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# The effect of inter cross-inoculation host group rhizobia on the growth and nitrogen fixation of Faba Bean (*Vicia faba L.*) varieties in North Showa, Amhara Regional State, Ethiopia

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**Faba bean (*Vicia faba* L.) is one of the most widely cultivated leguminous crops grown in the Highlands of Ethiopian. This study was aimed at evaluating the effect of *Rhizobium leguminosarum bv.viciae* isolates from other cross-inoculation hosts from field pea, grass pea, and lentil on faba bean varieties. Thus, 12 isolates were selected from faba bean, field pea, grass pea and lentil using host trap method on soil samples that was collected from North Shoa. All isolates were tolerated to pH (5 to 9), salt concentration (1 to 2%), and at a temperature of (15 to 35°C). Cross-inoculation experiments were conducted in the greenhouse to test the effects of *R. leguminosarum* isolates from faba bean, field pea, grass pea and lentil hosts on Hachallu and Wolki varieties of faba bean. The mean nodule number, nodule dry weight and mean shoot dry weight of the inoculated plants showed variations among the different cross inoculants ($p<0.01$). Symbiotic effectiveness varied from a minimum of 64% on Hachallu variety inoculated by isolate AUFBR5 (faba bean) and 52% on Wolki variety by isolate AUFPR1 (field pea) association to a maximum of 98% in Hachallu variety inoculated with isolate AUGPR13 (grass pea), and 117% in Wolki variety inoculated with isolate AUFBR5 (faba bean). The study showed physiological and symbiotic diversity of *R. leguminosarum* isolates of faba bean, field pea, grass pea and lentil. The effective isolates that have been tested with Hachallu and Wolki varities at green house level should be re-tested under field conditions to ascertain their performance *in vivo*.**

**Key words:** Field pea, grass pea, lentil, symbiotic effectiveness.

## INTRODUCTION

Legumes are one of the most diverse plants on earth which widespread in tropics and temperate zones (Tran and Nguyen, 2009). Leguminosae family comprises 800 genera and 20,000 species, and represents the third

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largest family of flowering plants (Siddique et al., 2012). Faba bean (*Vicia faba*) is one of the major cold season food crops grown in Ethiopian Highlands (CSA, 2005). Faba bean has different functions such as providing food and feed that is rich in protein improve soil fertility by symbiotic $N_2$ fixation with Rhizobium, diversifying the cropping system with crop rotation by reducing constraints on growth and yield of other crops (Nikfarjam and Aminpanah, 2015).

Ethiopia is the world's second-largest faba bean producer next to China (Salunkhe and Kadam, 1989) with annual production of 2,671,834.45 ton from 1,558,422.02 ha of land (CSA, 2015). However, the productivity of faba bean in Ethiopia is still, far below its potential due to biotic and biotic factors (Yohannes, 2000). Amongst the various limiting factors affecting plant growth and productivity, nitrogen deficiency in soil is one of the most common factors for a reduction in yield of legume crops such as faba bean particularly in Ethiopia (Adler, 2008). The supply of nitrogen into the soil must be sufficient to increase the yield of plants (Ihsanullah et al., 2008). Chemical nitrogen fertilizers are very expensive and had an adverse effect on the environment and human health. Hence, there is an urgent need to realize a vital and cheaper source of fertilizers having an eco-friendly approach. Biological nitrogen fixation with rhizobia-legumes symbiosis is one of the alternative solutions and the promising technologies (People, 1995).

Faba bean is the most effective nitrogen fixer of all cool-season pulse crops grown in association with root nodule bacteria (*Rhizobium leguminosarum* bv.*viciae*) (McVicar et al., 2005). Faba bean is nodulated by *Rhizobium leguminosarum bv. Viciae* that derives the highest percentage (96% Ndfa) of nitrogen from the atmosphere (Lo´ pez-Bellido et al., 2006). Jensen et al. (2010) reported that the crop comes after faba bean benefits up to 100 to 200 kg N $ha^{-1}$. It has been reported that the rhizobium inoculation increases the production and protein content of faba bean (Giller, 2001).

The specific rhizobium that nodulates faba bean is *Rhizobium leguminosarum* bv.*viciae* which also cross-nodulates field pea, grass pea, and lentils (McVicar et al., 2005). The finding showed that Rhizobia strain that is effective in nitrogen fixation in association with one plant species may also be effective with certain other legumes (Amara, 1990). This is demonstrated by isolates of pea and faba bean able to form nodules and fix nitrogen with faba bean host (Laguerre et al., 2003). However, due to the influence of host plant on diversity and the genetic structure, *R. lguminosarum bv.viciae* reflect differences in the degree of host specificity and effectiveness of nitrogen fixation within the *viceae* tribe cross-inoculation group (Handley et al., 1998).

