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Characterization of Symbiotic Effectiveness of Rhizobia Nodulating Faba bean (*Vicia faba* L.) Isolated from Central Ethiopia

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ABSTRACT

This study was performed to characterize the symbiotic effectiveness of rhizobia nodulating Faba bean (*Vicia faba* L.) isolated from central Ethiopia. A total of sixty isolates of rhizobia were isolated from as many sampling sites of central Ethiopia using plant infection method. Forty nine isolates were authenticated as rhizobia of faba bean. Results of analysis of variance indicated that significant variation of all parameters investigated except for root length. Based on Symbiotic Efficiency (SE), isolates were found to be very effective (6%), effective (88%) and only three ineffective isolates (NSFBR-2, NSFBR-9 and NSFBR-40) of rhizobia. Surprisingly, eight isolates showed better performance in SE exceeding 100% over N-treated plants. The data revealed that shoot dry weight showed positive significant correlation with all measured parameters except root length. Further evaluation of symbiotic effectiveness of selected isolates was conducted in pot experiment on Holleta and Kulumsa soils with acidic and slightly acidic soil pH, respectively. Most inoculation of rhizobia induced a significant improvement of growth of faba bean in both soils. Generally the growth performance of faba bean was better on Kulumsa soil than Holleta soil. N-fertilized plants produced statistically equal shoot dry weight, shoot length and plant total nitrogen with best performed isolate in Holleta soil. N-treatment did not have any effect on nodulation on both soils. Thus, the experiment finally shows central Ethiopia soils harbored with effective rhizobia of faba bean; soil pH and number of resident rhizobia are determinant factors affecting the effectiveness of inoculation of rhizobia in faba bean.

Key words: Faba bean (*Vicia faba* L.), rhizobia, symbiotic efficiency, Ethiopia

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most ancient food crops and originated in the Near East and quickly spread to Europe, North Africa, along the Nile to Ethiopia. It is one of the main pulse crops grown for dry seeds and green pods for consumption, or for animal feeding in developed countries (Telaye *et al*., 1994). It also serves as an important source of protein in the human diet. In modern agriculture, fertilization with Nitrogen is widely and increasingly practiced to improve the yields of crop plants. Average grain yield of faba bean have been significantly increased by the application of chemical nitrogen fertilizer in Vertisol and Nitisol soils at Holleta (Tsigie and Woldeab, 1994). However, most farmers have very low financial resources to combat nutrient depletion. Hence research should be directed to seek affordable and least risky to keep nutrient balance neutral. An alternative source for nutritional elements is required for improvement of faba bean production that minimizes environmental pollution and is economically feasible for farmers. Faba bean is a legume capable of fixing nitrogen in an endosymbiotic association with

Rhizobium leguminosarum var. *viciae* and thereby improve soil fertility. This has been used in crop rotation and traditional mixed low-in put agricultural systems. *Rhizobium leguminosarum* bv. *viciae*, also nodulates pea (*Pisum* spp.), vetch (*Vicia* spp), lentil (*Lens* spp.) and sweet pea (*Lathyrus* spp.) (Perret *et al*., 2000). The symbiotic effectiveness of different legume species and their microsymbionts has been found to be variable. Faba bean, however, has been found to be very efficient N fixers and can meet all of their N needs through BNF (Lindemann and Glover, 2003; Hardarson, 1993). According to Somasegaran and Hoben (1994), the amounts of N_2-fixed by faba bean have been 240-325 kg ha^{-1}. Limited numbers of works on the effectiveness of N_2 fixation of indigenous rhizobia of faba bean have been indicated promising results (Belay and Assefa, 2011; Minalku *et al*., 2009). These researches indicated that Ethiopia soil harbored with highly effective N_2-fixer rhizobia nodulating faba bean. In this present study isolation and characterization of the symbiotic effectiveness of rhizobia nodulating faba bean from central Ethiopia soils was carried out so as to screen the symbiotically effective rhizobia nodulating faba bean on Holleta and Kulumsa soils under greenhouse conditions.

MATERIALS AND METHODS

Soil sampling and trapping of nodules: Soils were sampled in more than seventy localities that ranged in seven different areas of central Ethiopia (Fig. 1). Soils were sampled from topsoil

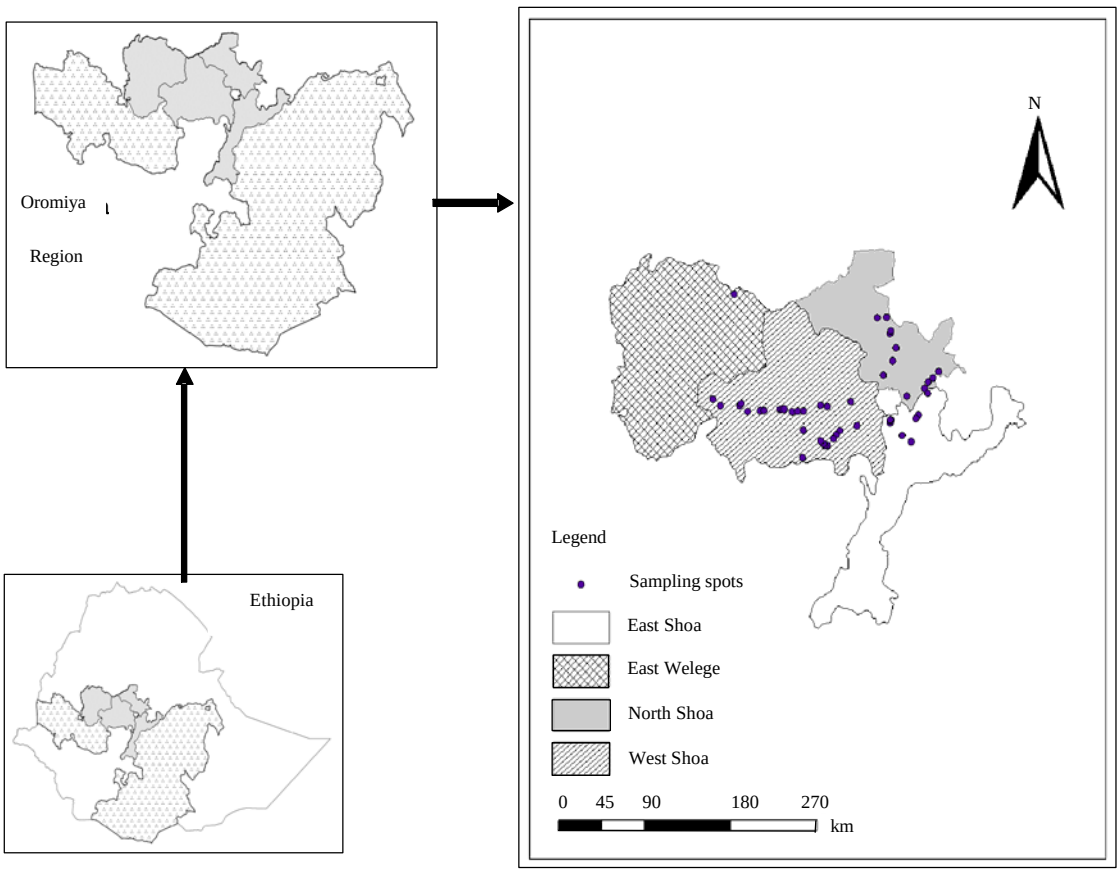

Fig. 1: Location of nodule and soil sampling sites

(0-30 cm) and stored at 4°C in the laboratory for rhizobia trapping in greenhouse experiment. Soil chemical properties (pH, EC, total nitrogen and organic matter) were determined following standard procedure indicated in Sertsu and Bekele (2000) and displayed with GPS in Table 1.

