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SYMBIOTIC EFFECTIVENESS OF INDIGENOUS RHIZOBIA NODULATING FIELD PEA (*PISUM SATIVUM* L.) ON SOILS OF HORRO GUDURU AND EAST WOLLEGA HIGHLANDS IN WESTERN ETHIOPIA

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ABSTRACT: Field pea (*Pisum sativum*) production in Ethiopia is low due to low soil fertility, particularly Nitrogen (N) deficiency Screening and selecting the most effective strain is important for effective biological nitrogen fixation. This study was initiated to characterize and evaluate symbiotic effectiveness of field pea rhizobia collected from East Wollega and Horro Guduru Wollega zones, Ethiopia. Thirty two rhizobial isolates from field pea collected from the zones were isolated and phenotypically characterized under laboratory conditions. The results showed that all 32 isolates exhibited typical colony characteristics of fast growing rhizobia. The majority of isolates displayed large mucoid (50.4%) and watery colony texture (40.6%) and attained colony sizes ranging from 2.8–6.0 mm with generation time between 1.8 and 4.5 h. A number of isolates (41%) displayed phosphorus solubilization ability with phosphate solubilization indices (SI) ranging from 1.1 to 2%. Most of the isolates grew at pH values ranging from 4.5–9.0, concentrations of NaCl (1–4%), and displayed tolerance to 10 to 45°C incubation temperatures. Twenty nine isolates were evaluated for their symbiotic effectiveness on sand pot experiment in green house in Holeta Research Centre in 2015. The data showed that 94.4% of the isolates performed best in symbiotic nitrogen fixation from which 36.4% were highly effective and 62% were effective. The inoculated plants also showed differences in plant tissue nitrogen content which ranged from 1.72 to 2.93%. The highly effective isolates were nutritionally versatile and ecologically competitive and hence deserve recommendation for further tests under field conditions to enhance field pea production.

Key words/phrases: Burkitu, Competence, Heterotrophic, Rhizobia.

INTRODUCTION

Field pea is one of the highland and cool-season grain legumes that are important sources of dietary protein, carbohydrates minerals and vitamins. It is cultivated in temperate, Mediterranean regions, and at high altitudes in sub-tropical and tropical countries including Burma, India, Ethiopia, Morocco,

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Columbia, Ecuador, Peru and Pakistan (Javaid *et al*., 2002).

In Ethiopia, field peas are mainly cultivated in highland and mid highlands with altitude ranging from 1800–3000 masl; mainly in the highlands of Gondar, Gojam, northwest Wollo, Tigray and central Highlands of Shewa (Asfaw Telaye *et al*., 1994), southern, western and central parts of the country (Yasin and Hussen, 2013) and Highlands of Hararghe (Kassa Baye *et al*., 2015). It is used for crop rotation in low-input agriculture in the country to replenish soil nutrient loss which is estimated to be equivalent to 80 kg of N per cultivated hectare from the highlands of Ethiopia (Stoorvogel and Smaling, 1990).

The integration of field pea in crop rotation is due its ability to fix atmospheric nitrogen (Biological Nitrogen Fixation (BNF) with root nodule bacteria known as *Rhizobium leguminosarum* bv. *viciae* to the tune of 121–175 kg ha^{-1} per year (McVicar *et al*., 2005). According to Schatz and Endres (2009), field pea is one of the most highly efficient nitrogen fixing leguminous crops that derives up to 80% of their total nitrogen requirements under good growing conditions. Consequently, the high return from the highly effective and low cost of Rhizobium inoculants are some of the reasons for the worldwide use of rhizobial inoculants to increase crop production and ensure food security using various legume crops. However, effectiveness in BNF is affected by low mineral contents such as P, K, Fe, Mo, salinity, drought, acidity, and soil temperature (Zahran, 1999).

In Ethiopia, a few researches have been done on the nodulation status and nitrogen fixation potential of field pea rhizobia from soils in eastern and western Hararghe Highlands (Kassa Baye *et al*., 2015), central Ethiopia (Aregu Amsalu *et al*., 2012), and southern Tigray (Fano Berhe, 2010). These studies showed that the Ethiopian soils are diverse in harbouring symbiotically effective rhizobia depending upon geographical locations and host varieties to improve field pea production.

Acidic soils limit crop production on 30–40% of the world's arable land and up to 70% of the world's potentially arable land (Taye, 2007). In Ethiopia 40% of the arable land is currently affected by acidity and this is particularly severe in western zones of Oromia, western parts of Ethiopia (Batjes, 1995; Abdenna *et al*., 2007; Mesfin Abebe, 2007). However, aluminum toxicity is a major problem constraining crop production in acid soils. This problem is exacerbated by the current extensive use of ammonium fertilizers and acid rain (von Uexkull and Mutert, 2005). Although some parts of Wollega are major field pea producing areas, the symbiosis could be inhibited by a very

low pH, a very high exchangeable acidity, low calcium, and potassium ion in the soil (Abdenna *et al*., 2013).

There are two most common ways to mitigate Al toxicity which are liming and use of tolerant cultivars or strains. Detoxification of Al by liming is possible in surface soil in the field to a pH 5.5 or above. However, liming does not remedy for sub soil acidity and it may not always be practical or cost effective (Mesfin Tesfaye *et al*., 2001). Under such situations, use of tolerant rhizobia strains may be a satisfactory solution to this problem.

There is variation in stress tolerance among different strains of *Rhizobium leguminosarum*. This variability is an important tool to measure the survival advantage of one strain over the other in the severe soil environment (Rice *et al*., 1977). Strains resistant to different soil stresses have potential to improve the production of legumes grown in the area, and extend the ranges of soils upon which legumes could be adapted to grow (Munns, 1978). Metabolic versatility and specialization among rhizobial strains enables some strains to persist in adverse environments and compete successfully with other bacteria (Mazur *et al*., 2013).

Use of acid tolerant rhizobium strains for aluminium tolerance is a reliable approach to enhance production of legume crops (field pea) on acidic soils (Wei *et al*., 2008). There is no any information on screening of acid tolerant rhizobium nodulating field pea in Ethiopia. However, limited work has been done on nodulation status and nitrogen fixation potential of field pea rhizobia from soils in eastern and western Hararghe Highlands (Kassa Baye *et al*., 2015), central Ethiopia (Aregu Amsalu *et al*., 2012), and southern Tigray (Fano Berhe, 2010). These studies showed that Ethiopian soils are diverse in harbouring symbiotically effective rhizobia depending upon geographical locations and host varieties to improve field pea production. These studies also showed that effectiveness of isolates under controlled environment may not necessarily be concurrent to their performance in the field because of the environmental conditions that govern the process of nitrogen fixation (Sanginga *et al*., 1995) which necessitates the need for screening *in vitro* the nutritionally versatile and stress tolerant isolates that could give an insight to their probable survival and persistence in the soil.

