*J. Agr. Sci. Tech. (2010) Vol. 12: 365-376*

# Characterization of Acid and Salt Tolerant *Rhizobial* Strains Isolated from Faba Bean Fields of Wollo, Northern Ethiopia

A. Keneni[1], F. Assefa[2], and P. C. Prabu[1*]

## ABSTRACT

**Density of *Rhizobium* population in faba bean (*Vicia faba* L.) fields of Wollo area (Asketema, Gashana, Kotem, Major and Sekota) northern Ethiopia was studied. The highest population of rhizobia was found at Major and the least at Sekota regions. The native *rhizobial* strains isolated from the northern parts of the country tolerated a higher salt concentration (5% NaCl) than the exotic *rhizobial* strains (Tall 1402 and Tall 1397). Both native and exotic strains failed to grow at pH 4 and 4.5 levels in the laboratory conditions. In the soil adjusted to pH 4-7, all the native *rhizobial* strains persisted while those of the exotic strain failed to survive at pHs below 5.5. The native strains were more versatile than the exotic ones in utilizing different carbohydrates as a sole carbon source and were found to be more resistant to many antibiotics (streptomycin, chloramphenicol, rimfampenicillin, oxytetracycline, penicillin and tetracycline) than the exotic strains which are found resistant to chloaramphenicol only. Percentage of nitrogen fixation is also higher for native *rhizobial* strains these isolates being found to be superior to the exotic strains in stimulating growth, dry matter yield, nodulation and nodule wet weight of faba bean in pouch culture.**

**Keywords**: Acid tolerant, Faba bean, Northern Ethiopia, *Rhizobial* strains, Salt tolerant.

## INTRODUCTION

Leguminous plants are able to establish nitrogen-fixing symbiosis with certain Gram-negative bacteria, collectively known as rhizobia. In the *Rhizobium*–legume symbiosis, the process of nitrogen fixation is strongly related to the physiological state of the host plant. Therefore, an efficient *rhizobial* strain is not expected to express its full capacity for nitrogen fixation if limiting factors impose limitations on the vigor of the host legume (Joaquina Nogales, 2002). Several environmental conditions are the limiting factors to the growth and activity of nitrogen-fixing plants. Typical environmental stresses faced by the legume nodules and the symbiotic partners may include water stress, salinity, soil pH, temperature, heavy metals, and so on (Cigdem Kucuk and Merih Kivanc, 2008).

Soil salinity is a significant problem facing agricultural production in many areas of the world and soil infertility in these areas is often due to the presence of large concentrations of salt. Nearly 40% of the world land surface can be categorized as suffering from potential salinity problem (Waraporn Payakapong, 2006). Most leguminous plants require a neutral or slightly acidic soil for growth, especially when they depend on symbiotic $N_2$ fixation and as well more sensitive to salinity than their *rhizobial* counterparts and consequently, the symbiosis being more sensitive to salt stress than free-living rhizobia (Zahran, 1999). The strategies employed in the last few years to reduce the

[1] Department of Applied Biology, Faculty of Natural Resources, Ambo University College, P. O. Box: 19, Ambo, Ethiopia.
[2] Department of Biology, Faculty of Science, Addis Ababa University, Addis Ababa, Ethiopia.
* Corresponding author; email:prabu_22@yahoo.com



[ Downloaded from jast.modares.ac.ir on 2024-05-03 ]

[ DOR: 20.1001.1.16807073.2010.12.3.10.0 ]

effect of salt stress on legume production have been focused on a selection of host genotypes that are tolerant to high salt conditions (Cordovilla *et al*., 1995). Thus, an increase of tolerance to salinity of *rhizobial* bacteria might constitute another approach to improve plant productivity under symbiosis. In free-living conditions some strains of *Rhizobium meliloti* (Breedveld *et al*., 1991) and *Rhizobium tropici* (Graham *et al*., 1992) can tolerate up to 500 mM of NaCl. It has been found out that some species of rhizobia adapt to saline conditions through the intracellular accumulation of low-molecular-weight organic solutes called osmolytes, such as glutamate, trehalose, glycine betaine and polyamines, or an accumulation of $K^+$ (Wei *et al*., 2004).

Nitrogen-fixing legumes tolerant to salinity represent an important alternative to improve fertility. In Ethiopia there is a widespread acidity and salinity problem, especially in the northern region. Many areas in this region are not suitable for agriculture because of either acidity or salinity. Faba bean (*Vicia faba* L.) grows successfully forming effective root nodules in these regions and ranks first in terms of heritage and production (Asfaw Telaye *et al*., 1994). From about 923,110 ha of land under pulses, 395,440 hectares were occupied by faba bean (CSA, 2002), which is about 42.83% of land under pulses and 20.6% of the land under total cultivation. In Ethiopia, it is more commonly found in the "*Weyna Dega*" i.e. between 1,800 and 2,400 meters of elevation with the greatest concentration in Shewa, Wollo, Tigray, Gojam and Gonder regions (Ayneabeba Adamu *et al*., 2001) and considered as a secondary center of diversity and also one of the nine major agro-geographical production regions (Asfaw Telaye *et al*., 1994). Wollo is one of the regions with soils being characterized by low pH, low P levels, and high concentrations of aluminum, which can limit N- fixation. The objectives of this investigation are to isolate and characterize, high acid and salt tolerant *rhizobial* strains from the nodules of faba bean and assess their ability to tolerate different constraints (high acidity and salt concentrations), to see the results on nitrogen fixation and dry matter production of faba bean.

