Original Research Article||

Evaluation of best performing indigenous *Rhizobium* strains on productivity of faba bean in Gumer District, south-eastern Ethiopia

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Abstract

Biological fixation of atmospheric nitrogen by legumes is a known way to recycle nitrogen into a plant-available form. The efficiency of nitrogen fixation depends on the legume genotype and requires a host-specific *Rhizobia* strain for nodule formation and yield enhancement. A field experiment was designed to evaluate the performance of Rhizobium strains on the yield and yield components of faba bean under rainfed conditions during two consecutive main growing seasons (2019 and 2020). Experiments consisted of a control, 121 kg NPS ha^{-1}, FB 04, FB 1018 and FB 1035, each strain treated separately with 60 kg ha^{-1} nitrogen, phosphorus and sulfer (NPS) and Triple superphosphate (TSP) and placed in a randomized complete block design with three replications. *Rhizobium* inoculation showed a highly significant ($p \leq 0.05$) effect on yield and yield attributes compared to un-inoculated (negative control) treatment. Over the years, the results showed that the inoculated plants gave a significant increase ($p \leq 0.05$) in nodule number and a benefit in grain yield compared to the un-inoculated plants (negative control). Among inoculated treatments numerically, the highest yield (5.87 t ha^{-1}) was recorded with FB 1018 inoculated together with 60 kg ha^{-1} TSP compared to those without inoculation (absolute control) which gave 2.48 t ha-1. All Rhizobium inoculants for faba bean showed better nodule formation, leading to increased yield, and it is recommended that farmers in the study area and similar agro-ecologies adopt this technology.

Key words: Faba bean, fertilizer, inoculation, *Rhizobium* strains

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INTRODUCTION

Faba bean (*Vicia faba* L.) is an important grain of the legume family and is grown for food and forage in many countries (Sillero et al., 2010). It is the main food and feed legume due to the high nutritional value of its seeds, which are rich in protein and starch (Duc et al., 2010). Faba bean plays an important role in fixing atmospheric nitrogen in the form available for the plants. Biological fixation of atmospheric nitrogen in legumes is known ecological practice to improve N-cycling resulted in higher shoot growth, higher number of pods and higher bean yield (Siczek and Lipiec, 2016). According to finding of Yadav and Verma, (2014), nitrogen fixation by legumes accounts for 50% of the 175 million tons of total biological annual N_2 fixation worldwide. However, nitrogen fixation depends on the legume genotype, the *Rhizobium* strain and their interactions with the biophysical environment and *Rhizobium* symbiosis nodule formation (Giller et al., 2013). Therefore, the amount of fixed nitrogen varies with the cultivar of legumes (Abdul-Aziz, 2013) and the effectiveness of associated microsymbionts (Argaw, 2012). The report of Ouma et al., (2016) also confirmed that the common bean and soybean host-specific *Rhizobium* strains are better adapted to local soil environmental conditions. To ensure successful establishment, the Rhizobium strain must be able to survive in the soil environment, as the best survival rate and persistence of Rhizobium in soil improve the possibility of effective nodulation and nitrogen fixation (Knezevic-Vukcevic, 2011). Otherwise, the low-efficiency *Rhizobium* strains can compete and gain an advantage over the efficient *Rhizobium* strains used for inoculation (Fujita et al., 2014). Of course, the soil can support certain native *Rhizobia* that form ineffective nodules; however, effective nodulation is highly dependent on the competitiveness of the inoculants (Laguerre et al., 2007). Inoculation of faba beans with a host-specific Rhizobial strain that is effective and appropriate is critical to enhance symbiotic nitrogen fixation and productivity (McKenzie et al., 2001). Inoculation affects the microbial community by increasing the population of desired *Rhizobial* strains in the rhizosphere (Siczek and Lipiec, 2016). The symbiotic performance of nodulation is largely determined by the abundance of effective *Rhizobium* strains and their competitiveness (Laguerre et al., 2003). Thus, inoculation with effective host-specific Rhizobial strains is required for effective nodulation and nitrogen fixation (Goss et al., 2002). Hence, the present study was initiated to identify the *Rhizobium* strains with the better performance in faba bean for nodulation and better yields for two consecutive main growing seasons under rainy conditions in Gummer district Gurage Zone, Southern Ethiopia.

