrogen contents (%) was significantly ($p<0.01$) affected by the application of chemical fertilizer on Awash Melka variety on Melkassa soil (Table 8). This correlated with Tsai *et al.*, (1993) verified at high fertility and at the highest N rate

(120 mg/kg soil), the stimulatory effect of N-fertilizer was still observed increasing the amounts of N_2 fixed from 37 up to 88mg N/plant. Moreover, the average SDW of negative control were generally lower than those obtained in inoculated treatments on Melkassa soil but not statistical significant, showing that importance of seed inoculation as indicated in Popescu (1998).

In general, both soils were once treated with fertilizer as recommended by Somagaran and Hoben (1994), response of the Babille soil to inoculation by selected effective strains was much pronounced (0-62%) than Melkassa soil(9-27%) compared to the uninoculated plants. Variation of effectiveness of inoculation between Babille and Melkassa soils was due to the variations of number of effective indigenous rhizobia as reported in (Thies *et al.*, 1991a, 1991b). The result indicated that enumeration of rhizobia population and evaluation of effectiveness of rhizobia would be essential in order to know the need for inoculation.

7. CONCLUSION AND RECOMMENDATION

This appears describes for the first time the phenotypic and symbiotic characterization of rhizobia nodulating common bean in Eastern Ethiopia. The preliminary phenotypic and its cluster analysis performed with the strains indicated that they have *Rhizobium leguminosarum*-like or *Rhizobium etli*-like, *Rhizobium gallicum*-like and *Bradyrhizobium*-like rhizobia nodulating common bean in Eastern Ethiopia, we recommend further work is needed with *Rhizobium* isolates from bean at the genetic level to confirm whether they represent different biovars or, perhaps different species.

The tested rhizobia metabolized a broader range of carbon and nitrogen sources. They also some of them tolerated low pH and survived at highest temperatures. While most of the isolates are able to grow at high pH and high salt concentration. These abilities may favour the establishment of the rhizobia and may represent an advantage of these over other inoculate use for common bean. Nevertheless, rhizobial strains that are tolerance to high salinity, pH and temperature levels in laboratory may not be effective in nitrogen fixation and survival these adverse condition under external environment. Thus, we recommend intensive evaluation are needed under field condition.

The initial nodulation experiments using Awash Melka variety, the collected rhizobial isolates revealed that there is remarkable difference of symbiotic effectiveness. This study showed that 89% isolates nodulating common bean isolated from Eastern Ethiopia soils are very effective and effective strains of rhizobia. We recommended that the very effective isolates could use as inoculum in other common bean growing area of the country.

In unsterilized soil, under greenhouse pot experiment of the selected isolates NSPVR-2, NSPVR-26, NSPVR-29, and NSPVR-31 exhibited different ability of symbiotic effectiveness on Awash Melka and Ayenew varieties on Babille soil. Isolate NSPVR-31 was exhibited critical difference of symbiotic effectiveness on Babille soil on Ayenew variety but not shown on Babille soil on Awash Melka variety. Nevertheless, isolate NSPVR-29 performed equally on Ayenew and Awash Melka varieties on Babille soil. This result revealed that isolate NSPVR-31 showed different performance of nitrogen

fixation between varieties. On the other hand, isolate NSPVR-29 had wide host range for symbiotic effectiveness. Nevertheless, all treatment including nitrogen-fertilized and uninoculated plants was performed equally on Melkassa soil on Ayenew and Awash Melka varieties. This is due to the presence of sufficient and effective rhizobial isolates nodulating common bean. Thus, we recommend inoculation is important for Babille soil. Moreover, `the need for selection of specific strain of inoculant for specific variety; further competitiveness study and field assay for selected isolates especially isolate NSPVR-29 would be important.

Surprisingly, NSPVR-8 and NSPVR-11 were shown high TCP solubilizer under solid medium and effective nitrogen fixers. This property is important to fill the need of phosphorus for both the plant and the symbionts. Then, we recommend that further evaluation TCP solubilizer strains under laboratory and field assays are essential to validate the result.

Finally, soils from other locations not covered in this study should be investigated in order to provide further information about the species of rhizobia that effectively nodulate common bean and developing a multi-inoculum for common bean in Ethiopia.