Therefore, the present study was designed with the objective of characterizing the potential of nitrogen fixing rhizobia from field pea from sampling sites in East and Horro Guduru Wollega, western parts of the country. The results will serve as baseline data for future endeavour to realize the full potential of biological nitrogen fixing system of field pea into the productivity of low-

input agriculture in the area.

MATERIALS AND METHODS

Description of the study sites

The study was carried out at Horro Guduru and East Wollega zones in western Ethiopia located between 36°30'44.28"–37°22'44.90" E and 08°44'29.66"– 09°13'3.95"N. The altitude, mean annual rainfall, and mean minimum and maximum temperature of these areas range from 1500 to 3140 metres above sea level, between 1000 and 2400 mm yr^{-1}, and 12.2 and 27°C, respectively (Demissu Hundie *et al*., 2013) (Fig. 1). The major crops grown in the zones include cereals (barley, wheat, teff, maize and oat), pulses (faba bean and field pea) and oil crops (niger seed, rapeseed and sesame). The pH of the study area ranged from pH 4.79–5.25.

Sample collection procedure and sample analysis

Nodule and soil samples were collected from six selected districts; Wayu-Tuqa, Leka-Dullecha, and Jimma-Arjo districts from East Wollega Zone and Jimma Rare, Horro and Guduru districts from Horro Guduru Wollega Zone (Table 1).

In each district, six farmlands were purposively selected based on production status of field pea. The nodule and soil collection spots were geo-referenced using geographic positioning system (GPS). Soil samples were collected from 0–30 cm depth in the rhizosphere of the same site; sub sampled, and collected in polyethylene bags and analyzed for selected chemical properties at Haramaya University soil laboratory. From each farmland, five plants were randomly taken from which 20 nodules were detached, composited, and collected in a vial containing silica gel covered with cotton and brought to Holeta Agricultural Research Centre, Ethiopia, for further analysis.

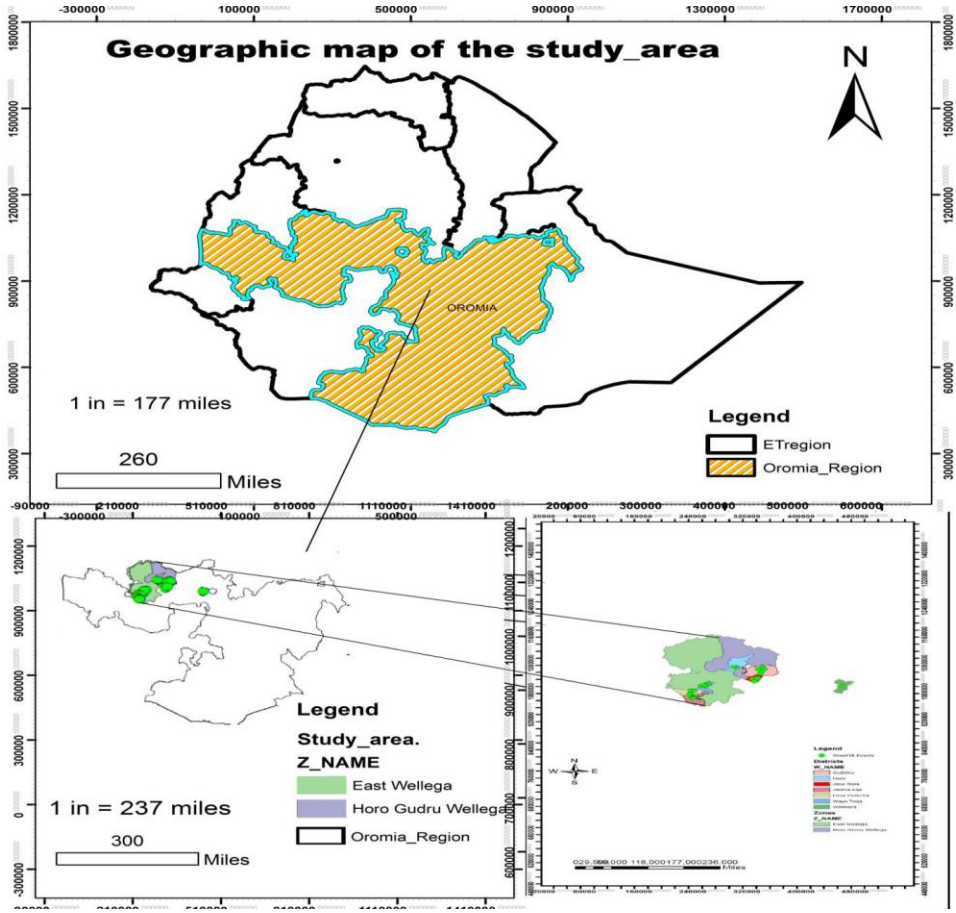

Fig. 1. Map of Oromia region in Ethiopia (A) and study zone of East and Horro Guduru Wollega in Oromia region (B), field pea nodules and rhizosphere soils collection and soil sampling points in the study area (C).

Organic carbon was determined using the wet oxidation method (Walkley and Black, 1934), while total N was determined following the modified micro-Kjeldahl procedure (Jackson, 1958). Available soil P was measured using the Olsen extraction method (Olsen *et al*., 1954), while pH was measured in the supernatant solution of 1:2 soil to water ratio suspension using pH meter (Carter, 1993).

Treatment and design of the experiment

From the 36 sampling sites, a total of 36 representative nodule samples were collected from field pea in East Wollega and Horro Guduru Wollega zones and isolation of rhizobia was done on YEMA medium at Holeta Research Centre in 2015. Out of the 36 nodule samples, only 32 rhizobia were successfully isolated and designated as Rhizobium of Haramaya University

(HU1-HU36). The 32 isolates were evaluated for their biochemical characterization (12 carbohydrate and 7 amino acid sources) on YEMA medium as a presumptive test. In addition, morphological and eco-physiological conditions [different levels of pH, temperature, salt concentrations, Intrinsic Antibiotic Resistance (IAR), and Heavy Metals Resistance (HMR)] of the isolates were tested on YEMA incubated for 3–5 days. Finally, 29 isolates were evaluated for their effectiveness and non-effectiveness with regard to symbiotic characteristics on sand pot experiment in 2015/16. Each evaluation was done in triplicates. The details of materials and procedures are explained in each section below.

Isolation and purification of isolates

After thorough washing with tap water, the nodules were soaked overnight in distilled water, and surface-sterilized using 95% ethanol and 3% hypochlorite, washed several times with sterilized water for three minutes and crushed in 0.1 N NaCl and a drop of distilled water (Somasegaran and Hoben, 1994). A loopful of the suspension from each nodule was streaked on Yeast Extract Mannitol Agar (YEMA) medium containing 0.0025% (w/v) Congo red and incubated at 28±2°C for 3 to 5 days. The colonies were purified by repeated sub-culturing on the same medium, gram stained, and grown on Hofer's alkaline broth (HAB) medium (Vincent, 1970) and Peptone-glucose-agar (PGA) medium (Subba Rao, 1999) as presumptive tests for identifying root nodule bacteria. The pure isolates were designated as (Rhizobium of Haramaya University – RHU1-RHU36) and preserved at +4°C for further studies.