Table 1: The GPS data such as altitude, latitude and longitudes and soil chemical properties

Code	Latitude	Longitude	Altitude	pH (H_2O)	EC (dS m^{-1})	Organic carbon (%)	Total nitrogen (%)
NSFBR-1	N08°53'45.6"	E038°49'43.6"	2199	7.9	0.304	1.076	0.103
NSFBR-2	N08°52'26.1"	E038°49'04.6"	2171	8.0	0.198	0.650	0.071
NSFBR-3	N08°56'16.4"	E039°05'04.4"	2360	8.3	0.253	0.609	0.070
NSFBR-4	N08°54'38.7"	E039°03'44.2"	2347	7.8	0.210	0.731	0.088
NSFBR-5	N08°42'40.5"	E039°01'02.6"	1876	7.5	0.149	0.812	0.081
NSFBR-6	N08°50'56.9"	E038°30'36.2"	2060	7.1	0.132	1.096	0.114
NSFBR-7	N08°48'28.4"	E038°20'51.1"	2073	7.1	0.099	0.954	0.096
NSFBR-8	N08°46'18.8"	E038°18'52.3"	2096	7.1	0.102	0.548	0.046
NSFBR-9	N08°44'20.3"	E038°17'05.0"	2122	7.1	0.111	0.508	0.061
NSFBR-10	N08°40'44.6"	E038°13'44.9"	2147	6.9	0.115	0.780	0.070
NSFBR-11	N08°40'46.9"	E038°12'26.9"	2163	6.8	0.162	0.508	0.036
NSFBR-12	N08°41'44.2"	E038°11'41.8"	2198	6.9	0.084	0.650	0.067
NSFBR-13	N08°43'01.9"	E038°10'00.9"	2181	6.5	0.126	1.746	0.140
NSFBR-14	N08°34'22.3"	E037°59'52.5"	2100	7.9	0.298	0.650	0.065
NSFBR-15	N09°06'09.5"	E038°58'46.0"	2500	6.8	0.064	0.751	0.079
NSFBR-16	N09°13'40.6"	E039°10'36.8"	2726	7.7	0.100	0.650	0.058
NSFBR-17	N09°15'42.8"	E039°13'22.6"	2820	6.6	0.093	0.995	0.145
NSFBR-18	N09°19'14.0"	E039°16'34.6"	2864	6.9	0.098	1.157	0.076
NSFBR-19	N09°10'22.3"	E039°08'35.8"	2627	7.5	0.101	0.853	0.154
NSFBR-20	N09°07'48.1"	E039°10'20.7"	2513	8.3	0.213	0.670	0.094
NSFBR-21	N09°04'40.8"	E037°09'12.5"	1633	6.3	0.146	1.980	0.179
NSFBR-22	N09°01'20.6"	E037°13'29.8"	1731	6.2	0.133	1.840	0.172
NSFBR-23	N09°59'38.4"	E037°21'20.4"	1767	6.3	0.135	3.060	0.314
NSFBR-24	N09°01'13.0"	E037°24'27.6"	2071	6.2	0.101	1.680	0.185
NSFBR-25	N09°02'18.6"	E037°25'10.5"	2281	5.8	0.154	1.920	0.204
NSFBR-26	N08°58'14.9"	E037°29'00.2"	2496	6.6	0.057	1.900	0.214
NSFBR-27	N08°58'41.1"	E037°36'01.4"	2374	6.1	0.155	2.400	0.232
NSFBR-28	N08°58'43.2"	E037°37'58.6"	2312	6.3	0.059	1.900	0.175
NSFBR-29	N08°59'20.4"	E037°47'08.0"	1954	6.4	0.147	3.220	0.230
NSFBR-30	N08°59'30.4"	E037°48'14.8"	1944	7.8	0.342	1.360	0.126
NSFBR-31	N08°59'29.7"	E037°49'25.5"	2050	8.0	0.345	0.800	0.098
NSFBR-32	N08°59'29.7"	E037°49'25.5"	2050	8.0	0.345	0.800	0.098
NSFBR-33	N08°58'05.6"	E037°53'57.8"	2187	7.0	0.106	0.800	0.097
NSFBR-34	N08°58'20.8"	E037°57'03.4"	2387	6.6	0.107	1.500	0.134
NSFBR-35	N08°58'30.5"	E038°00'20.5"	2454	7.9	0.258	0.940	0.111
NSFBR-36	N09°01'27.8"	E038°09'51.4"	2227	6.5	0.107	1.300	0.123
NSFBR-37	N09°00'48.9"	E038°13'41.2"	2165	7.8	0.393	1.220	0.123
NSFBR-38	N09°03'26.0"	E038°27'03.7"	2408	6.4	0.098	1.240	0.140
NSFBR-39	N08°53'49.8"	E038°49'13.1"	2199	5.7	0.132	2.180	0.234
NSFBR-40	N08°46'02.5"	E038°55'59.6"	1905	7.1	0.146	1.200	0.095
NSFBR-41	N08°48'47.7"	E039°00'17.5"	1892	8.1	0.232	0.781	0.071
NSFBR-42	N09°17'17.7"	E038°45'14.5"	2568	6.1	0.145	0.213	1.888
NSFBR-43	N09°24'48.6"	E038°50'40.8"	2607	6.7	0.166	0.133	0.944

Table 1: Continue

Code	Latitude	Longitude	Altitude	pH (H_2O)	EC (dS m^{-1})	Organic carbon (%)	Total nitrogen (%)
NSFBR-44	N09°31'40.6"	E038°52'22.3"	2658	6.2	0.078	0.252	2.258
NSFBR-45	N09°39'05.2"	E038°49'07.7"	2628	6.1	0.074	0.133	1.191
NSFBR-46	N09°40'30.3"	E038°49'28.7"	2667	6.2	0.073	0.125	1.067
NSFBR-47	N09°40'30.3"	E038°49'28.7"	2667	6.2	0.073	0.125	1.067
NSFBR-48	N09°44'35.5"	E038°47'11.6"	2660	7.2	0.109	0.108	0.862
NSFBR-49	N09°47'16.4"	E038°41'52.1"	2975	5.6	0.088	0.317	2.155

Nodulation was induced by 'plant trap' method using acid treated and sterilized river sand in pot experiment as described by Vincent (1970). Newly released Faba bean seed (var. Degaga) obtained from Kulumsa Agricultural Research Center, Arsi, was used as the plant material in the experiments. Uniformly sized faba bean seeds were surface sterilized by immersing in 70% (v/v) ethanol for one minute followed by three minutes in 4% (v/v) sodium hypochlorite and then rinsed six times with sterile distilled water. Sterilized seeds were germinated on a sterile petri-dish containing moistened filter paper and placed at 28°C in incubator. Five pre-germinated seedlings were transplanted and thinned to three after one week. Separate suspensions of collected soils were made in a sterilized test tube by adding 10 g of soil to 100 mL of distilled water and mixed for two minutes. At the end of the experiment, the fresh, red and large nodules were carefully collected from one healthy plant from each soil suspension treated pot.

