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Faba Bean (Vicia faba L) Yield Enhancement Using Elite Rhizobial Inoculants on Acidic Soils in Ethiopia's Central Highlands

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Abstract: The goal of this study was to assess how the faba bean responded to the inoculation of six indigenous rhizobial isolates in acidic field conditions at Damotu (in 2019) and Chiri (in 2020) in the Ejere district, Ethiopia. We laid out the experiments in a randomised complete block design (RCBD) with three replications and a plot size of 4 m x 3 m. The treatments consisted of six indigenous faba bean rhizobia isolates designated as FB-NS-02, FB-NS-03, FB-EM-02, FB-EM-05, FB-EM-07, and FB-EM-11. Additionally, we included a positive control that received 18 kg ha1 of urea and a non-inoculated negative control. The results revealed a significant difference ($p \leq 0.05$) between treatments on aboveground biomass, grain, and haulm yields. The inoculation of rhizobial isolate FB-EM-11 at both experimental sites yielded the highest grain yields (3378.3 kg ha-1 and 3286.7 kg ha-1). Rhizobial isolates FB-NS-03 (3376.7 kg ha-1) at Damotu and FB-EM-07 (3269.5 kg ha-1) at Chiri yielded the second highest grain yields. Based on the two successive years' grain yield responses, FB-EM-11 (3332.6 kg ha-^{1}) and FB-NS-03 (3198 kg ha^{-1}) were the first and second most performing isolates at Ejere district, respectively. Thus, these rhizobial isolates are the most promising candidates for the development of commercial faba bean rhizobial inoculants in acid-prone areas of Ethiopia.

***Keywords**: Central Highlands, Faba bean, inoculant, soil acidity, yield*

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INTRODUCTION

Faba bean (Vicia faba L.) is an annual legume crop that originated in the Middle East in the prehistoric period and is nowadays widespread in different parts of the world, including Ethiopia. It is a multipurpose crop that helps cropping systems last longer by adding nitrogen (N) to the system through biological fixation of N; changing production systems so that diseases, pests, and weeds don't get too big; and giving animals food and feed that is high in protein and carbs (Jensen et al., 2010).

Ethiopia ranks second in faba bean production next to China, and it is the fourth largest faba bean exporting country next to France, Australia, and the United Kingdom (FAO, 2016). Faba bean accounts for the largest share of the area (466,698 hectares) and production (172,739.9 tonnes) of pulses grown in Ethiopia (CSA, 2020). It plays a key role in improving the livelihood of smallholder farmers by serving as food, feed, and a means of income, while at the same time it also plays a great role in improving soil fertility. In Ethiopia, the national average yield of faba bean is about 2.1 t ha-1 (CSA, 2018), which is very low compared to the national average grain yield of 3.7 t ha-1 in major producer countries (FAO, 2018).Many biotic and abiotic factors, such as the frequent occurrence of parasitic weeds, the lack of stress-tolerant high-yielding faba bean varieties, poor agronomic practices, soil acidity, and fertility decline, are primarily responsible for the low yield of faba beans (Getachew and Rezene, 2006).On the other hand, ATA (2014) estimates that close to 28.1% of Ethiopia's arable land is strongly acidic. Such strong soil acidity (pH 4.1–5.5) has been highly constraining the production of grain legumes in the highlands of Ethiopia (Workneh, 2013). The primary manifestations of soil acidity on the growth and development of faba beans are believed to be common mechanisms such as deterring rhizobia's survival and nodulation, limiting symbiotic nitrogen fixation, and stunting crop growth and development (Graham et al., 1982; Munns, 1986). Aluminium and manganese toxicity, as well as P, Ca, Mg, and Mo deficiency, are the root causes of poor crop performance in highly acidic soils (Getachew et al., 2019). Chemical imbalances and aluminium toxicity irreversibly hinder the population size and effectiveness of native Rhizobium leguminosarum under such soil conditions (Andrade, 2002; Fageria, 2002; Endalkachew et al., 2018). The responses of cool-season grain legumes to soil acidity may vary between species. Among commonly cultivated pulses, the sensitivity to soil acidity is in the order of lentil > chickpea > faba bean and field pea > lupin (Tang and Thomson, 1996). This means root infection, colonisation, and nodulation, and in turn, the growth and productivity of faba beans will decline to a great extent (Kellman, 2008; Bationo and Waswa, 2011). Ethiopia has employed lime amendment as an effective chemical remedy to neutralise soil acidity and thereby enhance crop productivity. However, its higher dose requirement per unit area remained a challenge to farmers (Fairyhood, 2008). Lina et al. (2018) also noted the negative effect of long-term use of lime on the abundance, diversity, and community structure of diazotrophs. Integrating acid-tolerant strains of Rhizobia with other soil management methods in acidic soils is an environmentally friendly and economical option. When mixed with lime and host variety, they can work well together to help grain legumes grow better in very acidic soils in the long term (Muleta et al., 2017; Getahun and Abere, 2019). In this regard, the primary task would be to develop a relatively acid-tolerant Rhizobia inoculant under in vitro conditions. The goal of this study was to find out how well native faba bean rhizobia isolates work at increasing the yield of faba beans in Ethiopian areas that tend to be acidic.