Cultural and growth characteristics of isolates

Forty eight hours old YEM broth culture was inoculated on YEMA plate and incubated at 30°C for 3–5 days to determine colony characteristics like colour, texture and shape of isolates. The pure cultures were also inoculated into Bromothymol blue (0.0025% w/v) (BTB-YEMA) medium to detect acid/base production by the isolates according to Jordan (1984).

Growth rate (generation time) was also determined by transferring a single colony of each isolate into test tubes containing 10 ml YEM broth and placed on a rotary shaker (125 rpm) at room temperature for 24 h, from which one milliliter of suspension (1×10^8 cfu/ml) was transferred into 100 ml of YEM broth in 250 ml Erlenmeyer flasks and incubated at room temperature for 72 h. Samples (1 ml each) were drawn every 6 h to measure the turbidity (optical turbidity) using spectrophotometer (Jenway, 6405 UV/) at 540 nm against the

blank (sterilized un-inoculated YEM broth). The generation time was calculated for each isolates from the logarithmic phase (White, 1995) based on the following formula:

$$g = \frac{\log_2(t)}{\log X - \log X_0}$$

Where, g = generation time, t = time elapsed, X_0 = first optical density reading, X = second optical density reading

Utilization of C and N sources

The isolates were tested for their ability to utilize different carbon sources (Somasegaran and Hoben, 1994). The carbon sources were starch, cellobiose, dextrin, lactose, sucrose, galactose, maltose, fructose, glucose, arabinose, xylose and sorbitol. Ten percent of the sugar solutions were filter-sterilized (0.2 μm) and separately added to the autoclaved basal YEMA medium (without mannitol).

Similarly, the isolates were grown on the same basal medium containing filter-sterilized (0.22 μm) amino acids: L-lysine, L-leucine, L-alanine, Glycine, L-aspargine and L-cystine and L-Diphenylamine at concentrations of 0.5 g/l from which ammonium sulfate was omitted and mannitol was added at a concentration of 1 g/l. The substrates were filter-sterilized (Amarger *et al*., 1997).

Eco-physiological characteristics

For every experiment, about 30 μl of the inoculums suspensions (10^6/ml) was streaked onto Petri plates containing YEMA plates and incubated at 28±2°C for 5 days. Each test was done in triplicates. Growth was recorded as (+) for slight growth, (++) for abundant growth and (-) for no growth.

pH, salt and temperature tolerance of isolates

All the tests were undertaken according to Lupwayi and Haque (1994) and the ability of the isolates to grow on acidic or basic media was tested by streaking each isolate on separate Petri plates on YEMA with pH adjusted to 4.0, 4.5, 8.0 and 9.0 with sterile 0.1N HCl or 1N NaOH and inoculated onto YEMA incubated at 15, 40, 45, 50 and 55°C to test their temperature tolerance. The isolates were inoculated on the same medium adjusted to concentrations of 1, 2, 3 and 4% (w/v) sodium chloride. The inoculated plates were incubated at 30°C for 5–7 days. The parameters were recorded qualitatively either as + for growth or – for no growth.

Tests for inherent antibiotic (IAR) and heavy metal resistance (HMR)

The intrinsic antibiotic and heavy metal resistance of the isolates to different antibiotics were evaluated according to Zhang *et al*. (1991). Filter-sterilized (0.2 μm) antibiotics were supplemented to YEMA medium at two concentrations of 5 μg ml^{-1} and 10 μg ml^{-1}. The antibiotics were penicillin, kanamycin, streptomycin, amoxicillin, ampicillin, chloramphenicol, and spectinomycin. Most of the antibiotics were dissolved in water except chloramphenicol that was prepared in absolute alcohol (95%) and diluted to the required volume with distilled water. Resistances to heavy metals were also determined on solid YEMA medium containing the following filter-sterilized heavy metals at the concentration of (μg ml^{-1}) $MnCl_2$ 250, 500, $HgCl_2$ 5, 10, $ZnCl_2$ 50, 100 and $CuCl_2$ 50, 100. All the plates were incubated at 30°C for 5–7 days.

Screening for phosphorus solubilization

Rhizobial isolates were screened for tricalcium phosphate solubilization ability on Basal Sperber agar medium containing: 10 g/l glucose, 0.5 g/l yeast extract, 0.1 g/l $CaCl_2$, 0.25 g/l $MgSO_4.7H_2O$, 2.5g/l $Ca_3(PO_4)_2$, and 15g/l Agar and mixed in 1000 ml sterile distilled water and autoclaved for 15 minutes at 121 atm according to Alikhani *et al*. (2006). All isolates were tested with three replications. Twenty four hour cultures were spot inoculated on the medium and incubated at 28±2°C for 7 days to determine solubilization indices according to El-Yazeid *et al*. (2007):

$$PSI = \frac{DC + DHZ}{DC}$$

where, PSI is phosphate solubilizing index, CD is diameter of colony, and HZ is diameter of halo zone.

Authentication and evaluation of symbiotic effectiveness

After having presumptively tested for primary screening, the isolates were authenticated as root nodule bacteria and tested for their symbiotic effectiveness using the sand pot experiment following the procedure described by Somesegaran and Hoben (1994). Five seeds of "Burkitu" variety of *Pisum sativum* variety were surface sterilized and coated with powdered inoculants of 10% sugar solution at a rate of 20 ml inoculants kg^{-1} of seed. The coated seeds were sown into surface sterilized (95% alcohol) three-kg capacity plastic pots filled with river sand and soaked with sulfuric acid and well washed. All inoculations were done just before planting under shade to

maintain the viability of microbial cells. Plants were grown in a greenhouse with a 12/12 hour light/dark cycles and a maximum day and night time temperature of 27±1 and 20±1°C, respectively.

The experiment was laid out in complete randomized design (CRD) and replicated three times with two controls- the negative control (without N and Rhizobium) and the positive control (with N). At the beginning, all the plants were given 100 ml of 0.05% KNO_3 (W/V) once as starting nitrogen. The positive control was irrigated with 100 ml of 0.05% KNO_3 (w/v) solution once a week for 4 weeks. The N-free nutrient solution was given to all plants in 100 ml/pot once a week as full strength for the four consecutive weeks and supplied with distilled water every 2 days (Somasegaran and Hoben, 1994).

After 45 days of planting, plants were harvested to collect data on nodule number per plant, nodule colour, nodule dry weight (mg per plant), shoot dry weight (g per plant), Nitrogen content (percent N) and total N (g per plant) in plant shoot tissue was determined following the method outlined in Sahlemedhin Sertsu and Taye Bekele (2000), and multiplying the percent N content by the shoot dry weight.

The percent symbiotic effectiveness (SE%) of the isolates was computed using the formula (Beck *et al*., 1993).

$$\text{SE } (\%) = \frac{a}{b} x\ 100\%$$

Where, a = Shoot dry weight of inoculated plants, b = Shoot dry weight of N supplied plants. Finally, the symbiotic effectiveness (SE) values of the isolates were rated as highly effective (>80%), effective (50–80%), lowly effective (35–50%) and ineffective (<35%).

