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Evaluation of the Effects of Rhizobia on Nodulation, Yield and Yield Components of Faba Bean

Samrawit Mebrahtu [a*]

[a] *Department of Soil Fertility Research, Tigray Agricultural Research Institute, Shire Soil Research Centre, P.O. Box 40, Shire, Tigray, Ethiopia.*

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Leguminous plants are able to establish nitrogen-fixing symbiosis with certain Gram-negative bacteria, collectively known as rhizobia. Rhizobia are indeed highly efficient bacterial symbionts in legumes, playing a crucial role in biological nitrogen fixation (BNF). However, in the Tahtay Maychew area in the Tigray region of Ethiopia, no research has been done on the inoculation of grain legumes with rhizobium bacteria and the addition of phosphorus fertilizer. Therefore, during the 2015 rainy season, a study was carried out to investigate the effects of phosphorus fertilizer and rhizobium strains on the phenological parameters, nodulation, growth, yield, and yield-components of faba beans. A randomized complete block design with three replications was used to set up seven treatments: uninoculated, Strain1035, Strain1018, Strain EAL110, Strain1035 + 50 kg DAP ha^{-1}, Strain1018 + 50 kg DAP ha^{-1}, and Strain EAL110 + 50 kg DAP ha^{-1}.The data analysis showed that, almost in comparison to the other treatments, the combined effects of EAL110 and

**Corresponding author: E-mail: samrimeb19@gmail.com;*

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50 kg ha^{-1} DAP fertilizer produced a considerably larger difference in effective nodule number per plant, plant height, pod number per plant, and grain yield. The combination of 50 kg ha^{-1} DAP fertilizer with faba bean EAL110 strain increased grain yield by 52.17% compared to the control treatment. Hence, EAL110 and 50 kg ha^{-1} DAP fertilizer are recommended for use in the study area and other similar locations.

Keywords: Phosphorus fertilizer; faba bean; Rhizobium bacteria.

1. INTRODUCTION

The faba bean (*Vicia faba* L.) is a significant legume crop cultivated globally for food and forage [1]. This legume is highly valued for its substantial nutritional content, notably its high levels of protein and starch [2]. Beyond its nutritional benefits, the faba bean also contributes to sustainable agriculture through its ability to fix atmospheric nitrogen, converting it into forms accessible to plants. This process of biological nitrogen fixation enhances soil nitrogen levels, leading to increased shoot growth, a greater number of pods, and improved bean yields, which benefits overall nitrogen cycling and crop productivity [3].

"Leguminous plants are able to establish nitrogen-fixing symbiosis with certain Gram-negative bacteria, collectively known as rhizobia. In the *Rhizobium*–legume symbiosis, the process of nitrogen fixation is strongly related to the physiological state of the host plant. Therefore, an efficient *rhizobial* strain is not expected to express its full capacity for nitrogen fixation if limiting factors impose limitations on the vigor of the host legume" [4]. "Several environmental conditions are the limiting factors to the growth and activity of nitrogen-fixing plants. Typical environmental stresses faced by the legume nodules and the symbiotic partners may include water stress, salinity, soil pH, temperature, heavy metals, and so on" [5].

Rhizobia are indeed highly efficient bacterial symbionts in legumes, playing a crucial role in biological nitrogen fixation (BNF). They metabolize atmospheric nitrogen and convert it into forms usable by plants, housed within nodules where leghaemoglobin maintains the aerobic environment necessary for this process. In return, Rhizobia benefit from the carbon compounds provided by the host plant's photosynthesis. In agriculture, Rhizobia-legume symbiosis is responsible for around 80% of biologically fixed nitrogen, especially involving the Rhizobiaceae family [6]. This fixed nitrogen is advantageous not only for the immediate host plant but also for subsequent crops, as it enriches soil nitrogen content. Furthermore, Rhizobia can function as plant growth-promoting bacteria (PGPB) for certain non-leguminous crops such as rice and wheat, where they act as endophytes, offering growth-promoting benefits, as supported by studies like those of Biswas et al. [7].

"For all these reasons, the *Rhizobium*-legume symbiosis has been widely studied as a model of mutualistic associations and as a beneficial association for sustainable agriculture. With increasing use of *Rhizobium* and other beneficial microbes as bio fertilizers, reduction in the need for chemical fertilizers can be observed. Therefore, bio fertilization has great importance in decreasing environmental pollution and deterioration of nature" (Vessey, 2006, Erman et al., 2011). Rhizobia are among the most efficient bacterial symbionts in legumes, playing a key role in biological nitrogen fixation (BNF). Through this process, Rhizobia metabolize atmospheric nitrogen, converting it into a plant-usable form within specialized structures called nodules, where the aerobic conditions are controlled by leghaemoglobin. In return, Rhizobia receive carbon compounds from the plant's photosynthesis [8].