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9. APPENDICES

Appendix 1. Tolerance of rhizobia nodulating common bean at different pH range.

Isolates	PH 4.5	PH 5	PH 5.5	PH 6	PH 7	PH 8	PH 8.5	PH 9	PH 9.5	PH 10	PH 10.5	PH Tolerance Range
NSPVR 1	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR2	-	-	+	+	+	+	+	+	-	-	-	5.5-9
NSPVR3	-	+	+	+	+	+	+	+	+	-	-	5-9.5
NSPVR4	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR5	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR6	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR7	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR8	+	+	+	+	+	+	+	+	+	+	+	4.5-10.5
NSPVR9	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR10	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR11	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR12	+	+	+	+	+	+	+	+	-	-	-	4.5-9
NSPVR13	-	-	+	+	+	+	+	+	-	-	-	5.5-9
NSPVR14	+	+	+	+	+	+	+	+	-	-	-	4.5-10.5
NSPVR15	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR16	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR17	+	+	+	+	+	+	+	+	-	-	-	4.5-9
NSPVR18	+	-	+	+	+	+	+	-	-	-	-	4.5-8.5
NSPVR19	-	+	+	+	+	+	+	+	+	-	-	5-9.5
NSPVR20	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR21	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR22	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR23	-	-	+	+	+	+	+	+	-	+	+	5.5-10.5
NSPVR24	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR25	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR26	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR27	-	-	+	+	+	+	+	+	-	-	-	5.5-9
NSPVR28	+	+	+	+	+	+	+	+	+	+	+	4.5-10.5
NSPVR29	+	+	+	+	+	+	+	+	-	-	-	4.5-9
NSPVR30	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR31	+	+	+	+	+	+	+	+	+	+	+	4.5-10.5
NSPVR32	+	+	+	+	+	+	+	+	+	+	+	4.5-10.5
NSPVR33	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR34	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR35	-	-	+	+	+	+	+	+	+	+	+	5.5-10.5
NSPVR36	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR37	-	+	+	+	+	+	+	+	+	+	+	5-10.5

NSPVR38	-	-	+	+	+	+	+	+	+	+	+	5.5-10.5
NSPVR39	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR40	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR41	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR42	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR43	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR44	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR45	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR46	+	+	+	+	+	+	+	+	+	+	+	4.5-10.5
NSPVR47	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR48	-	-	+	+	+	+	+	+	-	-	-	5.5-9
NSPVR49	+	+	+	+	+	+	+	+	+	+	+	4.5-10.5
NSPVR50	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR51	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR52	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR53	-	-	+	+	+	+	+	+	+	+	+	5.5-10.5
NSPVR54	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR55	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR56	+	-	+	+	+	+	+	+	+	+	+	5.5-10.5
NSPVR57	+	-	+	+	+	+	+	+	+	-	-	5.5-10.5
NSPVR58	-	+	+	+	+	+	+	+	+	+	+	5-10.5
NSPVR59	-	-	+	+	+	+	+	+	-	-	-	5.5-9
NSPVR60	-	+	+	+	+	+	+	+	-	-	-	5-9
NSPVR61	+	-	+	+	+	+	+	+	+	+	-	5.5-10
NSPVR62	+	+	+	+	+	+	+	-	-	-	-	4.5-10
% of tolerated isolates	24%	79%	100%	100%	100%	100%	100%	98%	82%	77%	71%	

Key: + : Presence of growth.
- Absence of growth

Appendix 2. Tolerance of rhizobia nodulating common bean at different salt concentration.