Isolation of rhizobia from nodules: The nodules were treated by rinsing in absolute alcohol (10 s), surface sterilized in 3% NaOCl (2-5 min) and then rinsed five times with sterile distilled water following Vincent (1970). Nodules were crushed in normal saline solution (0.85%) and streaked onto yeast extract mannitol (YEM) agar medium containing Congo red (CR) (25 mg L^{-1}). Plates were incubated at 28+2°C for 3-5 days then restreaked to obtain pure culture. Single colony isolates were picked from plates, numbered and stored on YEM agar slants containing 0.3% (W/V) $CaCO_3$ at 4°C refrigerator for further characterization. The isolates were given names such as NSFBR (National Soil Faba bean *Rhizobium*) for identification and stored.

Presumptive screening of pure cultures: Cultures were examined for cell morphology and gram reaction after 2 days of growth in YEM- liquid medium. The colony morphology and purity of isolates were examined on YEMA agar-CR plates after an incubation of 5 days at 28+2°C. Individual colonies were characterized based on their color, shape, colony diameter, capacity to produce exopolysaccharide gum and their absorbance of the red color (Vincent, 1970). The production of acid or alkali was determined in YEM agar medium with bromo thymol blue (25 mg L^{-1}) (BTB) plates (Somasegaran and Hoben, 1994).

Characterization of symbiotic effectiveness in sand culture: This experiment was carried out at the National Soil Research Center, to authenticate and select elite isolates of rhizobia forming effective symbiotic association with faba bean following Somasegaran and Hoben (1994). Plants were grown in free-draining plastic pots (5 L) 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

95% ethyl alcohol (10 sec) and left for the complete evaporation of alcohol and then transferred into hydrogen peroxide solution of 3% (5 min) and rinsed six times with sterile deionized water. Five sterilized seeds were sown into each pot and when germinated, they were thinned to three seedling pot^{-1} after a week of planting. One milliliter of liquid inoculum (10^8 rhizobia cells mL^{-1}) of collected isolates was used separately to inoculate seeds at the sowing stage. The pots were arranged in randomized completed block design with four replicates, along with uninoculated and N-fertilized pots. All pots were treated with one fourth strength of micro-nutrient solution (Broughton and Dilworth N-free medium) with two days interval and the N-fertilized pot was received 70 mg KNO_3 L^{-1} $week^{-1}$. Likewise, all plants were regularly watered with sterile distilled water. At 60 days after sowing, all plants were harvested. Nodulation status: nodule number and nodule dry weight, shoot and root length, shoot dry weight were measured. The following effectiveness score was given i.e., very effective (VE) when the dry matter yield of the associated host was higher than the total mean of all isolates plus the standard deviation (more than 2.784); effective (E) when its yield was between that of the mean+the standard deviation (between 2.784+1.388) and ineffective (I) when its yield was smaller than the mean minus the standard deviation (less than 2.784-1.388) (Lalande *et al*., 1990). Total nitrogen of the harvested shoot was determined by Kjeldahl technique described by Sertsu and Bekele (2000).

Estimation of native rhizobia nodulating faba bean: The plant infection count, also known as most-probable number (MPN) counts was used to determine the number of viable and infective rhizobia following the procedure stated by Somasegaran and Hoben (1994). Ten grams of soil sample was diluted in aseptic condition in 90 mL sterilized distilled water. Then 1 mL from first dilution was transferred into 9 mL sterilized distilled water up to 10^{-10} and was used to inoculate a faba bean seedling adequately grown in acid treated and sterilized sand using plastic cups in four replication. Nodule observations were made 21 days after inoculation. Positive and negative nodulation of growth unit were recorded for all dilutions and converted into number of rhizobia g^{-1} using MPN table.

Characterization of selected rhizobia on soil culture under greenhouse condition: Isolates with highest nodulation (NSFBR-1, NSFBR-4 and NSFBR-48) and highest shoot dry weight (NSFBR-11, NSFBR-12, NSFBR-15, NSFBR-30, NSFBR-36 and NSFBR44) were considered as traits of efficient symbiosis for this experiment (Laguerre *et al*., 2007; Appunu *et al*., 2008). Two soils were used (Vertic luvisol soil from Kulumsa Agricultural Research Center had no previous history of inoculation and Nitosols soil from Holleta Agricultural Research Center, Holleta on which faba bean had grown for several years). Both soils were collected from the surface up to 30 cm and each consisted of a composite of ten samples taken at random from 1 ha area. These soils were stored at 4°C until used. The required physical and chemical properties (Table 2) of the surface soil (0-30 cm depth) used were determined following the procedure of Sertsu and Bekele (2000). The experimental design and procedures were identical to those described above.

Statistical analysis: Treatment effects were analyzed using the General Linear Model (GLM) procedure of SAS ver. 10. Differences between treatments were assessed by determining least significant differences at the 0.05 level of probability to separate means.

Table 2: Soil physicochemical properties and number of rhizobia per g of soil

Parameters	Holleta soils	Kulumsa soils
pH (H_2O)	5.5	6.0
pH ($CaCl_2$)	5.1	5.7
EC (dS m^{-1})	0.176	0.186
Sand (%)	7.0	11
Silt (%)	32	34
Clay (%)	61	55
Soil texture class	Clay	Clay
Na (cmol(+) kg^{-1})	0.39	0.43
K (cmol(+) kg^{-1})	2.61	2.71
Ca (cmol(+) kg^{-1})	1.20	2.42
Mg (cmol(+) kg^{-1})	4.93	5.18
CEC (cmol(+) kg^{-1})	27.29	34.51
Base saturation (%)	33	31.0
Exchangeable Al (me/100 g)	Trace	Trace
Exchangeable Acidity (me/100 g)	Trace	Trace
Total N (%)	0.228	0.202
Organic carbon (%)	2.053	2.463
C/N ratio	9.0	12.0
Available phosphorus (ppm)	31.60	16.52
MPN (Number of rhizobia/g of soils)	1.7×10^2	1×10^3

RESULTS AND DISCUSSION

In the present study, 60 isolates were isolated from the root nodules of species of faba bean (*Vicia faba* L.) collected from different sites of Central Ethiopia (Table 1). All isolates were found to be gram negative-rods did not absorb Congo red (data not shown). Almost all isolates were not grown on PGA medium except eleven isolates that grew well and changed the BCP into a yellowish color. The results obtained from Gram staining, growth on YEMA-CR medium and PGA-BCP medium preliminary confirming the standard cultural and morphological characteristics of *Rhizobium* sp. as described by Somasegaran and Hoben (1994), Kumar *et al.* (2011) and Vincent (1970). All tested isolates changed the BTB color into yellowish indicating a common characteristics of fast growing *Rhizobium* sp. (Somasegaran and Hoben, 1994). This result also confirms results of previous observation on faba bean nodulating rhizobia (Workalemahu, 2009; Belay and Assefa, 2011; Minalku *et al*., 2009). Almost all isolates formed dome-shaped colonies with shiny appearance, smoothed and circular colony margin and buttery texture. They displayed gelatinous colonies with colony diameter ranging from 3.5-4.5 within five days. These are the colony morphology of *Rhizobium leguminosarum* bv. *viciae* spp (Adiguzeli *et al*., 2010; Jordan, 2005). Similar results were reported on rhizobia of faba bean isolated from Ethiopia soils (Workalemahu, 2009; Belay and Assefa, 2011). Isolates with larger colony diameter of between 2-5 mm have been correlated with high copious production of exopolysaccharides (EPS) and development of yellowish colony on YEMA-BTB (Adamu *et al*., 2001).