MATERIALS AND METHODS

Nodule Sample Collection Sites

We collected the nodule samples for the isolation of acid-tolerant rhizobia isolates from acidic soil hot spot areas in the country, including Nejo, Bedi, Wolmera, Endibir, and Banja. These areas are located at 9° 30′ N and 35° 30′ E, altitude of 1821 masl; 9° 05' N and 38° 36' E, altitude of 2565 masl; 9°3′ N and 38° 30′ E, altitude of 2391 masl; 8° 7′ N and 37° 56′ E, altitude 2130–2164 masl; and 11.02° N and 36.75° E, altitude of 2099 masl These areas have a pH level below 5. We followed aseptic procedures to prevent contamination of the samples until they arrived at the microbial laboratory. The National Agricultural Biotechnology Research Centre's (NABRC) microbial biotechnology laboratory and greenhouse performed the isolation, purification, and authentication of the isolates in 2017–18..

Field Experimental Sites

We conducted the field experiment at Damotu, a farmers' training centre, and Chire, an on-farm location, during the main cropping seasons of 2019 and 2020, respectively. For the previous five years, the experimental sites had no inoculation history. Damotu and Chire are located at 09° 0353” N and 38° 26’ 22.7’’ E, at 2618 masl, and 09° 02’ 33” N and 38° 2545.5’’ E at 2527 masl, respectively. Nitisols, classified as strongly acidic soil (Murphy, 1968), dominated both experimental sites (Table 1). The commonly grown crops on the trial sites are wheat, barley, faba bean, field pea, and tef. Figure 1 displays the mean monthly minimum and maximum temperatures as well as the monthly rainfall of the sites during the trial .period. Trial period. During the trial period are shown in Figure 1.

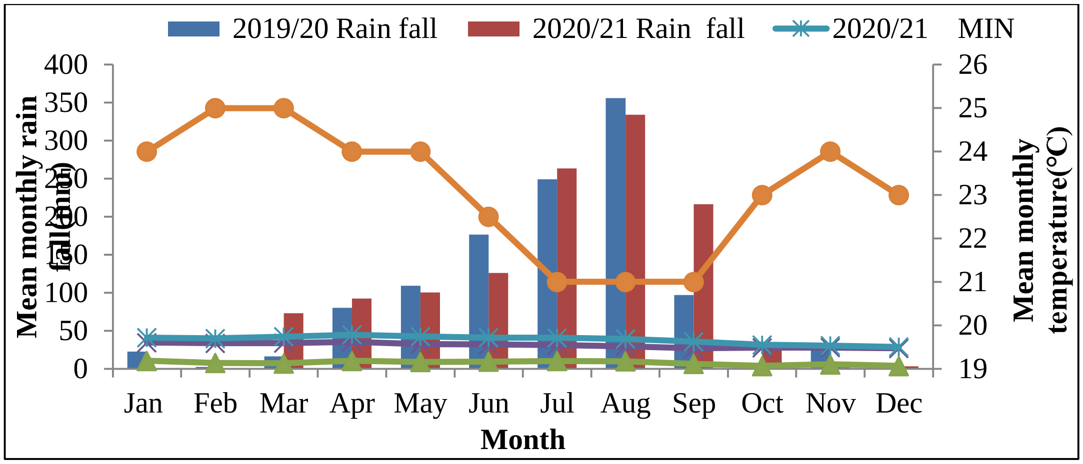

Figure 1: Rainfall and the mean monthly maximum and minimum temperature patterns at the experimental sites mental sites (Source: Holetta Agricultural Research Centre weather station)