Isolates	0.5% NaCl	1% NaCl	1.5% NaCl	2% NaCl	2.5% NaCl	3% NaCl	3.5% NaCl	4% NaCl	4.5% NaCl	5% NaCl	5.5% NaCl	6% NaCl	7% NaCl	8% NaCl	9% NaCl	10% NaCl	Salt Tolerance Range (%)
NSPVR 1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-9
NSPVR2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5
NSPVR3	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5
NSPVR4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0.5-10
NSPVR5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR12	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5
NSPVR13	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5
NSPVR14	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5
NSPVR15	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	0.5-5.5
NSPVR16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR17	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5-1
NSPVR18	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5
NSPVR19	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5
NSPVR20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR21	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR23	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	0.5-5.5
NSPVR24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0.5-10
NSPVR25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR26	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	0.5-5.5
NSPVR27	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5
NSPVR28	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	0.5-3
NSPVR29	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5
NSPVR30	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	0.5-5.5
NSPVR31	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR32	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR33	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR34	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	0.5-7
NSPVR35	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	0.5-6
NSPVR36	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR37	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	0.5-7
NSPVR38	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	0.5-7
NSPVR39	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	0.5-7
NSPVR40	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8

NSPVR41	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR42	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR43	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	0.5-4
NSPVR44	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR45	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR46	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR47	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR48	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5
NSPVR49	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR50	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	0.5-7
NSPVR51	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR52	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR53	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	0.5-3
NSPVR54	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR55	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	0.5-8
NSPVR56	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	0.5-3
NSPVR57	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5-1
NSPVR58	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0.5-10
NSPVR59	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5-1.5
NSPVR60	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5-1
NSPVR61	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5-1
NSPVR62	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5-1.5
% of tolerated isolates	100%	84%	77%	71%	71%	71%	69%	69%	68%	68%	68%	61%	60%	52%	5%	5%	

Key: + : Presence of growth.
- Absence of growth

Appendix 3. Tolerance of rhizobia nodulating common bean at different temperature range.

Isolates	5^{0}C	10^{0}C	15^{0}C	20^{0}C	30^{0}C	37^{0}C	40^{0}C	45^{0}C	Temperature Tolerance Range (^{0}C)
NSPVR 1	-	-	+	+	+	+	+	-	15-40
NSPVR2	-	+	+	+	+	-	-	-	10-30
NSPVR3	+	+	+	+	+	-	-	-	5-30
NSPVR4	-	-	+	+	+	+	+	+	15-45
NSPVR5	+	+	+	+	+	+	+	-	5-40
NSPVR6	-	-	+	+	+	+	+	-	15-40
NSPVR7	-	+	+	+	+	+	+	-	10-40
NSPVR8	-	-	+	+	+	+	+	-	15-40
NSPVR9	+	+	+	+	+	+	+	-	5-40
NSPVR10	+	+	+	+	+	+	+	-	5-40
NSPVR11	-	-	+	+	+	+	+	-	15-40
NSPVR12	-	-	+	+	+	-	-	-	15-30
NSPVR13	+	+	+	+	+	-	-	-	5-30
NSPVR14	+	+	+	+	+	-	-	-	5-30
NSPVR15	-	-	+	+	+	+	+	-	15-40
NSPVR16	-	-	+	+	+	+	-	-	15-37
NSPVR17	+	+	+	+	+	-	-	-	5-30
NSPVR18	+	+	+	+	+	-	-	-	5-30
NSPVR19	-	-	+	+	+	-	-	-	15-30
NSPVR20	-	+	+	+	+	+	+	-	10-40
NSPVR21	-	-	+	+	+	+	+	-	15-40
NSPVR22	+	+	+	+	+	-	-	-	5-30
NSPVR23	+	+	+	+	+	-	-	-	5-30
NSPVR24	-	+	+	+	+	+	+	+	10-45
NSPVR25	-	+	+	+	+	+	+	+	10-45
NSPVR26	+	+	+	+	+	-	-	-	5-30
NSPVR27	-	-	+	+	+	-	-	-	15-30
NSPVR28	-	-	+	+	+	-	-	-	15-30
NSPVR29	-	-	+	+	+	-	-	-	15-30
NSPVR30	-	-	+	+	+	+	+	-	15-40
NSPVR31	+	+	+	+	+	-	-	-	5-30
NSPVR32	-	-	+	+	+	+	+	-	15-40
NSPVR33	+	+	+	+	+	+	+	-	5-40
NSPVR34	-	-	+	+	+	+	+	-	15-40
NSPVR35	+	+	+	+	+	-	-	-	5-30
NSPVR36	-	-	+	+	+	+	+	-	15-40
NSPVR37	+	+	+	+	+	+	+	+	5-45
NSPVR38	-	-	+	+	+	+	+	-	15-40
NSPVR39	-	-	+	+	+	+	+	-	15-40
NSPVR40	-	-	+	+	+	+	+	-	15-40
NSPVR41	-	-	+	+	+	+	+	-	15-40

