



Productivity Improvement of Faba Bean (*Vicia faba* L.) through Elite Rhizobial Inoculants in the Central Highlands of Ethiopia

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Abstract

This study envisioned to assess the inoculation response of faba bean to six indigenous rhizobial isolates, 100 kg ha⁻¹ DAP as a positive control and non-inoculated negative control under farmers' field conditions at Welmera district, Ethiopia. The results revealed the presence of significantly different treatments ($p \leq 0.05$) in grain yield. The highest grain yields (3966 kg ha⁻¹ and 3694 kg ha⁻¹) were obtained from inoculation of rhizobial isolates (FB-AR-13 and FB-1018) during the 2019/20 and 2020/21 cropping seasons. Based on the two successive year's average grain yield response, FB-AR-13 (3966 kg ha⁻¹) became the first most performing isolate. The partial budget analysis results also showed that FB-AR-13 was the most promising isolate that showed a higher MRR of 64.22%, which is 43% higher than the MRR of the local standard check FB-1018. Therefore, isolate FB-AR-13 is the best promising candidate for the development of commercial faba bean rhizobial inoculant in the central high lands of Ethiopia after further verification over different farmers' fields at different agro-ecologies.



Article History

Received: 19 April 2021

Accepted: 27 May 2021

Keywords

Faba Bean;
Grain Yield;
Inoculant;
Strain.

Introduction

Faba bean (*Vicia faba* L.) is grown worldwide under different cropping systems as a dry grain (pulse), green grains/pods, and a green-manure legume. Faba bean is cultivated under rain fed and irrigated conditions and is distributed in more than 55 countries including Ethiopia. Faba bean is a multipurpose crop that contributes to the sustainability of cropping systems through its ability to contribute nitrogen (N) to the system by biologically fixing N₂; diversification

of production systems leading to decreased diseases, pests, and weed build-up, and potentially increased biodiversity; its capacity to reduce fossil energy consumption; providing food and feed rich in protein and carbohydrate (Jensen *et al.*, 2010).

Ethiopia ranks second in the production of faba bean 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 takes

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Doi: dx.doi.org/10.12944/CARJ.9.1.08

the largest share of area (466,698 hectares) and production (1006751.828 tones) of the pulses grown in Ethiopia (CSA, 2019/20).

It plays a key role in improving the livelihood of small holder farmers by serving as food, feed, and means of income and at the same time, it also plays a great role in improving soil fertility.

The average national yield of faba beans is about 2.2 t ha⁻¹ (CSA, 2019/20), which is very low compared to the average yield of 3.7 t ha⁻¹ in major producer countries (FAOSTAT, 2017). This low yield of faba bean production is mostly related to many biotic and abiotic factors like absence of adequate and effective indigenous rhizobia, frequent disease occurrence, parasitic weeds, lack of stress-tolerant high-yielding faba bean varieties, poor agronomic practices, soil acidity, and fertility decline could be mentioned (Getachew and Rezene, 2006; Endalkachew *et al.*, 2018).

Even though the productivity decline of faba bean due to the absence of adequate and effective indigenous rhizobia is strongly related to soil acidity, the natural distribution and number of the rhizobial strain also play their major role (Endalkachew *et al.*, 2018; Amarger, 2001). Rhizobia are very widespread as a result of natural distribution and legume cultivation. Despite this, there are still soils where strains of rhizobia specific for a legume crop are absent or are only present in low numbers (Amarger, 2001). Wherever a particular legume has long been established in an agricultural system or is considered a traditional crop, there will likely be an adequate number of indigenous rhizobia for

nodulation (Peoples *et al.*, 1995c). However, when a legume is grown for the first time in a particular soil, likely, compatible effective rhizobia will not be present. In such circumstances, the rhizobia must be introduced as inoculants (Graham, 2012).

Introduction of rhizobial inoculants also appropriates where soils harbor populations of rhizobia composed of a majority of strains which are symbiotically inactive with a particular legume (Moawad *et al.*, 1998; Deaker *et al.*, 2004) or when better-performing strains become available (Bosworth *et al.*, 1994). In connection with the point mentioned above, assessing the natural distribution and number of the rhizobia strains in faba bean growing area and selection of symbiotically most effective rhizobial inoculant at *in-vitro* condition would be the primary work. Hence, this specific study intended to evaluate how selected elite faba bean rhizobial isolates improve the productivity of faba bean in the central high lands of Ethiopia.