In Ethiopia, research was conducted on cool season legumes such as faba bean, lentil, and field pea (Hailemariam and Tsigie, 2006). However, most of the studies were limited to isolation, identification, and characterization of rhizobial isolates from faba bean in different parts of the country (Belay and Assefa, 2011). As a result, there is lack of sufficient information about cross-inoculation of rhizobia isolated from nodules of viceae tribe cross-inoculation group for their effectiveness in faba bean varieties. Hence, this study was directed to isolate and evaluate the effectiveness of inter cross-inoculation host group rhizobia on the growth and nitrogen fixation of faba bean (*Vicia faba* L.) varieties under greenhouse condition.

## MATERIALS AND METHODS

### Sample collection

Soil samples were collected from twenty sites in North Shoa Zone of Amhara Region, Ethiopia. From each site, two farmer fields were selected and samples from 10 to 15 cm depth were pooled and collected in alcohol sterilized polyethylene plastic bags. Types of legumes on the soil field were Faba bean, Field pea, Grass pea and lentil (Table1). The soil samples were brought to Applied Microbiology Laboratory of Addis Ababa University for further processing. The Seeds of Wolki and Hachallu varieties of faba bean (*Vicia faba*), Adi variety of field pea (*Pisum sativum*), and Alemaya variety of lentil (*Lens culinaris)* were provided by Holeta Agricultural Research Center whereas Wasse variety of grass pea *(Lathyrus sativus*) was gotten from Debrezeit Agricultural Research Center (Table 1).

### Isolation of *Rhizobium* from nodules

The soil samples were filled into 3 kg capacity plastic pots. The seeds of faba bean (*Vicia faba*), field pea (*Pisum sativum*), lentil (*Lens culinaris)* and grass pea *(Lathyrus sativus*) were surface sterilized and sown in each pot under greenhouse conditions. After 45 days, the pink and undamaged nodules were collected and surface sterilized (Lupwayi and Haque, 1994). Nodules were crushed with alcohol flamed sterile glass rod (Somasegaren and Hoben (1994). A loopful of crushed nodule saps were streaked on Yeast Extract Mannitol Agar (YEMA) plates and incubated at 28±2°C for 3 to 5 day. According to Somasegaren and Hoben (1994), Yeast Extract Mannitol Agar (YEMA) medium of volume 1000 ml consists of the following components (g/l):

Mannitol, 10; $K_2HP0_4$, 0.5;$MgS0_4$ .$7H_20$, 0.2;NaCl, 0.1;Yeast Extract, 0.5; Agar,15.

The purity and uniformity of colony types were carefully examined through repeated re-streaking. Pure culture was preserved on YEMA slant containing 0.3% (W/V) $CaCO_3$ at 4°C for future use (Jordan, 1984).

### Presumptive tests and designation of the isolates

The growth of isolates was evaluated on the peptone-glucose agar medium (Lupwayi and Haque, 1994). The purity of isolates was tested on Congo -Red (CR-YEMA) (Somasegaren and Hoben, 1994). All the isolates were gram stained, and their shapes and gram reaction were observed microscopically for rapid means of identification of contaminants as indicated in Lupwayi and Haque (1994). All the isolates were designated at Addis Ababa University Grass pea Rhizobia (AUGPR), Addis Ababa University Field pea

**Table 1.** Sites of soil sample collection and the types of legumes on the soil field.

| S/N | Sample sites | Collection zones | Types of legumes on the soil field |
|---|---|---|---|
| 1 | Cheki | North Shoa | Faba bean |
| 2 | Bedeyo | » | Faba bean |
| 3 | Jajo | » | Faba bean |
| 4 | Kulso | » | Faba bean |
| 5 | Dalota | » | Faba bean |
| 6 | Kulso | » | Field pea |
| 7 | Dalota | » | Field pea |
| 8 | Cheki | » | Field pea |
| 9 | Bedeyo | » | Field pea |
| 10 | Jajo | » | Field pea |
| 11 | Segeda | » | lentil |
| 12 | Amino | » | lentil |
| 13 | Adadi | » | lentil |
| 14 | Hawi | » | lentil |
| 15 | Folle | » | lentil |
| 16 | Adadi | » | Grass pea |
| 17 | Hawi | » | Grass pea |
| 18 | Folle | » | Grass pea |
| 19 | Amino | » | Grass pea |
| 20 | Segeda | » | Grass pea |

Rhizobia (AUFPR), Addis Ababa University Faba bean Rhizobia (AUFBR), and Addis Ababa University Lentil Rhizobia (AULR) representing each isolate.

**Authentication of isolates on sand culture**

In order to test the definitive purity of all rhizobial isolates, nodulation test was carried out for each of the purified isolates. They were inoculated into the host plant potted into 3 kg capacity plastic pots containing sterilized and nitrogen free sand (Somasegaren and Hoben, 1994). The sand was thoroughly washed with sulfuric acid whereas the pots were surface sterilized with 95% ethanol. Six seeds were sown in each pot and thinned down to three after germination. Each isolate was inoculated into Erlenmeyer flask for 3 days, and 1 ml of the culture suspension was inoculated into each seedling. The control plants were fertilized with 100 ml of 0.05% $KNO_2$ once every week. All plants were irrigated with N-free medium nutrients four times in their life (Broughton and Dilworth, 1971).

