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Morphophysiological and Symbiotic Characteristics of Rhizobia Nodulating Faba Bean (Vicia faba L.) from Bale, Ethiopia

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Abstract
Faba bean among legumes is the most important pulse crop in Ethiopia,and like other legumes can form symbiotic association with soil bacteria generally known as rhizobia. With the help of this association, it satisfies its nitrogen need to sustain life. Accordingly, the present study focused on isolation and selection of symbiotically effective rhizobia from unaddressed areas of Ethiopia. Thirty faba bean nodulating isolates trapped from soil collected from five districts (Agarfa,Dinsho,Gasera,Goba and Sinana) of Bale zone using plant infection method. The isolates were characterized for their cultural,nutritional,ecophysiological and symbiotic effectiveness.Almost all the isolates were large and mucoid,and all were acid producers and attained colony size that ranged 1 to 4mm with mean generation time ranging from 1.26 to 5 h and showed characteristics of fast growing rhizobia .Except for few isolates that tolerated extreme temperatures of 4 and 45^{o}C, many grew in temperature ranges of 15 to 35^{o}C, and were salt and acid tolerant, and relatively tolerated many of antibiotics except tetracycline. with respect to nutritional versatility, they grew on wide ranges of Carbon and Nitrogen sources. Pot experiments were conducted for parameters including; nodules number, nodule dry weight, shoot dry weight and total nitrogen were significantly improved by inoculation ($p< 0.05$). In cases of nitrogen accumulation, sixteen of isolates perform relatively better than the positive control and more than 76% of the isolates performed better than the negative control, in cases of shoot dry weight accumulation most of (83%) of the isolates are moderate to highly effective. Four test isolates (FBD5, FBS2, FBS5, FBA5) were found to be highly effective with their homologous host, faba bean which excelled the reference strain and were observed for better performance than all other isolates and the negative control. Isolate FBD5 which is recovered from soil of Dinsho is more and significantly accumulated higher dry weight than 70ppm KNO3 treated positive control and the reference strain. Taken together, our results suggest the presence of morphophysiologically diverse and symbiotically effective rhizobia nodulating faba bean growing in Ethiopia. In order to elucidate their exact taxonomic position, further characterization is needed based on molecular markers. Furthermore,their symbiotic performance should be tested under field condition in order to develop them into inoculants for faba bean production in Ethiopia and beyond.
Keywords: Bale, Biological Nitrogen Fixation, Faba Bean, *Rhizobium leguminosarum*, Symbiotic effectiveness

INTRODUCTION

Biological Nitrogen Fixation(BNF) is a central life supporting process that provides most of the fixed nitrogen needed to sustain life, which animals including humans and plants rely on(Fisher and Newton, 2002). BNF has advantages of lower costs and reduced environmental hazards, and is more consistent with the development of sustainable agriculture (Reddy et al., 2002). In this way, nitrogen fixation assumes significant importance in agriculture because good crop yields depend on an adequate supply of fixed nitrogen by which the biological process contributes about 65% of the total annual yield of fixed nitrogen(Fisher and Newton, 2002).

Faba Bean is the most important pulse crop in Ethiopia. It grows as field crop throughout the highlands between the altitudes 1800 m a.s.l and 3000m a.s.l where the need of chilling temperature is satisfied and also is most common in Weyena Dega in several regions with annual rainfall of 700-1000 mm (Gemechu Keneni et al., 2003). Faba Bean is a crop of high economic value with its edible seed serving as an important protein complement in the cereal based Ethiopian diet (Tamene Temesgen et al., 2015)and also contribute to smallholder income (FAO, 2014). Furthermore, it supplies an important added value to the crop by fixing atmospheric nitrogen in symbiosis, with root nodule bacteria known as Rhizobium leguminosarum bv. Viciae (Mutch and Young, 2004).An estimate of 240-235 amounts of N2 fixed (kilograms of N2 fixed per hectare) by Faba Bean with Faba Bean-rhizobial symbioses(Somasegaran and Hoben, 1994)thus, reducing costs by less fertilizer use and minimizing impact on the environment by natural soil maintenance (Alghamdi et al., 2012;IFPRI, 2010).

The rhizobium-legume association can be manipulated, through inoculation under N limiting field conditions, to enhance crop production easily and inexpensive. Where soils do not contain the specific rhizobia needed to establish an effective association, inoculation is essential, ensuring that a large and effective rhizobial

population is available in the rhizospher of the plant (Berkum; et al., 1995). Currently rhizobial inoculants are widely used in various parts of the world. They are the solution to dwindling soil fertility, inexpensive, environment friendly, and easy to use with no side effects in most cases(Wondwosen Tena et al., 2016). The technology, therefore, is good for Ethiopian soils where 85% are reported to have low levels of Nitrogen(EIAR, 2014). Several field demonstrations have confirmed that leguminous crops show remarkable growth and yield response to rhizobia inoculations in different agroecologies in Ethiopia. As a result, the use of rhizobia inoculants has shown spectacular growth in Ethiopia(EIAR, 2014).

Different research works made in recent years revealed that inoculation of Faba Bean with R.leguminosarum increase yield by 10-50% (Asfaw Hailemariam and Angaw Tsgie, 2003). In response to this, promising Faba Bean rhizobia screening activities were carried out during the past ten years in the country and revealed that there is diversity in different agroecologies (Abere Mnalku et al., 2009;Alemayehu Workalemahu, 2009; Zerihun Belay and Fassil Assefa, 2011;Anteneh Argaw, 2012; Solomon Legesse and Fassil Assefa, 2014;Dereje Tsegaye et al., 2015;Getahun Negash 2015) ,and this presence of diverse agroecological zones in the country and the dependence of rhizobial diversity and effectiveness on those diverse agroecologies call for isolation and selection of symbiotically effective indigenous rhizobia from unaddressed areas. Moreover, concerning symbiotic effectiveness no comprehensive study on indigenous population of rhizobia compatible to Faba Bean had been executed in all Woredas of Bale zone. This fact dictates investigation of rhizobia nodulating Faba Bean growing in different districts of Bale zone. The result will serve as baseline data for future endeavor of utilizing biological nitrogen fixing system of Faba Bean to increase productivity into low-input agriculture. In addition to that, in supporting the efforts of breaking the thriving mono cropping agricultural system of the zone and utilizing and supplementing the currently adopted few strains of rhizobia and generally in the region.

MATERIAL AND METHODS

Soil sampling and trapping of nodules

Soil samples from farmers field were collected from 28 locations in five woredas of Bale zone(Figure 1) considering all important descriptors (Site names, altitude, geographical points, growing history).Soils were sampled from top soils(0-30cm) and having been pooled, composited and stored at 4OC in the laboratory for rhizobia trapping in greenhouse experiment.Soil chemical properties (Soil types, pH, organic Carbon,total Nitrogen and available Phosporus) and displayed with GPS in Table 2.

Designiation of rhizobia isolates

The designation of isolates folowed FB the host Faba bean,and A,D,Ga,G,S to indicate districts of collection respectively of Agarfa,Dinsho,Gasera,Goba,Sinana, and different numbers are different isolates.