Treatments and Experimental Designs

We compared the treatments of six indigenous faba bean rhizobia isolates, designated as FB-NS-02, FB-NS-03, FB-EM-02, FB-EM-05, FB-EM-07, and FB-EM-11, against 18 kg N ha-1 (positive or standard) and an untreated or negative control. The experiments were laid out in a randomised complete block design (RCBD) with 3 replications on a plot size of 4 m x 3 m. To reduce cross-contamination of isolates, the space between plots and blocks was enlarged to 0.5 and 1m, respectively, and un-inoculated treatments were planted before inoculated treatments. The spaces between plants and rows were 10 and 40 cm, respectively. All experimental plots received basal application of 20 kg P ha^{-1} TSP at planting. The positive control received 18 kg N ha^{-1} from urea at planting. The negative control did not receive any form of external N source. The planting material was a Tumsa variety planted at 200 kg ha1. The experimental fields and experimental units were managed as per the recommended agronomic practices for faba beans.

Prepare the Carrier-Based Rhizobia Inoculant and Apply Seed Dressing.

The Microbial Biotechnology Laboratory of NABRC prepared Rhizobia isolates as carrier-based inoculants. The carrier material used for the study was powdered lignite (that passed through a 10^6-micrometre mesh size) adjusted to pH 7. One hundred twenty-five grammes of lignite were transferred to heat-resistant white polyethylene bags, sealed and sterilised at 121 OC for 30 minutes, and subsequently cooled. Then 25 ml of a quality broth culture of each rhizobia isolate containing more than 10^8 colony-forming units per millilitre of the broth culture was aseptically inoculated into the already sterilised and cooled lignite and homogenised in a laminar flow hood. Then, the inoculant was incubated at room temperature for two weeks for curing. The absence of contamination and minimum threshold rhizobial cell population were inspected via viable cell count (Vincent, 1970) and covered with yellow and opaque plastic bags to protect the inoculants from direct sunlight exposure.

We applied the carrier-based rhizobia inoculants at a rate of 500 g ha1. About 0.2 kg of faba bean seed was weighed, moistened with a table sugar solution, and dressed carefully with the respective inoculant until all the seeds in plastic bags were uniformly coated. The whole seed dressing procedure was carried out under shade. The fully-dressed and air-dried seeds were planted and immediately covered with soil.

Data Collection and Yield Determination

We collected and examined soil and agronomic data to assess how the bio-fertilizer responded to the progress of the faba bean harvest in the Ejere district of Ethiopia. The soil and agronomic parameters were soil pH, available phosphorus, organic carbon, total nitrogen, above-ground biomass yield (AGBY), Haulm yield (HY), and grain yield (GY). The data were subjected to analysis of variance by SAS statistical software version 9.3 (SAS Institute, 2011). Means were compared with the least significant difference (LSD) at a 5% probability level.

RESULTS AND DISCUSSION

Isolation, Purification, and Authentication of Rhizobia Isolates

In the isolation, purification, and authentication test, 60 isolates were obtained from nodules that were collected from acid soil hot spots in the country, such as

Nejo, Bedi, Wolmera, Emdibir, and Banja of Ethiopia. Among the 60 isolates, only 40 (66.7%) were found to be gramme-negative, rod-shaped bacteria that did not absorb Congo red on YEMA-CR medium and were not grown on Peptone Glucose Agar (PGA). According to Subba Roa (1999), the growth of bacteria on PGA media is a useful criterion for the identification of true rhizobia from the root nodule. These 40 isolates turned yeast extract mannitol agar medium containing bromothymol blue (YEMA-BTB) into a deep yellow colour after 48 hours of incubation, showing that all the isolates were fast-growing acid-producing rhizobia. Somasegaran, Hoben (1994), and Gupta (2000) also confirmed that colonies of fast-growing *Rhizobium spp.* show little or no Congo red absorption when incubated in the dark and should turn the YEMA-BTB yellow after 3-5 days of incubation.