NSPVR42	-	-	+	+	+	+	+	-	15-40
NSPVR43	-	-	+	+	+	-	-	-	15-30
NSPVR44	-	-	+	+	+	+	+	-	15-40
NSPVR45	+	+	+	+	+	+	+	-	5-40
NSPVR46	+	+	+	+	+	-	-	-	5-30
NSPVR47	-	-	+	+	+	-	-	-	15-30
NSPVR48	+	+	+	+	+	-	-	-	5-30
NSPVR49	-	-	+	+	+	+	+	-	15-40
NSPVR50	-	-	+	+	+	+	+	-	15-30
NSPVR51	-	-	+	+	+	+	-	-	15-37
NSPVR52	+	+	+	+	+	+	-	-	5-37
NSPVR53	+	+	+	+	+	-	-	-	5-30
NSPVR54	+	+	+	+	+	-	-	-	5-30
NSPVR55	-	-	+	+	+	+	+	-	15-40
NSPVR56	-	-	+	+	+	-	-	-	15-30
NSPVR57	-	-	+	+	+	-	-	-	15-30
NSPVR58	-	-	+	+	+	-	-	-	15-30
NSPVR59	+	+	+	+	+	+	+	-	5-40
NSPVR60	+	+	+	+	+	-	-	-	5-30
NSPVR61	-	-	+	+	+	-	-	-	15-30
NSPVR62	+	+	+	+	+	-	-	-	5-30
% of tolerated isolates	39%	48%	100%	100%	100%	55%	50%	6%	

Key: + : Presence of growth.
- Absence of growth

Appendix 4. Utilization of different nitrogen sources

Isolates	Glycine	L-tryptophan	L-tyrosine	L- aspargine	L-arginine	L-valine	L-phenyl alanine	L-isoleucine	L-alanine	L-glutamine	L-leucine	L-lysine
NSPVR 1	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR2	-	+	+	+	+	+	+	+	+	+	+	+
NSPVR3	-	+	+	+	+	+	+	+	+	+	+	+
NSPVR4	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR5	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR6	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR7	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR8	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR9	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR10	+	+	+	+	+	-	+	+	+	+	+	+
NSPVR11	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR12	-	+	+	+	+	+	+	+	+	+	+	+
NSPVR13	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR14	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR15	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR16	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR17	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR18	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR19	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR20	+	+	+	+	+	+	+	+	+	-	+	+
NSPVR21	+	+	+	+	+	+	+	+	+	+	+	-
NSPVR22	+	+	+	+	+	-	+	-	+	+	+	+
NSPVR23	+	+	+	+	+	+	-	+	-	-	+	+
NSPVR24	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR25	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR26	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR27	-	+	+	+	+	+	+	+	-	+	+	+
NSPVR28	-	+	+	+	+	+	+	+	+	+	+	+
NSPVR29	-	+	+	+	+	-	+	+	-	-	+	+
NSPVR30	+	-	+	+	+	-	+	-	+	+	+	+
NSPVR31	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR32	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR33	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR34	+	+	+	+	+	+	+	+	+	+	+	-
NSPVR35	+	+	+	+	+	+	+	+	-	+	+	+
NSPVR36	+	+	+	+	+	+	+	+	+	+	+	+