**Morphological and cultural characterization of isolates**

Colony morphology was characterized on YEMA as indicated in Martinez-Romero et al. (1991). The ability of isolates to produce acid/alkaline evaluated in the YEMA medium containing bromothymol blue (BTB) (0.025w/v) (Jordan, 1984). The growth rate of the isolates was determined as described by White (1995).

**Physiological characteristics of isolates**

Tolerance to acidity and alkalinity of each isolate was evaluated on YEMA media adjusted to pH levels of 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11 and 12 **(**Bernal and Graham, 2001). The ability of the rhizobial isolates tolerance to NaCl was tested by streaking on YEMA plates containing 1, 2, 3,4, 5, 6, 7, 8, 9 and 10% NaCl (w/v) as indicated in Bernal and Graham (2001). The growths of isolates were evaluated on YEMA plates at a different incubation temperature of 15°C, 20°C, 25°C, 30°C, 35°C, 40°C and 45°C (Lupwayi and Haque, 1994).

**Cross-inoculation and symbiotic effectiveness test on sand culture**

Rhizobial isolates of faba bean, field pea, grass pea, and lentil were selected as representative on the basis of the agronomic and economic importance of their legume host in the country (Table 2). They tested for their ability to form nodules and effective symbiosis with Hachalu and Wolki varieties of faba bean (*Vicia faba*). The sand was washed with sulfuric acid and autoclaved for sterilization whereas pots were surface sterilized with 95% ethanol (Lupwayi and Haque, 1994). The seeds of Hachalu and Wolki varieties of *Vicia faba* were rinsed in 95% ethanol for 10 s, and surface sterilized in 3% (v/v) solution of sodium hypochlorite for 3 min (Lupwayi and Haque, 1994).

Seeds were washed with sterile distilled water and planted into each pot. Starter cultures of selected test isolates were grown in test tubes containing 10 ml YEM broth on an orbital shaker for 3 days. 1 ml YEM broth culture of each test isolates was transferred into 100 ml sterilized YEM broth in 250 ml Erlenmeyer flask, and placed on an orbital shaker for 4 days. 1 ml of each 4 days of YEM broth culture was inoculated on to the base of seedlings of *Vicia faba* according to Vincent (1970).

The treatments were arranged in a randomized complete block design in triplicate. After 45 days of growth, the whole plants were carefully uprooted to determine modulation number, nodule dry weight, and shoot dry mass according to Vincent (1970) (Table 2). The shoots dry weight was determined as described by Somasegaren and Hoben (1994). The effectiveness of the isolates

**Table 2.** Selected isolates from viceae tribe host legumes for cross-inoculation test.

| S/N | Isolates | Site | Host of isolates |
|---|---|---|---|
| 1 | AUFBR2 | Bedyo | Faba bean |
| 2 | AUFBR3 | Jajo | Faba bean |
| 3 | AUFBR5 | Daloda | Faba bean |
| 4 | AUFPR6 | Kulso | Field pea |
| 5 | AUFPR7 | Dalota | Field pea |
| 6 | AUFPR10 | Jajo | Field pea |
| 7 | AUGPR12 | Amino | Grass pea |
| 8 | AUGPR13 | Adadi | Grass pea |
| 9 | AUGPR14 | Segeda | Grass pea |
| 10 | AULR16 | Adadi | Lentil |
| 11 | AULR17 | Hawi | Lentil |
| 12 | AULR18 | Fole | Lentil |

in accumulating plant shoot dry matter was calculated as described in Mulongoy (2004).

$$\%SE = \frac{\text{Inoculated plant DM}}{\text{N-fertilized plant DM}} \times 100$$

Where, DM = dry matter, N= nitrogen, SE= symbiotic effectiveness The rate of nitrogen-fixing effectiveness is evaluated as highly effective > 80%, Effective from 50 to 80%, lowly effective 35 to 49% and infective <35%.

#### Data analysis

Symbiotic effectiveness parameters such as nodule number, nodule dry weight, and shoot dry weight data were analyzed by one-way ANOVA (Tukey's HSD test) SPSS (Somasogaran and Hoben, 1994).

## RESULTS AND DISCUSSION

### Presumptive test of isolates

In this particular study, 20 isolates from faba bean, field pea, grass pea and lentil using host trap method on soil samples were collected from North Shoa. They were authenticated and characterized on the basis of their different phenotypic and symbiotic characteristics. None of the isolates grew on peptone-glucose-agar (PGA). Similarly, Somasegaren and Hoben (1994) stated that PGA does not allow the growth of rhizobia. All isolates were gram-negative and rod-shaped bacteria with no absorption of Congo-red from the YEM-CR medium (Jordan, 1984).