"In agriculture, it's estimated that approximately 80% of biologically fixed nitrogen originates from Rhizobia-legume symbiosis, particularly involving the Rhizobiaceae family. This symbiosis benefits not only the host legume crops but also subsequent crops planted in the same soil by increasing nitrogen availability. Additionally, Rhizobia can function as plant growth-promoting bacteria (PGPB) for non-leguminous crops like rice and wheat, where they act as endophytes, providing growth benefits as noted in studies" (e.g., [7]). The objective of this study is therefore to evaluate the effect of rhizobial inoculants on the nodulation and yield and yield components of faba bean.

2. MATERIALS AND METHODS

2.1 Description of Study Area

The field experiment was carried out in the 2015 growing season at the experimental sites of

Kewanit Tabia, Tahtaey Maichew district, Tigray, Ethiopia (Fig. 1). The altitude of the area is 2200 meter above sea level. The study area is located at distance of 17 km west of Axum historical town. The agro-ecology is sub humid where most of the middle altitude crops such as tef, wheat, faba bean are commonly grown. The area is characterized by monomodal rainfall pattern that the main rainy season, locally called *kiremty,* extends from July to end of August. The annual rainfall received by the experimental site during the main cropping season was 613.92 mm. The mean average annual minimum and maximum temperature was 12.16°C and 26.78°C, respectively.

2.2 Experimental Design and Treatments

The experiment was arranged in a randomized complete block design with three replicates. Seven treatment levels were tested: Control, Strain 1035, Strain 1018, Strain EAL110, Strain 1035 + 50 kg DAP ha^{-1}, Strain 1018 + 50 kg DAP ha^{-1}, and Strain EAL110 + 50 kg DAP ha^{-1}, each replicated across three different locations. The plot size was 4 m x 3 m, with spacing of 1 m between blocks, 0.5 m between plots, and 40 cm between rows. The seed rate was 250 kg ha^{-1} using the variety *Wolkit*. Inoculants were sourced from the soil microbiology lab at the Holeta Agricultural Research Center, and seeds were soaked in warm water to facilitate attachment to the Rhizobium strains. After air drying briefly, the inoculated seeds were planted in shaded conditions. Non-inoculated seeds were sown first to prevent cross-contamination. DAP fertilizer was applied at the time of planting by placing faba bean seeds in rows. Furrows were created between plots to minimize nutrient and bacterial leaching between plots or from external sources.

2.3 Data Collection Procedures

2.3.1 Phenological traits

Days to 50% flowering was calculated as the number of days after planting once 50% of the faba bean plants in each experimental plot had extruded flowers. The number of days after planting that 90% of the faba bean plants in each planting plot had yellowed leaves was used to calculate the days to 90% physiological maturity.

2.3.2 Nodulation

Nodulation sampling was conducted by excavating the roots of five randomly selected faba bean plants from each plot at the mid-flowering stage. Using destructive sampling, five plants were uprooted from each plot, and the number of nodules per plant was counted to assess effective nodulation. A hoe was used to loosen the soil around the roots, and a shovel was employed to dig approximately 20 cm deep, extending 12 cm from the central stem to encompass the entire root system. The roots were then cleaned with a washing bottle to remove the surrounding soil. The number of nodules per plant was recorded, and the average nodule count was calculated from the five sampled plants. Nodules with a pink color were classified as effective, while green nodules were considered non-effective.

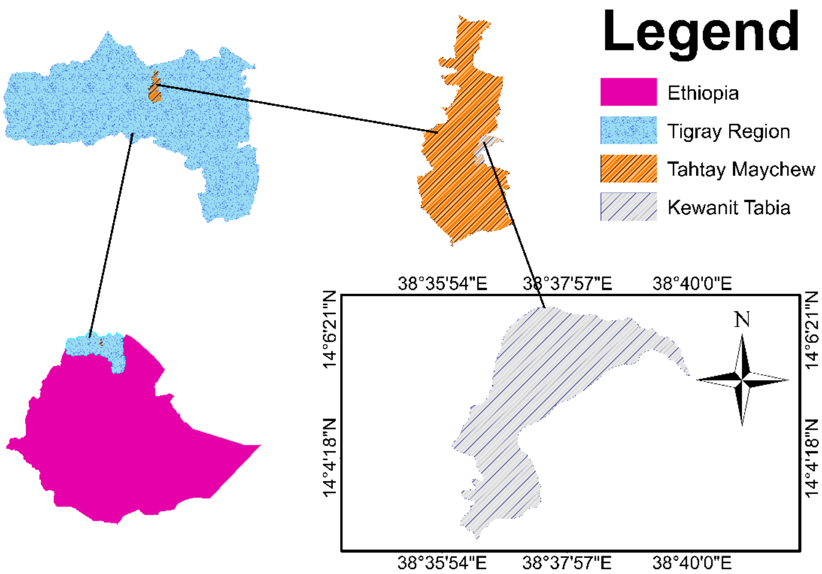

Fig. 1. Location of the study area

Table 1. Before planting soil physiochemical properties of the experimental sites.