## MATERIALS AND METHODS

### Sampling Areas and Isolation of *Rhizobium* sp.

Soil samples were collected from five regions of Wollo namely "Asketema, Gashana, Korem, Major and Sekota" in plastic bags. Faba bean seeds (variety *Bulga-70*) were surface sterilized with 95% ethyl alcohol and 0.2% acidified mercuric chloride for three minutes as per Vincent (1970). After being washed with several times with distilled sterilized water, five seeds were planted in each of a number of plastic bags with different soil samples and later thinned to three following germination. After 45 days of growth, plants were uprooted for a pick up a nodules. Nodules were surface sterilized and macerated with drops of sterile water in Petri dishes with aid of glass rods. Then 0.1 ml of the suspensions was streaked on plate containing Yeast Extract Mannitol Agar (YEMA). The isolates were purified through repeated streaking and being added congo red for differential diagnosis and stored on YEMA slants containing calcium chloride (3g $l^{-1}$). The isolated native strains were characterized on the basis of morphological and physiological characters according to Jordan, (1984). Some representative soil samples from each region were analyzed to correlate the differential availability of nutrients with the ability of the *Rhizobium* isolates to tolerate the different constraints and to see their effectiveness in stimulating growth and nodulation of the faba bean. The isolation of *rhizobial* strains, their test of tolerance to high acidity and the ability to grow in different salt concentrations were carried out at the department of Biology, Addis Ababa University. The physical and



[ Downloaded from jast.modares.ac.ir on 2024-05-03 ]

[ DOR: 20.1001.1.16807073.2010.12.3.10.0 ]

chemical analysis of soil and greenhouse experiments were done at National Soil Research Center of the Ethiopian Agricultural Research Organization (EARO).

### Cultural Characteristics and pH Changes

Cultures were examined for cell morphology and gram reaction after 1 to 2 days of growth in yeast extract mannitol liquid medium (Vincent, 1970). Colony purity and morphology were examined using cultures that were grown for three to five days on Yeast Extract Mannitol Agar (YEMA) containing 0 to 25 mg of BromoThymol blue $L^{-1}$. Change of the agar medium to yellow is indicative of acid production.

### Test for High Acidity and Salt Tolerance

Five *rhizobial* strains were isolated from the different localities of Wollo area and compared with two strains of Tall *Rhizobium* obtained from National Soil Research Center of the Ethiopian Agricultural Research Organization (EARO) that had known physiology and effectiveness. Gram reaction, cell shape, acid test, catalase and oxidase activity of these strains were studied. The capability of the *rhizobial* strains to grow in acidic media was tested by their being streaked on YEM agar plates of pH adjusted to 4, 4.5, 5.0, 5.5, 6.0, and 7.0 (Jordan, 1984). The ability of the isolates to grow in different concentrations of salt was tested by streaking isolates on YEM media containing 0.5%, 1%, 2%, 3%, 3.5%, 4%, and 5% (w/v) NaCl (El Sheikh and Wood, 1989). Acid tolerance was also studied in sterilized red soil (soil: sand 1:1 w/w) 10 g per tube/three tubes/treatment with isolated strains. Soil samples were sterilized by autoclaving and adjusted to field capacity. Limed soils were used one week after the addition of $CaCO_3$. The initial pH of the soil was 6.00 (in 2:5 w/v) and then adjusted to 4, 4.5, 5, 5.5, 6 or 7 using $CaCO_3$. After 15 days remaining at 28°C, 10 ml of distilled sterilized water was added to each one of the tube and 0.1 ml of the suspension streaked on Petri dishes containing yeast extract mannitol agar medium. The appearance of colonies after a lapse of three day time would indicate that the isolate had survived at a particular pH in the soil (Aurag and Sasson, 1992).

### Test for Ability to Persist at Low Moisture Content of the Soil

The five isolated *rhizobial* strains were inoculated into sterilized soil sand culture prepared, as previously described, at pH 5.5 and limed for pH 7. Twenty grams of each soil was dispensed in 100 ml glass flasks and incubated at 28°C. Soil humidity was either kept constant at field capacity during the experiment or only adjusted at the start and then left to fall, to mimic drought conditions. The population dynamics of the *rhizobial* strains was keenly followed through regular counting (the initial population number was $5\times10^7$ bacteria $g^{-1}$ of soil) (Aurag and Sasson, 1992).

### Carbohydrate Utilization

The basal medium was used with different carbohydrates (Cellobiose; Cellulose; Fructose; Galactose; Glucose; Inositole; Lactose; Sorbtols; Starch; Sucrose; Arabinose; Benzoate; Rhaminose, Acetate; Citrate; Succinate; Oxaloacetic acid) (1% w/v) substituted for mannitol. The medium was solidified with purified agar. Inocula were prepared by diluting the 24 hours young cultures with distilled sterilized water to a density of $10^6$ cells per ml then inoculating the surface of carbohydrate containing agar plates. Triplicate plates of each carbohydrate were incubated at 28°C for 7 days and scored for growth.