Among these 40 pure preliminarily screened rhizobial isolates, only 10 (25%) of them successfully nodulated the host plant and passed the symbiotic effectiveness test (Figure 2). However, among these 10 symbiotically efficient isolates, only six of them—FB-NS-02, FB-NS-03, FB-EM-02, FB-EM-05, FB-EM-07, and FB-EM-11—were considered for the field trial at Ejere district based on their superiority in symbiotic effectiveness.

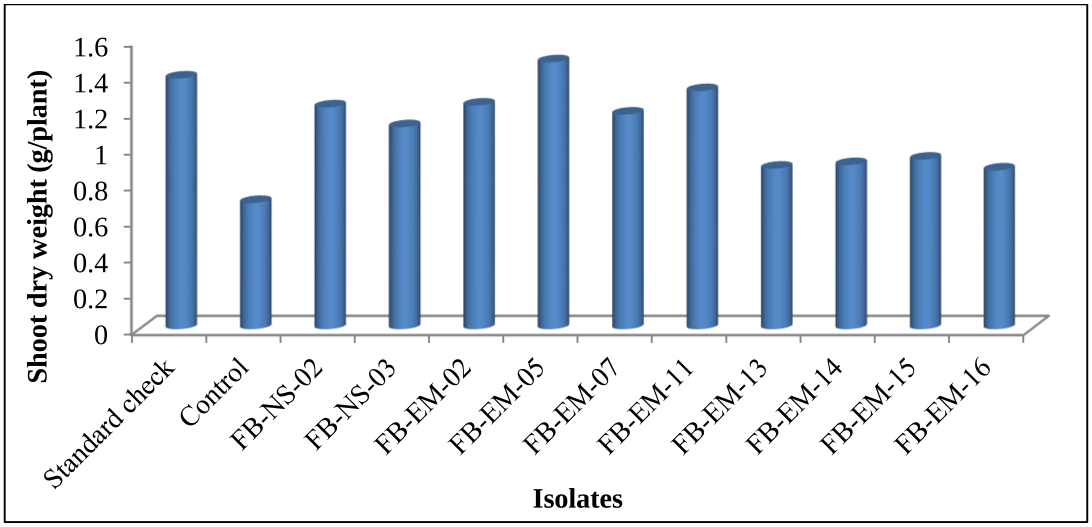

Figure 2. Symbiotic effectiveness test of Faba bean rhizobial isolates on sand, 2017/18

Soil test results

The soil chemical properties were found to be similar among the experimental sites (Table 1). The soil pH of the Damotu and Chire trial sites was 4.86-and 4.96, respectively. Therefore, Bruce and Rayment (1982) classified both locations as very strongly acidic soils. The organic carbon and available phosphorus of the sites were 1.34% and 1.4%, respectively, and 9.68 and 9.49 ppm for the Damotu and Chire trial sites, respectively. They can be categorised in the low range (Tekalign *et al*., 1991). The mean total nitrogen contents of the sites were found to be in a moderate range (Tekalign *et al*., 1991).

Table 1: Mean values of measured soil chemical properties of trial sites before planting

Parameter	Damotu	Chire	Range	Test Methods
Total N (%)	0.16	0.22	0.12-0.24	Modified Kjeldhal
pH	4.91	4.89	4.86- 5.0	1:2.5 H_2O
Available P (ppm)	9.68	9.49	6.84-12.52	Bray II
OC (%)	1.34	1.4	1.26-1.48	Walkley and Black (1934)

Faba Bean Response to Inoculation at Damotu

The results of the study (Table 2) indicated significant statistical differences ($p \leq 0.05$) between treatments on AGBY, GY, and HY. Even if no significant statistical difference was observed between isolate FB-EM-02 and the negative control, the isolate scored a 23% AGBY advantage over the positive control. In the same manner, even though there was no significant statistical difference on GY among FB-NS-03, FB-EM-(02, 07, and

11) inoculants, and the controls, FB-EM-11 showed the highest GY (3378.3 kg ha^{-1}). On the contrary, FB-EM-11 showed significant statistical GY superiority over FB-NS-02 and FB-EM-05 inoculants. Inoculant FB-EM-11 had 12 and 15% higher grain yields over positive and negative controls, respectively.

