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Symbiotic And Phenotypic Characteristics Of Rhizobia Nodulating Faba Bean *(Vicia Faba)* From Tahtay Koraro, Northwestern Zone Of Tigray Regional State, Ethiopia

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ABSTRACT: The ability of indigenous rhizobia to nodulate a legume crop effectively was critical to successful establishment and growth of legumes. This study was aimed to evaluate the symbiotic effectiveness along with growth responses to varied conditionslike pH, salt, temperature, antibiotics, and carbon and nitrogen sourcesfor rhizobial isolates nodulating faba bean from Tahtay Koraro, northwestern Zone of Tigray. For this matter, a total of thirty-six isolates of *Rhizobium* were isolated from as many sampling sites of Tahtay Koraro using plant infection method in Addis Ababa University, Applied Microbiology greenhouse.The isolates were characterized morphologically and physiologically and tested on sand to evaluate their symbiotic effectiveness.Results indicates that culturally almost all of them displayed large colonies with diameters of 2 to 4.5 mm, generation time of >3.91 hrs and showed characteristics of fast growing rhizobia.The symbiotic effectiveness results on sand culture indicated that, the isolates showed shoot dry matter ranging from 0.47 (AUFR-9) to 1.5 g/plant (AUFR-17), with negative control of 0.43 g/plant and positive control of 1.3 g/plant. All the tested isolates were able to grow well within the ranges of 6-9.5 and 15^0C-35^0C for pH and temperature, respectively. The highest and lowest nodule number score was 91 (AUFR-5) and 17 (AUFR-36), respectively. The preliminary screening of the authenticated isolates for symbiotic effectiveness on sand culture showed 74% of the isolates were found to be effective, whereAUFR-8, AUFR-13, AUFR-14, AUFR-17 and AUFR-25, were rated highly effective, of these AUFR-25 was found to be phosphate solubilizer. The numerical analysis on phenotypic features revealed the existence of diversity among the test isolates and categorized all isolates into six groups.Generally, the present work shows the physiological and symbiotic diversity of the isolates in the traditional agricultural areas of the study site and the potential of these rhizobia to be used as effective commercial inoculants in areas where the indigenous rhizobia fail to do so.

Keywords: nodulation, Rhizobia, Rhizobium leguminosarum, Tahtay Koraro, Tigray, Vicia faba.

INTRODUCTION

Faba bean (*Vicia faba*) is one of the most ancient food crops originated in the Near East and quickly spread to Europe, North Africa, along the Nile to Ethiopia [5]. It is one of the main pulse crops grown for dry seeds and green pods for consumption [5]. China is the largest faba bean producer (40.36%) with an average dry grain production of 1,720,000 mt from 945,400 hectares followed by Ethiopia (476, 026 mt), France (331,122 mt), Egypt (274,040 mt) and Australia (196,800 mt) [10]. Faba bean occupies about 3.9% of the total cultivated area of Ethiopia's agricultural land [4].It is grown as field crop throughout the highlands and is most common in mid altitudes between 1800 m.a.s.l and 2400 m.a.s.l. [4]. The major important production zones in the country includes the central high lands of Showa, all high lands of Gojam and Gondar, northwest Wallo and Tigry, Arsi, Bale and Wallega [5]. Faba bean is a legume capable of fixing nitrogen in an endosymbiotic association with *Rhizobium leguminosarum* biovar *vicieae* thus improves soil fertility. The dual contribution of faba bean as a source of protein for the majority of population, and its capability to fix nitrogen and to improve soil fertility has been used in crop rotation and traditional mixed low input agricultural systems [27].*Rhizobium leguminosarum* biovar *vicieae,* also nodulates pea (*Pisum* spp.), vetch (*Vicia* spp.), lentil (*Lens* spp.), and sweet pea (*Lathyrus* spp.)(Perret *et al.*, 2000).Among the grain legumes, faba bean is reported to derive the highest percentage of nitrogen from the atmosphere [18]. According to [24], the amount of nitrogen fixed by faba bean have been 240-325 kg/ha. The ability of faba bean to fix the desired amount of nitrogen depends on many factors such as the effectiveness of the symbiont strain, the genetic variation of the host plant, and other edaphic and environmental factors [19].In Ethiopia, attempts have been made to conduct research on rhizobiology of cool season legumes such as faba bean and field bean for the last two decades [3]. Limited number of works on the effectiveness of N_2 fixation of indigenous rhizobia of faba bean has been indicated promising results [28]; [11] and[12]. These researches indicated that Ethiopia harbored with highly effective N_2 fixer rhizobia nodulating faba bean. The present study was initiated with the aim of isolation and characterization of faba bean (*vicia faba*) rhizobial isolates collected from Tahtay koraro, northwestern Zone of Tigray Regional State under greenhouse condition.