In subsequent experiments, all isolates (60) were assessed for their infectiveness and symbiotic effectiveness using sterile and acid treated sand in pot experiment under greenhouse condition, of which, only 49 isolates were authenticated as nodules forming bacteria of faba bean. The remaining isolates (11) failed to nodulate the parent host. These isolates previously displayed in presumptive tests the characteristics of contaminants (Lupwayi and Haque, 1994). It is obvious

Table 3: The effect of inoculation of rhizobia nodulating faba bean and control condition for different characters on shoot length, root length, nodule number, nodule DW (dry weight), shoot DW, number of tiller per plant and plant total nitrogen of faba bean under control condition by using sand culture

Code	No. of nodule plant^{-1}	Nodule DW plant^{-1} (g)	Shoot length at 50% flowering stage (cm)	Root length at 50% flowering stage (cm)	Shoot DW (g plant^{-1})	No. of tiller plant^{-1}	Plant total nitrogen (%)	Symbiotic effectiveness efficiency (%)
NSFBR-1	325.3±93.3^{a}	0.148±0.029^{fl}	42.3±3.06^{bi}	22.0±4.58^{ad}	2.533±0.115^{bf}	2.7±0.58^{abc}	4.721±0.278^{be}	100.0
NSFBR-2	135.0±18.0^{eo}	0.059±0.015^{op}	35.0±1.00^{il}	20.7±2.08^{af}	1.233±0.252^{l}	1.7±0.58^{de}	2.200±0.257^{j}	48.7
NSFBR-3	222.3±154.7^{bh}	0.133±0.069^{hm}	42.7±0.58^{ai}	16.7±4.04^{efg}	2.333±0.577^{bj}	2.3±0.58^{bcd}	4.538±0.279^{bf}	92.1
NSFBR-4	288.7±96.7^{abc}	0.278±0.049^{a}	46.0±6.24^{ae}	19.7±2.08^{af}	2.467±0.467^{bh}	2.0±0.00^{cd}	4.870±0.312^{be}	97.4
NSFBR-5	114.7±42.3^{ko}	0.097±0.005^{lo}	40.3±0.58^{dk}	22.3±1.53^{ad}	1.600±0.400^{hl}	2.3±0.58^{bcd}	4.339±0.009^{bg}	63.2
NSFBR-6	165.0±22.9^{dn}	0.117±0.012^{jo}	48.7±5.13^{ab}	16.7±3.06^{efg}	2.333±0.503^{bj}	2.3±0.58^{bcd}	4.939±0.221^{ad}	92.1
NSFBR-7	182.7±4.6^{dm}	0.152±0.003^{fl}	46.7±6.03^{ae}	20.0±2.00^{af}	1.670±0.603^{fl}	2.0±0.00^{cd}	4.144±0.589^{ch}	65.9
NSFBR-8	183.7±34.9^{dm}	0.162±0.013^{ek}	43.3±4.16^{ah}	23.7±4.90^{ab}	2.400±0.529^{bi}	3.0±0.00^{ab}	4.834±0.740^{be}	94.7
NSFBR-9	130.0±52.9^{fo}	0.065±0.016^{no}	36.7±2.08^{hl}	18.0±0.00^{dg}	1.370±0.058^{kl}	2.0±0.00^{cd}	2.550±0.011^{ij}	54.1
NSFBR-10	176.7±70.9^{dm}	0.170±0.039^{dj}	45.0±8.66^{ae}	16.7±2.50^{efg}	2.277±1.193^{bj}	2.3±0.58^{bcd}	4.095±0.876^{ch}	89.9
NSFBR-11	296.3±46.5^{ab}	0.233±0.014^{abc}	42.7±2.08^{ai}	20.3±7.50^{af}	2.767±0.404^{bcd}	2.0±0.00^{cd}	4.805±0.567^{be}	109.2
NSFBR-12	147.7±30.2^{eo}	0.148±0.027^{gl}	44.0±5.29^{ag}	17.7±2.90^{dg}	3.000±0.36^{lb}	3.0±0.00^{ab}	4.244±0.476^{bg}	118.4
NSFBR-13	194.7±80.4^{cl}	0.124±0.028^{in}	43.7±2.89^{ah}	20.0±6.10^{af}	1.633±0.666^{gl}	1.7±0.58^{de}	3.980±0.625^{dh}	64.5
NSFBR-14	158.3±38.2^{do}	0.152±0.030^{fl}	46.7±1.53^{ae}	20.0±1.00^{af}	1.867±0.231^{el}	2.7±0.58^{abc}	4.447±0.325^{bg}	73.7
NSFBR-15	231.0±65.4^{abcd}	0.228±0.046^{ad}	39.7±5.51^{el}	20.0±1.00^{af}	2.667±0.513^{be}	3.0±0.00^{ab}	4.846±0.690^{be}	105.3
NSFBR-16	164.0±34.7^{dn}	0.139±0.020^{hl}	41.3±1.53^{cj}	22.7±5.50^{ad}	1.967±0.231^{dl}	2.7±0.58^{abc}	4.755±0.584^{be}	77.7
NSFBR-17	220.0±26.5^{bi}	0.148±0.005^{fl}	42.3±3.51^{bi}	20.0±1.00^{af}	1.933±0.551^{dl}	2.0±0.00^{cd}	4.114±0.394^{ch}	76.3
NSFBR-18	153.3±45.1^{do}	0.074±0.010^{mno}	34.0±3.00^{kl}	18.3±1.53^{eg}	1.467±0.416^{jkl}	2.0±0.00^{cd}	3.784±0.530^{eh}	57.9
NSFBR-19	216.3±86.7^{bj}	0.163±0.031^{ek}	46.0±3.61^{ae}	21.3±5.13^{ae}	2.200±0.361^{bk}	2.7±0.58^{abc}	4.958±0.109^{ad}	86.9
NSFBR-20	186.3±102.6^{dm}	0.159±0.026^{fk}	44.0±3.46^{ag}	24.7±4.04^{a}	2.333±0.379^{bj}	2.3±0.58^{abc}	4.310±0.356^{bg}	92.1
NSFBR-21	213.0±85.5^{bk}	0.154±0.040^{fl}	45.3±4.16^{ae}	18.7±3.06^{bg}	1.967±0.666^{dl}	2.0±0.00^{cd}	4.314±0.388^{bg}	77.7
NSFBR-22	114.3±43.8^{ko}	0.107±0.025^{ko}	42.3±6.81^{bi}	21.0±1.00^{af}	1.533±0.551^{il}	2.0±0.00^{cd}	3.915±0.641^{dh}	60.5
NSFBR-23	201.3±44.8^{bl}	0.143±0.026^{hl}	44.7±3.21^{ae}	23.7±2.52^{ab}	2.067±0.850^{cl}	2.3±0.58^{bcd}	4.053±0.128^{ch}	81.6
NSFBR-24	181.3±30.1^{dm}	0.166±0.029^{ej}	42.0±2.00^{bj}	23.3±1.15^{abc}	2.167±0.289^{bk}	3.0±0.00^{ab}	6.029±3.430^{a}	85.6
NSFBR-25	223.3±56.9^{bg}	0.191±0.023^{bh}	42.3±2.08^{bi}	20.7±1.15^{af}	1.800±0.100^{el}	1.7±0.58^{de}	4.111±0.012^{ch}	71.1
NSFBR-26	123.3±25.2^{ho}	0.135±0.015^{hl}	41.3±3.21^{cj}	22.3±2.08^{ad}	1.600±0.100^{hl}	2.7±0.58^{abc}	4.128±0.092^{ch}	63.2
NSFBR-27	148.3±47.5^{eo}	0.146±0.020^{gl}	40.3±0.58^{dk}	20.7±1.15^{af}	2.033±0.152^{cl}	2.0±0.00^{cd}	4.040±0.175^{ch}	80.3
NSFBR-28	118.7±47.5^{jo}	0.136±0.040^{hl}	42.7±4.04^{ai}	19.3±1.15^{bg}	1.467±0.230^{jkl}	2.0±0.00^{cd}	4.394±0.162^{bg}	57.9
NSFBR-29	144.0±48.3^{eo}	0.157±0.081^{fk}	41.0±4.00^{ck}	20.7±2.52^{af}	2.000±0.500^{dl}	2.7±0.58^{abc}	4.772±0.383^{be}	79.0
NSFBR-30	111.3±7.8^{mno}	0.107±0.061^{ko}	42.3±3.79^{bi}	18.7±2.31^{bg}	2.900±0.436^{bc}	2.3±0.58^{bcd}	4.902±0.360^{be}	114.5
NSFBR-31	143.3±34.9^{eo}	0.128±0.011^{im}	46.3±2.52^{ae}	19.7±0.58^{af}	1.900±0.100^{dl}	2.3±0.58^{bcd}	5.354±0.081^{ab}	75.0
NSFBR-32	250.0±132.3^{ad}	0.174±0.016^{ej}	44.3±1.53^{af}	19.7±0.58^{af}	2.500±0.624^{bg}	2.7±0.58^{abc}	4.549±0.658^{bf}	98.7