Statistical analysis

Data on nodule number, nodule dry weight, and shoot dry weight, plant tissue N content (in % and mg per plant) were tested using analysis of variance (ANOVA) SAS version 9.0 and a least significant difference (LSD) test was used for means separation. A probability level of $p<0.05$ was considered significant. Pearson's r values were also determined for the association of shoot dry weight with nodule number and nodule dry weight and with plant tissue N content (in % and mg per plant) and reported at $p<0.01$ level of significance.

RESULTS AND DISCUSSION

Soil chemical characteristics of the sampling sites

Selected soil chemical characteristics of the study sites are presented in Table 1. The percent of OC and TN varied from 0.93–2.9 and 0.08–1.8 among sampling sites, respectively. As per rating of Tekalign Tadese (1991), soil samples across the study area qualified as <0.5% very low, 0.5–1.5% low and 1.5–3.0% medium, and >3.0% as high levels of OC, about (43.75%) and (56.25%) of soil in the present study were found to be low and moderate in soil organic carbon, respectively. In comparing with the rating of Murphy (1968) qualified as low (<0.10), medium (0.10–0.15) and high (0.15–0.25), very high (>0.25), about (21.87%), (59.37%) and (18.75%) of soil in the present study were found to be low, medium and high in soil total nitrogen, respectively.

Long term cultivation without organic fertilizers leads to a decrease in soil OC and total N contents because organic forms generally account for more than 95% of soil N (Alexandra *et al*., 2013). The lower TN in the study area may be ascribed to complete removal of crop residues, less organic input application and more intensive cultivation. Frequent cultivation would accelerate a higher oxidation rate of soil OM. Tsehaye Gebrelibanos and Mohammed Assen (2013) reported lower soil TN content due to intensive cultivation, less input application, and higher mineralization rate in Ethiopian soils.

The soil pH varied from 4.79–5.25 among sampling sites indicating that the soils are strongly acidic (Table 1). The available phosphorus contents varied from 3.5 and 12 among sampling sites, respectively (Table 1). Based on the rating of EthioSIS (2014) as very low (<15), low (15–30) and optimum (30–80), high (80–150) and very high (>150), soils of the present study site were found to be very low (<15 mg kg^{-1}) in available phosphorus. The low contents of available P as a common characteristic in most of the cultivated soils of Ethiopia were indicated by Tekalign Mamo *et al*. (2002).

Table 1. Description of the study sites and soil characteristics of the sampling sites of East Wollega and Horro Guduru Wollega in western Ethiopia.

District/ Locality	Altitude (m)	OC (%)	TN (%)	AP (mg kg^{-1})	Soil pH
		Jimma Rare			
Sochosa	2373	1.51	0.16	8.2	4.92
Ilamu	2268	1.27	0.10	8	4.95
Goban	2548	1.39	0.12	9.4	5.17
Sochosa	2264	1.3	0.11	11	5.36
Gamada	2244	1.74	0.15	10	4.97

District/ Locality	Altitude (m)	OC (%)	TN (%)	AP (mg kg^{-1})	Soil pH
		Guduru			
Bantu	2301	1.74	0.13	9.7	4.85
Gabate	2302	1.47	0.09	7.6	4.8
Gabate	2375	1.77	0.11	8.3	5.29
Loha	2417	1.87	0.11	10	5.3
Didibe	2616	2.09	0.18	12	5.01
		Horro			
Cabir	2405	1.17	0.09	3.6	4.80
Akaji	2834	1.39	0.10	5	4.95
Kombolch	2754	1.86	0.16	9.3	4.94
Rifenti	2443	2.02	0.18	11	5.14
Akaji	2792	1.44	0.15	4.4	4.79
		Wayu-Tuqa			
Komto	2814	1.5	0.09	6.9	4.97
Lije sefer	2193	2.5	0.15	10	4.94
Dalo	2016	1.4	0.10	7.6	5.05
Dachasardo	1935	2.5	0.12	9.6	4.83
Dachasardo	2159	2.5	0.14	7	5.10
		Leqa-Dullecha			
Wanibo	2485	0.93	0.08	6.3	4.95
Gudina jilo	2168	1.27	0.11	9	5.25
Wanibo	2536	2.9	0.09	5	4.86
Getema	2405	2.09	0.18	11.4	5.15
Dabe	1875	1.86	0.11	4.2	4.73
Tume	2538	1.74	0.15	8	4.95
		Jimma Arjo			
Wayu-abono	2264	1.74	0.15	5.4	4.95
Gebrel sefer	2323	1.86	0.16	4	4.89
Odoro	2496	1.47	0.11	7	5.10
Odoro	2498	1.4	0.09	4	4.80
Odoro	2443	1.36	0.11	6	4.91
Geneti	2455	1.74	0.09	3.5	4.97

OC - Organic carbon, TN - Total Nitrogen, AP - Available Phosphorus

Cultural and growth characterization of isolates

A total of 32 field pea rhizobia were isolated from acidic soils (pH 4.79 to 5.36) of Jimma Rare (n = 5), Horro (n = 5), and Guduru (n = 5) of Horro Guduru Wollega and Wayu-Tuqa (n = 5), Leqa-Dullecha (n = 6), and Jimma Arjo (n = 6) of east Wollega zones (Table 2). All the isolates were gram negative and rod shaped bacteria, failed to absorb Congo red from YEMA + CR (Congo red) medium, and none of them were grown on Hoffer's alkaline broth (HAB) (Vincent, 1970) and Peptone glucose agar (PGA) medium that are the presumptive characteristic features of root nodule bacteria (Subba Rao, 1999). The isolates were authenticated as root nodule on sand culture.

Table 2. Growth, cultural characteristics of rhizobial isolates grown on YEMA medium and their p-solubilization capacity.

Isolates	Texture	Colony size	Generation time	P-Solubilization Index(SI)
RHU14	LM	3	2.4	-
RHU10	LW	3	1.8	-
RHU27	LW	3	2.5	1.3
RHU34	LM	4	3.1	-
RHU1	LW	3.6	4.0	-
RHU22	LM	4	1.8	1.25
RHU23	LW	3.2	2.9	-
RHU25	SD	2.8	2.1	1.3
RHU28	LW	3	3.4	-
RHU35	LM	6	2.0	1.3
RHU8	LW	3.7	3.8	-
RHU13	LW	3.5	3.3	1.3
RHU18	LM	5	1.9	1.8
RHU30	LW	3.9	3.4	-
RHU32	LW	3.8	3.8	-
RHU17	LM	4	2.8	1.1
RHU11	LM	3.8	2.6	-
RHU2	LM	3.6	3.4	-
RHU26	SD	2.9	4.1	1.25
RHU4	LM	3	2.9	-
RHU3	LM	5.5	1.8	1.7
RHU9	LM	3	3.1	1.3
RHU15	LW	4	2.3	-
RHU6	LW	3.4	2.0	-
RHU29	LM	4.3	2.1	1.25
RHU7	LM	4.4	2.6	-
RHU12	LW	4	2.9	-
RHU19	LM	3.5	3.9	2
RHU21	LM	4	2.3	-
RHU24	LM	3	4.5	1.3
RHU33	LW	4	3.9	-
RHU31	SD	2.8	4.1	-

LM (large mucoid colonies; LW (large watery colonies); SD (small dry colonies)

On the basis of colony characteristics, all isolates formed colony with circular shape; where most of them appeared as large mucoid colonies (50.4%) on YEMA medium with opaque, gummy and buttery texture and others (40.6%) displayed large watery (LW) colony texture with transparent texture and production of copious amount of exo-polysaccharides and a few isolates showed small dry (SD) colonies (9%) with flat surface with little or no mucoid production upon 5–7 days of incubation (Jordan, 1984).