MATERIALS AND METHODS

Soil sampling and trapping of nodules

Soils were sampled from more than 35 localities that ranged in twelve different kebeles of Tahtay Koraro (Fig 1). The study sites are characterized by mean annual temperature of 18 to 28°C and average annual rainfall of 900-1200 mm despite the fact that 75% of its topography is high land with total cultivated land of 18,577 ha. The soil type of the area includes 70% clay, 10% sandy loam and 20% loam with near neutral pH (Table 1). Soils were sampled from topsoil (0-30 cm) and collected in alcohol sterilized polyethylene plastic bags. The soil samples were

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brought to Applied Microbiology Laboratory, Addis Ababa University for rhizobia trapping in greenhouse experiment and for further *Rhizobium* isolation, identifications and pot experiments.

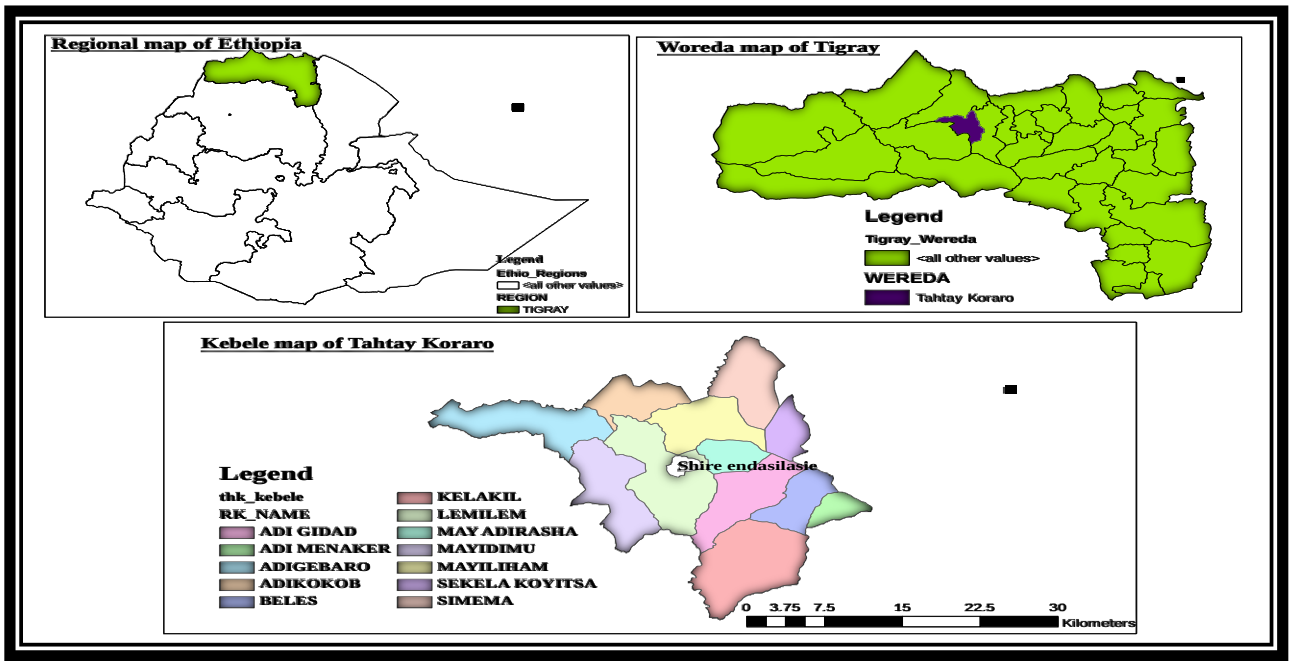

Figure 1: *Location map of the study area*

Isolation of rhizobia from nodules

Nodules were treated by rinsing in 70% ethanol (5 sec); surface sterilized in 3% sodium hypo-chlorate (3 min) and then rinsed five times with sterile distilled water following [17]. Crushing of nodules were done in normal saline solution (0.85%) and streaked onto yeast extract mannitol agar (YEMA) medium. Plates were incubated at 28±2^{o}C for 3-5 days then restreaked to obtain pure culture. Single colony isolates were picked from plates, numbered and stored on YEMA slants containing 0.3% (W/V) $CaCO_3$ at 4^{o}C refrigerator for further characterization. The isolates were given names such as AUFR (Addis Ababa University Faba bean *Rhizobium*) with different number representing each isolate.