NSPVR37	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR38	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR39	+	-	+	+	+	+	+	+	+	+	+	+
NSPVR40	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR41	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR42	-	+	+	+	+	+	+	+	+	+	+	+
NSPVR43	+	+	+	+	+	+	+	+	-	+	+	+
NSPVR44	+	+	+	+	+	+	+	-	+	+	+	+
NSPVR45	+	+	+	+	+	+	+	-	+	+	-	-
NSPVR46	+	+	+	+	+	-	+	-	+	+	-	+
NSPVR47	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR48	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR49	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR50	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR51	+	-	+	+	+	-	+	+	+	+	+	-
NSPVR52	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR53	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR54	+	+	+	+	+	-	+	-	+	+	+	+
NSPVR55	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR56	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR57	-	+	+	+	+	+	+	+	+	+	+	+
NSPVR58	+	+	+	+	+	+	+	+	+	+	+	-
NSPVR59	+	+	+	+	+	+	+	-	+	+	+	+
NSPVR60	+	+	+	+	+	+	+	+	-	+	+	+
NSPVR61	+	+	+	+	+	+	+	+	+	-	+	+
NSPVR62	+	+	+	+	+	+	+	+	-	+	+	+
% of grown isolates	87%	95%	100%	100%	100%	89%	98%	89%	89%	93%	97%	92%

Key: + : Presence of growth.
- Absence of growth

Appendix 5. Utilization of different carbon sources

Isolates	D-glucose	D-fructose	Lactose	D-glucoinic acid	Dextrin	Dulcitol	Raffinose	Mannose	L- arabonose	xylose	Citrate	Cellobiose	Galactose	L- rhaminose	Starch	Sorbitol	Malate	Glycerol	Trehalose	Eructose	Lactate	Tantarate
NSPVR 1	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
NSPVR2	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
NSPVR3	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
NSPVR4	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
NSPVR5	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR6	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
NSPVR7	+	+	+	+	-	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+
NSPVR8	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR9	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR10	+	+	+	+	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+
NSPVR11	+	+	+	+	-	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+
NSPVR12	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
NSPVR13	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+
NSPVR14	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
NSPVR15	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR16	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+
NSPVR17	+	+	+	-	-	-	+	+	-	+	-	+	+	+	-	+	+	+	+	+	+	-
NSPVR18	+	+	+	+	-	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+
NSPVR19	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
NSPVR20	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR21	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR22	+	+	+	+	-	+	+	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+
NSPVR23	+	+	-	+	-	-	-	-	-	+	+	-	+	-	+	-	+	+	-	+	+	+
NSPVR24	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
NSPVR25	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR26	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
NSPVR27	+	+	+	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	+	+	-	+
NSPVR28	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
NSPVR29	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
NSPVR30	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR31	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+
NSPVR32	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR33	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-

NSPVR34	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-
NSPVR35	+	+	-	+	-	-	-	+	-	+	+	+	+	+	+	-	+	+	-	+	+	+	+
NSPVR36	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR37	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR38	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
NSPVR39	+	+	+	+	-	-	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+
NSPVR40	+	+	+	+	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR41	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-
NSPVR42	+	+	+	+	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-
NSPVR43	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR44	+	+	+	+	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR45	+	+	+	+	-	-	-	-	-	+	-	+	+	+	-	+	+	+	+	+	+	+	-
NSPVR46	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR47	+	+	+	+	-	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR48	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR49	+	+	+	+	-	-	+	+	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+
NSPVR50	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR51	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR52	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR53	+	+	+	+	-	-	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	+	-
NSPVR54	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
NSPVR55	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-
NSPVR56	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR57	+	+	+	+	-	-	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+
NSPVR58	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR59	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR60	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-
NSPVR61	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-
NSPVR62	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
% of grown isolates	100%	100%	97%	92%	13%	53%	95%	97%	37%	100%	61%	98%	93%	95%	82%	92%	100%	98%	97%	100%	95%	69%	