### Authentication and relative effectiveness of rhizobia isolates on sand culture

On the basis of the nodule formation and symbiotic effectiveness of isolates in their respective host, nodule number, nodule dry weight and shoot dry weight were determined. All of the isolates except AUFBR4 (faba bean) and AUGPR11 (grass pea) were authenticated as root nodule bacteria from sand culture on the host plants according to Vincent (1970) and Brockwell (1998) (Table 3). The failure to re- nodulation could emanate from loss of symbiotic plasmids (Sym-plasmids) that govern the symbiotic interaction between the two partners (Zhange et al., 2001). As a result, those isolate that failed to re-modulate with their parent host may be due to other intruding bacteria that penetrated The shoots dry weight was determined as described by Somasegaren and Hoben (1994). The effectiveness of the isolates

### Morphological and cultural characterization of isolates

Colony morphology, colony diameter, acid-base reaction and generation time were used to evaluate the diversity of the test isolates. The different isolates showed variations amongst one another in their characteristics (Table 4). Isolates were fast growers with large mucoid and watery texture, and colony diameter of 2.5 to 6mm, the generation time of 1.0 to 4.3 h. They changed YEMA-BTB into yellow. Several works showed that *R. leguminosarum* bv.*viciae* nodulating faba bean (Adamu et al., 2001; Shimekit et al., 2008) were identified as fast-growing root nodule bacteria. Since they were isolated from faba bean, field pea, grass pea and lentil, they were also characterized as a cross nodulating group of *R. leguminosarum* bv. *viciae* (Jordan, 1984).

### Physiological characteristics of rhizobial isolates

The inhibitory effect of salt concentrations varied among isolates of faba bean, field pea, grass pea and lentil (Table 5). The result indicated that the percentage of tolerance continued to decrease as the concentrations of NaCl increases. All 100% of the isolates grew at 1 to 2% salt concentrations. Some isolates which included AUFBR2 (faba bean), AUGPR13 and AUGPR14 (grass pea) were found to grow at all tested salt (1 to 10%) levels of concentrations as described in Adal (2009). Adal (2009) work on grass pea rhizobia reported that some isolates of *R. leguminosarum* can survive in the presence of extremely high levels of salt concentrations up to 13% of NaCl. In contrast, Teshome (2006) reported that faba bean isolated strains of *R. leguminosarum bv. Vicieae* managed to grow at up t o 6% NaCl from the Northern Ethiopia.

Isolates were grown at a different range of pH (pH 4-12) (Table 5). All isolates were tolerant to pH 5 to 9 according to Demissie (2006). Demissie (2006) reported that the *R. leguminosarum biovar Vicieae* of *Vicia faba* and *Pisum sativum* from Northwestern Ethiopia grew at pH more than 4.75. Isolates AUFBR5 (faba bean),

**Table 3.** Authentication and relative effectiveness of rhizoidal isolates.

| Isolates | Site | Host | Nodule number/ plant | Nodule dry weig ht/plant (mg) | Shoot dry weight/plant (g) | Percentage (%) SE | Effectiveness |
|---|---|---|---|---|---|---|---|
| AUFBR1 | Cheki | Faba bean | 25±2.88b | 6±0.001$^{a}$ | 2.8±0.12$^{a}$ | 76 | E |
| AUFBR2 | Bedeyo | ″ | 70±5.77$^{c}$ | 89±0.006$^{b}$ | 3.01±0.11$^{bc}$ | 83 | HE |
| AUFBR3 | Jajo | ″ | 118±6.11$^{e}$ | 90±0.004$^{b}$ | 3.17±0.05$^{cd}$ | 87 | HE |
| AUFBR4 | Kulso | ″ | - | - | 1.50±0.10$^{a}$ | 41 | LE |
| AUFBR5 | Dalota | ″ | 90±5.77$^{d}$ | 88±0.006$^{b}$ | 3.52±0.18$^{d}$ | 96 | HE |
| (+)Control | | ″ | - | - | 3.65±0.09$^{de}$ | | |
| AUFPR6 | Kulso | Field pea | 105±5.51$^{d}$ | 37±0.009$^{c}$ | 1.13±0.40$^{a}$ | 73 | E |
| AUFPR7 | Dalota | ″ | 120±2.65$^{d}$ | 54±0.006$^{d}$ | 1.56±0.28$^{a}$ | 101 | HE |
| AUFPR8 | Cheki | ″ | 25±5.13$^{b}$ | 10±0.001$^{ab}$ | 1.01±0.31$^{a}$ | 65 | E |
| AUFPR9 | Bedeyo | ″ | 20±5.29$^{b}$ | 7±0.001$^{ab}$ | 0.54±0.37$^{a}$ | 35 | LE |
| AUFPR10 | Jajo | ″ | 85±8.66$^{c}$ | 21±0.009$^{bc}$ | 1.36±0.72$^{a}$ | 88 | HE |
| (+) Control | - | ″ | - | - | 1.55±0.04$^{a}$ | | |
| AUGPR11 | Hawi | Grass pea | - | - | 0.09±0.02$^{a}$ | 36 | LE |
| AUGPR12 | Amino | ″ | 67±8.54$^{b}$ | 47±0.006$^{c}$ | 0.16±0.02$^{bc}$ | 64 | E |
| AUGPR13 | Adadi | ″ | 90±7.21$^{b}$ | 62±0.002$^{d}$ | 0.20±0.01$^{cd}$ | 81 | HE |
| AUGPR14 | Segeda | ″ | 135±18.87$^{c}$ | 86±0.006$^{e}$ | 0.21±0.02$^{cd}$ | 84 | HE |
| AUGPR15 | Folle | ″ | 24±4.16$^{a}$ | 35±0.002$^{b}$ | 0.11±0.01$^{ab}$ | 44 | LE |
| (+) Control | | ″ | - | - | 0.25±0.29$^{d}$ | | |
| AULR16 | Adadi | lentil | 30±7.64$^{b}$ | 15±0.002$^{b}$ | 0.06±0.010$^{a}$ | 55 | E |
| AULR17 | Hawi | ″ | 18±4.04$^{bc}$ | 2±0.001$^{a}$ | 0.07±0.02$^{a}$ | 64 | E |
| AULR18 | Folle | ″ | 55±4.58$^{d}$ | 17±0.003$^{b}$ | 0.1±0.01$^{a}$ | 91 | HE |
| AULR19 | Amino | ″ | 16±3.28$^{ab}$ | 2±0.001$^{a}$ | 0.05±0.01$^{a}$ | 45 | LE |
| AULR20 | Segeda | ″ | 18±4.73$^{bc}$ | 6±0.006$^{bc}$ | 0.06±0.11$^{a}$ | 55 | E |
| (+) Control | - | ″ | - | - | 0.11±0.01$^{a}$ | - | - |