Isolation of rhizobia from nodules

Nodulation was induced by the use of host plant for trapping isolates as described in Vincent (1970) using acid treated and sterilized sand in pot experiment. Seeds of newly released Faba bean variety(Ashebeqa) obtained from Kuliumsa Agricultural research Center was used. The sterilized seeds were germinated on sterile petri-dish containing moistened filtered paper and placed at 28OC in incubator. Five pregerminated seeds were planted in each pot with soil samples and later trimmed to three.

Plants were carefully uprooted following germination after 55 days of growth to recover the nodules (Tolera Abera et al., 2015).For isolation of rhizobia, root nodules were transported to Ambo Agricultural Research Center, Bacteriology laboratory where treated by rinsing in sterilizing solutions of 70% (V/V) ethanol (5sec) surface sterilized in 3% sodium hypochlorite (3 min) and then rinsed five times with sterile distilled water. They were crushed in normal saline solution (0.85%) and streaked onto yeast extract mannitol agar (YEMA) medium which contains g/l of: Mannitol, 10; K2HPO4, 0.5; MgSO47H2O, 0.2; NaCl,0.1; yeast extract, 0.5; agar, 15.Plates were incubated at 28±20C for 3-5 days then restreaked to obtain pure culture. Single colony isolates were picked from plates, numbered and stored in YEMA slants containing 0.3% (W/V) CaCO3 at 40C refrigerator for further characterization(Howieson and Dilworth, 2016;Somasegaran and Hoben, 1994)

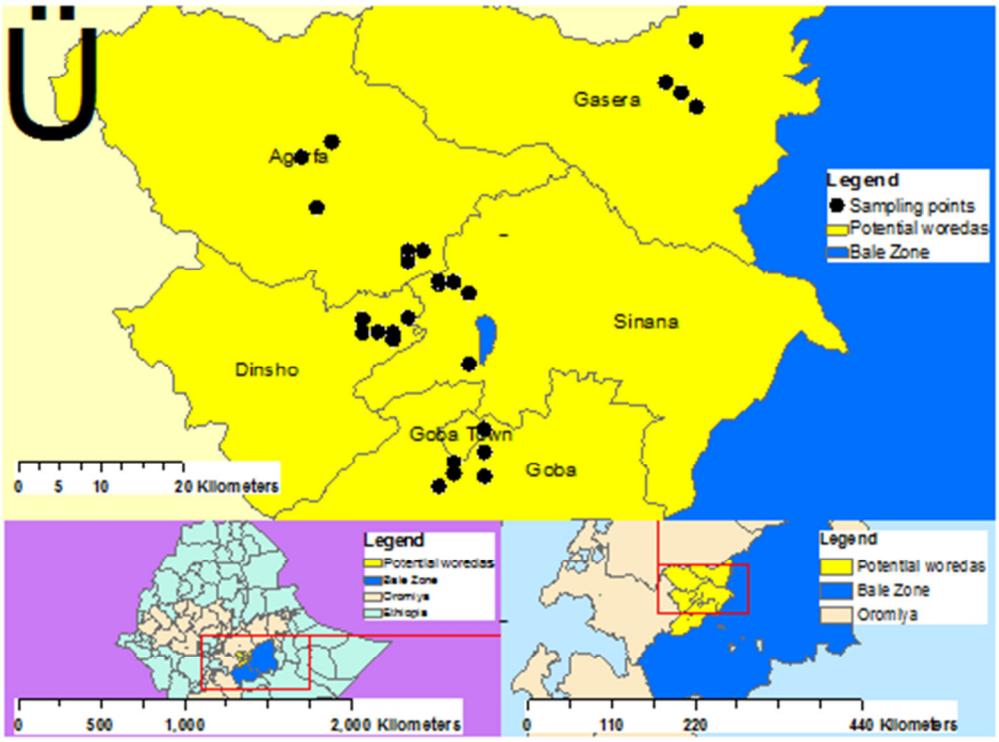

Figure 1 Potential Faba Bean production sites in Bale zone

Presumptive screening of pure cultures

Cultures were examined for cell morphology and gram reaction after 3 days of growth in YEM broth medium. 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) and the production of acid or alkali was also determined in YEMA medium with Bromothymol blue (BTB) (25 mg L-1) plates(Somasegaran and Hoben, 1994).

Stress tolerance tests

Tolerance tests on temperature(40C,100C,150C, 350C,450C,500C),salt(0.1, 0.3,0.5,0.8,1,2,3,4, 5 and 6%) and intrisic antibiotic resistance(concentration of 2.5, 5 and 10µg/ml :Ampicillin, Streptomycin, Penicillin, Tetracycline, Erythromycin and Chloramphenicol) was performed according to Lupwayi and Hague (1994) and tests on pH(4,4.5,5,5.5,8 and 9 adjusted with sterile HCl or NaOH) according to CIAT (1988).

Substrate utilization test

Growth of isolates on different nitrogen and Carbon source utilization was determined following the method of Somasegaran and Hoben (1994). Carbohydrates were prepared as 10% (W/V) solution in water. The carbohydrates free medium, which are essentially similar to YEMA medium, was modified by reducing the yeast extract to 0.05g/ liter. Heat labile carbohydrates (Galactose, Maltose, Starch, Sorbitol, Arabinose) already sterilized by membrane filtration using milli pore with pore size of 0.22um and added to the autoclaved carbohydrate free basal medium. The heat-stable carbohydrates (Glucose, Lactose, Manitol and Sucrose) were autoclaved together with the medium. YEMA medium without Mannitol and with Mannitol was used as a negative and positive controls, respectively.Amino acids such as L-alanine, L-arginine,L-glutamate, L-leucine, L-lysine, L-phenylalanine, L-tryptophan and L-tyrosine were used to determine the ability of the isolates to utilize amino acids The membrane filter sterilized amino acids were added to the autoclaved and cooled (approximately 550C)

Authentications and symbiotic efficiency tests

After conducting of presumptive test for rhizobia, each of the selected strains were authenticated as root nodulating bacteria by reinoculating them on the host as described by Somasegaran and Hoben (1994) in glasshouse on three kilogram capacity sand filled pots which were acid washed and heat sterilized (1210C, 151b/ for 15 min). Isolates effectiveness in accumulating plant shoot dry weight was calculated according to the equation used in Purcino (2000).As a starter, 20 ppm of N solution was included in each pot before planting

(Alemayehu Workalemahu, 2009).Five pre-germinated seeds were transferred into each pot. Each seedling inoculated with 1 ml of each isolate with an inoculum size of 109cells/ml(Somasegaran and Hoben, 1994).After a week, the seedlings were reduced into three per pot. Two treatments were used as control: one both without inoculation and nitrogen (N-) and the other un-inoculated but contains 70mg/liter of N applied with 0.05% (W/V) KNO3 per week (N+) ,for the +N control treatment it was considered N available at a non-limiting rate, such that growth can be compared with the N2-fixing treatments and relative effectiveness of strains can be assessed by comparison(Howieson and Dilworth, 2016). The pots were arranged in Randomized Complete Block Design (RCBD) and Plants were supplied with distilled water every two days, and fertilized with quarter strength Broughton and Dilworth N-free medium (Somasegaran and Hoben, 1994) and uprooted after 55 days of growth, and data for nodulation score, nodule dry weight, shoot dry mass and total nitrogen (oven dried at 700C for 48hrs to determine the dry weight) was determined according to the methodology used in Musa Adal (2009) using the modified Kjeldhal method.