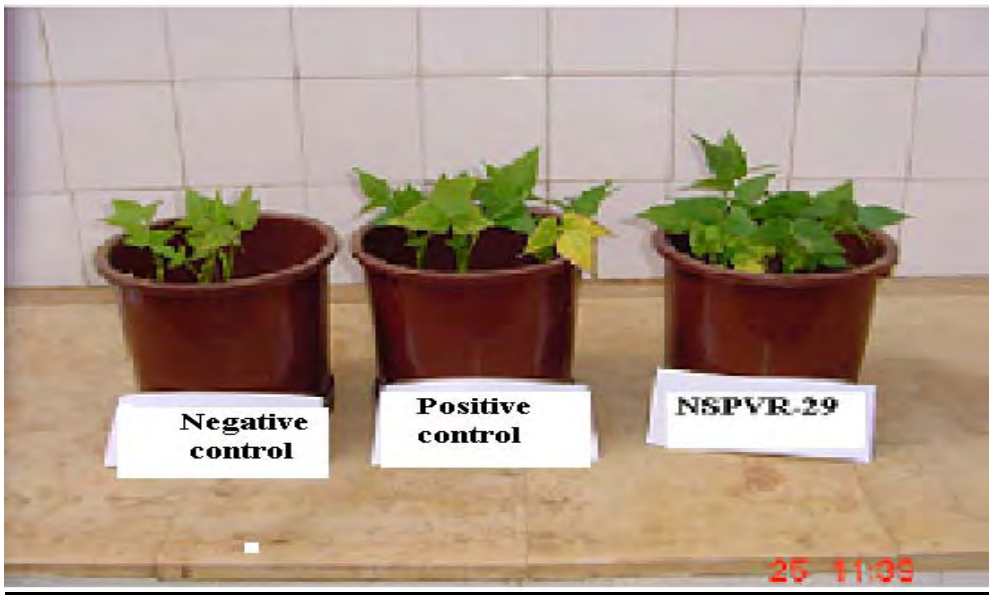
Key: + : Presence of growth
- Absence of growth

Appendix 6.Effect of different antibiotics on growth of common bean rhizobia.

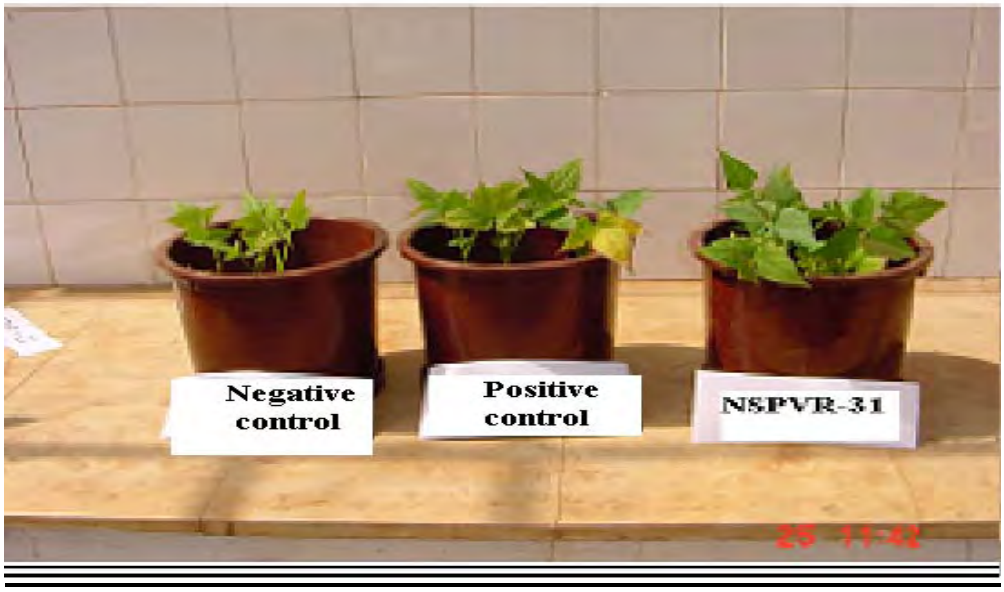
Isolates	Streptomycin (3ug/ml)	Streptomycin (10ug/ml)	Novobiocin 0.5ug/ml)	Novobiocin (2 ug/ml)	Kanamycin (5ug/ml)	Kanamycin (15ug/ml)	Spectinomycin (2.5ug/ml)	Spectinomycin (5ug/ml)	Oxytetracycline(0.1ug/ml)	Oxytetracycline(0.2ug/ml)	Naldixic acid(40ug/ml)	Naldixic acid (60ug/ml)
NSPVR 1	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR2	-	+	+	+	+	+	+	+	+	+	+	+
NSPVR3	+	-	+	+	+	+	+	+	+	+	+	+
NSPVR4	+	+	+	+	+	-	+	+	+	+	+	+
NSPVR5	+	-	+	+	-	-	+	+	+	+	+	+
NSPVR6	-	-	+	+	-	-	+	+	+	+	+	+
NSPVR7	-	-	+	+	-	-	+	+	+	+	+	-
NSPVR8	-	-	+	+	-	-	+	+	+	+	+	+
NSPVR9	-	-	+	+	-	-	+	+	+	+	+	+
NSPVR10	-	-	+	+	-	-	+	+	+	+	+	+
NSPVR11	-	-	+	+	+	-	+	+	+	+	+	-
NSPVR12	-	-	+	+	-	-	+	+	+	+	+	+
NSPVR13	+	+	+	+	+	-	+	+	-	-	+	+
NSPVR14	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR15	+	-	+	+	-	-	+	+	+	+	-	-
NSPVR16	-	-	+	+	-	-	+	+	+	+	+	+
NSPVR17	-	-	+	+	+	+	+	+	+	+	+	-
NSPVR18	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR19	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR20	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR21	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR22	-	-	+	+	-	-	+	+	+	+	+	+
NSPVR23	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR24	+	+	+	+	-	-	+	+	+	+	+	+
NSPVR25	-	-	+	+	-	-	+	+	+	+	+	-
NSPVR26	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR27	+	+	-	-	-	-	-	-	-	-	-	-
NSPVR28	-	-	+	+	-	-	+	+	+	+	+	-
NSPVR29	+	+	+	+	-	-	+	+	+	+	+	+
NSPVR30	-	-	+	+	-	-	+	-	+	+	+	-
NSPVR31	+	+	+	+	+	+	+	+	+	+	+	+