Presumptive screening of pure cultures

Cultures were examined for cell morphology and gram reaction after 3 days of growth in YEM broth medium. The colony morphology and purity of isolates were examined on YEMA containing Congo red plates after an incubation of 5 days at 28±2^{o}C. Individual colonies were characterized based on their color, shape, colony diameter, capacity to produce exopolysaccharide gum and their absorbance of the red color on YEMA-CR (Vincent, 1970). The production of acid or alkali was determined in YEMA medium with Bromothymol blue (BTB) (25 mg L^{-1}) plates [24].

Intrinsic antibiotic resistance

This intrinsic resistance of isolates was determined by inoculating(10^{9}cells ml^{-1}) on solid YEMA medium containing five filtersterilized (0, 22 mm Millipore filters) antibiotics at concentrations of2.5, 5, 10 and 20 µg/ml of water according to [16].

Tolerance to acidity, alkalinity, salinity and temperature

All experiments on tolerance to acidity, alkalinity, salinity and temperature were performed according to [18]. The capacity of each rhizobial isolate to grow on acidic and alkaline media was determined by inoculating a loopful of each isolate on YEMA adjusted at a pH of 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0, using 1N HCl and NaOH before autoclaving. For salt tolerance, the isolates were transferred to YEMA plates supplemented with NaCl at concentrations of 0.1, 0.3, 0.5, 0.8, 1, 2, 3, 4, 5, 6, and 7% w/v). The ability of bacterial isolates to grow at high and low temperatures was monitored at incubation temperatures of 4, 10, 15, 35, 40, 45 and 48 ^{0}C.

Nitrogen and carbon sources utilization

For nitrogen source utilization tests, 13 nitrogen sources were taken. They were filter sterilized and added at a final concentration of 0.5g/l to a basal media containing 1 g of KH_2PO_4; 1 g K_2HPO_4; 0.01 g $FeCl_3.6H_2O$; 0.2 g $MgSO_4.7H_2O$; 0.1 g $CaCl_2$; 15 g agar and supplemented with 1g/l of mannitol. The plates were incubated at 28±2^{0}C for 3-5 days. Carbon utilization of isolates was determined following the method of[24] on 15 carbohydratesprepared as 10% (w/v) solution in water. The carbohydrate free YEMAmedium was modified by reducing the yeast extract to 0.05 gl^{-1} liter.

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Table 1. *Sample location, altitude, soil pH and types of legumes grown*

Isolates	Sampling sites	Altitude	Type of legume	Soil pH
AUFR-2	Beles	1883	Faba bean	7.04
AUFR-3			Faba bean	7.06
AUFR-5	Koyetsa	1890	Faba bean	7.01
AUFR-6			Faba bean	7.02
AUFR-7	Kelakil	1870	Faba bean	6.73
AUFR-8			Faba bean	6.63
AUFR-9			Faba bean	6.87
AUFR-10	Semema	1889	Faba bean	6.68
AUFR-11			Faba bean	6.76
AUFR-12			Faba bean	6.54
AUFR-13	Adi-Gebaro	1883	Faba bean	6.75
AUFR-14			Faba bean	6.94
AUFR-15			Faba bean	6.81
AUFR-17	Adi-Menabir	1893	Faba bean	7.14
AUFR-18			Faba bean	7.11
AUFR-20	May-Dimu	1877	Faba bean	7.11
AUFR-21			Faba bean	7.22
AUFR-23	Adi-Kokob	1888	Faba bean	6.87
AUFR-24			Faba bean	6.87
AUFR-25	Lemlem	1883	Faba bean	6.99
AUFR-27			Faba bean	6.98
AUFR-28	May-Liham	1881	Faba bean	6.55
AUFR-30			Faba bean	6.93
AUFR-31	Adi-Gidad	1885	Faba bean	6.95
AUFR-32			Faba bean	6.97
AUFR-34	May-Adrasha	1895	Faba bean	7.12
AUFR-36			Faba bean	7.1