Soil properties	Experimental sites		
	Site 1	**Site 2**	**Site 3**
% Clay	27	41	25
% Silt	47	29	49
% Sand	26	30	26
Textural class	**Loam**	**Clay**	**Loam**
pH	6.68	6.31	6.27
EC (dS m^{-1})	0.63	0.75	0.44
OC (%)	0.984	0.984	0.703
Ava. P (ppm)	23.48	46.21	20.30
CEC (cmol (+) kg^{-1})	40.8	43.6	36

2.3.3 Plant height

Five plants from central rows were randomly selected for measuring their height at physiological maturity using measuring tape. The average height of five plants was taken from each plot and was considered as plant height.

2.3.4 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 number of pods per plant.

2.3.5 Grain yield

At physiological maturity, plants from middle rows were manually harvested close to the ground surface. The harvested plant biomass was sun-dried in an open air, and then threshed in order to separated grain yield of each plot from the total biomass. Finally, grain yield per plot was converted to hectare basis grain yield.

2.3.6 Soil physico-chemical analysis

Composite soil samples were analyzed for various physico-chemical properties, including texture, electrical conductivity, pH, organic carbon, cation exchange capacity (CEC), and available phosphorus (P), before planting. The soil pH was measured using the potentiometric method with a soil-to-water ratio of 1:2, following Van Reewijk LP [9]. CEC was determined using the 1 M ammonium acetate method at pH 7, as per Chapman HD, [10], while organic carbon was assessed through the dichromate oxidation method [11]. Available P was analyzed using the Olsen method, as described by Olsen SR et al., [12].

2.4 Statistical Analysis

The data were analyzed using analysis of variance (ANOVA) following standard procedures, utilizing SAS (16th edition) software for the analysis. After conducting the ANOVA, the treatment means were compared, and differences were separated using the Least Significant Difference (LSD) test at a 5% probability level

3. RESULTS AND DISCUSSION

3.1 Phenological Attributes

The application of either a single strain or a combination of DAP and Rhizobial strains did not significantly impact the flowering and maturity of faba beans (Table 2). This is explained by the natural tendency of legumes to produce enough nitrogen through biological N fixation to start flowering and maturing [13]. Plant species' genetic makeup and ability to respond to environmental stressors have a greater influence on phenological parameters than management techniques (Souri et al., 2019), [14]. For plants to germinate and attain flowering and maturity within their typical periods, minimum requirements for growth are required [15].

3.2 Plant Height

According to Table 2's mean results, faba bean plant height is considerably increased by Rhizobium inoculation in combination with 50 kg DAP ha^{-1}. The faba bean plots inoculated with Strain EAL110 plus 50 kg DAP ha^{-1} exhibited the tallest plants, measuring 151.78 cm, indicating a significant increase in height when compared to the control treatment (102.84 cm). This demonstrates the benefits of inoculating faba beans with robust Rhizobium strains, especially when done in conjunction with DAP fertilizer, which probably improves the plants' ability to fix and use nitrogen. This effect is due to the increased nitrogen supply caused by Rhizobium inoculation, which encourages overall plant development. Nitrogen is a crucial nutrient for plant development since it is necessary for cell division and elongation, both of which lead to increased plant height. Bejandi et al. [16] and Gedamu et al. [17] discovered that Rhizobium seed inoculation significantly increases nitrogen uptake, enhancing plant growth and yield, which supports present findings. The increased availability of nitrogen in the infected plants most likely promoted better vegetative growth, enabling them to grow higher and possibly boosting their total yield. The results suggest that inoculation with Rhizobium strains is a practical and effective way to improve faba bean development, especially when paired with phosphorus fertilization.

3.3 Effective Nodule Number

The rhizobium strain had a significant ($P \leq 0.05$) impact on the effective nodule number (Table 2). The combination of applying 50 kg ha^{-1} DAP fertilizer and inoculating faba beans with strain EAL 110 produced the highest effective nodule number (174.84/plant); however, this was not statistically significant when applying 50 kg ha^{-1} DAP fertilizer and inoculating faba beans with strain 1018. The control plots, which were not given either inoculants or DAP fertilizer, had the lowest nodule number (89.51/plant). This illustrates how nodulation can be improved by inoculating faba beans with robust Rhizobium strains. This result is consistent with earlier study by El-Khateeb et al. [18], Desta et al. [19], Woldekiros et al. [20], and Gedamu et al. [17], all of whom reported a significant increase in nodules following faba bean inoculation with Rhizobium strains. It implies that by enhancing nitrogen fixation, selected strains may have great promise for faba bean yield enhancement in the study location.