In line with Aregu Amsalu *et al*. (2012), similar pattern of cultural characteristics of field pea rhizobia where 69% exhibited large mucoid colony texture with opaque, gummy and buttery characteristics but 21% isolates showed large watery and small dry colony texture of field pea

rhizobia isolates of central and southern Ethiopia. However, Girmaye Kenasa *et al*. (2014) have reported that all the isolates (100%) of faba bean rhizobia isolated from acidic soils of Wollega displayed large mucoid, colony diameters that ranged 2–5 mm.

All the isolates changed the color of BTB-YMA to yellow due to production of acid and had generation time of 1.8–4 h with colony size of 2.8–6 mm upon 3–5 days of incubation indicating acid production and fast growth of rhizobia (Jordan, 1984). The results of colony shape, size, colour, texture and acid production in the growth medium were typical of the characteristics of fast-growing rhizobia and similar to field pea rhizobia isolated from southern Tigray (Fano Berhe, 2010), eastern and western Hararghe (Kassa Baye *et al*., 2015).

Eco-physiological characteristics

pH tolerance

The isolates showed wide differences in their pH tolerance (Table 3). All the isolates grew on media adjusted at pH of 6–8 which could be considered as the optimum pH for the growth of the isolates. However, 65% of the rhizobial isolates were acid tolerant (pH 4.5), whereas a number of isolates (78%) were capable of growing only between pH range of 6.8 and 9.0. Likewise, a few isolates (44%) were tolerant to a wide range of acidic and alkaline pH range of 4.5 and 9.0 but none of the isolates grew at low pH 4.0 (Table 3).

The current result *Rhizobium leguminosarum* var *viciae* were tolerant on the alkaline growth media as compared to acidic growth media. Jordan (1984) showed that the majority of these bacteria can tolerate up to pH 9.0. The change in the lipopolysaccharides composition (Vriezen *et al*., 2007), and the presence of several genes such as ActA, actP, actS, actR, phrR and exoR are important features that help rhizobia to grow at low pH (Abd-alla *et al*., 2014).

Salt tolerance

The isolates showed variations in tolerance to different concentrations of salt. The majority (more than 70%) of the isolates were able to grow well on YEMA plates amended with 1–2% NaCl but the number of isolates growing decreased as the concentration of salt increased in the medium. Accordingly, some isolates (47%) were able to grow at salt concentration of 4%. In contrary, elasticity of salt tolerance in this study was lower than the wide range of tolerance of 1–7% recorded from field pea isolates collected from southern Tigray (Fano Berhe, 2010), and that of 0.5–6% of NaCl tolerance

recorded from field pea rhizobia collected from different pulse growing regions of Ethiopia (Aregu Amsalu *et al*., 2012).

With regard to salt tolerance from the same cross inoculating rhizobia from faba bean, different studies showed similar pattern of high salt tolerance of rhizobia from Wollega (4–6%) (Girmaye Kenasa *et al*. (2014), northern Tigray (4–7%) (Solomon Legesse and Fassil Assefa, 2014). However, the pattern of salt tolerance in this study was similar to the ones reported from faba bean rhizobia from northern parts of Ethiopia (Zerihun Belay and Fassil Assefa, 2011; Mulisa Jida and Fassil Assefa, 2012).

All taken together, salt tolerance is influenced by the host and site of isolation (soil pH) and type of medium used for screening (Fitouri *et al*., 2012). The high salt tolerance of some rhizobial strains could be associated with their ability to limit adverse effects as a result of accumulation of protective organic osmolytes and to changes in the cell morphology in order to maintain the cell turgor (Thami-Alami *et al*., 2010).

Temperature tolerance

All the rhizobial isolates (100%) grew well between 15°C and 30°C, and many isolates were also tolerant to 40°C (78%) and 45°C (47%), but could not grow at 50°C (Table 3). Isolates RHU10, RHU9, RHU15, RHU6, and RHU31 were the most tolerant within the range of 10–45°C. Kassa Baye *et al*. (2015) have reported that pea rhizobial isolates were tolerant to a range of temperature values (15–40°C). Similarly, Girmaye Kenasa *et al*. (2014) and Solomon Legesse and Fassil Assefa (2014) have also demonstrated the same pattern of temperature tolerance by faba bean rhizobial isolates within the range of 5–45°C and 10–45°C, respectively, thus indicating an analogous pattern between field pea rhizobia and faba bean rhizobia as opposed to their diversity in salt tolerance by the two endo-symbionts.

Utilization of C and N source

The data on C and N utilization properties showed that the isolates were capable of utilizing 67–100% of the tested carbohydrates and 86–100% of the amino acid substrates (Table 3). Almost all the isolates utilized glucose, maltose and fructose, lactose, sucrose and galactose. Several isolates utilized starch (63%) and cellobiose (69%) as carbon sources.

The most versatile isolates (RHU25, RHU 35, RHU18, RHU3, RHU9, RHU22 and RHU29) were able to utilize all the carbon sources. Aregu Amsalu *et al*. (2012) have showed that the field pea rhizobia were capable of utilizing 76% and 100% of carbon sources, and the ones collected from

southern Tigray were capable of utilizing 87% of the 13 carbon sources (Fano Berhe, 2010). Sadowsky *et al*. (1983) have earlier showed that fast-growing rhizobia in general, were able to grow on a large variety of carbon substrates.

All the isolates were able to metabolize L-alanine, L-glycine and L-asparagine, and L-leucine but fewer isolates utilized L-Diphenylamine (Table 3). In general, the isolates utilized 86–100% of the tested nitrogen sources indicating that field pea rhizobia were more versatile in utilizing N-resources than the carbon sources. Thus, isolates RHU 23, RHU 35, RHU 13, RHU 18, RHU 11 and RHU 24 were able to utilize all the N sources. The versatility of the isolates to utilize amino acids (86–100%) was more pronounced than the fewer faba bean rhizobial isolates (40–48%) that were capable of utilizing all the amino acids (Girmaye Kenasa *et al*., 2014; Dereje Tsegaye *et al*., 2015). The differences might be associated with type of strains that are genetically endowed with the capacity to utilize a wide variety of amino acids and the type of amino acids used for the test.