Materials and Methods

Nodule Sample Collection

The nodule samples were collected from major faba bean growing areas of the central and northern highlands of Ethiopia in late July of 2016/17. In the same year period, isolation and purification of the isolates were done at National Agricultural Biotechnology Research Center (NABRC) microbial biotechnology laboratory. Authentication of the isolates was also done at NABRC greenhouse in 2017/18. Nodule collection spots were geo-referenced. Aseptic precautions were exercised to avoid contamination of the samples until they reached the microbial laboratory.

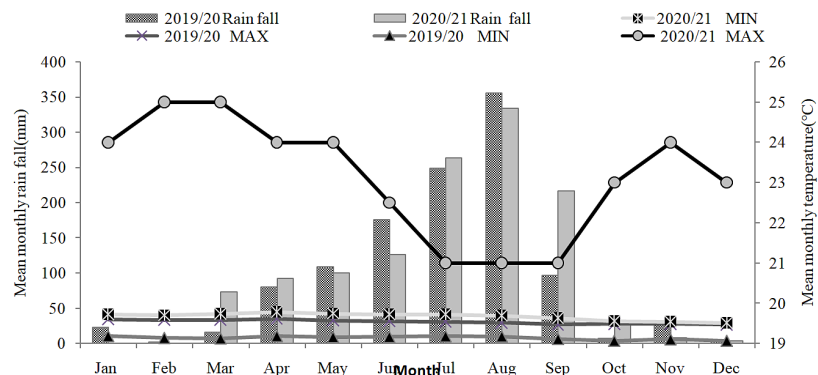


Fig.1: Mean monthly rainfall, and mean monthly maximum and minimum temperature patterns of the experimental sites (Source: Holetta Agricultural Research Center weather station).

Major characteristics of the Experimental Site

The field experiment was conducted at the farmers' fieldat Damotu (farmers' training center) and Robgebeya (on-farm) in the Welmera district of Ethiopia during the main cropping seasons of 2019/20 and 2020/21, respectively. These experimental sites did not have any inoculation history for the last five years. Damotu and Robgebeya are located at 090 03'53" N and 380 26'22.7" E, at an altitude of 2618 m, and at (90 7'54" N and 380 26'22" E, at an altitude of 2608 m above sea level, respectively. Both experimental sites were dominated by Nitisols, which are categorized under, moderately acidic to acidic characteristics (Table 1). The commonly grown crops in the experimental sites are wheat, barley, faba bean, field pea, and teff. The average minimum and maximum temperatures and rainfall of the experimental sites recorded during the implementation of the trial are indicated in Fig. 1.

Soil Sampling and Analysis

Composite soil samples were collected from random spots of the experimental plots at a depth of 0-20 cm before land preparation. The soil samples were air-dried and ground to pass through a 2 mm sieve. Soil pH and exchangeable acidity was measured in 1:2.5 soils to water ratio and L.P. Van Reeuwijk 1N KCl leaching-titration method, respectively. The Walkley and Black (1934) wet digestion method was used to determine soil organic carbon. Total nitrogen content of the soil was determined by the wet-digestion procedure of the Kjeldahl (1883) and available phosphorus was determined by the Bray-II extraction method.

Experimental Conditions

Six indigenous faba bean rhizobial isolates FB-EAR-05, FB-EAR-15, FB-AR-13, FB-AR-06, FB-AR-11 and FB-1018 (local standard check) were evaluated at Damotu and Robgebeya against 18 kg N ha⁻¹ (positive or standard) and untreated or absolute check (negative control).

The experiments were carried out in a randomized complete block design (RCBD) with three replications and a plot size of 4m x 3m. To reduce cross-contamination of isolates, the space between plots and blocks was enlarged to 0.5 and 1m, respectively. The space between plants and rows was 10 and 40 cm, respectively. Based on the result of soil exchangeable acidity, the experimental fields were

treated with a rate of 2.58 t ha⁻¹ limes (CaCO₃) 15 days before planting to amend the soil acidity to meet the survival and performance requirement of the isolates. Each of the experimental plots received a basal application of 20 kg P ha⁻¹ at the time of planting. The positive control received 18 kg N ha⁻¹ from diammonium phosphate (DAP). However, the negative control did not receive any form of external nitrogen source. The planting material was Tumsa variety planted at 200kg ha⁻¹. The experimental fields and experimental units were managed as per the recommended agronomic practices for faba beans.