[ Downloaded from jast.modares.ac.ir on 2024-05-03 ]

[ DOR: 20.1001.1.16807073.2010.12.3.10.0 ]

### Intrinsic Antibiotic Resistance

The five isolated strains and standard strains were treated with selected antibiotics to determine their intrinsic antibiotics resistance pattern. The antibiotics (mg $l^{-1}$) used were: chloramphenicol (2, 20), sterptomycin sulphate (2.5, 10), rimfampenicillin (3, 6), oxytetracycline (10, 20), penicillin (20, 30) and tetracycline (10, 20). Stock solution of the antibiotics was prepared immediately before use in sterile distilled water with the exception of rimfampenicillin which was dissolved in a small volume of 95% ethanol. Appropriate quantities of the antibiotic stock solutions mentioned above were added to molten YEMA at 48°C, mixed thoroughly and then poured on petri dishes. Isolates were grown in YEM broth for 48 hours. A portion of each culture was diluted in sterile distilled water to prepare for solutions so that 0.1 ml of each isolate inoculated on a Petri dish would finally contain about $10^7$ CFU $ml^{-1}$ of the culture.

### Test for N-fixing Capacity

The N-fixation efficiency of strains graded as 2 and 3 according to their pink color was further tested using the variety *Bulga-70* of faba bean cultivated in pouch. For this experiment, seeds were surface sterilized and pre germinated as described before. Each pouch was planted with three seedling inoculated with each seedling receiving 1 ml of a solution containing approximately $10^8$ cells per ml of *rhizobial* strains. Non-inoculated pouchs and pouchs fertilized with mineral nitrogen (3 mg of N as $KNO_3$) were employed as control. The all pouches received 200 ml of macro and micro N-free nutrients each week. The experiment was carried out in a greenhouse and the experimental design was a randomized complete block one, with each treatment in triplicate. After eight weeks of growth, the plants were harvested and separated into their roots and shoots. Nodules were removed from roots and counted. Dry weight was determined for shoots, roots and nodules. Strain effectiveness was assessed according to the equation proposed by Purcino *et al.* (2000) as (100× inoculated plant DM/N-fertilized plant DM) and nitrogen fixing efficiency being classified as ineffective <35%, moderately effective 35-50%, effective 50-80% and highly effective >80% (Purcino *et al.,* 2000). An analysis of variance was carried out for each variable, mean value being analyzed through cluster analysis.

## RESULTS

Many *rhizobial* strains were isolated from each sampling field. The isolates were fast growing acid producing, similar to the type strain *Rhizobium leguminosarum* biovar. *viciae*. They were differentiated from one another on the basis of morphological, cultural and some such important biochemical characters as carbohydrate utilization and growth on different concentrations of sodium chloride to test for the ability to resist various antibiotics. From among the many isolates, five native ones (R301, R302, R303, R306, and R309 of each region of Wollo area) showed more growth and could be used for further study along with exotic *rhizobial* cultures (Tall 1402 and Tall 1397).

### Population Density

The number of *Rhizobia* at each site were found to differ from $2.2\times10^5$ Sekota and $3.5\times10^{10}$ at Major per gram of soil (Table 1). *Rhizobial* numbers were highly correlated with pH of the soil and also significantly associated with available P (r= 0.75). Great variability was observed in some nutrients tested in the different sampling sites. Gashana and Major showed low total soil nitrogen, while those of Asketama, Korem and Sekota identified as containing medium total soil nitrogen. The soil phosphorus



[ Downloaded from jast.modares.ac.ir on 2024-05-03 ]

[ DOR: 20.1001.1.16807073.2010.12.3.10.0 ]

[ DOR: 20.1001.1.16807073.2010.12.3.10.0 ] [ Downloaded from jast.modares.ac.ir on 2024-05-03 ]



**Table 1.** Soil physical and chemical analysis and population density of *Rhizobia* at each study site**.**

| Site | pH | Exchangeable base (meq 100 $g^{-1}$) | | | | | CEC (meq 100 $g^{-1}$) | T. N. [a] (%) | O. C. [b] (%) | C/N | Av. P (mg $kg^{-1}$) | EC (dS $m^{-1}$) | Population density |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Na | K | Ca | Mg | Sum | | | | | | | |
| Asketema | 6.14 | 0.19 | 0.22 | 18.66 | 9.27 | 28.15 | 30.0 | 0.129 | 1.269 | 10 | 18.0 | 0.151 | $2.25\times10^{6}$ |
| Gashana | 6.3 | 0.29 | 0.13 | 15.27 | 7.32 | 23.03 | 28.0 | 0.09 | 0.635 | 7 | 13.4 | 0.034 | $2.5\times10^{7}$ |
| Korem | 6.4 | 0.33 | 0.2 | 17.64 | 10.95 | 29.11 | 36.9 | 0.109 | 0.778 | 7 | 7.0 | 0.048 | $2.8\times10^{9}$ |
| Major | 7.05 | 0.12 | 0.23 | 8.23 | 6.92 | 15.38 | 18.0 | 0.045 | 0.404 | 9 | 11.2 | 0.040 | $3.5\times10^{10}$ |
| Sekota | 6.55 | 0.21 | 0.17 | 11.23 | 13.5 | 24.89 | 29.4 | 0.109 | 0.779 | 7 | 6.0 | 0.078 | $2.2\times10^{5}$ |

[a] Total Nitrogen, [b] Organic Carbon.