Data Analysis

Comparison between treatments, (data on nodule number, nodule dry weight, shoot dry weight, and total nitrogen) were subjected to multivariate analysis of variance (MANOVA) (Field and Miles, 2010;SAS, 2009).Pearson's r values were also determined for the association of shoot dry weight with nodule number and nodule dry weight in sand culture (Tukeys HSD tests) (SAS 9) at p= 0.05 level of significance.

Table 2 Descriptions of sampling sites of isolates and soil chemical properties

Soil sample	Woreda name	Site name	Geographical references				Soil type	pH (1:2)	Available P (ppm)	Nitrogen (%)	OC (%)	OM (%)	Mean annual temperature (^{0}C) (min-max)	Mean annual rain fall (mm)
			Altitude (masl)	Latitude (DMS)	Longitude (DMS)	Growing history								
1	Agarfa	Asano	2505	N07^{0}15'19.3''	E039^{0}49'40.3''	Faba Bean	Vertisol	5.697	154.225	0.172	1.85	3.189	13.1-15.6	1244.63
2	Agarfa	Asano	2507	N07^{0}15'39.1''	E039^{0}50'14.5''	Faba Bean	Vertisol	5.72	7.884	0.162	1.778	3.066	13.1-15.6	1244.63
3	Agarfa	Sebeja	2460	N07^{0}18'40.5''	E039^{0}48'22.9''	Faba Bean	Clay	5.89	10.769	0.15	2.31	3.983	13.1-15.6	1244.63
4	Agarfa	Welteyelbdu	2452	N07^{0}12'27.6''	E039^{0}55'38.2''	Field pea	Clay	5.968	8.458	0.176	2.78	4.793	13.1-15.6	1244.63
5	Agarfa	Welteyelbdu	2397	N07^{0}11'43.6''	E039^{0}55'42.5''	Faba Bean	Clay	5.705	2.336	0.223	3.048	5.254	13.1-15.6	1244.63
6	Agarfa	Welteyelbdu	2414	N07^{0}12'27.6''	E039^{0}56'42.0''	Field pea	Clay	6.249	2.9	0.186	2.838	4.893	13.1-15.6	1244.63
7	Dinsho	Meo	2604	N07^{0}06'38.8''	E039^{0}54'31.9''	Faba Bean	Vertisol	5.812	2.5	0.204	3.091	5.329	(-3)-24	1401.44
8	Dinsho	Meo	2558	N07^{0}07'04.4''	E039^{0}54'54.5''	Faba Bean	Vertisol	5.514	6.538	0.295	3.328	5.738	(-3)-24	1401.44
9	Dinsho	Abakeran	2772	N07^{0}07'39.7''	E039^{0}52'25.9''	Faba Bean	Clay	5.263	8.654	0.218	1.956	3.371	(-3)-24	1401.44
10	Dinsho	Abakeran	2806	N07^{0}07'57.7''	E039^{0}52'05.9''	Faba Bean	Clay	5.802	2.819	0.225	2.8	4.893	(-3)-24	1401.44
11	Dinsho	Abakeran	2659	N07^{0}07'87.5''	E039^{0}53'26.0''	Faba Bean	Clay	5.832	1.933	0.2	2.951	5.088	(-3)-24	1401.44
12	Dinsho	Homa	2533	N07^{0}08'03.0''	E039^{0}55'14.3''	Faba Bean	Clay	6.615	27.467	0.155	3.017	5.201	(-3)-24	1401.44
13	Goba	welteyqupi	2582	N06^{0}57'37.9''	E040^{0}01'44.0''	Faba Bean	Vertisol	5.228	8.269	0.235	2.446	4.217	11.7-14.2	1250
14	Goba	Aliso tilo	2568	N06^{0}59'15.4''	E040^{0}00'35.3''	Faba Bean	Vertisol	5.83	37.883	0.148	1.428	2.461	11.7-14.2	1250
15	Goba	Welteymgda	2495	N07^{0}00'44.6''	E040^{0}03'38.5''	Faba Bean	Clay	6.23	14.23	0.143	2.155	3.716	11.7-14.2	1250
16	Goba	Fasil angeso	2811	N06^{0}58'34.2''	E039^{0}58'12.0''	Faba Bean	Vertisol	6.333	4.188	0.308	2.903	5.006	11.7-14.2	1250
17	Goba	Fasil angeso	2853	N06^{0}57'49.5''	E039^{0}58'44.9''	Faba Bean	Vertisol	6.029	24.567	0.363	3.247	5.597	11.7-14.2	1250
18	Goba	Fasil angeso	2971	N06^{0}56'58.9''	E039^{0}57'45.7''	Faba Bean	Vertisol	5.98	2.497	0.363	2.831	4.881	11.7-14.2	1250
19	Gassera	Chifaro	2402	N07^{0}22'46.7''	E040^{0}13'39.2''	Faba Bean	Vertisol	6.002	106.342	0.214	2.989	5.153	14-28	1191.05
20	Gassera	Chifaro	2435	N07^{0}21'52.4''	E040^{0}14'53.5''	Faba Bean	Vertisol	5.942	12.691	0.181	1.518	2.617	14-28	1191.05
21	Gassera	Balo amiga	2412	N07^{0}23'29.5''	E040^{0}12'49.4''	Faba Bean	Vertisol	5.682	9.423	0.227	2.099	3.618	14-28	1191.05
22	Gassera	Guranda	2394	N07^{0}26'15.9''	E040^{0}14'13.2''	Faba Bean	Clay	5.717	23.46	0.202	2.088	3.599	14-28	1191.05
23	Sinnana	Kebira	2404	N07^{0}10'24.0''	E039^{0}58'24.4''	Faba Bean	Vertisol	6.226	11.538	0.139	1.352	2.331	24-Apr	897.75
24	Sinnana	Weltey berisa	2412	N07^{0}10'13.3''	E039^{0}57'56.1''	Faba Bean	Clay	6.116	10.384	0.172	1.401	2.416	24-Apr	897.75
25	Sinnana	Oroboka	2525	N07^{0}05'00.8''	E039^{0}59'38.0''	Faba Bean	Clay	5.786	20.961	0.193	2.616	4.509	24-Apr	897.75
26	Sinnana	Oroboka	2429	N07^{0}09'39.0''	E039^{0}59'16.3''	Field pea	Clay	6.029	4.39	0.176	2.643	4.556	24-Apr	897.75
27	Sinnana	Weltey berisa	2399	N07^{0}10'26.7''	E039^{0}57'44.7''	Field pea	Clay	6.377	5.397	0.171	2.828	4.875	24-Apr	897.75
28	Sinnana	Weltey berisa	2410	N07^{0}10'21.6''	E039^{0}58'27.8''	Faba Bean	Vertisol	6.498	13.774	0.176	2.588	4.461	24-Apr	897.75