Carrier-Based Rhizobial Inoculant Preparation and Seeds Dressing

Rhizobial isolates were prepared in carrier-based inoculants at the Microbial Biotechnology Laboratory of NABRC. The carrier material used for the study was powdered (able to pass through 106-micrometer mesh size) lignite adjusted to pH 7. One hundred twenty-five grams of lignite was transferred to heat-resistant white polyethylene bags, sealed and sterilized at 121°C for 30 minutes, and subsequently cooled. Then 25 ml of a quality broth culture of each rhizobial isolates (containing more than 10⁸ colony-forming units per milliliter of the broth culture) was aseptically inoculated to the already sterilized and cooled lignite, and homogenized in a laminar flow hood. Then, the inoculant was incubated at room temperature for curing in two weeks. 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 prevent the inoculants from direct sunlight exposure.

The carrier-based rhizobial inoculants were applied at a rate of 500 g ha⁻¹. About 0.2 kg of faba bean seed was weighed, moistened with sticker solution; 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 the shade. The fully-dressed and air-dried seeds were planted and immediately covered with soil.

Data Collection and Analysis

Biological and economic datasets were collected and analyzed to determine the best faba bean yield improving inoculants at Welmera district of Ethiopia. The agronomic datasets used to measure the actual

biological responses were above-ground biomass yield (AGBY), Haulm yield (HY), and grain yield (GY). The mean value of five representative plants per plot was considered to evaluate the effect of rhizobial isolates on the number of pods per plant and the number of seeds per pod (after converted into hectare base). The collected data were subjected to analysis of variance using the General Linear

Model Procedure of SAS statistical package version 9.3 (SAS Institute, 2002). Means were compared with Least Significance Difference (LSD) at a 5% probability level. To measure the monetary benefit of the intervention, farm gate prices of inputs and outputs were considered and marginal rate of return (% MRR) was computed for each treatment, and values ≥ 100 was set as profitable in absolute terms.

Table 1: Major chemical properties of the experimental sites before planting

Parameter	Damotu (Mean)	Robgebeya (Mean)	Range	Test Method
Total N (%)	0.16	0.18	0.12-0.24	Modified Kjeldhal
pH	4.86	4.90	4.8- 5.2	1:2.5 H ₂ O
Ex.Acidity (meq100g ⁻¹)	1.25	1.29	1.22-1.31	L.P. Van Reeuwijk 1N KCl leaching titration
Available P (ppm)	9.68	7.85	6.84-12.68	Bray II
OC (%)	1.34	1.32	1.24-1.48	Walkley and Black (1934)

Result and Discussion

Soil Test Result

As the result of Table 1 shows soil chemical properties uniformity was obtained among the experimental sites. The soil pH and exchangeable acidity of Damotu and Robgebeya trial sites were (4.86 and 4.9) and (1.25 and 1.29meq100g⁻¹ soil), respectively. Therefore, both sites can be grouped in the range of very strong pH status of the soil (Bruce and Rayment, 1982; Tekalign *et al.*, 1991).

The organic carbon and available phosphorus of the sites were 1.34 and 1.32%, and 9.68 and 7.85ppm, respectively. The values were found in low ranges for each site (Jones, 2002; Tekalign *et al.*, 1991; Charman *et al.*, 2007; Tekalign *et al.*, 1991). Moreover, the average total nitrogen contents of the sites were 0.16 and 0.18%; found in moderate ranges (Bruce and Rayment, 1982; Tekalign *et al.*, 1991), respectively.

Table 2: Response of faba bean to rhizobial inoculation at Damotu in 2019/20

Treatments	AGBY (kg ha ⁻¹)	GY (kg ha ⁻¹)	HY (kg ha ⁻¹)
FB-EAR-05	2386ab	485abc	109
FB-EAR-15	1990b	409c	120
FB-1018	3387ab	532a	106
FB-AR-06	2388ab	426c	120
FB-AR-11	2785ab	474abc	131
FB-AR-13	2908ab	422c	121
18 kg Nha ⁻¹	3726a	516ab	112
No inoculation	2521ab	436bc	109
LSD (5%)	1418	88	ns
CV (%)	29	11	27
Mean	2762	463	116

AGBY= above ground biomass yield, GY= grain yield, HY= Haulms, ns = none significant

Faba Bean Response to Inoculation at Damotu

As results of the study in Table 2 below indicate treatments showed significant statistical differences ($p \leq 0.05$) on above ground biomass and grain yields. Even if it scored a higher AGBY (3726 kg ha^{-1}), the positive control did not show any significant statistical differences with other treatments, except with FB-EAR-15. In the same manner, even though there was no significant statistical difference on GY among FB-1018, FB-EAR-05 and FB-AR-11, and the positive control, FB-1018 showed a higher GY (532 kg ha^{-1}).