**Table 2.** Some morphological and physiological features of the *rhizobial* strains and effect of the different concentrations of sodium chloride on their growth.

| Isolate code | Gram reaction | Cell shape | Acid test | Catalase activity | Oxidase activity | Different NaCl concentration (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 0.5 | 1 | 2 | 3 | 4 | 5 |
| R301 | - | rod | yellow | + | - | + | + | + | + | + | + |
| R302 | - | rod | yellow | + | - | + | + | + | + | + | + |
| R303 | - | rod | yellow | + | - | + | + | + | + | + | + |
| R306 | - | rod | yellow | + | - | + | + | + | + | + | + |
| R309 | - | rod | yellow | + | - | + | + | + | + | + | + |
| Tall 1402 | - | rod | yellow | + | + | + | + | + | - | - | - |
| Tall 1397 | - | rod | yellow | + | + | + | + | + | - | - | - |

**Table 3.** Effect of different pH of soil and lab media on growth of *rhizobial* strains.

| Isolate code | Different pH value of laboratory media | | | | | | Different pH value of Soil | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH 4 | pH 4.5 | pH5 | pH 5.5 | pH 6 | pH 7 | pH4 | pH 4.5 | pH 5 | pH 5.5 | pH 6 | pH 7 |
| R301 | - | - | - | - | - | + | + | + | + | + | + | + |
| R302 | - | - | - | - | - | + | + | + | + | + | + | + |
| R303 | - | - | - | + | + | + | + | + | + | + | + | + |
| R306 | - | - | + | + | + | + | + | + | + | + | + | + |
| R309 | - | - | - | - | - | + | + | + | + | + | + | + |
| Tall 1402 | - | - | - | - | - | + | - | - | - | + | + | + |
| Tall 1397 | - | - | - | - | - | + | - | - | - | + | + | + |

content of Asketema site is categorized as high, Gashana and Major sampling sites identified as medium while soils of Korem and Sekota are of low phosphorus content.

### Ability to Grow in Different Concentration of Sodium Chloride on Laboratory Media as Well as in Soil Conditions

All *Rhizobia* isolated from different sampling sites were able to grow in all ranges of the various sodium chloride concentrations tested. The ability to tolerate high salt is far greater for the native strains than for the two exotic strains, which failed to grow in above 2% sodium chloride concentrations of the medium (Table 2). But the native *rhizobial* strains isolated from the Wollo region are able to grow variably throughout the different sodium chloride range tested from 0.5% to 5% indicating the fact that native *rhizobial* strains were more adapted to soils highly concentrated with various forms of cations and anions.

### Effect of pH on Growth of *Rhizobial* Strains on Laboratory Media and in Soil Conditions

No *rhizobial* strains were able to grow at pH of the medium adjusted to 4 and 4.5 including the exotic strains. Only one strain (R306) was able to grow on the medium adjusted to pH 5, whereas another *rhizobial* strain (R303) grew on the medium adjusted to 5.5. Except for these two strains of the native *rhizobial* isolates, all the rest including the exotic *Rhizobia* were able to grow only on the medium adjusted to pH 7 (Table 3). In the soil environment the condition is highly different. All the native *rhizobial* strains were able to survive well in the various soils adjusted to pH 4 up to 7, while the two exotic strains were unable to survive pHs up to 5.5 of the soil.

Any of the *rhizobial* strains tested showed significant differences in their ability to utilize different carbohydrates as a sole carbon source. Of all the carbohydrates tested, only three (acetate, cellulose, succinate) were completely not utilized by all the *rhizobial* isolates (Table 4). All the *rhizobial* strains tested were able to utilize cellobiose, fructose, inositole and starch as a sole carbon source. Three of the native *rhizobial* strains were unable to utilize five carbohydrates as a sole carbon source. One native isolate (R309) was unable to utilize 9 out of the seventeen carbohydrates tested as a sole carbon source. The exotic *rhizobial* strains were less efficient in utilizing various carbohydrates as a sole carbon source than the native, indicating the fact that native isolates are more versatile in utilizing various carbohydrates as a sole carbon source which in turn indicates their more competitiveness in nodulation and nitrogen fixation in soil conditions.

The native *rhizobial* strains isolated from the northern parts of Ethiopia showed great versatility to the different antibiotics. Two (R303 and R309) out of five native *rhizobial* strains tested showed resistance to streptomycin sulphate, chloramphenicol, penicillin and tetracycline, whereas another native *rhizobial* strain (R301) showed resistance to chloramphenicol and penicillin (Table 5). The native isolate R302 and the two exotic type strains were able to resist chloramphenicol only. In general, the native *rhizobial* strains were found to be more resistant to the tested antibiotics than the exotic ones. All the strains tested were 100% sensitive to rimfampencilline and oxytetracycline. There were significant differences observed in strain effectiveness among both exotic and native isolates.