MATERIALS AND METHODS

Description of the Study Areas

A field experiment was conducted for two years (2019 and 2020) in the main growing seasons under rainy conditions in Gummer, Guraghe Zone, Southern Nations Nationalities and Ethiopian Peoples Regional State (SNNPRS). The test site is at 8°01'56.2" N and 38°01'58.3" E and at an altitude of 2767 meter above sea level. The geographical location of the study area is highlighted in Figure 1.

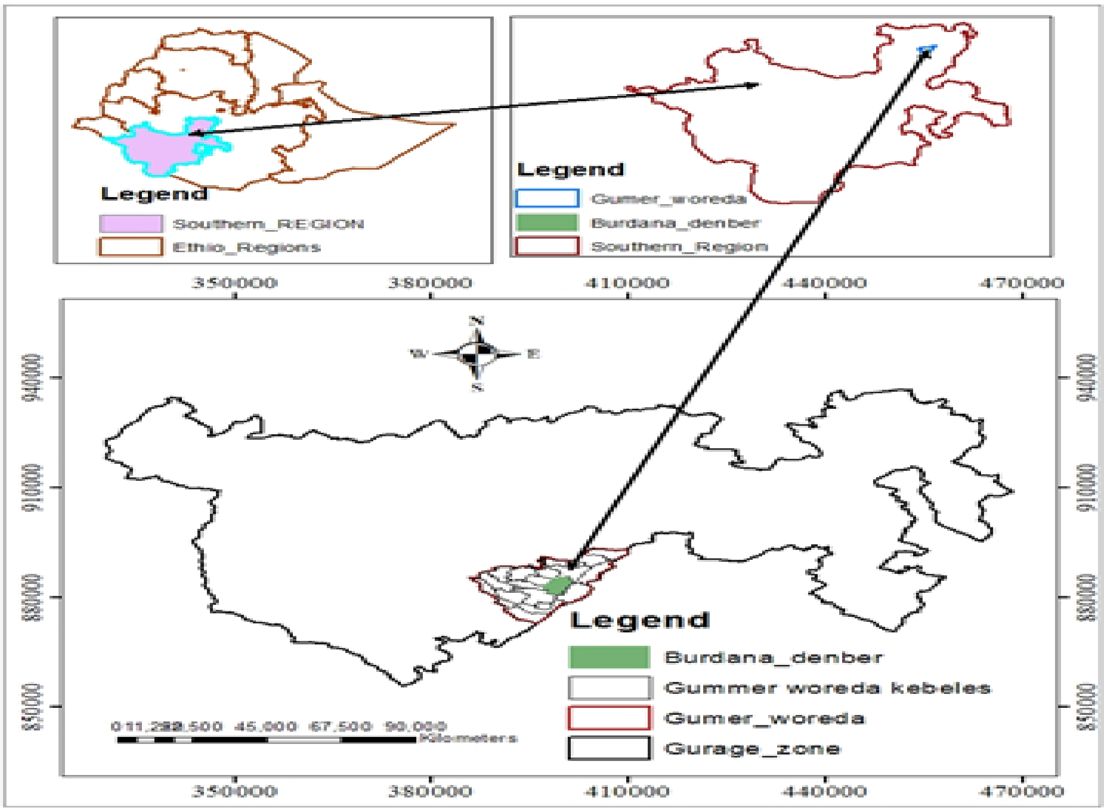

Figure 1. Location map of the study area

Experimental Design and Treatments

The experiment was established in a randomized complete block design with three replications. Eight treatment levels were (control, 121 kg NPS ha^{-1}, FB 04, FB 1018 and FB 1035, each strain treated separately with 60 kg ha^{-1} NPS and TSP fertilizer). The size of the plot was 3 x 3 m (9 m^{2}) and the improved Dosha variety was used for the trials at spacing of 0.1 and 0.4 m distance between plants and rows, respectively. The distance between plots and blocks was 1 m each. Inoculants were obtained from the soil microbiology laboratory from Holeta Agricultural Research Center. Seeds were dipped in warm water to anchor themselves to the *Rhizobium* strains. The sugar suspension was used as an adhesive for the carrier-based inoculants, ensuring that the inoculants adhered to and coated the seeds. The inoculated seeds were allowed to air dry for a few minutes and were planted in the shade immediately after drying. The un-inoculated treatments were sown before the starts of inoculation to thoroughly avoid cross-contamination. Nitrogen and phosphorus fertilizers were applied at sowing in rows. Furrows were made between each plot to reduce the movement of bacteria and the leaching or addition of nutrients from one plot to another or from the external environment.

Data Collection Procedures

Soil Physicochemical Analysis

Composite soil samples were analyzed for bulk density, particle size distribution, pH, organic carbon, cation exchange capacity, total nitrogen and available P of the representative soil before planting. The bulk density of the soil was estimated by the core method to a depth of 30 cm and calculated as:

$$= \rho_b = \frac{M_s}{V_t}$$