**Table 4.** Morphological and cultural characteristics of selected isolates from faba bean, field pea, grass pea and lentil.

| Isolates | Collection site | Host of isolates | Colony diameter | Colony morphology | Mean generation time (MGT) (h) |
|---|---|---|---|---|---|
| AUFBR2 | Benyo | Faba bean | 4 | LM, translucent | 1.3 |
| AUFBR3 | Jajo | Faba bean | 4.5 | LW, transparent | 2.6 |
| AUFBR5 | Daloda | Faba bean | 6 | LW, transparent | 1.7 |
| AUFPR6 | Kulso | Field pea | 3 | LM, translucent | 3.3 |
| AUFPR7 | Dalota | Field pea | 2.5 | LW, transparent | 2.9 |
| AUFPR10 | Jajo | Field pea | 4.1 | LM, translucent | 4 |
| AUGPR12 | Amino | Grass pea | 2.5 | LM, translucent | 3.9 |
| AUGPR13 | Adadi | Grass pea | 5.1 | LM, translucent | 2.2 |
| AUGPR14 | Segeda | Grass pea | 3.3 | LM, translucent | 2.8 |
| AULR16 | Adadi | Lentil | 3 | LW, transparent | 4.3 |
| AULR17 | Hawi | Lentil | 3.3 | LM, translucent | 1.9 |
| AULR18 | Fole | Lentil | 4 | LM, translucent | 1.0 |

NS=north Shoa, LM= Large mucoid, LW= Large watery.

AUFPR6 (field pea), AUGPR12, AUGPR13, AUGPR14(grass pea) and AULR18 (lentil) were able to grow at pH 4, in contrast with Keneni et al. (2010). Keneni et al. (2010) showed that faba bean rhizobia from Wollo, Northern Ethiopia, could not tolerate pH 4. Isolates AUFBR5 (faba bean), AUGPR13 (grass pea) and AUGPR14 (grass pea) showed a wide range of pH tolerance (pH 4 to12).

The isolates developed within a varied range of temperature 5 to 45°C (Table 5). All the isolates (100%)

**Table 5.** Physiological properties of Isolates from faba bean, field pea, grass pea, and lentil.

| Isolates | Host | Range of salt (%) | Range of pH | Range of T (°C) |
|---|---|---|---|---|
| AUFBR2 | Faba bean | 1-10 | 4.5-12 | 5-40 |
| AUFBR3 | Faba bean | 1-3 | 4.5-9 | 5-45 |
| AUFBR5 | Faba bean | 1-6 | 4-12 | 10-35 |
| AUFPR6 | Field pea | 1-8 | 4-9.5 | 15-35 |
| AUFPR7 | Field pea | 1-7 | 5-12 | 5-35 |
| AUFPR10 | Field pea | 1- 2 | 4.5-11 | 15-40 |
| AUGPR12 | Grass pea | 1-3 | 4-10.5 | 5-45 |
| AUGPR13 | Grass pea | 1-10 | 4-12 | 10-40 |
| AUGPR14 | Grass pea | 1-10 | 4-12 | 5-45 |
| AULR16 | Lentil | 1-7 | 5-11 | 15-35 |
| AULR17 | Lentil | 1-4 | 4.5-9.5 | 15-40 |
| AULR18 | Lentil | 1-9 | 4-11 | 5-45 |

grew well between 15 and 35°C as reported in (Belay (2006), Amsalu (2007), Tesfaye (2008) and some isolates were found to tolerate 5°C of temperature. This result correlated with (Belay (2006) and Tesfaye (2008) from faba bean isolates, Fano (2010) on field pea and Adal (2009) from grass pea. They also reported that the tolerance of some *R. leguminosarum biovar viciae* isolates at 4, 5, and 10°C.