Also, inoculant FB-NS-03 showed significant statistical differences in HY compared to FB-NS-02 and FB-EM-05, as well as positive control treatments that showed the list HY kg ha-1. The higher HY (3668.7 kg ha^{-1}) scored by FB-NS-03 was 44% and 17% higher than the HY recorded by both positive and negative controls, respectively.

Table 2: Response of faba bean to rhizobial inoculation at Damotu in 2011/12 EC season

Treatments	AGBY (kg ha^{-1})	GY (kg ha^{-1})	HY (kg ha^{-1})
FB-NS-02	1808b	2573b	2752.7b
FB-NS-03	2186.7b	3376.7a	3668.7a
FB-EM-02	2400.7ab	3353a	2980.3ab
FB-EM-05	1910.3b	2504.7b	2637.7b
FB-EM-07	2055.7b	2993ab	2872ab
FB-EM-11	2280.7b	3378.3a	2929.7ab
Positive control (18 kg N ha^{-1})	1953.7b	3011.3ab	2540.7b
Negative control	3021.7a	2931.7ab	3123.3ab
CV (%)	16.5	15	16.58
LSD (5%)	636.3	776.25	852.98
Mean	2202.2	3015.2	2938.1

AGBY= above ground biomass yield, GY= grain yield, HY= Haulms yield

Significantly superior ($p \leq 0.05$) grain yield was obtained from rhizobial isolates FB-EM-11 and FB-NS-03 as compared to the rest of the inoculants and the negative control. Moreover, isolate FB-NS-03 showed relatively significant superiority over the other treatments on HY. This relative superior performance of the inoculants in such acidic soil conditions could be attributed to their capability to provide high N to the host through BNF (Getachew *et al*., 2019; Andrade, 2002). In line with these results, Youseif *et al.* (2017) reported that rhizobia strain inoculation and application of starter nitrogen to faba bean increased AGBY and GY significantly as compared to the un-inoculated and unfertilized control.

Faba bean response to inoculation at Chiri

At Chire, treatments showed significant statistical differences ($p \leq 0.05$) in AGBY, GY, and HY (Table 3). Even if no significant statistical difference was observed among isolates of FB-NS-(02, 03) and FB-EM-(07, 11), they showed significantly higher AGBY, GY, and HY than FB-EM-(02, 05). The high value of AGBY (2472.7kg ha^{-1}) was 129 and 145% higher than the AGBY obtained in the positive control (534.57kg ha^{-1}) and negative control (395.8 kg ha^{-1}). Similarly, the highest GY scored by FB-EM-11 (3286.7 kg ha^{-1}) was 58 and 81% superior to the grain yield obtained by the positive and negative control, respectively. Although FB-NS-02, 03, and FB-EM-07 and 11 did not show any significant statistical differences, FB-EM-11 showed a higher HY (2238 kg ha^{-1}) than the other treatments. Yet, this inoculant showed significant statistical differences on HY with FB-EM (02, 05), and both controls and treatments showed the same yield kg ha^{-1} in the study. The higher HY (2238 kg ha^{-1}) scored by FB-EM-11 was 41% and 105% higher than the haul yield scored by both positive and negative controls, respectively.

The rhizobial isolate FB-EM-11 showed relative significant superiority ($p \leq 0.05$) as compared to the rest of the treatments on AGBY, GY, and HY. This relative superior performance of FB-EM-11 in such acidic soil conditions in Chiri district could be attributed to its competence in providing high N to the host through BNF (Getachew *et al*., 2019; Andrade, 2002) or its role through other plant growth promotion. Similar results were also obtained from the inoculation of faba beans with indigenous rhizobial isolates in eastern Ethiopia (Anteneh and Abere, 2017).