where, ρ_b is the bulk density of the soil (g cm^{-3}), Ms = mass of dry soil (g) and Vt = total volume of Soil sample (cm^3) (Black, 1965). The pH of the soil was determined using the potentiometric method in a soil: water ratio of 1:2 (Van Reewijk, 1992). Cation exchange capacity was determined using the 1M ammonium acetate method at pH 7 (Chapman, 1965), while organic carbon was determined using the dichromate oxidation method (Walkley, 1934), and total nitrogen using the micro-Kjeldhal method (Jakobsen, 1985), and available P was analyzed using the Olsen method (Olsen, 1954). The particle size distribution of the soil was determined using the hydrometer method (Bouyoucos, 1951).

Table 1. Chemical and physical properties of the soil before sowing

pH	BD	%OC	%TN	AP	CEC	Textural class (%)			
						Sand	Clay	Silt	Texture
5.9	0.99	1.1	0.094	1.28	41.2	70	14	16	Sandy loam

BD = bulk density; OC = organic carbon; TN = total nitrogen; AP = available phosphorus; CEC = cation exchange capacity of the samples

Nodulation

Sampling for nodulation was performed by digging up the roots of five randomly selected plants from each plot at the mid-flowering stage of faba beans. A destructive sampling from border rows was used. A hoe was used to dig up the root surrounding the soil and the spade was used to excavate at a depth of about 20 cm, which is about the root depth of faba beans. The radius excavation extended 12 cm from the central stem to contain the entire root system of the faba bean. The excavated soil was washed off the roots with a washing bottle. Nodules from the crown region and lateral roots were then removed from the roots and were collected in a plastic bag for counting. The total number of nodules was counted on five sample plants, considering the intensity of pink color (visual observation), which was regarded as the nodule count.

Plant Height

Five plants were randomly selected from the middle rows to measure their height at physiological maturity using a tape measure. The average height of these five plants was calculated for each plot and considered as the plant height.

Number of Pods per Plant

Five plants were randomly selected from harvestable rows of each plot. The pods were collected and counted separately from each plant and their average was taken and reported as the number of pods per plant.

Number of Seeds per Pod

After counting the pods from each of the five randomly selected non-border plants, the grains were separated from the pods to obtain the number of seeds per plant. For each plant, the number of grains per pod was calculated by dividing the total number of grains per plant by the number of pods per plant. The average of five plants was used as the number of grains per pod.