High variability was observed particularly in comparing the native isolates with the exotic ones. All the *rhizobial* strains of the northern region of Ethiopia are also highly effective in stimulating nodulation and in biomass increase of the faba bean. The plant shoot height showed highly significant differences due to inoculation of different *rhizobial* strains in the pouch culture, indicating that different *rhizobial* strains



[ Downloaded from jast.modares.ac.ir on 2024-05-03 ]

[ DOR: 20.1001.1.16807073.2010.12.3.10.0 ]

[ DOR: 20.1001.1.16807073.2010.12.3.10.0 ] [ Downloaded from jast.modares.ac.ir on 2024-05-03 ]





**Table 4.** Ability of isolated *rhizobial* strains to utilize different carbohydrates as a sole carbon source.

| Isolate code | Source of carbon | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cello [a] | Cellu [b] | Fru [c] | Gala [d] | Glu [e] | Inosi [f] | Lac [g] | Sorb [h] | Star [i] | Sucr [j] | Benzo [k] | Arabi [l] | Rhami [m] | Citr [n] | Aceta [o] | OAA [p] | Succi [q] |
| R301 | + | - | + | + | + | + | + | + | + | + | - | + | + | + | - | - | - |
| R302 | + | - | + | + | + | + | + | + | + | + | - | + | + | - | - | + | - |
| R303 | + | - | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| R306 | + | - | + | + | + | + | + | + | + | + | - | + | + | + | - | - | - |
| R309 | + | - | + | - | - | + | + | - | + | + | + | - | + | - | - | - | - |
| Tall 1402 | + | - | + | + | - | + | - | + | + | - | + | + | - | - | - | - | - |
| Tall 1397 | + | - | + | + | - | + | - | + | + | - | + | + | + | + | - | - | - |

[a] Cellobiose; [b] Cellulose; [c] Fructose; [d] Galactose; [e] Glucose; [f] Inositole; [g] Lactose; [h] Sorbtols; [i] Starch; [j] Sucrose; [k] Arabinose; [l] Benzoate; [m] Rhaminose; [n] Acetate; [o] Citrate; [p] Succinate, [q] Oxaloacetic acid.

**Table 5.** Effect of different concentrations of antibiotics on growth of *rhizobial* strains.

| Isolate code | Different concentrations of antibiotics (mg $L^{-1}$) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Streptomycin sulphate | | Chloramphinicol | | Rimfampenicillin | | Oxytetracycline | | Penicillin | | Tetracycline | |
| | 2.5 | 10 | 12 | 20 | 3 | 6 | 3 | 10 | 20 | 30 | 10 | 20 |
| R301 | - | - | + | + | - | - | - | - | + | + | - | - |
| R302 | - | - | + | + | - | - | - | - | - | - | - | - |
| R303 | + | + | + | + | - | - | - | - | + | + | + | + |
| R306 | - | - | + | + | - | - | - | - | + | + | - | - |
| R309 | + | + | + | + | - | - | - | - | + | + | + | + |
| Tall 1402 | - | - | + | + | - | - | - | - | - | - | - | - |
| Tall 1397 | - | - | + | + | - | - | - | - | - | - | - | - |

**Table 6:** Effect of different *rhizobial* strains on faba bean height, dry matter yield nodule number and nodule wet weight.

| Isolate code | Plant height (cm) | Dry matter (g) | Nodule No. | Nodule weight (g) |
|---|---|---|---|---|
| R301 | 48 ± 3.0[a] | 3.93±.0.40 [a] | 152±15.0 [b] | 3.5±0.40 [b] |
| R302 | 48± 3.0 [a] | 4.27±0.24 [a] | 205±12.0 [a] | 4.0 ±0.35 [a] |
| R303 | 49±4.51[a] | 3.53±0.75 [b] | 209± 14.0 [a] | 4.0±0.23 [a] |
| R306 | 42±2.8 [c] | 3.4±0.32 [b] | 215±16.0 [a] | 4.2±0.32 [a] |
| R309 | 45±3.0 [b] | 3.26±0.23 [b] | 103±13.0 [c] | 4.1±0.75 [a] |
| Tal 1402 | 36±2.5[d] | 2.13±0.35 [d] | 120±12.0 [b] | 2.8±0.24 [c] |
| Tal 1397 | 36±3.0 [d] | 2.9±0.40 [c] | 121±11.0 [b] | 2.7±0.40 [c] |

were different in stimulating faba bean growth (Table 6).

## DISCUSSION

Soils of the different study sites highly varied in some nutrients like nitrogen and phosphorus. Gashana and Major regions are characterized by having low total soil nitrogen while the rest of the sites were characterized by having medium total soil nitrogen (Keneni *et al*., 2008). Significant variations were also observed in the phosphorus content of the soils from the different study areas, Asketma region being characterized as having high phosphorus content, Gashana and Major being characterized by having medium phosphorus contents, while Korem and Sekota characterized by containing low phosphorus content (Dibabe, 2000).