Table 3: Continue

Code	No. of nodule plant^{-1}	Nodule DW plant^{-1} (g)	Shoot length at 50% flowering stage (cm)	Root length at 50% flowering stage (cm)	Shoot DW (g plant^{-1})	No. of tiller plant^{-1}	Plant total nitrogen (%)	Symbiotic effectiveness efficiency (%)
NSFBR-33	121.3±72.0^{io}	0.116±0.046^{jo}	37.3±7.09^{fl}	21.0±5.57^{af}	1.733±0.757^{fl}	2.0±1.00^{cd}	3.096±1.251^{hij}	68.4
NSFBR-34	93.3±20.8^{mp}	0.117±0.019^{jo}	35.7±4.16^{jl}	14.3±0.58^{g}	1.467±0.306^{ikl}	1.0±0.00^{e}	3.407±0.142^{ghi}	57.9
NSFBR-35	186.3±14.0^{dm}	0.135±0.061^{hl}	47.7±7.02^{abc}	16.0±1.00^{fg}	1.967±0.850^{dl}	2.0±0.00^{cd}	4.842±0.075^{be}	77.7
NSFBR-36	186.0±110.5^{dm}	0.207±0.090^{bf}	43.7±11.20^{ah}	19.7±2.50^{af}	4.300±1.900^{a}	3.3±0.58^{a}	4.615±0.099^{be}	169.8
NSFBR-37	153.3±75.7^{eb}	0.137±0.027^{hl}	44.0±2.00^{ag}	19.3±2.30^{bg}	1.833±0.208^{el}	2.3±0.58^{bcd}	4.162±0.726^{eh}	72.4
NSFBR-38	189.0±1.7^{cm}	0.167±0.030^{ej}	44.0±2.65^{ag}	22.0±3.60^{ad}	2.233±0.404^{bk}	2.3±0.58^{bcd}	4.823±0.142^{be}	88.2
NSFBR-39	67.3±46.9^{nop}	0.151±0.032^{fl}	42.3±1.53^{bi}	20.7±1.20^{af}	2.000±0.529^{dl}	2.3±0.58^{bcd}	3.957±0.321^{dh}	79.0
NSFBR-40	60.3±32.5^{op}	0.097±0.012^{lo}	37.0±5.57^{gl}	20.0±3.60^{af}	1.267±0.351^{l}	1.0±0.00^{e}	2.498±0.257^{ij}	50.0
NSFBR-41	229.3±70.7^{af}	0.118±0.020^{jo}	45.3±6.50^{ae}	22.3±4.04^{ad}	2.533±0.252^{bf}	2.0±0.00^{cd}	4.250±0.134^{bg}	100.0
NSFBR-42	114.3±28.9^{ko}	0.180±0.084^{bj}	43.7±1.53^{ah}	19.3±2.30^{bg}	2.200±0.872^{bk}	2.0±0.00^{cd}	4.646±0.392^{be}	86.9
NSFBR-43	127.3±37.4^{go}	0.148±0.008^{fl}	47.0±5.0^{ad}	19.7±4.70^{af}	1.967±0.058^{dl}	2.3±0.58^{bcd}	4.373±0.228^{bg}	77.7
NSFBR-44	185.7±75.9^{dm}	0.221±0.024^{ae}	41.7±1.53^{bj}	23.3±4.20^{abc}	2.667±0.058^{dl}	2.7±0.58^{abc}	5.164±0.304^{abc}	105.3
NSFBR-45	113.3±15.3^{ko}	0.154±0.011^{fl}	39.7±1.53^{el}	21.0±1.00^{af}	2.367±0.416^{bi}	2.7±0.58^{abc}	5.031±1.226^{ad}	93.4
NSFBR-46	199.3±86.4^{bl}	0.140±0.012^{hl}	41.3±5.03^{el}	21.3±2.10^{ae}	1.533±0.643^{il}	2.0±0.00^{cd}	4.384±0.410^{bg}	60.5
NSFBR-47	173.7 ±66.9^{dm}	0.147±0.033^{gl}	44.0±7.81^{ag}	23.3±5.00^{abc}	2.167±0.208^{bk}	2.0±0.00^{cd}	4.470±0.338^{bg}	85.6
NSFBR-48	325.0±75.0^{a}	0.238±0.024^{ab}	49.7±3.51^{a}	18.7±1.50^{bg}	2.267±0.306^{bj}	2.0±0.00^{cd}	5.135±0.477^{abc}	89.5
NSFBR-49	173.3±25.2^{dm}	0.204±0.076^{bg}	43.3±5.69^{a}	20.7±3.80^{af}	2.100±0.100^{cl}	3.0±0.00^{ab}	3.481±0.181^{fi}	82.9
N	0.0±0.0^{p}	0.0±0.0^{p}	43.7±4.16^{ah}	19.7±0.58^{af}	2.533±0.208^{bf}	2.7±1.15^{abc}	2.580±0.284^{ij}	-
Control	0.0±0.0^{p}	0.0±0.0^{p}	33.0±3.61^{l}	22.0±3.00^{ad}	1.267±0.321^{l}	1.0±0.00^{e}	2.162±0.151^{j}	-
Mean	167.8	0.145	42.50	20.30	2.086	2.300	4.327	-
SEM	61.6	0.037	4.45	3.13	0.547	0.461	0.694	
CV(%)	36.7	25.400	10.50	15.40	26.200	20.400	16.000	
LSD	99.8	0.059	7.20	5.06	0.886	0.746	1.124	

Values are Mean±SE of 3 replicates, Same letters are not significantlydifferent at LSD $p<0.05$ level, Control: Without chemical and biological fertilizers, N: With optimum amount of nitrogen fertilizer

that no nodulation in case of inoculation have been observed in some isolates of lentil (Zafar-ul-Hye *et al.*, 2007). This might be contaminated saprophytic soil bacteria that penetrate the nodule (Johnston and Beringer, 1976).