In this study, isolates AUFBR3 (from faba bean), AUGPR12, AUGPR14 (from grass pea) and AULR18 (from lentil) without a field pea isolates were tolerated with high temperature of 45°C as described by Adal (2009) on grass pea rhizobia in contrast with earlier works on cool season food legumes (Gebremariam, 2007) on faba bean and (Amsalu, 2007; Fano, 2010) on field pea rhizobia (Table 5).

## Cross-inoculation and symbiotic effectiveness test

The cross-inoculation of hachallu and wolki varieties of faba bean (*Vicia faba*) with isolates from viceae variety hosts were determined. The ability to form nodules and enough nitrogen of the isolates were evaluated on the basis of nodule number, nodule dry weight and shoot dry weight as described by Brockwel (1998). All of the rhizobial isolates from faba bean, field pea, grass pea, and lentil were successfully cross inoculated and formed nodules on two varieties of Faba bean (*Vicia faba*). The result correlated with the work of McVicar et al. (2005) who reported that *R. leguminosarum bv. viciae* have broad host range than cross-nodulate field pea, lentils, grass pea and faba bean.

Nodules number ranged between 45 to 131 and 41to122 nodules/plant on Hachallu and wolki varieties respectively. The highest number of nodule/ plant (131) was recorded on Hachallu variety inoculated with isolate AUGPR14 (grass pea) and 122 nodules/plant on wolki variety inoculated with isolate AUFBR5 (faba bean). The least number of nodules (45 nodules/pl) was recorded from plants inoculated with isolate AUFPR6 (field pea) from Hachallu variety and 41 nodules/plant from wolki variety inoculated with isolate AUGPR13 (grass pea) (Table 6).

The finding showed that strains isolated from vicieae tribe's cross-inoculation group were able to form nodules with faba bean plant but, the number, color, and shape of nodule varied according to the strain inoculated (Laguerre et al., 2003). Nodules produced by strain on one legumes host may have no resemblance to nodules produced by the same strain on another host in the same cross-inoculation group (Mahajan and Gupta, 2009) (Table 6).

The nodule dry weight was ranged between 0.091 to 0.043 g, and plant inoculated with isolates AUGPR12 (grass pea) and AULR1 (lentil) on wolki variety whereas, 0.017 to 0.001gm/ plant by isolates AUFBR2 and AUFBR3 (faba bean) on Hachallu variety accordingly (Table 6). There was a discrepancy in nodule number and weight among the inoculated plants at $P<0.01$.

Maximum mean shoot dry mass (4.04g/plant) was recorded by isolate AUGPR13 (grass pea) on Hachallu variety and 3.11 g/plant with isolate AUFBR5 (faba bean) on wolki variety. The minimum shoot dry mass was recorded by plant inoculated with isolates AUFBR5 (faba bean) and AULR17 (lentil) (2.60g/Pl) on Hachallun and 1.74 g/pl by isolate AULR17 (lentil) on wolki variety. This result supported by previous findings of Talukder et al. (2008) in such a way that shoot dry weight was influenced by rhizobium inoculation. In this experiment, a significant difference in shoot dry weight among the isolates from cross inoculated hosts was recorded at $p<0.01$(Table 6).

The relative symbiotic effectiveness among the test isolates from heterogeneous cross-inoculation hosts were evaluated on the basis of the shoot dry matter accumulation by the infected host plants in reference to the nitrogen-fertilized control (Purcino et al., 2000) (Table7). From faba bean, isolate AUFBR2 was highly

**Table 6.** Comparative evaluation of symbiotic effectiveness of the modulated isolates with both Hachallu and Wolki varieties of faba bean.