Symbiotic effectiveness on sand culture

This experiment was carried out at Addis Ababa University, applied microbiology greenhouse to authenticate and select elite isolates of rhizobia forming effective symbiotic association with faba bean following [24]. Plants were grown in free-draining plastic pots (3 kg capacity) that had been surface disinfected by soaking in 70% ethanol and drying. Sterile paper towels were inserted aseptically in the base of the pots to prevent loss of nutrients and filled with acid treated sterile moisten sand. Faba bean seeds were treated with 70% ethanol (5 sec), surface sterilized in 3% sodium hypo-chlorate (3 min) and then rinsed five times with steriledistilled water following [17]. Five sterilized seeds were sown in each pot and when germinated, they were thinned to three seedlings per pot after a week of planting. One milliliter of liquid inoculum (10^9 rhizobia cells ml^{-1}) of collected isolates was used separately to inoculate seeds at the sowing stage. Treatments were arranged in a randomized block design with three replicates each. All pots were treated with ¼ strength of micro-nutrient solution (Broughton and Dilworth N-free medium) with two days interval and the N-fertilize Pot was received 70 mg KNO_3 L^{-1} $week^{-1}$. Likewise, all plants were wateredevery two days with sterile distilled water. Plants were harvested 45 days after planting. Nodulation status; nodule number and nodule dry weight, and shoot dry weight were measured. Relative effectiveness of isolates was calculated according to the equation proposed by [8] in [20] (inoculated plant dry matter x 100 / N-fertilized plant dry matter) with Nitrogen fixing effectiveness classified as ineffective <35%; lowly-effective, 35 to 50%; effective, 50 to 80%; and highly effective, >80%.

Statistical analysis

One way analysis of variance (ANOVA) was used for data analysis using the statistical program (SPSS) ver.20. Mean separation was calculated using Duncan test value when the F-test was significant at p≤0.05 %.

RESULTS AND DISCUSSION

In the present study, 36 isolates were isolated from the root nodules of faba bean grown on the soil samples collected from different sites of Tahtay Koraro.All isolates were identified as gram negative-rods with no absorption of Congo-red on YEMA-CR medium under dark incubation and turned the BTB supplemented YEMA medium into yellowish (Table 2). These results obtained from gram staining, growth on YEMA-CR, YEMA-BTB and PGA medium preliminary confirm the standard cultural and morphological characteristics of *Rhizobium* species as described by [24] and [26]. None of the isolates showed growth on peptone-glucose-agar medium and this is in line with the description given by [24] that states PGA does not allow the growth of rhizobia but other contaminants. The isolates displayed colony diameter of 1.5-4.5 mm within 3-5 days of incubation (Table 2). All of the isolates formed dome-shaped with circular colony margin.Majority of the test isolates (81%) were large and mucoid with less translucent appearances, whereas others which constitute 19% of the tested isolates, formed large watery colonies(Table 2). Similar results were reported on rhizobia of faba bean isolated from Ethiopian soils [28]; [11] and [12].

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Table 2. *Morphological characterization of isolates*

Isolates	Sampling Sites	Colony characteristics after 4-5 days	Growth on YEMA-BTB	Growth on YEMA-CR	Colony diameter	MGT
AUFR-2	Beles	LM	Deep yellow	Colorless	2 mm	3.9
AUFR-3		LM	Deep yellew	Colorless	3 mm	2.8
AUFR-5	Koyetsa	LW	Yellow	Colorless	2.5 mm	3.7
AUFR-6		LM	Deep yellow	Colorless	4.5 mm	3.6
AUFR-7	Kelakil	LM	Moder. Yellow	Colorless	2 mm	2.2
AUFR-8		LM	Deep yellow	Colorless	3.5 mm	1.3
AUFR-9		LW	Deep yellow	Colorless	2 mm	2.6
AUFR-10	Semema	LW	Moder. Yellow	Colorless	4.5 mm	2.3
AUFR-11		LW	Yellow	Colorless	2 mm	2.2
AUFR-12		LM	Yellow	Colorless	2 mm	3.2
AUFR-13	Adi-Gebaro	LM	Moder. Yellow	Colorless	3 mm	2.7
AUFR-14		LM	Deep yellow	Colorless	2 mm	1.7
AUFR-15		LM	Yellow	Colorless	2.5 mm	2.8
AUFR-17	Adi-Menabir	LM	Moder. Yellow	Colorless	3 mm	3.1
AUFR-18		LM	Yellow	Colorless	2 mm	2.2
AUFR-20	May-Dimu	LM	Moder. Yellow	Colorless	2 mm	2.9
AUFR-21		LM	Yellow	Colorless	2 mm	1.5
AUFR-23	Adi-Kokob	LM	Yellow	Colorless	2.5 mm	2.3
AUFR-24		LM	Deep yellow	Colorless	2.5 mm	2.1
AUFR-25	Lemlem	LM	Yellow	Colorless	3 mm	2.2
AUFR-27		LM	Yellow	Colorless	2 mm	2.3
AUFR-28	May-Liham	LW	Deep yellow	Colorless	2mm	3.9
AUFR-30		LW	Yellow	Colorless	2 mm	1.6
AUFR-31	Adi-Gidad	LM	Yellow	Colorless	2 mm	2.0
AUFR-32		LM	Yellow	Colorless	1.5 mm	2.9
AUFR-34	May-Adrasha	LM	Moder. Yellow	Colorless	2 mm	2.8
AUFR-36		LM	Yellow	Colorless	1.5mm	3.2