Results shown in Table 3 suggest that bacterial inoculations significantly ($p<0.05$) influenced all the parameters investigated compared with control (no inoculation and chemical N). Likewise, measured parameters displayed significant variability among *Rhizobium* treated plants at $p<0.05$. The mean nodule number plant^{-1} record ranged from 60 for isolate NSFBR-40 to 325 for the two isolates NSFBR-48 and NSFBR-1. This result was much higher than previously reported by Minalku *et al.* (2009) that isolates of faba bean from eastern Ethiopia produced less number of nodules (181 plant^{-1}). The experiment recorded the mean nodules dry mass between 0.059 and 0.278 g plant^{-1} for isolates NSFBR-2 and NSFBR-4, respectively, which was greater than that previously reported in Gondor and eastern Ethiopia soils (Belay and Assefa, 2011; Minalku *et al.*, 2009). This could be due to the fact that the study areas are the major faba bean growing region of the country thereby harboring symbiotically effective isolates of rhizobia. All inoculated plant, except NSFBR-2, NSFBR-9 and NSFBR-40 treatments, formed red and pink nodules with dark green leaves (data not shown). Somasegaran and Hoben (1994) suggested that nodules with a pink colour indicate an effective nodule, whereas white and greenish nodules infer ineffective symbiosis. This nodule coloration is evidence of the presence of effective N_2-fixation and an indication of leghemoglobin (Amara *et al.*, 1995). Both uninoculated treatments did not form nodules.

It was also evident that inoculation induced significant improvement of mean shoot height at flowering stage of faba bean comparing the control plants (Table 3). The highest mean shoot height (49.7 cm) was noted with isolate NSFBR-48, which showed pronounced improvement in shoot height i.e., 51 and 14% over negative and N-treated plants, respectively. NSFBR-48 displayed statistically equal shoot height with N-treated plants. Lowest mean shoot height was 33 cm with the control which was lower than total mean shoot height (42.5 cm). This result was in accordance with Argaw (2012) who worked on soybean inoculation with *Bradyrhizobium japonicum* which produced significantly higher plant height over the untreated plants. This enhancement of shoot height could be attributed to the fact that rhizobia may augment plant growth by providing products of dinitrogen fixation. In addition to fixed nitrogen, mobilization of insoluble nutrients followed by enhancement of uptake of nutrients by the plant, production of toxic substances to soil-born pathogens and production of plant growth regulators may also stimulate plant growth (Kumar *et al.*, 2011). However, data in tables show clearly that the mean root lengths were not significantly enhanced by inoculation. Plant dry weight was used indirectly to estimate N_2 fixation in the present study. This method is the best for screening large number of plants for nitrogen fixation in nitrogen free media (Halliday, 1984). According to Lalande *et al.* (1990), the symbiotic effectiveness efficiency (SE) of isolates was found to be very effective (6%) which were 3 isolates and effective (88%) which were 43 rhizobia and three isolates (NSFBR-2, NSFBR-9 and NSFBR-40) grouped as ineffective N_2-fixers (Table 3). This proportion was quite higher than what Minalku *et al.* (2009) found (55%) from 40 rhizobial isolates from Eastern Ethiopia. In fact, similar observation has been reported by Belay and Assefa (2011) and Adamu *et al.* (2001) who found that >80 and 87% of the collected isolates, respectively, were symbiotically effective. This result underlines the importance for local screening of *Rhizobium* isolates in order to improve N_2 fixation in faba bean. The properties of soils (Table 1) from which the rhizobia were isolated had no correlation with the SE of tested rhizobia.

The highest symbiotic efficiency (SE %) were noted with NSFBR-36, NSFBR-12 and NSFBR-30 as 169.8, 118.4 and 114.5%, respectively, comparing the N-treated plants. It was interesting to note that, 16.3% of authenticated isolates scored their SE% greater than 100%. A similar report was obtained with rhizobia of faba bean isolated from Eastern Ethiopia (Minalku *et al*., 2009). Ogutco *et al*. (2008) indicated that 33% of tested rhizobia of chickpea isolated from Turkish soils performed better than the KNO_3 treated plants. These could be due to the fact that some strains of rhizobia have produced plant growth promoting hormones (Gulati *et al*., 2007). In contrast, Beyene and Tsige (1987) found that only 23 isolates of rhizobia were found to be effective out of 108 tested isolates of faba bean.

It can be seen from Table 3, that the maximum shoot dry weight (4.300 g plant^{-1}) was noted by NSFBR-36 as 239 and 70% over the control and N-treated plants, respectively. Number of tillers plant^{-1} was significantly increased by inoculation of rhizobial isolates. NSFBR-36 treatment scored highest number of tiller plant-1 (>3 tillers/plant). While NSFBR-2 treated plant produced lowest shoot dry weight (1.233 g plant^{-1}). A pronounced accumulation in total plant nitrogen was recorded in case of inoculation of NSFBR-24, 1.83 and 1.34 folds over those of control and N-treated plants, respectively. The lowest total plant nitrogen (2.162%) was estimated with the negative control. Very effective isolates accumulated the total plant nitrogen ranging from 0.96 to 1.27 folds over the control. Ineffective isolates lowly accumulated between 2.200 and 2.550%. Moreover, inoculation produced plants with dark green leaf color even better compared with N-treated plants. This result could be an indication of effective symbiotic association between most tested rhizobia with faba bean (Pimratch *et al*., 2004). Nodulation showed positive correlation with all measured parameters except root length (Table 4). Nodule number was positively correlated ($r = 0.7147$, $p<0.001$) with nodule dry weight, as reported by Fening and Danso (2002). Shoot dry weight was positively correlated with nodule number ($r = 0.3717$, $p<0.01$) and nodule dry weight ($r = 0.4986$, $p<0.001$) indicating the determinant factor of nodulation on nitrogen fixation and efficient symbiosis in faba bean (He *et al*., 2011). Nodulation was also positively correlated with symbiotic effectiveness as was reported by Denton *et al*. (2000). This is in agreement with previous work on rhizobia of faba bean (Minalku *et al*., 2009). Shoot dry matter yield and total nitrogen were also positively correlated ($r = 0.4981$, $p<0.001$) (Table 6). In general, nodule and shoot dry matter yield can therefore be used as an accurate measure of N_2-fixation in symbiotic nitrogen fixation experiments (Fening and Danso (2002). Neither total plant nitrogen nor shoot dry weight showed any significant correlation