Growth of the five native *rhizobial* strains from the Wollo area is not much affected as the concentration of the salt increases from 0.5 to 5% as compared with that of exotic *rhizobial* strains, which were unable to grow in the sodium chloride concentrations above 2%. This ability of growth of the native *rhizobial* strains in high concentrations of sodium chloride solution can give high competitive value in the rhizosphere to survive and nodulate the host plants in harsh environmental conditions particularly at high concentrations of salt in the soil. This finding is in line with the report of Saraf and Dhandhukia (2005), who found that *Sinorhizobium meliloti* growth was not completely inhibited by 5% of sodium chloride concentration. Rabie and Alamadini (2005) also stated that the growth of *Rhizobium* was not affected by low and moderate levels of salinity. Acidity factors (high Al, low Ca and low $PO_4$) have a direct impact on either *rhizobial* growth, persistence or nodule initiation and nitrogen fixation effectiveness (Covertry and Evans, 1989). Soil acidity limits *Rhizobium* survival and persistence in soils and its subsequent root colonization, infection and nodule activity (Graham *et al*., 1992). There was a greater variability observed in the ability of the *Rhizobium* isolates to grow on laboratory media of low pH and to persist in acid soil conditions of lower pHs. All the five native *rhizobial* strains were able to persist in the soil adjusted to pH 4 well for fifteen days, while the exotic strains were unable to persist in soil adjusted to pH 4, 4.5 and 5. This result indicates that in soil environment, the effect of acidity could be buffered by the edaphic factors in which the *Rhizobium* is surviving. As a result the *rhizobial* strains that survived in pH 4 of the soil for 15 days are very important candidates as inoculants for the highly acidic soils of most faba bean fields to improve the yield. The ability to grow at an acidic pH would provide these isolates with competitive advantage over other rhizosphere organisms. These observations are in line with the reports of Aurag and Sasson (1992), who indicated that strains of *Rhizobium leguminosarum* bv. *phaseoli* grew in liquid media of pH 5. Evans *et al.* (1989) have cited critical pH ranges for



[ Downloaded from jast.modares.ac.ir on 2024-05-03 ]

[ DOR: 20.1001.1.16807073.2010.12.3.10.0 ]

*Rhizobium* activity as: at pH 7 *Rhizobium* root symbiosis unaffected by pH, at pH range of 7-6 suppression of nod gene occurs, in a pH range between 5 to 6, decreased multiplication and infection of *Rhizobium* occurs. Poor persistence of *Rhizobium leguminosarum* biovar. *viciae* in acidic soils has also been demonstrated and is reflected by a low nodulation score along with poor plant growth (Slattery *et al.,* 2001).

Survival, persistence and competitiveness of the *rhizobial* strains are the major factors determining their successful use as inoculants (Purcino *et al*., 2000). To determine these properties, the inoculated strains must be distinguished from the indigenous *Rhizobia* present in the soil. A large number of methods have been described for these purposes, but because of their complexity most of these methods are of limited applications. Use of intrinsic antibiotics resistance is the simplest and most commonly used method for strain identification (Cigdem Kucuk *et al*., 2006). In the present investigation it was found that, 100% of the isolates tested were able to resist chloramphenicol and 28.5% were resistant to the four antibiotics of: chloramphenicol, streptomycin sulphate, penicillin and tetracycline while 28.5% were found to be resistant to two antibiotics of: chloramphenicol and penicillin. Broad ranges of intrinsic antibiotic resistance pattern were found in strains isolated from chickpea nodules in Eskisehir areas of Turkey with a high level of resistance shown against streptomycin, erythromycin, kanamycin, penicillin and chloramphenicol (Kucuk and Kivanc, 2008).

Taking into consideration the percentage of nitrogen fixation effectiveness in pouch culture, R302 strain was found to be superior all the rest of the rhizobia tested, followed by R301 isolate. In general, the native *rhizobial* strains of the Wollo region were far more responsive than the two exotic *rhizobial* strains. Great variations were observed among the different rhizobia isolated from different fields of northern Ethiopia and as well between them and the exotic *rhizobial* strains tested as on the increase of faba bean growth, dry matter and nitrogen fixation (Figure 1). The *rhizobial* strain R302 was superior in increasing the dry matter yield of faba bean followed by R301 and there was no significant difference observed among the three other native *rhizobial* strains. Ability to form nodules on host plant is one of the parameters that makes a *Rhizobium* either effective or ineffective. As compared to other parameters tested, the variation

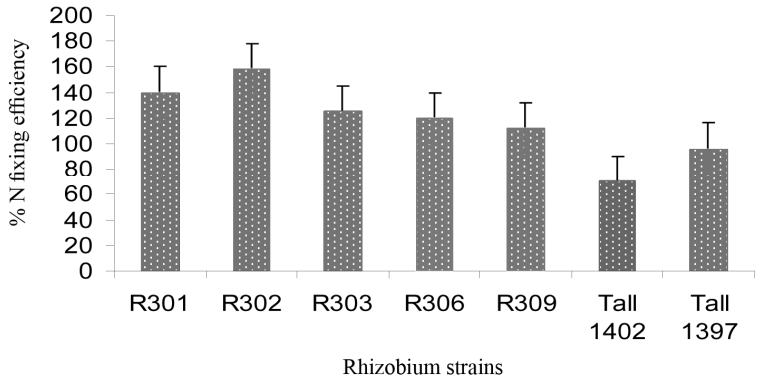


**Figure 1.** Percentage of N fixing efficiency of native and exotic *rhizobial* strains.



[ Downloaded from jast.modares.ac.ir on 2024-05-03 ]