Biomass and Grain Yields

At physiological maturity, plants from 5 rows were harvested manually near the soil surface. The harvested plant samples were sun-dried and weighed to determine above-ground plant biomass yield. The grain yield of each plot after threshing was also determined and converted into grain yield per hectare.

Statistical Analysis

Collected data were subjected to analysis of variance (ANOVA) using SAS 9.4 software. Mean separation was carried out using the least significant difference (LSD) at a 5% probability level.

RESULTS AND DISCUSSION

Effect of Rhizobium Inoculation on Grain Yield

Mean over the two years of trial showed that *Rhizobium* inoculation significantly ($p<0.05$) affected faba bean grain yield at the study site. Statistically, the highest yields were recorded on inoculated plants compared to un-inoculated counterparts. As shown in Table 2, the maximum grain yield (5.875 tons ha^{-1}) was obtained from the inoculation of FB 1018 followed by FB 1035 yielding 5.29 and 5.078 ton ha^{-1}, respectively together with 60 kg ha^{-1} TSP, while the lowest yield of grain corresponded to the non-inoculated (2.48 ton ha^{-1}). The increase in yield of the inoculated plants could be effective nodules formation of the rhizobium strains thereby improving nitrogen supply through biological fixation (Kutafo and Alemneh, 2020). This study is also consistent with the results of Rugheim and Abdelgani, (2012), who reported that inoculation of rhizobia strains significantly increased faba bean yield. Desta et al., (2015) also confirmed that application of effective rhizobia strains alone and/or in combination with zinc significantly increases faba bean yield. The report by Youseif et al., (2017) also shows that application of effective strains increases faba bean grain yield by up to 47%.

Aboveground Biomass

Inoculation of *Rhizobium* strains significantly affected biomass yield ($p \leq 0.05$). Samples treated with with FB 1018, FB 1035 and FB 04 together with 60 kg ha^{-1} TSP had the higher biomass compared to the un-inoculated (control) ones, which statistically gave the lowest biomass yield (Table 2). Effective *Rhizobium* nodulation contributes to increased faba bean growth and yield parameters by supplying nitrogen to plants by fixing it from the atmosphere and converting it into plant-available nutrient froms. This result is consistent with the reports of El-Azeem et al., (2007) who reported that inoculation of bacterial rhizobia strains resulted in significant above ground biomass in faba beans. Gedamu et al., (2021) also showed that inoculation of rhizobia strains significantly increased the weight of faba bean biomass compared to non-inoculated counterparts. The difference in biomass yield obtained from inoculation of faba bean is that *Rhizobium* strains could be due to the additional supply of nitrogen through the remarkable biological nitrogen fixation by the inoculated strains.

Table 2. Mean of biomass and grain yield affected by inoculation of rhizobium strain

Treatments	Biomass yield (ton ha^{-1})	Grain yield (ton ha^{-1})
T1: Control	5.758^{d}	2.48^{c}
T2: 121 kg ha^{-1} NPS	11.462^{ab}	5.635^{a}
T3: 60 kg ha^{-1} NPS+ FB 04	9.452^{bc}	4.375^{b}
T4: 60 kg ha-1 NPS + FB 1035	8.962^{c}	4.406^{b}
T5: 60 kg ha-1 NPS + FB 1018	10.05^{abc}	5.035^{a}
T6: FB 04+60 kg ha^{-1} TSP	11.518^{ab}	5.293^{a}
T7: FB 1035+60 kg ha^{-1} TSP	10.558^{abc}	5.078^{ab}
T8: FB 1018+60 kg ha^{-1} TSP	11.868^{a}	5.875^{a}
Mean	9.95	4.77
LSD (0.05)	2.384	1.123
CV (%)	20.4	20.1

LSD (0.05%): least significant difference at 5% level; CV: coefficient of variation; means in a column followed by the same letters are not significantly different at 5% level of significance.