Tests for intrinsic antibiotic and heavy metal resistance

Most of the isolates were tolerant to high concentration (10 µg) of ampicillin, chloramphenicol, amoxicillin, penicillin and spectinomycin. However, some isolates were resistant to kanamycin (54%) and streptomycin (47%) (Table 3). The most resistant isolates to different antibiotics were RHU 10, RHU 22, RHU 35, RHU 18, RHU 4, RHU 3 and RHU 33. Different studies showed that field pea rhizobia were sensitive to kanamycin (Fano Berhe, 2010; Aregu Amsalu *et al*., 2012). In general, the cross-inoculation rhizobia from faba bean and field pea showed similar pattern of resistance to chloramphenicol, ampicillin, and sensitivity to streptomycin and kanamycin (Zerihun Belay and Fassil Assefa, 2011; Solomon Legesse and Fassil Assefa, 2014; Girmaye Kenasa *et al*., 2014). Intrinsic antibiotic resistance (IAR) could be partly due to bacterial impermeability to antibiotics through hydrophobicity, electrical charge and amount of the antibiotic (Hungaria *et al*., 2000).

The study also showed that isolates were resistant to heavy metal at lower concentrations (Table 3). However, only half of the isolates were resistant to high concentration of $MnCl_2$ (500 µg ml^{-1}), $HgCl_3$ (10 µg ml^{-1}), $ZnCl_2$ (100 µg ml^{-1}) and $CuCl_3$ (100 µg ml^{-1}). Isolates RHU27, RHU22, RHU35, RHU21 and RHU29 were relatively tolerant to the tested heavy metals (Table 3). Kassa Baye *et al*. (2015) showed that all the tested isolates exhibited high resistance to ZnCl, MnCl and HgCl at concentrations of 50, 250 and 5 µg ml^{-1}, respectively and even higher concentrations of $CuCl_2$ (50 and 100 µg ml^{-1}), $ZnCl_2$ (100 µgml^{-1}) and $MnCl_2$ (500 µg ml^{-1}). Another study also

showed that field pea rhizobia were resistant to manganese, but sensitive to copper chloride, zinc chloride and mercuric chloride (Aregu Amsalu *et al*., 2012).

Table 3. Pattern of pH, NaCl, temperature tolerance, intrinsic antibiotic and heavy metal resistance and utilization of carbon and nitrogen sources of field pea rhizobia.

Isolates	Location	pH	NaCl%	Temp	CU	AAU	IAR	HM
RHU14	Sochosa	4.5–8.0	1–3	15–40	91	85.7	71	62.5
RHU10	Ilamu	4.5–9.0	1–3	10–45	91	85.7	86	75
RHU27	Goban	4.5–8.0	1–4	15–45	91	85.7	71	87.5
RHU34	Sochosa	8.0–9.0	1–2	15–40	83	85.7	78	50
RHU1	Gemeda	4.5–8.0	1–2	10–40	91	85.7	71	50
RHU22	Bantu	4.5–9.0	1–4	10–40	83	100	86	87
RHU23	Gabate	4.5–9.0	1–2	10–15	66.6	85.7	71	62.5
RHU25	Gabate	4.5–9.0	1–4	10–15	100	100	78	62.5
RHU28	Loha	4.5–9.0	1	15–40	83	85.7	78	62.5
RHU35	Didibe	4.5–9.0	1–4	10–40	100	100	93	87.5
RHU8	Cabir	4.5–8.0	1–3	15–40	91	85.7	71	62.5
RHU13	Akaji	4.5–8.0	1–4	15–45	91	100	78	75
RHU18	Kombo	4.5–9.0	1–4	10–40	100	100	93	75
RHU30	Rifenti	8.0–9.0	1–3	10–15	91	100	71	62.5
RHU32	Akaji	8.0–9.0	1	10–40	91	85.7	78	50
RHU17	Lije	8.0–9.0	1–2	15–40	83.	85.7	71	75
RHU11	Lije	4.5–9.0	1–4	10–45	66.6	100	71	75
RHU2	Dalo	4.5–8.0	1–3	15–40	83	100	78	75
RHU26	Dachasa	8.0–9.0	1–3	15–40	91	85.7	78	75
RHU4	Dachasa	8.0–9.0	1–3	10–15	83	85.7	86	50
RHU3	Wanibo	4.5–9.0	1–2	10–45	100	100	93	75
RHU9	Gudina	8.0–9.0	1–4	10–45	100	85.7	78	75
RHU15	Wanibbo	8.0–9.0	1–3	10–45	91	100	86	62.5
RHU6	Getema	8.0–9.0	1–4	10–45	91	100	71	62.5
RHU29	Dabe	4.5–9.0	1–4	10–15	100	100	93	87.5
RHU7	Tume	8.0–9.0	1–3	10–15	83	100	78	75
RHU12	Wayuabo	4.5–9.0	1–4	10–40	91	85.7	78	62.5
RHU19	Gebreal	4.5–9.0	1–2	10–15	75	100	78	50
RHU21	Odoro	4.5–9.0	1–4	10–40	91	100	78	87.5
RHU24	Odoro	4.5–9.0	1–2	15–40	91	100	78	75
RHU33	Odoro	4.5–9.0	1–4	10–40	75	85.7	86	62.5
RHU31	Genenti	8.0–9.0	1–4	10–45	91	85.7	71	62.5

IA-Intrinsic Antibiotic, HM-Heavy Metal, CU- Carbon Utilization, AAU - Amino Acid Utilization

Authentication and evaluation of symbiotic effectiveness

The characteristics of investigated shoot length, number of nodules per plant, nodule dry mass, shoot dry weight and plant total nitrogen showed statistically significant difference ($p<0.01$) (Table 4). The inoculated plants significantly ($p<0.01$) increased shoot length, number of nodules per plant, nodule dry mass, shoot dry mass and plant total nitrogen as compared to the negative control treatment (Table 4).

Inoculation has significantly increased the shoot length ranging from 116.6 cm/plant to 158.3 cm/plant (Table 4). The highest shoot height 166 cm/plant was recorded from plant inoculated with isolate RHU3. The mean nodule number was in the range of 30/plant (RHU6) to 102/plant (RHU35). This variation might be associated with plant hormone ethylene which is produced by plant during stress resulting in inhibition of nodulation. Gresshoff *et al*. (2009) reported that many nitrogenous compounds are strong inhibitors of nodule formation. The other important factor that determines the nodule number is reported to be the number of lateral roots (Miransari *et al*., 2006). Caetano-Anollés *et al*. (1991) also reported that the formation of both lateral roots and nodules was controlled by the auto-regulation mechanism.

The same pattern of field pea nodulation between 25–93 nodules per plant was recorded from southern Tigray (Fano Berhe, 2010) and 29 to 108 nodules/plant was recorded from pea rhizobia from northern and central parts of Ethiopia (Aregu Amsalu *et al*., 2012). However, Kassa Baye *et al*. (2015) reported that field pea plants were abundantly nodulated to the tune of 84–246 nodules/plant from eastern and western Hararghe. Relatively similar nodule number of 56 to 169 nodules per plant was also recorded from faba bean rhizobia of acidic soils of Ethiopia (Dereje Tsegaye *et al*., 2015).