| Isolates | Host of isolate | NN/pl | | NDW/pl (g) | | SDW/pl (g) | | % SE | | Rate | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | HV | WV | HV | WV | HV | WV | HV | WV | HV | WV |
| AUFBR2 | Faba bean | 112$^{e}$ | 106$^{f-h}$ | 0.001$^{b-e}$ | 0.045$^{b}$ | 3.32$^{a-d}$ | 2.63$^{a}$ | 82 | 99 | HE | HE |
| AUFBR3 | Faba bean | 65$^{bc}$ | 103$^{e-h}$ | 0.017$^{b-f}$ | 0.074$^{c-e}$ | 3.14$^{a-d}$ | 2.98$^{a}$ | 77 | 112 | E | HE |
| AUFBR5 | Faba bean | 112$^{e}$ | 122$^{h}$ | 0.004$^{a-c}$ | 0.058$^{b-d}$ | 2.60$^{a-d}$ | 3.11$^{bc}$ | 64 | 117 | E | HE |
| (+) control | - | - | - | - | - | 4.06$^{d}$ | 2.66$^{a}$ | - | - | - | - |
| (-) control | - | - | - | - | - | 1.58$^{ab}$ | 0.72$^{a}$ | - | | - | - |
| AUFPR6 | Field pea | 45$^{b}$ | 91$^{d-g}$ | 0.002$^{ab}$ | 0.068$^{b-e}$ | 3.25$^{a-d}$ | 2.08$^{a}$ | 89 | 52 | HE | E |
| AUFPR7 | Field pea | 73$^{b-d}$ | 110$^{gh}$ | 0.012$^{a-d}$ | 0.074$^{c-e}$ | 2.91$^{a-d}$ | 2.94$^{a}$ | 81 | 115 | HE | HE |
| AUFPR10 | Field pea | 67$^{bc}$ | 87$^{d-g}$ | 0.016$^{a-e}$ | 0.065$^{b-e}$ | 3.09$^{a-d}$ | 2.65$^{a}$ | 85 | 104 | HE | HE |
| (+) control | - | - | - | - | - | 3.61$^{b-d}$ | 2.55$^{a}$ | - | - | - | - |
| (-) control | - | - | - | - | - | 1.68$^{a-c}$ | 1.03$^{a}$ | - | - | - | - |
| AUGPR12 | Grass pea | 105$^{de}$ | 90$^{d-g}$ | 0.008$^{b-f}$ | 0.091$^{e}$ | 3.36$^{a-d}$ | 2.90$^{b}$ | 82 | 94 | HE | HE |
| AUGPR13 | Grass pea | 70$^{b-d}$ | 41$^{b}$ | 0.005$^{d-f}$ | 0.053$^{bc}$ | 4.04$^{d}$ | 2.13$^{a}$ | 98 | 69 | HE | E |
| AUGPR14 | Grass pea | 131$^{e}$ | 46$^{bc}$ | 0.015$^{ef}$ | 0.058$^{b-d}$ | 3.67$^{b-d}$ | 2.71$^{a}$ | 89 | 87 | HE | HE |
| (+) control | - | - | - | - | - | 4.11$^{d}$ | 3.10$^{bc}$ | - | - | - | - |
| (-) control | - | - | - | - | - | 1.57$^{ab}$ | 1.07$^{a}$ | - | - | - | - |
| AULR16 | Lentil | 60$^{b}$ | 63$^{b-d}$ | 0.010$^{b-f}$ | 0.043$^{b}$ | 2.82$^{a-d}$ | 2.50$^{a}$ | 74 | 92 | E | HE |
| AULR17 | Lentil | 54$^{b}$ | 72$^{c-e}$ | 0.014$^{c-f}$ | 0.086$^{e}$ | 2.60$^{a-d}$ | 1.74$^{a}$ | 68 | 64 | E | E |
| AULR18 | Lentil | 103$^{c-e}$ | 76$^{c-f}$ | 0.003$^{f}$ | 0.081$^{de}$ | 2.77$^{a-d}$ | 2.64$^{a}$ | 72 | 97 | E | HE |
| (+) control | - | - | - | - | - | 3.82$^{cd}$ | 2.73$^{a}$ | - | - | - | - |
| (-) control | - | - | - | - | - | 1.31$^{a}$ | 0.83$^{a}$ | | - | - | - |

HV=hachallu variety, WV=wolki variety of faba bean, Pl=plant, NN=nodule number, NDW=nodule dry weight, SDW=shoot dry weight, SE=symbiotic effectiveness

**Table 7.** The relative symbiotic effectiveness of the isolates from heterogeneous cross-inoculation host on two varieties of faba bean.

| Host of isolate | Hachallu variety of faba bean | | Wolki variety of faba bean | |
|---|---|---|---|---|
| | Effective | Highly effective | Effective | Highly effective |
| Faba bean | AUFBR3, AUFBR5 | AUFBR2 | - | AUFBR2, AUFBR3 and AUFBR5 |
| Total | 2 | 1 | - | 3 |
| Percentage (%) | 66.7 | 33.3 | - | 100 |
| Field pea | - | AUFPR6, AUFPR7, AUFPR10 | AUFPR6 | AUFPR7, AUFPR10 |
| Total | | 3 | 1 | 2 |
| Percentage (%) | | 100 | 33.3 | 66.7 |
| Grass pea | - | AUGPR12, AUGPR13, AUGPR14 | AUGPR13 | AUGPR12, AUGPR14 |
| Total | | 3 | 1 | 2 |
| Percentage (%) | | 100 | 33.3 | 66.7 |
| Lentil | AULR16, AULR17, AULR18 | - | AULR17 | AAULR16, AAULR18 |
| Total | 3 | - | 1 | 2 |
| Percentage (%) | 100 | - | 33.3 | 66.7 |

effective; isolates AUFBR3 and AUFBR5 were effective on Hachallu variety whereas, all isolates were highly effective on wolki variety. All isolates of Field pea were highly effective on Hachallu variety whereas, isolates AUFPR7 and AUFPR10 were highly effective and isolate AUFPR6 was effective on Wolki variety.