LM= Large Mucoid LM=Large Watery

All isolates were able to grow well up to 0.8 % NaCl on YEMA plates and 40% of the isolates were able to grow at 7% NaCl concentration (Table 3). This result is comparable to the findings of [11] and [29]who reported the existence of faba bean rhizobial isolates from different part of Ethiopia could tolerate up to 6-7% of NaCl concentration. [2] also reported that faba bean rhizobia were assessed on salt concentrations 0.5, 1, 1.5 and 2% and the isolates grew on these concentrations. However the result is contrary to the report by[28] from North Gonder where only a few isolates (14%) from faba bean were found to resist 2% NaCl concentration. All rhizobial isolates (100%) grew well between 15^0C and 35^0C with decreasing percentage of growth above and below these values (Table 3). This result is found to be similar with the previous work on faba bean by [28] and [11] in which all of their isolates showed tolerance to 15^0C-35^0C. Even though the optimum growth temperature for most isolates of pulse rhizobia ranges from 25-35^0C [24], 63% of the tested isolates were shown growth at 40^0C and 26% of the isolates were capable of growing at 45^0C (Table 3). The isolates also grew on a wide range of moderate acidity and alkalinity (pH 5.5 to 9) except AUFR-31 which failed to grow at pH 10 (Table 3). [2] also reported almost all isolates were found to grow at pH 6.0-8.0. This is concurrent to the finding that *R. leguminosarum* bv. *Viciae* strains are generally sensitive to low pH and grow well on near neutral and basic pH [14]. The isolates were found to utilize 89 to 100% of the tested carbon sources, while no isolate did grow on citrate (data not shown). This finding corroborates with previous reports of carbohydrate metabolism of *R.leguminosarum* bv. *viciae* [28]; [11] and [12]. With regard to nitrogen sources, isolates were found to utilize 67-100%. The lowest utilization percentage (67%) was found from DL-threonine. It has been reported that certain isolates of *Rhizobium* can solubilize both organic and inorganic phosphates [1]. Accordingly, six isolates were capable of solubilizing inorganic tricalcium phosphate (Table 3). The isolates were AUFR-(6, 25, 27, 28, 32 and 34) which accounted about 22% of the total isolates. [12] also retrieved twelve (12) isolates from faba bean that have the ability to solubilize inorganic tricalcium phosphate from acidic soils of Wollega. Others, [28] and [11] were not found anyrhizobial isolate from faba bean that had the competence to solubilize inorganic phosphate. All isolates exhibited variations in their intrinsic antibiotic resistance (IAR) to different concentrations and types. The majority of

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the isolates were found to be more sensitive to streptomycin followed by Erythromycin and Chloramphenicol than the other antibiotics. On the contrary, almost all of the isolates were resistant to ampicillin and nalidixic acid at the final concentrations of 2.5, 5 and 10 µg/ml. AUFR-6 was the only isolate which was tolerant to concentrations of 5, 10 and 20 μgml^{-1} for streptomycin (Table 3).As compared to the current work, isolates from Wollega [12] showed more sensitivity to erythromycin and ampicillin, whereas isolates from north Gondar [28] displayed the highest resistance to streptomycin and chloramphenicol.