Field pea plants were also different in nodule dry weights, with the highest mean nodule dry weight recorded from plants inoculated with isolate RHU22 with 135.3 mg/plant nodule dry weight followed by inoculated plants with isolates RHU35 and RHU18 with nodule dry weight of 129.7 mg/plant and 123 mg/plant, respectively (Table 4). This shows that there is a huge difference between the isolates in terms of nodule dry weight than nodule number.

Table 4. Authentication and symbiotic effectiveness of rhizobial isolates on the host pea plant (Variety Burkitu) after 45 days of growth under greenhouse conditions on sand culture.

Isolates	**SL (cm/p)**	**NN/p**	**NDW (mg/p)**	**SDW (gm/p)**	**%PN**	**%SE**	**Rate**
RHU14	146.33^{bf}	51.66^{hj}	78.3^{hi}	1.47^{hm}	2.21^{fj}	58.8	E
RHU10	130^{fi}	50.66^{hj}	82^{gh}	1.72^{gi}	2.31^{ei}	68.8	E
RHU27	126.66^{hi}	49.33^{ij}	84.66^{gh}	1.66^{g-j}	2.32^{ei}	65.2	E
RHU34	155^{ac}	48.66^{ij}	102.33^{f}	1.88^{e-g}	2.41^{eg}	75.2	E
RHU1	152.66^{ad}	30^{m}	9.6^{pq}	0.77^{q}	1.15^{l}	30.8	IE
RHU22	140^{c-h}	83.66b	135.33^{a}	2.68^{a}	3.16^{ab}	107	HE
RHU23	131.66^{fi}	68^{df}	114.333^{ce}	2.06^{df}	2.39^{eh}	82.6	HE
RHU25	133.33^{ei}	73.3^{cf}	108.333^{df}	2.28^{bc}	2.80^{bd}	91.5	HE
RHU28	128.33^{gi}	33.66^{lm}	64.66^{jk}	1.32^{kn}	2.26^{fi}	52.8	E
RHU35	159.66^{ab}	102^{a}	129.667^{ab}	2.58^{ab}	3.30^{a}	103	HE
RHU8	116.66^{i}	39^{kl}	49^{l}	1.2^{1o}	1.85^{k}	50	E
RHU13	159.66^{ab}	58.3^{gh}	69.667^{ij}	1.58^{gk}	2.33^{ei}	63.2	E

Isolates	SL (cm/p)	NN/p	NDW (mg/p)	SDW (gm/p)	%PN	%SE	Rate
RHU18	130^{fi}	101.33^{a}	123^{bc}	2.61^{ab}	3.16^{ab}	104	HE
RHU30	146.66^{bf}	43j^{k}	58^{kl}	1.53^{hl}	2.23^{fj}	61.2	E
RHU32	146.66^{bf}	37.6^{k-m}	80^{h}	1.77^{fh}	2.36^{ei}	71	E
RHU17	136.66^{dh}	51.33^{h-j}	66.333^{jk}	1.37^{jn}	2.36^{ei}	54.8	E
RHU11	130^{fi}	58.33^{gh}	80.333^{h}	1.41^{in}	2.23^{fj}	56.6	E
RHU26	133.33^{ei}	51.33^{hg}	91.333^{g}	1.7^{gj}	2.24^{fj}	68	E
RHU3	166 ^{a}	76.66^{b-d}	116.333^{cd}	2.56^{ab}	3.17 ^{a}	102.4	HE
RHU9	136.66^{dh}	67.66^{ef}	35^{m}	1.74^{fi}	2.45^{ef}	69.7	E
RHU15	150^{ae}	55.66^{hi}	28^{mn}	1.67^{gj}	2.30^{fi}	66.9	E
RHU6	151.66^{ad}	30.66^{lm}	19.33^{no}	1.17^{mo}	2.07^{gk}	50	E
RHU29	136.66^{dh}	82^{bc}	107.667^{d-f}	2.53^{ab}	3.17 ^{a}	101.2	HE
RHU7	146.66^{bf}	48^{ij}	20no	1.50^{hm}	1.90^{jk}	60.2	E
RHU12	150^{ae}	51^{hj}	14.33^{op}	1.19^{lo}	2.03^{hk}	47.8	E
RHU19	145^{bg}	76.33^{be}	105.667^{ef}	2.17^{ce}	2.62^{ce}	85.7	HE
RHU21	128.33^{gi}	69.66^{df}	121.667^{bc}	2.33^{bc}	2.53^{df}	93.3	HE
RHU24	131.66^{fi}	70.3^{d-f}	110.667^{df}	2.14^{de}	2.39^{eh}	85.7	HE
RHU31	158.33^{ab}	66.6^{fg}	87.333^{gh}	1.68 ^{gj}	2.40^{eg}	67.2	E
Positive	164.33 ^{a}	-	-	2.50 ^{ac}	2.97^{ac}	-	
Negative	139.66^{ch}	-	-	0.78^{q}	1.01^{l}	-	
Mean	142.17	55.6	73.9	1.79	2.39		
CV	7.46	9.9	8.41	12.1	9.5		
LSD	17.53	9.07	10.1	0.36	0.35		

SDW - Shoot Dry Weight, SL - Shoot Length, NDW (mg/p) - Nodule Dry Weight in milligram per plant, NN/p - Nodule Number per plant, PN - Plant Nitrogen, SE - Symbiotic Effectiveness, HE - Highly effective, E - Effective, LE - Less effective, I – Ineffective. Means followed by the same letters in a column are not significantly different at $p<0.05$ level. CV- Coefficient of variation, LSD - least significant difference

In this study, the mean nodule dry weight recorded was 68.24 mg/plant. This variation could be associated with the nodule number and sizes. A few large size nodules can increase nodule dry weight over small size ones (Lin *et al*., 2012). This result was slightly higher than that 30–90 mg/plant recorded from field pea from southern Tigray (Fano Berhe, 2010).

Almost all of the tested isolates showed higher shoot dry matter accumulation compared to negative control. The mean shoot dry weight of the inoculated plants showed variations among the treatments. The highest shoot dry matter of 2.68 g/plant was recorded from the plant inoculated with isolate RHU22 (Guduru) followed by plant treated with isolates RHU35 (Guduru), RHU18 (Horro), RHU3 (Leqa-Dullecha) and RHU29 (Leqa-Dullecha) that accumulated 2.61 g/plant, 2.58 g/plant, 2.56 g/plant and 2.53 g/plant, respectively. These values were comparable to the shoot dry weight of N-fertilized positive control plant (2.50 g/plant). This implies that the isolates can be good as the N-fertilized plant if they are used as inoculant at low input of N and soil acidity problem from which area they were isolated. The lowest shoot dry weight of 0.77 g/plant was recorded from the host plant inoculated

with isolate RHU1 (Jimma Rare) which was similar to the shoot dry weight of negative control plants (0.78 g/plant) (Table 4). This implies that different rhizobial isolates vary in their ability to improve shoot dry weight when inoculated with field pea. The result was similar to the shoot dry matter of 0.68 g/plant and 3.35 g/plant recorded from inoculation field pea rhizobia from northern and central parts of Ethiopia (Aregu Amsalu *et al*., 2012).