Similarly, all isolates of grass pea were highly effective on Hachallu variety, and isolate AUGPR12 and AUGPR14 were highly effective with 1isolate (AUGPR13) effective on wolki variety. All isolates of lentil were effective on Hachallu variety and 2 isolates (AULR16 and AULR18) and 1 isolate (AULR16) were highly effective and effective on wolki variety respectively (Table 7). This result indicated that *R*. *leguminosarum* bv.*viciae* isolated

**Table 8.** Summary of physiological and symbiotic properties of Isolates from faba bean, field pea, grass pea and lentil.

| Isolates | Host | Range of salt (%) | Range of pH | Range of T (°C) | % SE | | Rate | |
|---|---|---|---|---|---|---|---|---|
| | | | | | HV | WV | HV | WV |
| AUFBR2 | Faba bean | 1-10 | 4.5-12 | 5-40 | 82 | 99 | HE | HE |
| AUFBR3 | Faba bean | 1-3 | 4.5-9 | 5-45 | 77 | 112 | E | HE |
| AUFBR5 | Faba bean | 1-6 | 4-12 | 10-35 | 64 | 117 | E | HE |
| AUFPR6 | Field pea | 1-8 | 4-9.5 | 15-35 | 89 | 52 | HE | E |
| AUFPR7 | Field pea | 1-7 | 5-12 | 5-35 | 81 | 115 | HE | HE |
| AUFPR10 | Field pea | 1- 2 | 4.5-11 | 15-40 | 85 | 104 | HE | HE |
| AUGPR12 | Grass pea | 1-3 | 4-10.5 | 5-45 | 82 | 94 | HE | HE |
| AUGPR13 | Grass pea | 1-10 | 4-12 | 10-40 | 98 | 69 | HE | E |
| AUGPR14 | Grass pea | 1-10 | 4-12 | 5-45 | 89 | 87 | HE | HE |
| AULR16 | Lentil | 1-7 | 5-11 | 15-35 | 74 | 92 | E | HE |
| AULR17 | Lentil | 1-4 | 4.5-9.5 | 15-40 | 68 | 64 | E | E |
| AULR18 | Lentil | 1-9 | 4-11 | 5-45 | 72 | 97 | E | HE |

T=temperature, HV=hachallu variety of faba bean, WV=wolki variety of faba bean; SE=symbiotic effectiveness, HE=highly effective, E=effective.

from different host legumes showed variation in symbiotic effectiveness on faba bean varieties. The result is similar with that of Shimeket et al. (2008) who reported variation in symbiotic effectiveness from a population of *R. leguminosarum* bv.*viciae* on vetch from different soils. Laguerre et al. (2003) reported that specific symbiotic plasmid-encoded genes showed variation in competition for modulating of faba beans and nitrogen-fixing effectiveness.

Generally, isolates of AUFBR2 (faba bean) and AUGPR14 (grass pea) were highly effective in both hachallu and wolki varieties, and showed tolerance wide range of salt, pH, and temperature than their cross-inoculation group of *R. leguminosarum var viciae* (Table 8). These remarkable features of isolates are very important for screen, and it's shown to be competitive for future inoculants legumes production under extreme environmental conditions at the field trials in the soil ecosystem.

## Conclusion

The present study shows the physiological and symbiotic diversity of *R. leguminosarum bv.viciae* isolates of faba bean, field pea, grass pea, and lentil. Some of the isolates showed tolerant to a wider range of salt, pH, and temperature. Twelve isolates of faba bean, field pea, grass pea, and lentil were cross inoculated and induced nodules on faba bean host. Isolates from different cross-inoculated were effective irrespective of their host from which they isolate. The symbiotic effectiveness of isolates showed a significant difference on Hachallu variety (64 to 98% SE) and Wolki variety (52 to 117% SE). Isolates of AUFBR2 (faba bean) and AUGPR14 (grass pea) were best matched with both hachallu and wolki varieties followed by isolates AUFPR2 (field pea) and AUGPR3 (grass pea). This matching of rhizobial isolates to host legume is one of the most important factors in maximizing productivity and full expression of $N_2$ fixation in stressed environmental condition.

## RECOMMENDATIONS

Isolates of AUFBR2 (faba bean), isolate AUGPR14 (grass pea), AUGPR13 (grass pea) and AUFPR7 (field pea) must be used to develop inoculants as they are ecologically competitive. Screening isolates genetically using molecular techniques of REP/PCR or RFLP/PCR to evaluate their effectiveness under different environmental conditions.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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