RESULTS AND DISCUSSIONS

Thirty isolates (Table 4) has been recovered from the trapping experiment of soil samples from different woredas of Bale zone. The isolates were diverse in shape and size (most of the isolates were large and all were mucoid in appearance). Their range of size was estimated from <1mm to 4mm. Observation on reaction to acidity and alkalinity showed that none of the isolated absorbed the Congo red dye and it was observed yellow color for the BTB incubation in all isolates. The results of doubling time indicated that it is of the range of 1.26hrs to 5.00hrs. The growth of the isolates was similar to elite commercial strain FBEAL_110 that was also included in the study.In that the strains fall into fast growing rhizobia based on doubling time, yellow color indicating the acid producing ability of the isolates on BTB-YEMA.This result is consistent to results of other researchers on fast growing Faba Bean nodulating isolates(Abere Mnalku et al.,2009;Zerihun Belay and Fassil Assefa, 2011;Getahun Negash 2015).Moreover generation times of 2-4hours with large colony diameter of 1-5mm) and

production of copious exopolysaccharide will put the isolates into cross-nodulation group of Rhizobium leguminosarum var. viceae((Berkum; et al., 1995;Somasegaran and Hoben, 1994).In addition to that they preliminarily responded similar to the already authenticated and commercial elite strain FBEAL-110.

According to Figure 2d variation in growth temperature was observed, in that case only 5 (16.7%) of isolates succeeded to grow at lower incubation temperature(4oC) and 7(23.3%) isolates grew at temperature of 45OC but none managed to grow at the highest growth temperature (50oC). Generally ,isolates FBA5,FBA6 and FBS4 are more tolerant and grew over wide range of temperature(4OC to 45OC). During symbiosis ,temperature affects root hair infection, bacteroid differentiation, nodule structure, and the functioning of the legume root nodule and now a days several strains that tolerate higher temperature regime are being described (Zahran, 1999). Strains that could tolerate adverse conditions like elevated temperature were mentioned by Zahran (2001) in that case strains with this inherent characteristics exploited as a hot spot for future biotechnology tools. The cold adaptation observed, which is apparent because the source soil was from Bale zone which most of its districts under this investigation were humid to cool. It is worthy of further investigation with respect to the improvement of nitrogen fixation under cool climates and for studies on the mechanisms of cold adaptation (Pascal et al., 1996). Climatic predictions are apparent in that future trends are inhabitable and such resources with possessions of extreme stress tolerant make ups can be compromised. The work of Benidire et al. (2018) also highlighted abiotic stresses in his study to Moroccan Faba Bean that was produced in marginal lands.

All isolates tolerated salt concentration of 0.1% to 1% and 66.7 % of isolates tolerated concentration of 0.1% to 6%.From the results 3(10%) isolates (FBA6,FBG2a and FBS4) are relatively less tolerant to different salt concentrations(Figure 2b). These observations might highlight the concomitant rhizobium-host efficiency which is hampered by high levels of salinity which decreases the Ca2+content of rhizobium cells, and the outer membrane structure of the rhizobium cells was greatly distorted (Zahran, 1999). In that regard Giller (2001) mentioned that salt stress limits legume growth, especially when the crop relies on symbiotically fixed nitrogen. But the response of both rhizobia and host plant may differ and so a detrimental effect on soil microbial populations as a result of direct toxicity as well as through osmotic stress will be resulted. Simon et al. (2014) also reported in that some different combination of factors besides to salt and osmotic stress can affect the initial stage of legume-rhizobial interaction and nodule formation. When it came to above 1% of concentration, even though further increment was not investigated it was observed that growth at higher salt concentration was in decrement trend which is similarly reported by the work of Zerihun Belay and Fassil Assefa (2011) and Amha Gebremariam and Fassil Assefa (2018).From this fact that the development of new nodules, the activity of nodules and the formation of the nitrogenase enzyme are reduced by salinity (Ahmed and Elsheikh, 1998) so the observed diversity in tolerance for these isolates has paramount importance.

All isolates are resistant to all the different concentrations (2ug/ml,5ug/ml and 10ug/ml) of antibiotics (Table 3 and Figure 2a). Relative to others they are less resistant to tetracycline at all concentrations but generally very similar in their resistance. With respect to individual isolates (data not shown) isolates resistance ranged from 38.9%(FBS1) to 100%(FBA3,FBG4 and FBS5).Rhizobia are influenced in edaphic situations of their micro environment. In such situations an increase in the antibiotic-resistant rhizobia population and association with an increase in soil P and Al contents was revealed in studies of Xavier et al. (1998) on Faba Bean host and he further elaborated that isolates which were sensitive to spectinomycin, ampicillin, streptomycin, chloramphenicol and tetracycline were present at higher rates in soils devoid of Al. Relatively trends in growth observed to be affected when concentration increased and tetracycline was the least according to isolates performance at all concentration. Result is similar to the work of Zerihun Belay and Fassil Assefa (2011) and Girmaye Kenasa et al. (2014). Intrinsic antibiotic resistance and persistence as major factors to competition during natural invasion and it was stated that strains to vary in their success to cope up environmental contests and grow well on diverse antibiotics and loads (Tolera Abera et al., 2015).

Table 3 percent of resistance to different rates of antibiotics

	Isolates' % of resistance to antibiotics rates(ug/ml)			
Antibiotics	2.5	5	10	Average % resistance
Ampicillin	93.3	83.3	70.0	82.2
Streptomycin	93.3	83.3	73.3	83.3
Tetracycline	66.7	46.7	36.7	50.0
Erythromycin	96.7	90.0	73.3	86.7
Penicillin	93.3	80.0	73.3	82.2
Chloramphenicol	86.7	83.3	80.0	83.3

Tolerances to the micro environment with respect to acidity/alkalinity has been revealed (Figure 2c) by the respective isolates. 23(76.7%) of isolate were tolerant to pH=4 to 9 and 3(10%) isolates (FBS1,FBS2 and FBGa1) tolerated all pH ranges except 4, and only few isolates(FBA2 and FBGa2) did not tolerate lower (4 to 5) pH ranges. 3 (10%) other isolates (FBD5,FBD6 and FBGa2) are less tolerant to pH variations. Growth of isolates in a range of pH=4 to 9 indicated that isolates grew in divers ranges from which >76% of isolates grew at lower pH =4 .This trend in growth is similar to the work of Alemayehu Workalemahu (2009) and Getahun Negash (2015) in contrast to the assertion of Zerihun Belay and Fassil Assefa (2011) that general sensitivity to low pH . The attribute indicated in morphology of colonies i.e. the mucus production observed in almost all of the isolates might have enabled to tolerate low pH this manifestation can be taken as an adaptation of acid soils was reported in Brazilian tropical Savannah by Teixeira; et al. (2010). Girmaye Kenasa et al. (2014) emphasized strains resistant to different soil stresses such as pH have potential to improvement in the production of legumes grown on the area and extend the ranges of soils upon which legumes adapted to grow. And this diverse opportunity can be further utilized in future production of biofertilizers from which extreme condition tolerant strains may be tried and used.