[ DOR: 20.1001.1.16807073.2010.12.3.10.0 ]

in the nodule number is high in *Rhizobium* isolated from the Major site (R306) and as well superior in forming nodules in pouch culture followed by Korem (R303) and Gashana (R302). Both exotic strains of rhizobia carried lesser nodule numbers and were of less nodule-wet weight. This result is in conformity with the results obtained by Ademu *et al*. (2001) reporting significant variations in shoot length, dry matter and nodule fresh weight of faba bean when fertilized and inoculated differently in various soil types. Dibabe (2000) also reported significant differences among the treatments for dry matter and grain yields of faba bean treated with various concentrations of fertilizers in the field conditions.

## CONCLUSIONS

The highest population of rhizobia was found at Major and the least at Sekota regions of northern Ethiopia.

The native *rhizobial* strains isolated from the northern parts of the country tolerated higher salt concentrations (5% NaCl) than the exotic *rhizobial* strains did.

The native strains were more versatile than the exotic ones in utilizing different carbohydrates as a sole carbon source and were found to be more resistant to many antibiotics (streptomycin, chloramphenicol, rimfampenicillin, oxytetracycline, penicillin and tetracycline) than the exotic strains which are resistant to chloramphenicol only.

Percentage of nitrogen fixation is also higher for native *rhizobial* strains with these isolates found to be superior to the exotic ones in stimulating growth, promoting dry matter yield, nodulation and nodule wet weight of faba bean in pouch culture.

## REFERENCES


1. Keneni, A., Assefa, F. and Hailemariam, A. 2008. Associative Effect of *Rhizobium* and Phosphate Solubilizing Bacteria on Growth and Nutrient Uptake of Faba Bean (*Vicie faba* L).
2. Telaye, A., Bejiga, G. and Berhe, A. 1994. Role of Cool Season Food Legumes and Their Production Constraints in Ethiopian Agriculture, In: "Cool Season Food Legumes of Ethiopia", Telaye, A. (Ed.). Proceeding of the First National Conference, 16-20, December 1993. Addis Ababa, Ethiopia. PP. 3-17.
3. Dibabe, A. 2000. Effect of Fertilizers on the Yyield and Nodulation Pattern of Faba Bean on Nitosol os adet North Western Ethiopia. *Ethiopian J. Nat. Resource*, **2**: 237-244.
4. Aurag, J. and Sasson, A.1992. Tolerance of *Rhizobium leguminosarum* by Phaseoli to Acidity and Drought. *World J. Microbiol. Biotechnol*., **8**: 532-535.
5. Adamu, A., Assefa, F., Hailemariam, A. and Bekele, E. 2001. Studies of *Rhizobium* Inoculation and Fertilizer Treatments on Growth and Production of Faba Bean (*Vicia faba*) in Some Yield Depleted and Yield Sustained Region of Semien Shewa. *SINET, Ethiopia J. Sci*., **24**: 197-211.
6. Breedveld, M. W., Zevenhuizen, L. P. T. M. and Zehnder, A. J. B. 1991. Osmotically Regulated Trehalose Accumulation and Cyclic Beta (1, 2) Glucan Excretion by *Rhizobium leguminosarum* Biovar. *trifolii* TA–1. *Arch. Microbiol.,* **156**: 501–506.
7. Cordovilla, M. P., Ligero, F. and Lluch, C. 1994. The Effect of Salinity on $N_2$ Fixation and Assimilation in *Vicia faba. J. Exp. Bot.,* **45**: 1483–1488.
8. Kucuk, C. and Kivanc, M. 2008. Preliminary Characterization of *Rhizobium* Strains Isolated from Chickpea Nodules. *Afr. J. Biotechnol*., **7(6)**: 772-775.
9. Kucuk, C., Kivanc, M. and Kinaci, E. 2006. Characterization of *Rhizobium* sp. Isolated from Bean. *Turk. J. Biol*., **30**:127:132
10. Covertry D. R. and Evans J. 1989. Symbiotic Nitrogen Fixation and Soil Acidity, In: *"Soil Acidity and Plant Growth*", Robson, A. D. (Ed.). Academic Press, Sydney, PP. 103-136.
11. CSA (Central Statistical Authority), 2002. *Agricultural Samples Survey, 2001/2002*. Main Season Statistical Bulletin No. 114.
12. Evans, J., Hockman, Z., O`Connor, G. E. and Osborne, G. J. 1989. Soil Acidity and *Rhizobium*: Their Effect on Nodulation of Subterranean Clover on the Slops of




[ Downloaded from jast.modares.ac.ir on 2024-05-03 ]

[ DOR: 20.1001.1.16807073.2010.12.3.10.0 ]