Table 4: Correlation coefficients among investigated parameter of faba bean

	NN	NDW (g)	SL (cm)	RL (cm)	SDW (g)	NTP	TN (%)	SE (%)
NN	1.00000							
NDW (g)	0.71474***	1.00000						
SL (cm)	0.46517***	0.48945***	1.00000					
RL (cm)	0.05927^{ns}	0.07736^{ns}	-0.06307^{ns}	1.00000				
SDW (g)	0.37171**	0.49859***	0.41971**	0.02387^{ns}	1.00000			
NTP	0.23146^{ns}	0.36877**	0.36412**	0.26009^{ns}	0.64115***	1.00000		
TN (%)	0.55615***	0.66647***	0.60241***	0.12063^{ns}	0.49814***	0.53007***	1.00000	
SE (%)	0.29905*	0.41963**	0.34357*	0.05115^{ns}	0.97481***	0.57530***	0.42502**	1.0000

*, **, ***Significant at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, ns: Not significant at $p<0.05$, SL: Shoot length (cm), RL: Root length (cm), NN: Nodule number, NDW: Nodule dry weight (g plant^{-1}), TN: Plant total nitrogen (%), NTP: Number of tillers per plant, SE (%): Symbiotic efficiency

with root length. Similar findings reported by Indrasumnar *et al.* (2011) found that root growth reflects both N_2-fixation and the plant's status with respect to other nutrients. Root growth is strongly respondent to deficiencies of nutrients such as P by increasing the root to shoot ratio (Raghothama, 1999).

As indicated in Table 2 previously, similar soil parameters were exhibited except soil pH and available P. Holleta soil contained two fold higher available P than Kulumsa soil. More acidic pH was noted with Holleta soil and slightly acidic in Kulumsa soil. The most likely number of rhizobia specific to faba bean was calculated following the method of Somasegaran and Hoben (1994). Naturalised soil rhizobial population size is one important factor in determining a response to inoculation with a commercial rhizobial strain (Thies *et al.*, 1991). Both the Kulumsa and Holleta soils contained compatible native rhizobia of faba bean, making them suitable for testing the competitive abilities of selected rhizobia isolates against native field soil rhizobia. The Holleta soils with long cropping history of faba bean harbored a lower number of rhizobia nodulating faba bean g^{-1} of soil (1×10^2). Relatively, higher number of rhizobia was estimated in Kulumsa soil which was 1×10^3 with no history of cropping of faba bean. This could be indicated none effect of the prevalence of host plant on naturalized rhizobia. Instead, it is exclusively indicating the close negative correlations between the sizes of rhizobial populations and increasing soil acidity (Brockwell *et al.*, 1991; Yang *et al.*, 2001). Ballard *et al.* (2003), Fening and Danso (2002) and Andrade *et al.* (2002) showed that host plant has less effect on rhizobial population than soil pH. The optimum pH for rhizobial growth is considered to be between 6.0 and 7.0 (Jordan, 2005) and relatively few rhizobia grow well at pH less than 5.0 (Graham *et al.*, 1994). However, the presence and continuing cultivation of appropriate host is a major determinant rhizobial population (Abaidoo *et al.*, 2007). Based on the result indicated on sand culture, NSFBR-1, NSFBR-4, NSFBR-11, NSFBR-12, NSFBR-15, NSFBR-30, NSFBR-36, NSFBR-44 and NSFBR-48 isolates were selected for further experimentation on Kulumsa and Holleta soils under greenhouse condition. Inoculation improved all investigated parameters except root length and plant total nitrogen in Kulumsa soils (Table 5). On the other hand, all parameters were significantly improved by inoculation of rhizobia in Holleta soils at $p<0.05$ (Table 6). Generally, the performance of faba bean was better in Kulumsa soil than Holleta soil in all measured parameters. These differences might be due to natural harbor of higher background rhizobia in soil and had optimum soil pH as compared to Holleta soils (Ibekwe *et al.*, 1997; Ballard *et al.*, 2003). Isolates NSFBR-1 and NSFBR-44 enhanced significantly higher nodule number $plant^{-1}$ (186.7 and 172, respectively) over the control in Kulumsa and Holleta soils, respectively. NSFBR-1 and NSFBR-15 inoculation scored significant amount of nodule dry weight, 0.263 and 0.197 g $plant^{-1}$ in Kulumsa and Holleta soils, respectively, (Table 4, 5). These finding are higher than those reported on rhizobia of faba bean isolated from Gondor and Eastern soils of Ethiopia (Belay and Assefa, 2011; Minalku *et al.*, 2009). Chemical N-fertilizer application did not show any significant effect on nodulation on both soils which agrees with the previous report of Albareda *et al.* (2009). All selected isolates produced higher nodule number and dry weight in sand culture than in soil culture. A similar finding was reported previously by Belay and Assefa (2011). It is obviously known that soil harbored with microorganisms could be competent and antagonistic with rhizobia. Inoculation of NSFBR-48 and NSFBR-44 produced significantly higher shoot length over the control in Kulumsa and Holleta soils, respectively (Table 4, 5). However, data indicates none of the inoculated plants produced

Table 5: Symbiotic effectiveness of selected rhizobia nodulating fababean on Kullumsa soil under greenhouse condition

Code	No. of nodule plant^{-1}	Nodule DW plant^{-1} (g)	Shoot length at 50% flowering stage (cm)	Root length at 50% flowering stage (cm)	Shoot DW (g plant^{-1})	No. of tiller plant^{-1}	Plant total nitrogen
NSFBR-1	186.7±27.5^{a}	0.263±0.053^{a}	54.0±17.09^{ab}	39.33±4.04^{a}	11.000±1.230^{a}	3.0±1.00^{a}	3.900±0.759^{abc}
NSFBR-4	152.7±12.5^{ab}	0.140±0.025^{bc}	52.3±5.51^{ab}	33.00±11.53^{abc}	7.300±2.070^{bcd}	1.7±0.58^{b}	3.470±0.396^{bcd}
NSFBR-11	73.3±23.1^{e}	0.097±0.020^{cd}	55.7±3.21^{ab}	34.67±3.06^{ab}	9.100±0.260^{abc}	2.0±0.00^{ab}	4.140±0.295^{abc}
NSFBR-12	102.7±28.6^{cde}	0.183±0.065^{b}	53.7±8.50^{ab}	30.00±5.00^{abc}	7.300±0.610^{bcd}	1.7±0.58^{b}	4.270±0.572^{ab}
NSFBR-15	124.7±27.5^{bcd}	0.067±0.013^{d}	47.3±7.09^{ab}	23.33±6.03^{c}	7.830±2.510^{bcd}	2.0±1.00^{ab}	4.250±0.212^{abc}
NSFBR-30	91.0±21.0^{de}	0.095±0.022^{cd}	45.7±4.50^{b}	27.67±2.9^{bc}	5.900±2.020^{d}	1.7±0.58^{b}	3.760±0.008^{abc}
NSFBR-36	99.7±16.5^{cde}	0.136±0.067^{bc}	43.3±6.11^{b}	28.33±6.66^{bc}	6.530±1.620^{cd}	1.7±0.58^{b}	3.640±0.987^{bcd}
NSFBR-44	130.0±36.0^{bc}	0.067±0.012^{d}	45.3±7.51^{b}	29.00±5.57^{abc}	7.570±2.400^{bcd}	1.7±1.15^{b}	3.420±0.193^{cd}
NSFBR-48	172.0±13.9^{a}	0.130±0.032^{bc}	60.3±4.16^{a}	28.33±2.89^{bc}	9.370±1.50^{ab}	1.7±0.58^{b}	4.500±0.530^{a}
N	83.3±10.4^{e}	0.116±0.006^{cd}	52.7±4.62^{ab}	34.00±5.29^{abc}	7.770±0.150^{bcd}	1.3±0.58^{b}	3.370±0.867^{d}
Control	93.3±11.5^{cde}	0.132±0.018^{bc}	46.7±5.77^{b}	32.33±9.29^{abc}	6.430±1.920^{d}	1.7±0.58^{b}	3.840±0.346^{abc}
Mean	119.0	0.129	50.6	30.91	7.827	1.8	3.825
SEM	21.97	0.036	7.81	6.42	1.51	0.71	0.48
CV (%)	18.45	28.11	15.43	20.77	19.26	39.01	12.67
LSD	37.41	0.062	13.31	10.93	2.57	1.21	0.825