All isolated exhibited no diversity (Figure 2e) in utilization of sugar sources. Only few isolates (FBA1,FBA6 ,FBD2,FBG1 and FBS4) identified that did not grow on Arabinose and Sorbitol .Generally almost all (97.9%) isolates grew on listed sugars.Utilization in carbon and nitrogen source was assessed and isolates' pattern to utilization of sugar is very similar. Except very few(five) isolates which did not grow on either sorbitol or arabinose all isolates grew on almost all sugars. This competence in nutrient utilization is useful trait in which the efficiency of the symbiosis is affected (Somasegaran and Hoben, 1994) by the levels of the various nutrients in the soil and variability in the response to inoculation. This similarity is corroborated with the work of Getahun Negash (2015) and Dereje Tsegaye et al. (2015) also in agreement with Zerihun Belay and Fassil Assefa (2011) who reported the isolates from North Gondar were found to utilize 80 to 100% of the tested monosaccharide and disaccharides, and similar claim with other researcher (Fano Berhe, 2010) on similar cross inoculating group. Growth on different nitrogen sources was assessed and according to which almost all isolates managed to grow to the different sources. But tyrosine(87.1% of isolates) and leucine(90.3% of isolates) were relatively less utilized sources(Figure 2 f).Viewing isolates with respect to performances, almost all isolates grew on all nitrogen sources except for few isolates (FBD1,FBG5,FBGa2,FBGa4a,FBS2,FBS3 and FBS4) that failed to grow.

The results of utilization of nitrogen sources indicated that more than 78% of all isolates metabolized all the nitrogen sources which exhibited relative similarity with Girmaye Kenasa et al. (2014) in their work that all sources of nitrogen utilized which is in contrast to the work of Getahun Negash (2015) who stated that 37.1% of the isolates and commercial strains were able to metabolize all the nitrogen sources. This nutritional versatility is important in exploitation of any environment(O'Hara et al., 2002).

Table 4 Cultural and symbiotic characteristics of isolates

Isolates	Colony characteristics (4-5days)	Colony diameter (mm)	Growth on YEMA+BTB	on YEMA+CR	MGT (hours)	NN	NDW(gm)	SDW(gm)	TN(%)	SE(%)
FBA1	Small Mucoid,circular to ovoid,translucent	1.5	Yellow	colorless	1.26	20.33^{ab}	0.0205^{bc}	0.5167^{c}	3.66^{a}	29.6
FBA2	Large Mucoid,irregular,translucent	2.5	Yellow	colorless	3.09	36.00^{ab}	0.0118^{bc}	0.8061^{b}	2.35^{bc}	46.2
FBA3	Large Mucoid,circular,translucent	3	Yellow	colorless	3.00	31.67^{ab}	0.0122^{bc}	0.7750^{b}	1.96^{bc}	44.4
FBA4	Large Mucoid,circular,translucent	3	Yellow	colorless	2.63	35.33^{ab}	0.0124^{bc}	0.9750^{ab}	2.40^{bc}	55.9
FBA5	Large Mucoid,circular,translucent	3.5	Yellow	colorless	3.41	57.00^{a}	0.0231^{bc}	1.3111^{ab}	1.83^{bc}	75.2
FBA6	Small,raised,mucoid,translucent	1	Yellow	colorless	3.53	49.00^{ab}	0.0281^{abc}	0.7000^{b}	2.37^{bc}	40.1
FBD1	Large Mucoid,circular,translucent	4	Yellow	colorless	3.35	24.50^{ab}	0.0201^{bc}	0.8250^{b}	2.56^{abc}	47.3
FBD2	Large Mucoid,circular,translucent	3.5	Yellow	colorless	2.45	28.00^{ab}	0.0093^{bc}	0.6444^{c}	2.18^{bc}	36.9
FBD3	Large Mucoid,irregular,translucent	2.5	Yellow	colorless	1.41	69.50^{a}	0.0228^{bc}	0.7778^{b}	2.89^{abc}	44.6
FBD4	Small Mucoid,irregular,translucent	1.5	Yellow	colorless	3.93	35.00^{ab}	0.0178^{bc}	1.0000^{ab}	2.45^{abc}	57.3
FBD5	Large Mucoid,circular,translucent	2.5	Yellow	colorless	3.13	30.00^{ab}	0.0440^{ab}	1.8950^{a}	1.97^{bc}	108.6
FBD6	Large mucoid,irregular,translucent	2	Yellow	colorless	3.01	24.00^{ab}	0.0112^{bc}	1.0000^{ab}	1.97^{bc}	57.3
FBG1	Large mucoid,irregular,translucent	2.5	Yellow	colorless	4.8	53.50^{a}	0.0247^{abc}	0.7444^{b}	2.38^{bc}	42.7
FBG2a	Large Mucoid,irregular,translucent	2	Yellow	colorless	1.39	41.00^{ab}	0.0127^{bc}	0.6861^{b}	2.40^{bc}	39.3
FBG2b	Large mcoid,irregular,translucent	3 .5	Yellow	colorless	2.61	22.00^{ab}	0.0187^{bc}	1.1875^{ab}	2.23^{bc}	68.1
FBG3	Large Mucoid,circular,translucent	2.5	Yellow	colorless	2.26	67.00^{a}	0.0153^{bc}	0.9167^{ab}	2.47^{abc}	52.6
FBG4	Large mucoid,irregular,translucent	3	Yellow	colorless	3.88	25.33^{ab}	0.0070^{c}	0.5333^{c}	2.81^{abc}	30.6
FBG5	Large Mucoid,irregular,translucent	2.5	Yellow	colorless	1.35	50.50^{a}	0.0565^{a}	0.9708^{ab}	2.64^{abc}	55.7
FBG6	Small Mucoid,irregular,translucent	1.5	Yellow	colorless	1.63	22.50^{ab}	0.0166^{bc}	0.2833^{c}	2.95^{abc}	16.2
FBGa1	Large Mucoid,circular,translucent	2	Yellow	colorless	3.25	43.00^{ab}	0.0104^{bc}	0.8956^{ab}	2.29^{bc}	51.3
FBGa2	Small mucoid,raised,translucent	<1	Yellow	colorless	3.56	20.00^{ab}	0.0097^{bc}	0.3333^{c}	2.28^{bc}	19.1
FBGa3	Mucoid,circular,translucent	1.5	Yellow	colorless	4.53	30.67^{ab}	0.0270^{abc}	0.8700^{ab}	2.51^{abc}	49.9
FBGa4a	Large Mucoid,circular,translucent	2.5	Yellow	colorless	3.68	29.00^{ab}	0.0201^{bc}	1.1667^{ab}	2.27^{bc}	66.9
FBGa4b	Large mucoid,translucent	4	Yellow	colorless	4.04	31.50^{ab}	0.0168^{bc}	0.6500^{c}	2.23^{bc}	37.3
FBS1	Large Mucoid,circular,translucent	3	Yellow	colorless	2.67	25.00^{ab}	0.0254^{abc}	0.8278^{ab}	2.11^{bc}	47.5
FBS2	Large Mucoid,circular,translucent	2.5	Yellow	colorless	5.00	24.67^{ab}	0.0149^{bc}	1.6667^{ab}	2.47^{abc}	95.5
FBS3	Large Mucoid,circular,translucent	2.5	Yellow	colorless	3.08	41.50^{ab}	0.0253^{abc}	0.7000^{ab}	2.15^{bc}	40.1
FBS4	Small Mucoid,circular,translucent	1	Yellow	colorless	3.41	37.33^{ab}	0.0122^{bc}	0.9389^{ab}	2.36^{bc}	53.8
FBS5	Small mucoid,circular,translucent	1.5	Yellow	colorless	4.33	31.00^{ab}	0.0157^{bc}	1.5167^{ab}	3.04^{ab}	86.9
FBS6	Large mucoid,circular,whitish translucent	2.5	Yellow	colorless	3.47	46.00^{ab}	0.0098^{bc}	0.4972^{c}	2.15^{bc}	28.5
FBEAL_110	Large mucoid translucent	3	Yellow	colorless	2.5	50^{a}	0.0126^{bc}	1.3083^{ab}	3.05^{ab}	75.0
+ve control						-	-	1.7444^{ab}	2.36^{bc}	100