Number of nodules per plant

Inoculation with *Rhizobium* showed a significant increase in the number of nodules per plant. Table 3 shows that stem inoculation significantly affected the number of nodules/plant ($p \leq 0.05$). A greater number of nodules were obtained from all inoculated plants compared to the un-inoculated plants, since inoculation with rhizobia improves effectiveness for nodulation. This result indicated that the inoculation of these strains in the study area could be more appropriate and competitive than the existing native strains of faba bean rhizobia. Woldekiros et al., (2018) reported that inoculation of the *Rhizobium* strains on faba bean seeds produced highest nodules. Likewise, the report of Gedamu et al., (2021) and (El-Khateeb et al., (2012) confirmed that inoculation of *Rhizobium* strains in faba beans significantly increased the number of nodules. Desta et al., (2015) also reported that inoculation of rhizobia on faba bean significantly increases the number of nodules per plant.

Number of Pods per Plant and Seeds per Pod

The number of pods per plant was affected by inoculation of all *Rhizobium* strains (FB04, FB1035 and FB1018), which increased in growth parameters of faba bean (Table 3). Inoculation of faba bean seeds with *Rhizobium* strains also had a statistically significant effect on the number of seeds per pod compared to un-inoculated treatment. Woldekiros et al., (2018) reported that the number of pods per plant was significantly ($p < 0.05$) increased due to inoculation by rhizobia. According to Desta et al., (2015) and Gedamu et al., (2021) the rhizobia strain alone could significantly increase the number of pods plant. The result of the present study disagrees with those reported by Zerihun and Abera (2014), who showed that the number of seeds per faba bean pod was not significantly affected by fertilizer rate and Rhizobia inoculation.

Plant Height

The result of the present study showed that inoculation of seeds with *Rhizobium* increases plant height (Table 3). Rhizobium inoculation increases faba bean growth parameters by increasing nitrogen supply. Bejandi et al., (2012) confirmed that seed inoculation by *Rhizobium strains* significantly increased nitrogen uptake, thus improving plant growth and yield, and possibly increasing the potential of plants to produce more height.

Table 3. Mean of growth and yield parameters of faba bean as affected by *Rhizobium inoculation*

Treatments	Number of nodules	Plant height (cm)	Number of pods per plant	Number of seed per plant
T1: Control	69.6^{d}	90^{b}	14.5^{b}	33^{b}
T2: 121 kg ha^{-1} NPS	89.4^{c}	111^{a}	27.5^{a}	49^{a}
T3: 60 kg ha^{-1} NPS + FB 04	109^{c}	110^{a}	25.3^{a}	47^{a}
T4: 60 kg ha^{-1} NPS + FB 1035	118^{c}	112^{a}	29^{a}	49^{a}
T5: 60 kg ha^{-1} NPS + FB 1018	137^{a}	110^{a}	30.5^{a}	51^{a}
T6: FB 04 + 60 kg ha^{-1} TSP	121^{bc}	105^{a}	29.8^{a}	49^{a}
T7: FB 1035 + 60 kg ha^{-1} TSP	121^{bc}	121^{a}	29.5^{a}	50^{a}
T8: FB 1018 + 60 kg ha^{-1} TSP	135^{ab}	112^{a}	31.3^{a}	55^{a}
Mean	112	107	27.5	48
LSD (0.05)	15.5	12.2	5.62	9.53
CV (%)	11.8	9.7	17.4	16.9

LSD (0.05%): least significant difference at 5% level; CV: coefficient of variation; means in a column followed by the same letters are not significantly different at 5% level of significance.

CONCLUSIONS

Rhizobium inoculation significantly improved the faba bean grain yield. The inoculated plants gave the greater yield benefit compared to the un-inoculated ones. All tested rhizobia strains performed better showing greater potential being ecologically competent and symbiotically effective in nodule formation and yield increase. It is therefore recommended that farmers use the technology in the study area and others with similar agro-ecologies.

CONFLICTS OF INTEREST

Authors declare no conflicts of interest regarding the publication of this paper.

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