Table 3: Response of faba bean to rhizobial inoculation at Chiri in 2012/13 cropping season

Treatments	AGBY (kg ha-1)	GY (kg ha^{-1})	HY (kg ha^{-1})
FB-NS-02	1612.6ab	2856a	1712ab
FB-NS-03	1399.5b	3019a	2181a
FB-EM-02	841.2c	1761bc	935cd
FB-EM-05	723.1cd	2240b	1403bc
FB-EM-07	1357.4b	3270a	2234a
FB-EM-11	1862.8a	3287a	2238a
18 kg N ha^{-1}	541.5d	1811bc	1472bc
Negative control	442.5d	1389c	699d
LSD (5%)	289	514	605
CV (%)	22	12	22
Mean	1098	2454	1609

AGBY= above ground biomass yield, GY= grain yield, HY= Haulms yield

Cost-benefit analysis

The partial budget analysis result (Table 5) indicated that the application of 500 g of FB-EM-11 ha-1 yielded the highest net benefit (ETB 76163 ha-1). The dominance analysis showed that except for the positive control, all inoculants were not dominant. That means all inoculants are economically feasible one after the other in the following descending order: FB-EM-11, FB-NS-03, FB-EM-07, FB-NS-02, FB-EM-02, and FB-EM-05. Since no beneficiary will prefer an alternative that gives lower net benefits than one with a higher net benefit and lower total variable costs, the dominated treatments, the positive control in this study, were eliminated from further economic analysis.

Table 4. Partial budget analysis of rhizobial isolates experiment on faba bean, 2019-2021

Treatment	GY (kg/ha)	Adj. yield -15% (kg/ha)	Gross benefit (Birr ha-1)	TVC (Birr ha - 1)	Net benefit (Birr ha-1)	Dominance (Birr ha-1)	MC (Birr ha-1)	MNB (Birr ha - 1)	MRR (%)
No inoculation	2160.1	1836.085	49574.295	0	49574.295				
FB-EM-05	2372.4	2016.54	54446.58	320	54126.58	ND	320	4552.285	1422.589
FB-EM-02	2557.1	2173.535	58685.445	320	58365.445	ND	320	8791.15	2747.234
FB-NS-02	2714.7	2307.495	62302.365	320	61982.365	ND	320	12408.07	3877.522
FB-EM-07	3131.3	2661.605	71863.335	320	71543.335	ND	320	21969.04	6865.325
FB-NS-03	3198	2718.3	73394.1	320	73074.1	ND	320	23499.81	7343.689
FB-EM-11	3332.6	2832.71	76483.17	320	76163.17	ND	320	26588.88	8309.023
18 kg N/ha	2411	2049.35	55332.45	1400	53932.45	D			

GY=grain yield, Adj= adjusted yield, TVC= total variable cost, MC=marginal cost, MNB=marginal net benefit, MRR= marginal rate of return, ND=none dominated D= dominated.

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The result from the MRR promises that for each ETB 1.00 investment in faba bean production using FB-EM-11, FB-NS-03, FB-EM-07, FB-NS-02, FB-EM-02, and FB-EM-05 inoculation on acidic soil, the producer can get an additional return of ETB 83, 73, 69, 39, 27.5, and 14, respectively. The minimum acceptable rate of return assumed in this experiment was 100%, and as a result, all strains were profitable options. However, in relative terms, inoculation of faba bean with FB-EM-11 gave the highest marginal rate of return (839%). Thus, this rhizobial isolate is the most promising candidate for extra confirmation in the farmers' field at diverse agro-ecologies to consider it a nominee for the preparation of marketable faba bean rhizobial inoculants in acid-vulnerable faba bean cultivating parts of Ethiopia.

CONCLUSIONS AND RECOMMENDATIONS

Based on the two consecutive main cropping season's field trial results on faba bean inoculation under acidic soil at Ejere district, isolates FB-EM-11, FB-NS-03, and FB-EM-07 were ranked first, second, and third regarding grain and commercial yields, respectively. Due to this reasonable superiority, the strains became the most likely isolates for the production of faba beans in such an acidic soil in Ethiopia. Consequently, it is proposed that additional authentication of the isolates should be done in replicated settings in wider soil and weather conditions.

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Conflict of Interest

The authors do not have any conflicts of interest.

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