On the contrary, FB-1018 showed significant statistical GY superiority over FB-EAR-15, FB-AR-06, FB-AR-13, and the negative control. Inoculant FB-1018 had 3 and 21% higher GY over positive and negative controls, respectively. Even though there were no significant statistical differences between treatments, FB-AR-11 scored a higher HY (131 kg ha^{-1}). This higher HY scored by FB-AR-11 was 17% and 21% higher than the HY recorded by both positive and negative controls; respectively.

The rhizobial isolates FB-1018 showed relative significant superiority ($p \leq 0.05$) over the other treatments on GY and AGBY except for the positive

control. This relative superior performance of the inoculant in Damotu soil condition is certainly attributed to their capability of availing high N to the host through BNF (Mitiku and Mnalku, 2003; Getachew *et al.*, 2019; And rade *et al.*, 2002). In line with these results, Mitiku and Mnalku (2003) and Youseif *et al.* (2017) reported that rhizobial strains inoculation and application of starter nitrogen to faba bean increased AGBY and GY significantly as compared to the un-inoculated and unfertilized control. Similar results were also obtained from inoculation of faba bean with indigenous rhizobial isolates in Eastern Ethiopia (Mitiku and Mnalku, 2003; Anteneh and Abere, 2017).

Faba Bean Response to Inoculation at Robgebeya

According to Table 3, none of the treatments showed any significant statistical differences ($p \leq 0.05$) in response to AGBY, GY, and HY. Even if no significant statistical difference was observed among treatments, FB-EAR-05, FB-AR-13, and FB-AR-06 showed higher AGBY (2923 kg ha^{-1}), GY (3639 kg ha^{-1}) and HY (4461 kg ha^{-1}), respectively. The higher GY, AGBY, and HY scored by FB-AR-13, FB-EAR-05 and FB-AR-06 were 2% and 19%, 44% and 8%, and 11% and 7% greater than the respective yields of positive and negative controls, respectively.

Table 3: Response of faba bean to rhizobial inoculation at Robgebeya in 2020/21

Treatments	AGBY (kg ha^{-1})	GY (kg ha^{-1})	HY (kg ha^{-1})
FB-EAR-05	2923	3074	3469
FB-EAR-15	2710	3245	4070
FB-1018	2257	3288	4445
FB-AR-06	2574	3616	4461
FB-AR-11	2832	3068	3809
FB-AR-13	2818	3639	3988
18 kg N ha^{-1}	2033	3579	4019
No inoculation	2698	3057	4181
LSD (5%)	ns	ns	ns
CV (%)	23	20	21
Mean	2606	3321	4055

AGBY= above ground biomass yield, GY= grain yield, HY= Haulms,
ns=none significant

The rhizobial isolates FB-AR-13, FB-EAR-05 and FB-AR-06 showed relative significant superiority

($p \leq 0.05$) as compared to the rest of the treatments, on GY, AGBY and HY. This relative superior

performance of the inoculants in Robgebeya soil condition is certainly credited to their capability of availing high N to the host through BNF (Getachew *et al.*, 2019; And rade *et al.*, 2002). Similar to the Damotu case, Mitiku and Mnalku (2003) and Youseif *et al.* (2017) reported that rhizobial strains inoculation and application of starter nitrogen to faba bean increased AGBY, GY and HY significantly as compared to the uninoculated and unfertilized control. Similar results were also obtained from inoculation of faba bean with indigenous rhizobial isolates in Eastern Ethiopia (Anteneh and Abere, 2017).

The Average Response of Faba Bean to Inoculation at Welmera District

As the two consecutive years, statistical analysis results in Table 4 showed, there were significant statistical differences ($p \leq 0.05$) among the treatments on GY. Rhizobial isolate FB-AR-13 showed superior performance on GY (3965 kg ha⁻¹).

At the same time, it attained the second higher score on AGBY (2863 kg ha⁻¹).

Even though there were no statistical differences between treatments, the positive control and isolate FB-AR-06 showed a higher AGBY (3148 kg ha⁻¹) and HY (2290 kg ha⁻¹). The higher GY (3965 kg ha⁻¹) scored by isolate FB-AR-13 was 5%, 30%, and 7% higher than the GY of the positive control (3785 kg ha⁻¹), negative control (3056 kg ha⁻¹) and the local standard check FB-1018 (3694 kg ha⁻¹), respectively. And the second higher AGBY (2863 kg ha⁻¹) obtained by this isolate was 10% higher than the AGBY of the negative control (2610 kg ha⁻¹). In general, the combined analysis confirmed that FB-AR-13 showed superior GY of faba bean at Welmera district as compared to the controls. Consequently, the above-mentioned elite indigenous faba bean rhizobial isolate is the best candidate for the central high lands of faba bean growing areas of Ethiopia in terms of biological yield.