Southern New South Wales. *Aust. J. Agr. Res.*, **38**: 605-618.
13. El Sheikh, E. A. E. and Wood, M.1989. Response of Chickpea and Soybean Rhizobia to Salt: Osmotic and Specific Ion Effects of Salts. *Soil Biol. Biochem*., **21**: 889-985.
14. Graham, P. H., Viteri, S. E., Mackie, F., Vargus, A. T. and Palacios, A. 1992. Variation in Acid Soil Tolerance among Strains of *Rhizobium phaseoli*. *Field Crops Res.*, **5**: 121-128.
15. Nogales, J., Campos, R., Ben Abdelkhalek, H., Olivares, J., Liuch, C. and Sanjuan, J. 2002. *Rhizobium tropici* Genes Involved in Free Living Salt Tolerance Are Required for the Establishment of Efficient Nitrogen Fixing Symbiosis with *Phaseolus vulgaris*. *MPMI,* **15(3)**: 225-232.
16. Jordan, D. C. 1984. Family III. Rhizobiaceae. In: "*BergeyÕs Manual of Systematic Bacteriology*", Krieg, N. R. and Holt, J. G. (Eds.). Williams and Wilkins, Baltimore, PP. 234-242.
17. Purcino, H. M. A., Festin, P. M. and Elkan, G. H.2000. Identification of Effective Strains of *Bradyrhizobium* for *Archis pintoi*. *Trop. Agric*., **77**: 226-231.
18. Rabie, G. H. and Almadini, A. M. 2005. Role of Bioinoculants in Development of Salt-Tolerance of *Vicia faba* Plants under Salinity Stress. *Afr. J. Biotechnol*., **4(3)**: 210-222.
19. Saraf, M. and Dhandhukia, P. 2005. Response of *Sinorhizobium meliloti* to High Salt Concentration and Effect of Added Osmotica*. J. Microb. World*, **7**: 250-257.
20. Slattery, J. F, Conventry, D. R. and Slattery, W. J. 2001. *Rhizobial* Ecology as Affected by the Soil Environment. *Aust. J. Exp. Agric*., **41**: 289-298.
21. Vincent, S. P. 1970. *A Manual for the Practical Study of the Root Nodule Bacteria.* IBP Hand Book, No. 15, Black Well Scientific Publications, Oxford.
22. Payakapong, W., Tittabutr, P., Teaumroong, N., Boonkerd, N., Singleton, P. W. and Borthakur, D. 2006. Identification of Two Clusters of Genes Involved in Salt Tolerance in *Sinorhizobium* sp. Strain BL3. Symbiosis, **41**:47-53.
23. Wei, W., Jiang, J. and Yang, S. S. 2004. Mutagenesis and Complementation of *relA* from *Sinorhizobium meliloti* 042BM as a Salt Tolerance Involvement Gene. *Annals. Microbiol.*, **54(3)**: 317-324
24. Zahran, H. H. 1999. *Rhizobium*-legume Symbiosis and Nitrogen Fixation under Severe Conditions and in an Arid Climate. *Microbiol. Mol. Biol. Rev.,* **63**: 968–989.

## بررسی ویژگی‌های سوش های ریزوبیومی مقاوم در برابر شوری و اسیدیته جدا شده از مزارع لوبیا در منطقه وولو - شمال اتیوپی

**ع. کننی، ف. عاصفا و پ. س. پرابو**

### چکیده

تراکم جمعیت میکروبی ریزوبیوم لوبیا در منطقه وولو در شمال اتیوپی (اسکتما، گاشانا،کوتم، میجور و سکوتا ) بررسی گردید. بیشترین جمعیت ریزوبیوم در منطقه میجور و حداقل جمعیت در سکوتا یافت شد. سوش‌های بومی ریزوبیوم جداسازی شده از مناطق تحت بررسی در مقایسه با سوش های غیر بومی( Tall 1402وTall 1397 ) تحمل غلظت بالای نمک (۵٪ کلرید سدیم )را داشتند. رشد سوش های بومی و غیر بومی در شرایط آزمایشگاهی در pHهای ۴ و ۴/۵ متوقف گردید. در pH های تنظیم شده در خاک بین ۴ الی ۷، سوش های بومی قادر به رشد بودند در حالی که سوش های غیر بومی در pH های پایین‌تر



[ Downloaded from jast.modares.ac.ir on 2024-05-03 ]
[ DOR: 20.1001.1.16807073.2010.12.3.10.0 ]

ار ۵/۵ قادر به رشد نبودند. سوش‌های بومی در بهره‌برداری از مواد هیدروکربنی و منابع کربنی نسبت به سوش های غیر بومی موثرتر عمل کرده و نیز مقاومت آنها در برابر آنتی بیوتیک‌های (استرپتومایسس،کلروم‌فنیکول، ریفامپنسیلین، اکسی تتراسایکلین، پنی سیلین و تترا سایکلین) نسبت به سوش‌های غیر بومی که فقط در برابر آنتی‌بیوتیک کلروم‌فنیکول مقاوم بودند، بیشتر بود. درصد نیتروژن تثبیتی در سوش‌های بومی به مراتب بیشتر از سوش های غیر بومی بوده و این سوش‌ها توانمندی بیشتری در تحریک رشد، عملکرد ماده خشک و گره‌زایی لوبیا در محیط کشت کیسه‌ای (Pouch culture ) نشان دادند.

[ Downloaded from jast.modares.ac.ir on 2024-05-03 ]

[ DOR: 20.1001.1.16807073.2010.12.3.10.0 ]