MGT=Mean Generation Time ;NN=nodule number,NDW=nodule dry weight,SDW=shoot dry weight,TN=total nitrogen.Means with the same letter are not significantly different at p=0.05

All of isolates managed to nodulate their respective host plant, Faba Bean up on re infection. According to Table 4 isolates had scored different performances and marked variation at $p<0.05$ based on nodulation scores, nodule dry weight, shoot dry weight total nitrogen.The highest and least NN (nodule number) was observed respectively in isolates FBD3(69) and FBGa2(20) the result of NDW(nodule dry weight) indicated that a range of 0.0070 to 0.0565 gm from isolate FBG4 and FBG5 when SDW(shoot dry weight) was also assessed and isolates have displayed variation according to which isolates FBG6 and FBD5 scored the least and highest (0.28 to 1.89 gm) SDW.It was observed that more than 76% of the isolates performed better than the negative control. In cases of nitrogen accumulation sixteen of isolated perform relatively better than the positive control. The highest and lowest shoot dry weight was recorded by isolates FBD5 and FBG6 with respectively mean shoot dry weight of (0.28 to 1.89 gm) shoot dry weight as a good indicator of efficiency(Somasegaran and Hoben, 1994) and a host inoculated with different strains might respond differently in that case one isolate (FBD5,SE=108.6%) is greater than the N fertilized control. From Table 5 it can be seen that only 17% of isolates are ineffective from which most of the remaining are promising in presumably in inoculant production and three of isolates are more effective than the reference strain. And more than 78% of the isolates surpassed that of the negative control. The relative shoot dry weight recorded in this study(0.52±0.04gm) is similar to that of the work of Getahun Negash (2015) , (0.5 g plant-1) and less than (1.63 g plant-1) obtained by Zerihun Belay and Fassil Assefa (2011) less than (1.38g plant-1) obtained by , Abere Mnalku et al. (2009) and much less than that of Anteneh Argaw (2012), (2.08 gm plant -1) This observation happened due to relatively low shoot dry weights (Ballarda et al., 2004) that could be attributed with delayed or erratic nodulation caused by low numbers of rhizobia.

The mean number of nodules plant-1 nodule ,dry weight and shoot dry weight showed variation at $p<0.05$ for the isolates ranges from 20(FBA5) to 69(FBGa2). The positive correlation existed with much of nodule number might not serve as a good indicator of effectiveness because from the result highly effective isolates had few nodules. The process of nodule formation requires a number of highly specific signaling interactions between the plant and bacterial partners(Ferguson, 2017). In this respect some micro environmental factors might have affected in some way,Else from this result the mean number of nodules which is 33.8±2.6 is comparably similar to the work of Getahun Negash (2015),25 nodules plant-1 but very low when compared to the result of other works by Abere Mnalku et al. (2009);Anteneh Argaw (2012) which they recorded respectively of 128,56,124,87, nodules per plant.

With regard to inter correlational relationship among parameters it was found that all parameters (NN,NDW,SDW) are correlated(Table 6).Strong correlation between SDW and NDW, $r= 0.6275$, $p<0.0001$ and other combinations (NN and NDW) are positively correlated ,($r=0.3839$,$p<0.05$).But none of them were correlated to nitrogen content (TN) of samples which the major assumption of assimilation of the same amount of soil N, However, differences in N uptake because of differences in root morphologies that might arise during initiation of symbiosis expected to correlate with dry matter accumulation (Pre'vost and Antoun, 2006) but the

result is not as such significant. The promising isolates (FBD5,FBS2,FBS5,FBA5) which are highly effective in artificially axenic conditions indicated this general notion and measures of effectiveness by Somasegaran and Hoben (1994).In line to this, similar observation was made in the work of Abere Mnalku et al. (2009) and Getahun Negash (2015) so checking for further verification in field trial would be important.

Isolates were listed based on their symbiotic efficiency and effectiveness (Table 5) and it was observed that more than 43% of the isolates are effective and highly effective.

Table 5 summary of symbiotic effectiveness of isolates by districts of soil source

	Symbiotic effectiveness				
Districts	Ineffective	Mildly effective	Effective	Highly effective	Total
Agarfa	1	3	2	0	6
Dinsho	0	3	2	1	6
Gassera	1	2	3	0	6
Goba	2	2	2	0	6
Sinana	1	2	1	2	6
Total	5	12	10	3	30