NSPVR32	-	-	+	+	-	-	+	+	+	+	-	-
NSPVR33	-	-	+	+	-	-	+	+	+	+	+	-
NSPVR34	-	-	+	+	-	-	+	+	+	+	+	+
NSPVR35	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR36	+	-	+	+	-	-	+	+	+	+	+	+
NSPVR37	+	+	+	+	-	-	+	+	+	+	+	+
NSPVR38	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR39	+	+	+	+	+	-	+	+	+	+	+	+
NSPVR40	-	-	+	+	-	-	+	+	+	+	+	+
NSPVR41	-	-	+	+	-	-	-	+	+	+	-	-
NSPVR42	-	-	+	+	-	+	+	+	+	+	+	-
NSPVR43	+	+	+	+	-	-	+	+	+	+	+	+
NSPVR44	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR45	-	-	+	+	-	-	+	+	+	+	+	+
NSPVR46	-	-	+	+	-	-	-	-	+	-	+	-
NSPVR47	-	-	+	+	-	-	+	+	+	+	+	-
NSPVR48	+	+	+	+	-	-	+	+	+	+	+	+
NSPVR49	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR50	+	+	+	+	-	-	+	+	+	+	+	+
NSPVR51	-	-	+	+	-	-	+	+	+	+	-	-
NSPVR52	+	+	+	+	-	-	+	+	+	+	+	+
NSPVR53	+	+	+	+	+	-	+	+	+	+	+	+
NSPVR54	-	-	+	+	-	-	+	+	+	+	-	-
NSPVR55	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR56	+	+	+	+	-	-	+	+	+	+	+	+
NSPVR57	-	-	+	+	-	-	+	+	+	-	-	-
NSPVR58	-	-	+	+	+	+	+	+	+	+	+	+
NSPVR59	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR60	-	-	-	-	+	+	+	+	+	-	-	+
NSPVR61	+	+	+	+	+	+	+	+	+	+	+	+
NSPVR62	+	-	+	+	+	-	+	+	-	+	+	-
% of tolerated isolates	56%	48%	97%	97%	42%	35%	95%	95%	95%	92%	87%	71%