Table 4: Response of faba bean to rhizobial inoculation at Welmera district in 2019/20-2020/21

Treatment	AGBY (kg ha ⁻¹)	GY (kg ha ⁻¹)	HY (kg ha ⁻¹)
FB-EAR-05	2655	3643ab	1789
FB-EAR-15	2350	3514ab	2108
FB-1018	2822	3694ab	2276
FB-AR-06	2481	3683ab	2300
FB-AR-11	2808	3607ab	1970
FB-AR-13	2863	3965a	2052
18 kg N ha ⁻¹	3148	3785ab	2058
No inoculation	2610	3056b	2145
LSD (P<0.05)	ns	812	ns
Year			
2019/20	2762a	3916a	119b
2020/21	2673b	3321b	4055a
LSD (P<0.05)	453	406	437
CV (%)	28	18	35
Mean	2717	3618	2087.3

AGBY= above ground biomass yield, GY= grain yield, HY= Haulms, ns=none significant

Cost-Benefit Analysis

The partial budget analysis results of Table 5 revealed that the highest net benefit (ETB 90688

ha⁻¹) was obtained from the application of FB-AR-13 @ 500 g ha⁻¹ at the Welmera district. The dominance analysis showed that no treatments were dominated.

The marginal rate of return of FB-AR-13 and FB-1018 was found to be 6422 % and 4476 %, respectively. Isolate FB-AR-13 was scored 43% higher MRR than that of the MRR of the local standard check;

FB-1018. The result of MRR also showed that except isolate FB-AR-13 the other non-dominated treatments were economically less feasible than the local standard check; FB-1018.

Table 5: Partial budget analysis of rhizobial isolates experiment on faba bean at Welmera, 2019- 2021

Treatment	GY (kg ha ⁻¹)	Adj. yield -15% (kg ha ⁻¹)	Gross benefit (Birr ha ⁻¹)	TVC (Birr ha ⁻¹)	Net benefit (Birr ha ⁻¹)	Domi- nance (Birr ha ⁻¹)	MC (Birr ha ⁻¹)	MNB (Birr ha ⁻¹)	MRR (%)
No inoculation	3056	2598	70137	0	70137				
FB-EAR-05	3643	3097	83614	320	83294	ND	320	13156	4111
FB-EAR-15	3514	2987	80637	320	80317	D			
FB-1018	3694	3140	84782	320	84462	ND	320	14324	4476
FB-AR-06	3683	3130	84516	320	84196	D			
FB-AR-11	3607	3066	82787	320	82467	D			
FB-AR-13	3965	3371	91008	320	90688	ND	320	20551	6422
18kg Nha ⁻¹	3785	3217	86861	1400	85461	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.

This MRR result promises that for each ETB 1.00 investment in faba bean production the producer can get ETB 1.00 and additional ETB 64.22 and ETB 44.8, respectively. Since the minimum acceptable rate of return assumed in this experiment was 100% therefore all strains had cost-effective options regardless of their economic feasibility. However, in relative terms, inoculation of faba bean with FB-AR-13 at Welmera district gave the highest marginal rate of return (6422%). Therefore, faba bean producers on the central highlands of Ethiopia can go for the application of FB-AR-13 rhizobial strain.

Conclusion and Recommendations

Based on the consecutive two main cropping seasons' field trial results on faba bean inoculation at Welmera district, due to their reasonable superiority in biological and economic yield FB-AR-13 became the best promising isolate for the production of faba bean in central highlands of Ethiopia. The analytical results of the soil were found to be sub-optimal for the production of faba bean except for phosphorus. This confirms that producing fababeans using rhizobial

isolate FB-AR-13 at Welmera district along with 46 kg P₂O₅ on such soil condition is reasonable for feiting in terms of yield and yield benefit. Therefore, it is suggested that further verification of the isolates should be carried out in replicated conditions in similar soil and weather conditions.

Acknowledgments

The Holetta Biological and Organic Soil Fertility Management Research teams' sincere acknowledgment goes to the Ethiopian Institute of Agricultural Research (EIAR) for funding this activity. The team also wants to express thanks to all the technical and field assistants of the program for their spectacular contribution in conducting the microbiological laboratory tasks, managing the experimental fields, and data collection.

Funding

The author(s) received no financial support for publication of this article.

Conflict of Interest

No conflict of interest.

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