Table 3. *In vitro ecological competence and symbiotic effectiveness of isolates collected from faba bean of Tahtay Koraro*

Isolates	**Site**	C & N Sources Utili.(%)		Salt Tole. (%)	T^{o} Tolerance (^{o}c)	pH Tolerance	Antibiotic Tolerance (µg/ml)					P	SE
		C	N				Amp	Strept	Eryth	Nalid	Chloro		
AUFR-2	Beles	93	92	0.1-1	15-35	4.5-10	2.5-20	2.5	NG	2.5-20	2.5-10		LE
AUFR-3		93	92	0.1-7	10-40	4.5-10	2.5-20	NG	NG	2.5-20	2.5-20		E
AUFR-5	Koyetsa	93	100	0.1-2	15-40	5.5-10	2.5-10	2.5	2.5-10	2.5-10	2.5		E
AUFR-6		93	100	0.1-4	15-40	4.5-10	2.5-20	2.5-20	2.5	2.5-20	2.5-10	*p	LE
AUFR-7	Kelakil	93	100	0.1-7	10-35	4.5-10	2.5-20	2.5	NG	2.5-20	2.5-10		E
AUFR-8		93	100	0.1-1	15-40	5.5-10	2.5-20	2.5	2.5-10	2.5-20	2.5-10		HE
AUFR-9		93	92	0.1-1	15-40	4.5-10	2.5-20	2.5	2.5-10	2.5-20	2.5-20		LE
AUFR-10	Semema	93	69	0.1-1	10-35	4.5-10	2.5-20	2.5	NG	2.5-20	2.5-20		E
AUFR-11		93	100	0.1-7	15-40	4.5-10	2.5-20	2.5	2.5-10	2.5-10	NG		E
AUFR-12		93	92	0.1-7	15-40	4.5-10	2.5-20	2.5	NG	2.5-20	2.5-20		E
AUFR-13	Adi-Gebaro	93	100	0.1-7	15-45	4.5-10	2.5-20	2.5	2.5-10	2.5-10	2.5-20		HE
AUFR-14		93	100	0.1-7	15-40	4.5-10	2.5-20	2.5	2.5-10	2.5-20	2.5-20		HE
AUFR-15		93	92	0.1-7	15-35	4.5-10	2.5-20	2.5	2.5-10	2.5-20	2.5-10		E
AUFR-17	Adi-Menabir	93	77	0.1-6	10-35	4.5-10	2.5-20	2.5	NG	2.5-20	2.5-10		HE
AUFR-18		93	69	0.1-4	15-35	4.5-10	2.5-20	2.5	NG	2.5-20	2.5-20		LE
AUFR-20	May-Dimu	87	92	0.1-5	10-45	4.5-10	2.5-20	2.5	2.5-10	2.5-10	NG		E
AUFR-21		93	100	0.1-6	15-45	4.5-10	2.5-20	2.5	2.5-10	2.5-10	NG		E
AUFR-23	Adi-Kokob	93	100	0.1-0.8	15-45	4.5-10	2.5-20	2.5	2.5-10	2.5-20	NG		E
AUFR-24		93	92	0.1-3	10-35	4.5-10	2.5-20	2.5	2.5-10	2.5-20	2.5-20		E
AUFR-25	Lemlem	93	100	0.1-3	15-40	4.5-10	2.5-20	2.5	2.5-10	2.5-20	2.5-10	*p	HE
AUFR-27		93	69	0.1-7	10-35	4.5-10	2.5-20	2.5	2.5-10	2.5-20	2.5-20	*p	E
AUFR-28	May-Liham	93	85	0.1-7	15-45	4.5-10	2.5-20	2.5	NG	2.5-20	2.5-10	*p	LE
AUFR-30		93	100	0.1-5	10-45	4.5-10	2.5-20	2.5	2.5-10	2.5	2.5-5		LE
AUFR-31	Adi-Gidad	93	100	0.1-3	15-40	5.5-9.5	2.5-5	2.5	2.5-10	2.5	NG		E
AUFR-32		87	100	0.1-7	15-45	4.5-10	2.5-20	2.5	2.5-10	2.5-20	2.5-20	*p	E
AUFR-34	May-Adrasha	87	100	0.1-0.8	15-40	4.5-10	2.5-20	2.5	2.5-10	2.5-20	2.5-5	*p	E
AUFR-36		93	100	0.1-4	10-40	4.5-10	2.5-20	2.5	2.5-10	2.5-10	2.5		LE

*C=Carbon; N= Nitrogen; P= phosphorus; *p=solubilize tri-calcium phosphate; NG= no growth at all antibiotic doses*