3.4 Pod Number

Table 2 indicates that the tested treatment had a significant effect on the number of pods per plant. As a result, the combination of applying 50 kg DAP ha^{-1} and inoculating faba beans with Strain EAL 110 produced the greatest number of pods per plant (19.89). The plots that were not provided with DAP or strain inoculation, however, had the fewest pods per plant (9.64). The research by Desta et al. [19] and Gedamu et al. [17] revealed that rhizobial strain could considerably enhance the number of pods per plants, which is consistent with the current findings. This can be attributed to enhanced nitrogen fixation from the inoculated rhizobia, which supports better plant growth and reproductive development. The present study, however, runs contrary to the findings of Zerihun and Abera, [21], who found that neither fertilizer rates nor rhizobia inoculation significantly affected the number of seeds per pod. This disparity may result from variations in the conditions for the study, including soil fertility, the variety of faba beans used, or the strains of Rhizobium employed. Considering these variations, the current study shows that inoculating faba beans with Rhizobium, especially in conjunction with DAP, has an obvious benefit in terms of enhanced pod development and overall yield.

Table 2. Days to 50% flowering (DF), days to 90% physiological maturity (DM), plant height (PH), number of nodules per plant (NNP), number of pods per plant (NPP), and grain yield of faba bean as influenced by different Rhizobial inoculants

Treatments	DF	DM	NNP	PH (cm)	NPP	GY (kg ha^{-1})
Control (Un Inoculated)	48.89	104.44	89.51^{e}	102.84^{c}	9.64^{d}	2371.3^{d}
Strain 1035	48.33	104.78	108.73^{de}	111.82^{c}	13.80^{c}	2868.1^{bc}
Strain 1018	48.44	105.11	125.69^{cd}	125.93^{b}	13.87^{c}	2963.0^{bc}
Strain EAL110	48.44	106.11	134.18^{bc}	128.18^{b}	16.31^{cb}	3137.0^{b}
Strain 1035 + 50 kg DAP/ha	48.33	105.11	128.44^{bcd}	128.24^{b}	15.11^{cb}	2719.4^{cd}
Strain 1018 + 50 kg DAP/ha	48.78	105.22	150.67^{ab}	136.36^{b}	16.42^{b}	3069.4^{bc}
Strain EAL110 + 50 kg DAP/ha	48.78	105.11	174.84^{a}	151.78^{a}	19.89^{a}	3608.3^{a}
Mean	48.57	105.13	130.30	126.45	15.01	2962.37
LSD (0.05)	NS	NS	24.63	10.81	2.5	413.71
CV (%)	2.25	1.69	13.07	5.91	11.60	9.66

LSD- least significant difference, CV- coefficient variance, NS- non-significant.

3.5 Grain Yield

The inoculation of strains has a considerable impact on the grain production of faba beans. Table 2 indicates that the inoculation of EAL110 in combination with 50 kg DAP ha^{-1} produced the highest grain production (3608.3 kg ha^{-1}), while the control produced the lowest grain yield. According to the results, applying the faba bean rhizobium strain EAL110 along with 50 kg DAP ha^{-1} increased grain yield by 52.17% compared to plots that did not receive phosphate fertilizer or strain inoculants. This result is consistent with research by Rugheim and Abdelgani [22], Genetu et al. [23], and Gedamu et al. [17] that showed a notable increase in faba bean grain production following rhizobial strain inoculation. In a comparable way, Desta et al. [19] found that applying an efficient rhizobium strain either alone or in conjunction with zinc greatly enhanced faba bean grain yields. Contrary to recent findings, Zerihun and Abera [21] discovered that rhizobium inoculation did not increase faba bean grain yield than the control. Increases in grain production brought about by strain inoculation showed that nitrogen in the soil is a limiting factor and that soil existing rhizobium bacteria might not be able to provide nitrogen through biological nitrogen fixing. Therefore, strain inoculation can significantly increase faba bean grain production.

4. CONCLUSION

Rhizobium inoculation had a significant impact on effective nodule, plant height, pod number of the faba bean, leading to improved grain yield. Inoculating legumes with rhizobia can help maintain soil health by improving nitrogen fixation. This can help prevent the soil structure from deteriorating and reduce the amount of nutrients and water the soil can hold . Using chemical fertilizers can damage the soil and its fauna and flora. Biological nitrogen fixation can help reduce this damage while still increasing crop production and minimizing environmental damage The rhizobia strains tested, especially when combined with DAP fertilizer, were effective in both nodule formation and yield enhancement, and the same time proving ecologically suitable and symbiotically beneficial. Thus, the faba bean strain110 combined with DAP fertilizer is recommended for use in the study area and similar agroecological regions.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author hereby declare that NO generative AI technologies such have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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