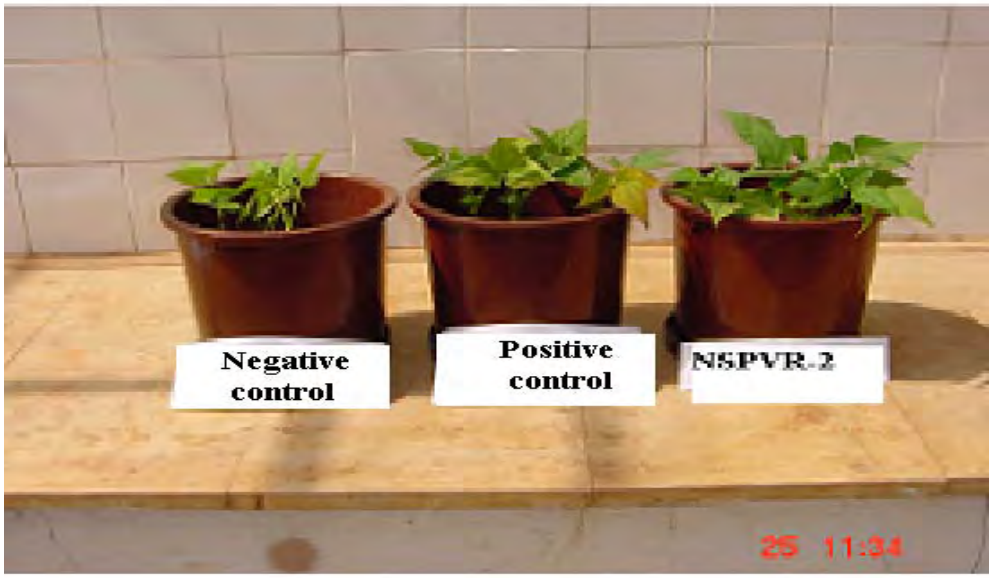
Key: + : Presence of growth.
- Absence of growth



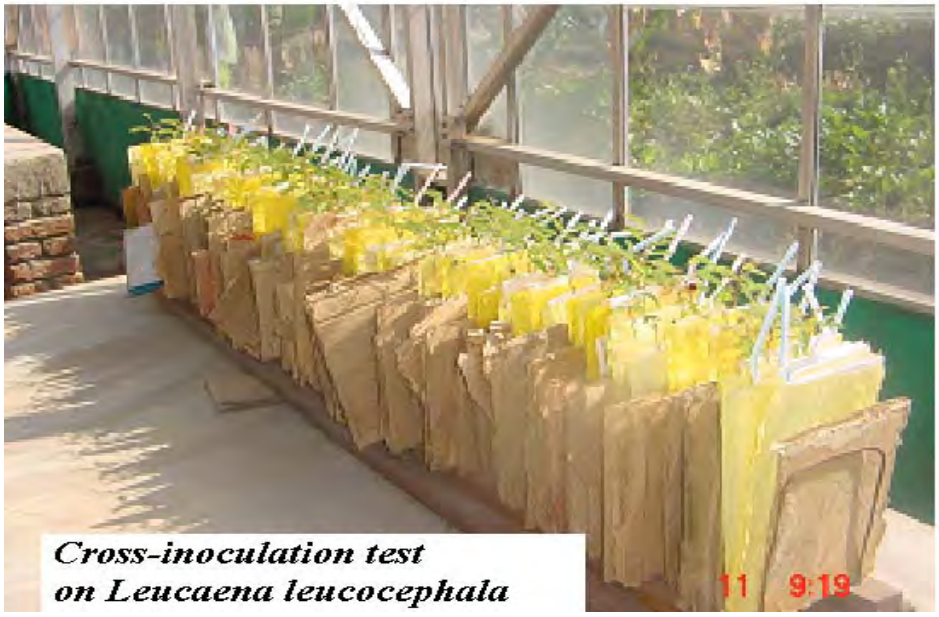
Appendix 7. Variety Awash Melka Inoculated with NSPVR-29.



Appendix 8. Variety Awash Melka inoculated with NSPVR-31.



Appendix 9. Variety Awash Melka inoculated with NSPVR-2



Appendix 10. Cross inoculation pouch experiment on *Leucaena leucocephala*

Appendix 11. Phosphorus solubilization efficiency of isolate NSPVR-8.

Appendix 12. Phosphorus Solubilization efficiency of isolate NSPVR-11.

Appendix 13. Pouch experiment of enumeration for Melkassa soil on Ayenew variety.

Appendix 14. Pouch experiment of enumeration for Babille soil on Ayenew variety.