Based on the relative plant shoot dry matter accumulation of inoculated plants and nitrogen-fertilized control, the rate of effectiveness of the isolates were rated as 94.4% of the isolates performed as good as the N-fertilized plant from which 34.4% and 62% of the isolates were highly effective and effective, respectively. Some of the highly effective isolates were RHU22, RHU18, RHU35, RHU3, and RHU29. The symbiotic efficiencies of isolates were also compared among the sampling sites (districts) where 60–83% of the isolates were highly effective and effective at the different sampling sites indicating that sampling sites harbour diverse and effective isolates that may not require inoculation under the circumstances.

Similar patterns of effectiveness of 67% was also recorded from pea rhizobia from southern Tigray (Fano Berhe, 2010), similar pattern of effectiveness of 90% from western Hararghe (Kassa Baye *et al*., 2015) and 76% was recorded from pea rhizobia from northern and central parts of Ethiopia (Aregu Amsalu *et al*., 2012). Ballard *et al*. (2004) also reported 61–98% effectiveness of inoculated field pea plant compared to the control plant in South Australia.

The plant tissue nitrogen (PN) content of the inoculated plant was within the range of 1.2% inoculated by isolate RHU1 (Jimma Rare) to the highest value of 3.3% inoculated by isolates RHU35 (Guduru). Aregu Amsalu *et al*. (2012) reported that the plant tissue nitrogen content of inoculated field pea plant was between 1.52% and 2.91% from pea rhizobia of northern and central parts of Ethiopia.

In this study, shoot dry weight was strongly and positively correlated ($r = 0.66$ and $r = 0.72$, $p<0.001$) with nodule number and nodule dry weight. However no significant relationship was observed with plant height ($r = 0.11$, $p<0.26$). This implies that inoculation might impact on shoot biomasses than shoot length of field pea. The percentage of plant total nitrogen was strongly and positively correlated ($r = 0.68$, $r = 0.69$, $p<0.0001$ and $r = 0.87$, $p<0.0001$) with nodule number, nodule dry weight and shoot dry weight, respectively (Table 5). Similarly, Fano Berhe (2010) reported strong coefficient of correlation ($r = 0.6$) amongst nodule number, nodule dry weight and shoot dry weight. Similar relationship ($r = 0.73$ and 0.81; $p<0.001$) was

reported with shoot dry weight and percent N of faba bean, respectively (Abere Mnalku *et al*., 2009). Reports showed that shoot dry weight and nodule dry weight are usually highly correlated, thus shoot dry weight is used routinely as an indicator of relative effectiveness (Somasegaran and Hoben, 1994). The strong association between N content and shoot dry weight substantiate the use of N content to measure symbiotic N fixation under field as well as in greenhouse conditions (Atici *et al*., 2005). Reports have shown that shoot dry weight and nodule dry weight are usually highly correlated, thus shoot dry weight is used routinely as an indicator of relative effectiveness (Somasegaran and Hoben, 1994).

Although most of the rhizobial isolates (92.6%) from the different sampling sites were effective and very effective in nitrogen fixation of field pea, all of them may not be competitive if they are inoculated in the soil under different environmental stresses. The data showed isolates which displayed good performance (highly effective and effective) in symbiotic relation showed diverse phenotypic characters *in vitro* (Tables 3 and 4). Accordingly, 34% (RHU22, RHU25, RHU35, RHU13, RHU18, RHU11, RHU3, RHU29, RHU21, RHU27 and RHU24) of the isolates were tolerant to many eco-physiological factors (Tables 3 and 4). The remaining isolates (66%) did not show persistent pattern even though they showed good symbiotic performance.

Table 5. Pearson correlations matrix of effect of inoculation of rhizobium on shoot length, nodule number (NN), nodule dry weight (NDW), shoot dry weight (SDW) and percent of plant tissue nitrogen (% PTN).

	SL	**NN**	**NDW**	**SDW**	**PTN**
SL					
NN	-0.09				
NDW	-0.15	0.79**			
SDW	0.082	0.66*	0.72*		
PTN	0.11	0.68	0.69	0.87**	

Significant*, highly significant **

This indicates that fewer isolates were versatile in assimilating different carbon sources and heterotrophically and ecologically competent that would ensure their survival in the soil. Purcino *et al*. (2000) reported that survival, persistence and competitiveness of rhizobial strains are the major factors to determine their successful use as inoculants. To this end, diversity in tolerance to salt, pH and temperature show the possibility of screening best performing strains as inoculants from the soil where they are naturally selected (O'Hara *et al*., 2002).

Table 6. Mean square value of Rhizobium inoculation and control treatment on plant height (PH), nodule number (NN), shoot dry weight (SDW) and plant tissue nitrogen (of field pea plant on sand experiment under green house (n = 31).

		Mean square					
ANOVA	**df**	**PH**	**NN**	**NDW**	**SDW**	**PTN**	**Probability level**
Treatment	30	470.28*	1670.81**	5041.22**	0.86**	0.82**	p<0.5
Error	62	114.559	30.9355	2399.33	0.04723		
RMSE		10.7032	5.56197	6.22085	0.21733	0.22821	

PH = plant height, NNPP = nodule number per plant, NDW = nodule dry weight, PTN = plant tissue nitrogen

CONCLUSION

The study showed that field pea rhizobia are abundant in soils of East Wollega and Horro Guduru Wollega Ethiopia. All the 32 isolates exhibited typical colony characteristics of fast growing rhizobia. The majority of the isolates, 50% and 40.6% displayed large mucoid and watery colony texture, respectively. They showed significant variations in their symbiotic effectiveness in nitrogen fixation and enhancing growth of the pea cultivar Burkitu compared to the uninoculated and non-fertilized control plants. The data also showed that 94.4% of the isolates performed best in symbiotic nitrogen fixation from which 36.4% were highly effective and 62% were effective.

Although the study was very limited to a number of isolates and one host variety (Burkitu), it gave an insight that most of the soils in the major field pea-growing areas in the sampling sites harboured symbiotically effective and very effective rhizobia that nodulate field pea. Thus, the best performing isolates RHU22, RHU18, RHU35, RHU3 and RHU29, and the effective isolates RHU10 and RHU13 were nutritionally versatile and ecologically competitive and deserve recommendation for further testing on several pea varieties under field trials to validate their ability to enhance biological nitrogen fixation, growth and production of field pea. In general, if the selection of rhizobial isolates from pea could be properly followed with the appropriate genetic and environmental studies, more isolates can be screened for integrated soil fertility management in the low-input agriculture in the area and the country at large.

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