Isolates from western Shewa and Hararghe [11] showed comparatively similar resistance ability to chloramphenicol but better in resisting streptomycin. Two features, infectivity and symbiotic effectiveness are commonly used to assess the ecological and evolutionary relationship between rhizobia and their host [7]. Thus, the glasshouse trial of this

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study on potted sand revealed that 27 of the 36 tested isolates (75%) induced nodulation (Table 4). It is possible to suggest that those isolates that failed to re-nodulate their parent host are either rhizobia that lost their nodulation capacity due to a loss of plasmids [22]; [30] or some other intruding soil bacteria that penetrated the nodule [13]. The inoculated plants showed diversity in their physical appearances; nodule number, nodule dry weight and shoot dry weight (Table 4). The nodule number recorded from the authenticated plants ranged from 17 per plant for isolate AUFR-11 (Semema) to 91 per plant for isolate AUFR-5 (Koyetsa) and the range of nodule dry mass was between 15 mg/p and 57 mg/p for isolates AUFR-27 (Lemlem) and AUFR-18 (Adi-menabr), respectively (Table 4). Studies indicated that variation in nodulation could be due to low rhizobial density, incompatibility of the rhizobia and edaphic factors that hinder the effectiveness of the rhizobia [27]; [23]; [16]). Maximum and minimum mean shoot dry mass of 1.5 g/pl and 0.5 g/pl was scored by AUFR-17 and AUFR-6/AUFR-28 (Tabe 4). Isolates from acidic soils of Wollega [12], western Shewa and Hararghe [11] and north Gonder [28] showed maximum and minimum mean shoot dry mass of 1.95 g/pl and 0.54 g/pl, 1.95 g/pl and 0.38 g/pl and, 2.3 g/pl and 0.4 g/pl, respectively. Shoot dry weight and nodule dry weight are usually highly correlated, thus shoot dry weight is used routinely as an indicator of relative effectiveness [24]. In this experiment, a significant ($p=0.05$) difference was recorded in shoot dry weight, nodule number and nodule dry weight among the infective isolates (Table 4). On the basis of relative shoot dry matter accumulation by the inoculated plants in reference to the nitrogen-fertilized control, 18% of the isolates; AUFR-8 (Kelakil), AUFR-13 (Adi-gebaro), AUFR-14 (Adi-Menabir), AUFR-17 (Adi-menabir) and AUFR-25 (Lemlem) were rated as highly effective with symbiotic effectiveness between 82% and 115% (Table 4). Likewise, 56% of the isolates were rated effective by scoring relative effectiveness scale between 50-80%. The rest of the isolates (26%) were rated lowly effective with relative effectiveness scale between 35%-50% (Table 4). The ratio of effective to lowly effective symbiotic effectiveness of the current work was 74%: 26%. Previously, [28], [11] and [12] reported the percentage of effective and highly effective faba bean rhizobial isolates showed to be 80%, 60% and 70% from north Gonder, western Shewa and Hararghe, and acidic soils of Wollega, respectively. This was contrary to the report of [9] that showed only 23 symbiotically effective isolates (21%) among the 108 isolates collected from Central Shewa. Such variability in symbiotic effectiveness of faba bean *Rhizobium* was found to be widespread in Ethiopia [25].[6] also reported 66-87% effectiveness of faba bean rhizobia from Ankober, Molale, Keyt, and Mehalmeda sites of northern Shewa. Generally the result of this study and other studies suggest the existence of effective naturally occurring faba bean rhizobial diversity in different agro ecological zones of Ethiopia where faba bean production takes place.

CONCLUSION

From this study, it can be concluded that the selection of highly performed isolates are worthy of further investigation from different faba bean growing regions of Ethiopia. Given that Tahtay Koraro is one of the important sites of faba bean production, 74% of the isolates were found to be effective indicating the sampling sites harbor effective rhizobia, where as some of faba bean growing areas evaluated in this study are most presumably in need of inoculants like May-Liham (Table 4). Though the presence of diversity among the isolates revealed the possibility of getting potentially effective adaptable rhizobial isolate that enhance faba bean productivity, the weak symbiotic properties observed during isolation and nodulation status survey might partly be responsible for yield variation in the cropping systems of Tigray. Hence, studies on a need for inoculation and factors responsible for poor nodulation need to be undertaken to realize the role of biological nitrogen fixation in Tigray cropping systems.