Table 6 summary of correlation of parameters

	NN	**NDW**	**TN**
SDW	0.3538*	0.6275***	0.0296
NN		0.3839**	0.0750
NDW			-0.0171

*** = strong significance at p<0.0001, **=significance at p<0.01 and *=significance at p<0.05

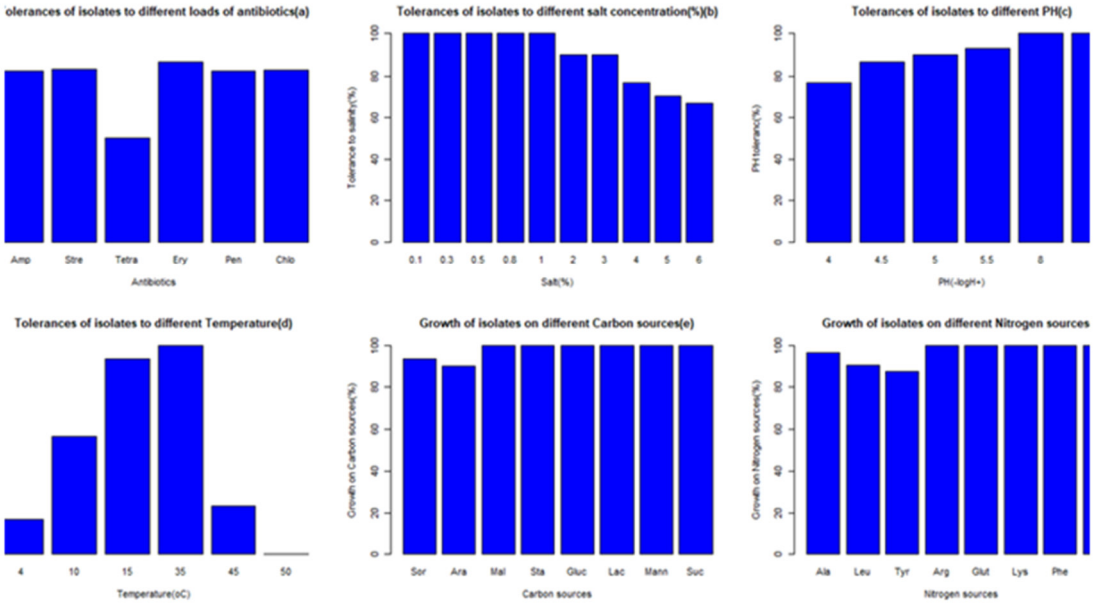

Figure 2 Responses of isolates to different(a-f) Eco physiological characteristics

N:B;Amp=Ampicilin,Str=Streptomycin,Tetra=Tetracycline,Ery=Erythromycin,Pen=Penicilin,Chlo=Chloramenicol,Sor=Sorbitol,Ara=Arabinose,Mal=Maltose,StStarch,Gluc=Glucose,Lac=Lactose,Mann=Mannitol,Suc=Sucrose,Ala=Alanine,Leu=Leucine,Tyr=Tyrosine,Arg=Argenine,Glut=Glutamine,Lys=Lysine,Phe=Phenylalanine,Try=Tryptophane

CONCLUSIONS

The present study showed the physiological and symbiotic diversity of Faba Bean nodulating isolates which is proved after the reinfection. This remarkable physiological characteristic expressed (i.e resistance to antibiotics, high salt tolerance, diverse carbohydrate and nitrogen source assimilation, extreme pH and temperature

tolerances) can be further expounded and utilized. Moreover most (83%) of the isolates are moderate to highly effective which is un explained resource to the zone which suffered with monoculture production system.

In this study symbiotically effective Faba Bean isolates FBD5, FBS2,FBS5,FBA5 which were checked in sterile sand pot experiment under controlled condition should be tested on the field in multi climatic and locations basis to check their competency with already commercial elite strains and used as inoculants. Generally, future climate scenarios predict an enhanced variability in rainfalls leading to more extreme events that develop faster and with greater intensity, so considering global climatic trends would be wise while screening isolates.

For the bulk of observed phenotypic diversity will not signify the reality; molecular bases for the tolerance and attributes of the legume microbe symbiotic systems to environmental stress should be taken through which is important to elucidate relationships among these strains.

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REFERENCES

Abere Mnalku, Heluf Gebrekidan and Fassil Assefa (2009). Simbiotic effectivness and characterization of rhizobium strains of faba bean (*Vicia faba L.*) collected from Eastern and Wstern Hararghe highlands of Ethiopia. *Ethiopian Journal of Natural Resources,* 11(2):223-244.

Ahmed, A. E. and Elsheikh, E. A. E. (1998). Effects of Biological and Chemical Fertilizers on Growth and Symbiotic Properties of Faba Bean (Vida/aha L.) Under Salt Stress. *University of Khartoum Journal of Agricultural science,* 6(1):150-164.

Alemayehu Workalemahu (2009). The Effect of Indigenous Root-Nodulating Bacteria on Nodulation and Growth of Faba Bean (*Vicia faba l.*) in the Low-Input Agricultural Systems of Tigray Highlands, Northern Ethiopia. *Momona Ethopian Journal Of Science,* 1(2):30-43.

Alghamdi, S. S., Ammar, M. H., Siddique, K. H. M., Migdadi, H. M. and Paull, J. G. (2012). Faba bean genomics: current status and future prospects:A Review. *Euphytica* 186::609–624.

Amha Gebremariam and Fassil Assefa (2018). 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. *Journal of Agricultural Biotechnology and Sustainable Development,* 10(2):25-33.

Anteneh Argaw (2012). Characterization of symbiotic effectivness of rhizobia nodulating faba bean(Vicia faba L.) isolted from central Ethiopia. *Research Journal of microbiology*:1-17.

Asfaw Hailemariam and Angaw Tsgie (2003). BNF Research on food legumes in Ethiopia. In: Ali, K., Gemechu Keneni, Seid, A., Malhotra, R., Beniwal, S., Makkouk, K. andHalia, M. H. (eds.) Food and forage legumes of Ethiopia:Progress and prospects,proceedings of the workshop on food and forage legumes,22-26 September 2003,Addis Ababa ,Ethiopia.: Ethiopian Institute of Agricultural Research(EIAR) and Internationational Center for Agricultural Research in Dry land Areas (ICARDA),Aleppo,Syria.

Atlas, R. M. (2010). Hand book of Microbiological Media, Washington D.C, CRC press.

Ballarda, R. A., Charmana, N., McInnesb, A. and Davidson, J. A. (2004). Size, symbiotic effectiveness and genetic diversity of field pea rhizobia (Rhizobium leguminosarum bv. viciae) populations in South Australian soils. *Soil Biology & Biochemistry,* 36 1347–1355.

Benidire, L., Lahrouni, M., Daoui, K., Fatemi, Z. e. A., Gomez Carmona, R., Göttfert, M. and Oufdou, K. (2018). Phenotypic and genetic diversity of Moroccan rhizobia isolated from Vicia faba and study of genes that are likely to be involved in their osmotolerance. *Systematic and Applied Microbiology,* 41(1):51-61.

Berkum;, P. V., Beyene;, D., Vera;, F. T. and H.Keyser;, H. (1995). Variability among Rhizobium Strains Originating from Nodules of Vicia faba. *African and Envronmental Microbiology,* 61(7):2649–2653.

CIAT (1988). Legume- Rhizobium symbiosis: Methods Manual for Evaluation, Selection and Agronomic Management, Cali: CIAT.

Dereje Tsegaye, Fasil Assefa, Heluf Gebrekidan and Gemechu Keneni, W. (2015). Nutritional, eco-physiological and symbiotic characteristics of rhizobia nodulating faba bean (Vicia faba L.) collected from acidic soils of Ethiopia. *African Journal of Environmental Science and Technology,* 9(7):646-654.

EIAR (2014). Rhizobia-based bio-fertilizer:Guidelines for smallholder farmers. EIAR.

Fano Berhe (2010). Phenotypic and Symbiotic characteristics of Rhizobia nodulating field Pea (Pisum sativum L.) in southern Tigray, Ethiopia. MSc. Thesis, Addis Ababa University.81 p.