Values are Mean±SE of 3 replicates, Same letters are not significantly different at LSD $p<0.05$ level, Control: Without chemical and biologicalfertilizers, N: With optimum amount of nitrogen fertilizer

Table 6: Symbiotic effectiveness of selected rhizobia nodulating fababean on Holleta soil under greenhouse condition

Code	No. of nodule plant^{-1}	Nodule DW plant^{-1} (g)	Shoot length at 50% flowering stage (cm)	Root length at 50% flowering stage (cm)	Shoot DW (g plant^{-1})	No. of tiller plant^{-1}	Plant total nitrogen
NSFBR-1	130.0±39.6^{ab}	0.145±0.026^{abc}	47.0±7.21^{ab}	25.0±1.73^{bcd}	5.900±0.300^{ab}	3.0±0.00a	4.720±0.333^{ab}
NSFBR-4	96.3±23.2^{bc}	0.096±0.035^{cd}	47.7±5.69^{ab}	33.7±3.06^{ab}	6.400±0.300^{a}	2.7±0.58^{ab}	5.395±1.058^{a}
NSFBR-11	72.3±22.4^{cd}	0.137±0.007^{abc}	42.7±6.43^{ab}	25.0±2.65^{bcd}	5.430±1.457^{ab}	2.0±1.00^{bc}	4.431±0.194^{ab}
NSFBR-12	66.7±11.55^{cd}	0.131±0.047^{bc}	46.0±3.00^{ab}	26.7±6.50^{a-d}	6.730±0.208^{a}	1.3±0.58^{cd}	4.678±0.379^{ab}
NSFBR-15	93.0±20.7^{bc}	0.197±0.050^{a}	44.3±2.08^{ab}	31.7±13.32^{abc}	6.970±0.764^{a}	2.0±1.00^{bc}	4.076±0.394^{b}
NSFBR-30	150.33±35.64^{a}	0.194±0.034^{ab}	41.7±6.65^{ab}	28.0±0.00^{ad}	5.270±0.379^{ab}	2.0±0.00^{bc}	4.627±0.300^{ab}
NSFBR-36	129.3±27.5^{ab}	0.133±0.015^{abc}	40.7±5.00^{ab}	34.7±4.60^{a}	5.900±0.400^{ab}	2.0±1.00^{bc}	4.254±0.669^{ab}
NSFBR-44	145.3±30.7^{a}	0.141±0.022^{abc}	48.0±3.00^{a}	27.0±5.57^{ad}	5.600±0.954^{ab}	1.3±0.58^{cd}	3.983±0.784^{b}
NSFBR-48	137.0±28.7^{ab}	0.156±0.027^{abc}	39.0±9.64^{b}	29.3±4.04^{ad}	6.670±0.810^{a}	2.3±0.58^{ab}	4.576±0.370^{ab}
N	43.33±15.27^{d}	0.064±0.070^{d}	51.7±10.60^{a}	22.3±3.10^{cd}	6.900±1.179^{a}	1.3±0.58^{cd}	4.832±1.617^{ab}
Control	44.67±13.28^{d}	0.097±0.070^{cd}	41.7±11.40^{b}	20.3±5.70^{d}	4.300±2.551^{b}	1.0±0.00^{d}	3.999±0.815^{b}
Mean	100.76	0.136	44.4	27.6	6.006	1.9	4.507
SEM	26.74	0.039	6.53	5.64	1.07	0.57	0.749
CV (%)	26.54	28.46	14.72	20.43	17.84	29.69	16.63
LSD	45.541	0.066	11.12	9.60	1.824	0.966	1.276

Values are Mean±SE of 3 replicates, Same letters are not significantly different at LSD $p<0.05$ level, Control: Without chemical and biologicalfertilizers, N: With optimum amount of nitrogen fertilizer

significant amount of shoot length over N-treated plants. On the other hand, inoculation did not show significant variation of root length in Kulumsa soil. Plants inoculated with NSFBR-36 scored statistically higher root length in Holleta soil at $p<0.05$.

Significant amount of shoot dry weight was scored in plants inoculated with NSFBR-1 over the control and N-treated plants in Kulumsa soil. A similar result was obtained with inoculation of faba bean on the same soil (Amanuel *et al*., 2000). NSFBR-4, NSFBR-12, NSFBR-15, NSFBR-48 and N-treatments scored significantly higher shoot dry weight over the control in Holleta soil (Table 4). The difference in performance of N-treated plants could be due to the differences in number of rhizobia nodulating faba bean which is lower in Holleta soil than Kulumsa soil (Thies *et al*., 1991). Surprisingly, all selected isolates produced very high shoot dry weight in soil culture comparing with the performance in sand culture. This may be due to the fact that few rhizobia are known to produce certain metabolites which play a significant role in rhizosphere affecting the growth and activity of the other microbes and plant health as well (Chandra *et al*., 2007; Belay and Assefa, 2011). All isolates except NSFBR-36 and NSFBR-44 accumulated significantly higher plant total nitrogen over N-treatment but did not show any significant difference with the control in Kulumsa soil. Whereas, only NSFBR-4 accumulated significantly higher plant total nitrogen over the control in Holleta soil. While, the remaining treatments showed non significant difference among each other. NSFBR-1 produced a significantly higher number of tiller plant^{-1} over the N-treatment and the control in both soils. From the study, it could be observed that soil pH rather than host plants affect the presence of rhizobia. The soil pH and number of indigenous rhizobia are major factors affecting the effectiveness of inoculated rhizobia nodulating faba bean in central Ethiopian soils. Central Ethiopian soils are haboured with symbiotically effective rhizobia of faba bean which performed higher than N-treated plants. N-fertilization improved the faba bean growth statistically equal with inoculation in soil with low number of indigenous rhizobia. N-application did not affect the nodulation in both soils. Inoculation improved all growth parameters excluding root length in sand and soil cultures. Therefore, the conclusion argues that rhizobia from a major legume growing area could be potentially utilized for biofertilizer production.

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