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Table 4. *Nodulation and relative effectiveness in nitrogen fixation of Rhizobium leguminosarum biovar viciae isolates on Degaga variety of faba bean on sand culture*

Isolates	Site	Nodule No. / plant	Nodule dry weight / plant (g)	Shoot dry weight / plant (g)	% SE	Effectiveness	% of site effective
AUFR-2	Beles	38±20.55^{d-h}	0.0317±0.0170^{a-f}	0.600±0.265^{e-i}	46	LE	50
AUFR-3		71±21.38^{a-e}	0.0423±0.0121 $^{a-e}$	0.867±0.116^{c-g}	67	E	
AUFR-5	Koyetsa	91±39.32^{a}	0.0533±0.0137^{ab}	1.033±0.252^{b-d}	79	E	50
AUFR-6		24±8.15^{ab}	0.0193±0.0047^{ef}	0.500±0.000^{g-i}	38	LE	
AUFR-7	Kelakil	48±32.35^{b-h}	0.0277±0.0086^{d-f}	0.867±0.153^{bc-g}	67	E	77
AUFR-8		78±12.70^{a-d}	0.0359±0.0096^{a-f}	1.200±0.173^{a-c}	92	HE	
AUFR-9		24±11.24^{gh}	0.0207±0.0098^{ef}	0.467±0.153^{hi}	36	LE	
AUFR-10	Semema	40±11.93^{c-h}	0.0376±0.0032^{a-f}	0.767±0.058^{d-i}	59	E	100
AUFR-11		23±9.85^{gh}	0.0170±0.0087^{f}	0.833±0.058^{d-h}	64	E	
AUFR-12		68±39.39^{a-f}	0.0396±0.0225^{a-f}	0.800±0.346^{d-i}	62	E	
AUFR-13	Adi-Gebaro	90±20.66^{ab}	0.0379±0.0072^{a-f}	1.200±0.346^{a-c}	92	HE	100
AUFR-14		85±39.51^{ab}	0.0563±0.0107^{a}	1.067±0.252^{b-d}	82	HE	
AUFR-15		58±11.15^{a-h}	0.0379±0.0706^{a-f}	0.700±0.100^{d-i}	54	E	
AUFR-17	Adi-Menabir	86±19.93^{ab}	0.0573±0.0107^{a}	1.500±0.300^{a}	115	HE	50
AUFR-18		34±10.02^{e-h}	0.0276±0.0114^{d-f}	0.567±0.058^{f-i}	44	LE	
AUFR-20	May-Dimu	42±13.65^{c-h}	0.0282±0.0106^{d-f}	0.867±0.379^{c-g}	67	E	100
AUFR-21		27±12.77^{f-h}	0.0360±0.0165^{a-f}	0.867±0.058^{b-f}	67	E	
AUFR-23	Adi-Kokob	83±46.06^{a-c}	0.0490±0.0223^{a-c}	0.833±0.351^{c-h}	64	E	100
AUFR-24		37±11.53^{d-h}	0.0302±0.0085^{c-f}	0.767±0.116^{d-i}	59	E	
AUFR-25	Lemlem	64±14.36^{a-g}	0.0567±0.0289^{a}	1.467±0.252^{a}	112	HE	100
AUFR-27		78±7.64^{a-d}	0.0423±0.0070^{a-e}	0.967±0.058^{b-e}	74	E	
AUFR-28	May-Liham	40±27.62^{c-h}	0.0220±0.0137^{ef}	0.500±0.100^{g-i}	38	LE	0
AUFR-30		51±20.03^{a-h}	0.0316±0.0096^{b-f}	0.600±0.100^{e-i}	46	LE	
AUFR-31	Adi-Gidad	42±7.51^{c-h}	0.0280±0.0087^{d-f}	0.800±0.200^{d-i}	62	E	100
AUFR-32		49±12.17^{a-h}	0.0340±0.0044^{a-f}	0.767±0.153^{d-i}	59	E	
AUFR-34	May-Adrasha	49±14.84^{a-h}	0.0367±0.0080^{a-f}	0.900±0.100^{c-f}	69	E	50
AUFR-36		17±6.43^{h}	0.0153±0.0070^{f}	0.567±0.058^{f-i}	44	LE	
+Ve control		-	-	1.3±0.1.0^{ab}	100		
-Ve control		-	-	0.433±0.116^{i}	33		

- *Numbers are the means of variables of three replicates of three plants*
- *Levels not followed by the same letter/letters are significantly different at p<0.05 (Dunkan HSD test)+Ve=Posetive -ve= Negative*

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