FAO (2014). Country fact sheet on food and agriculture policy trends :Ethiopia, p.1.

Ferguson, B. J. (2017). Rhizobia and Legume Nodulation Genes. Reference Module in Life Sciences. Elsevier.

Field, A.andMiles, J. (2010). Discovering Statistics Using SAS, UK,London, SAGE Publications Ltd.753.p.

Fisher, K.andNewton, W. E. (2002). Nitrogen Fixation- A General Overview. In: G. Jeffery Leigh (ed.) Nitrogen Fixation at the Millenniu. Elsevier.

Gemechu Keneni, Musa Jarso and Tezera Wolabu (2003). Faba bean(Vicia faba L.):Genetic and breeding research in Ethiopia. In: Ali, K., Gemechu Keneni, Seid, A., Malhotra, R., Beniwal, S., Makkouk, K. andHalia, M. H. (eds.) Food and forage legumes of Ethiopia:Progress and prospects,proceedings of the workshop on food and forage legumes,22-26 September 2033,Addis Ababa ,Ethiopia.: Ethiopian Institute of Agricultural Research(EIAR) and Internationational Center for Agricultural Research in Dry land Areas(ICARDA),Aleppo,Syria.

Getahun Negash , T. (2015). Symbiotic and phenotypic characteristics of indigenous rhizobia nodulating faba bean (*Vicia faba L.*) growing in some parts of Wello, Northern Ethiopia. MSc thesis Hawassa University.111 p.

Giller, K. E. (2001). Nitrogen fixation in tropical cropping systems, CABI

Girmaye Kenasa, Jida, M. and Assefa, F. (2014). Characterization of Phosphate Solubilizing Faba Bean (*Vicia faba l.*) Nodulating Rhizobia Isolated from Acidic Soils of Wollega, Ethiopia. *Science,Technology and Arts Research,* 3(3):11-17.

Howieson, J. G. and Dilworth, M. J. (2016). Working with rhizobia*,* Canberra,Australia, Australian Centre for International Agricultural Research.314.p.

IFPRI (2010). Pulses Value Chain in Ethiopia Constraints and Opportunities For Enhancing Exports, p.44.

Lupwayi, N.and Hague, I. (1994). Legume-Rhizobium Technology Manual*,* Addis Ababa, Ethiopia, Environmental Science Division, International Livestock Center for Africa.97.p.

Musa Adal (2009). Phenotypic and Symbiotic characterization of grass pea (Lathyrus sativus) rhizobial isolates from some major growing areas of South Wollo and West Shoa , Ethiopia. MSc. Thesis, Addis Ababa University.102 p.

Mutch, L. A. and Young, J. P. W. (2004). Diversity and specificity of Rhizobium leguminosarum biovar viciae on wild and cultivated legumes. *Molecular Ecology,* 13:2435–2444.

O'Hara, G. W., J.G.Howmson and P.H.Graham (2002). Nitrogen Fixation and Agricultural Practice. In: G. Jeffery Leigh (ed.) Nitrogen Fixation at the Millennium*.* Elsevier.

Pascal, D., Prevost, D. and Antoun, H. (1996). Classification of Bacteria Nodulating Lathyrus japonicus and Strains of Lathyrus pratensis in Northern Quebec as Rhizobium leguminosarum biovar viciae *International Jornal of Systematic Bacteriology,* 46(4):1016-1024.

Pre'vost, D. and Antoun, H. (2006). Root Nodule Bacteria and Symbiotic Nitrogen Fixation. Taylor & Francis Group, LLC.

Purcino, H. M. A., Festin, P. M. and Elkan, G. H. (2000). Identification of effective strains of Bradyrhizobium for Archis Pintoi. *Trop. Agric.,* 77:226-231.

Reddy, P. M., James, E. K. and K.Ladha, J. (2002). Nitrogen Fixation in Rice. In: Leigh, G. J. (ed.) Nitrogen Fixation at the Millennium*.* Elsevier.

SAS 9 (2002). SAS(Statistical Analysis software),Version 9. 9.0 ed.: SAS Institute Inc., Cary, NC, USA.

SAS (2009). SAS/STAT 9.2 ® User's Guide*,* Cary, NC, USA, SAS Institute Inc., .7886.p.

Simon, Z., Mtei, K., Amare Gessesse and Ndakidemi, P. A. (2014). Isolation and Characterization of Nitrogen Fixing Rhizobia from Cultivated and Uncultivated Soils of Northern Tanzania. *American Journal of Plant Sciences,* 5:4050-4067.

Solomon Legesse and Fassil Assefa (2014). Symbiotic And Phenotypic Characteristics Of Rhizobia Nodulating Faba Bean (Vicia Faba) From Tahtay Koraro, Northwestern Zone Of Tigray Regional State, Ethiopia *IJTEEE.,* 2(11):1-9.

Somasegaran, P. and Hoben, H. J. (1994). Handbook for Rhizobia:Methods in Legume-Rhizobium Technology*,* USA, Springer-Verlag.455.p.

Tamene Temesgen, Gemechu Keneni, Tadese Seferaa and Mussa Jarso (2015). Yield stability and relationships among stability parameters in faba bean (Vicia faba L.) genotypes. *T he Crop Journal,* 3:258 – 268.

Teixeira;, F. C. P. e., Borges;, W. L., Xavier;, G. R. and Rumjanek, N. G. (2010). Characterization of indigenous rhizobia from caatinga. *Brazilian Journal of Microbiology,* 41:201-208.

Tolera Abera, Semu, E., Debele, T., Wegar, D. and Kim, H. (2015). Determination Soil Rhizobium Populations, Intrinsic Antibiotic Resistance,Nodulation and Seed Yield of Faba Bean and Soybean in Western Ethiopia. *World Journal of Agricultural Sciences,* 11(5):311-324.

Vincent, J. M. (1970). A Manual for the Practical Study of Root Nodule Bacteria. *,* Oxford and Edinburgh, Blackwell.

Wondwosen Tena, EndalkachewWolde-Meskel and FranWalley (2016). Symbiotic Efficiency of Native and Exotic Rhizobium Strains Nodulating Lentil (Lens culinaris Medik.) in Soils of Southern Ethiopia. *Agronomy,* 6(11):10.

Xavier, G. R., L.M.V, M., M.C.P., N.andG., R. N. (1998). Edaphic factors as determinants for the distrbution of intrisic antibiotic resistance in a cow pea rhizobia population. *Biology and Fertility of Soils,* 27:386-392.

Zahran, H. H. (1999). Rhizobium-Legume Symbiosis and Nitrogen Fixation under Severe Conditions and in an Arid Climate. *MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS,* 63(4):968–989.

Zahran, H. H. (2001). Rhizobia from wild legumes: diversity, taxonomy, ecology,nitrogen fixation and biotechnology. *Journal of Biotechnology,* 91:143–153.

Zerihun Belay and Fassil Assefa (2011). Symbiotic and phenotypic diversity of Rhizobium leguminosarum bv. viciae from Northern Gondar,Ethiopia. *African Journal of Biotechnology,* 10(21):4372-4379.