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Evaluation of Effective Rhizobia Nodulating Faba bean in Western Amhara, Moretna Jiru District

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ABSTRACT

This on farm experiment was conducted at Mortina Jiru district, Amhara Region, Ethiopia during the main rainy seasons of 2016/17 and 2017/18. The objective of the study was to evaluate the effectiveness of different commercial and new rhizobia strains and to evaluate the effect phosphorus on nodulation and grain yield of faba bean in the area. The experiment comprised of three Rhizobial strain (EAL 110, 1035 and 1018), one Phosphorus level (10 kg P ha) and control (with-out inoculation and P application) which were laid out in randomized block design with three replications. The analysis of variance showed that except nodule size in 2017/18, all the tested nodulation parameters were not affected by the treatment. Plant height which was obtained from both years was significantly affected by the treatment. In 2016/17, significantly the highest plant height (86.4 cm) was observed from combined application of EAL 110 strain with 10 kg P ha^{-1}. The lowest plant height (78.4 cm) was observed with inoculation of the seed of faba bean with strain 1018. In 2016/17 the highest plant height was observed with sole application of strain 1035. Number of pod per plant also responded for the treatment. In 2016/17, significantly the highest number of pod per plant (21.4) was observed with combined application of strain EAL 110. In 2017/18, significantly the highest number of pod per plant (26.8) was observed with combined application of EAL 110 with 10 kg P. The analysis of variance also showed that, Seed yield exhibited a significant response to the treatment. In 2016/7, significantly the highest seed yield (2537 kg ha^{-1}) was observed with combined application of EAL 110 with 50 kg ha^{-1}. The lowest seed yield (1931.6 kg ha^{-1}) was observed from the control plot. In 2017/18, significantly the highest seed yield was observed with inoculation of the seed of faba bean with strain EAL 110 alone. Hence, Rhizobium inoculation with strain EAL with 10 kg P ha^{-1} could be recommended for faba bean production at the experimental locations in Mortina Jiru district.

Keywords : Faba bean, Rhizobium Inoculation, Nodulation, Yield

I. INTRODUCTION

Faba bean (Vicia fabae L.) is a major cool season food legume occupies about 34% of the total cultivated land from pulses in Ethiopia (CSA, 2012). Amhara and Oromia are the two areas in Ethiopia where production of faba bean is highest. Those two regions account for 85% of the national faba bean production (IFPRI, 2010b). Faba bean is grown in the main season, on both red and black soils.

The crop has been an important protein source for human diet. The straw of the crop is also used as animal food. Faba bean is a legume capable of fixing nitrogen by forming association with root nodulating bacteria group called *Rhizobium* leguminosarum biovar vicieae. As a result it improves fertility status of the soil and make N for subsequent crops (Ammanuel *et al.*, 2000;

Habtegebriel *et al.*, 2007). Some report indicated faba bean derive the highest percentage of N from the atmosphere (Hardarson *et al.*, 1991) and the amount of nitrogen fixed by faba bean have been 240-325 kg ha^{-1} (Somasegaran and Hoben, 1994).

Even though faba bean is such importance in Ethiopia, the national yield has remained low and According to Central Statistics Agency of Ethiopia 2012/13, the national average yield of faba bean is 1.5 tones ha-1 (CSA, 2013). Several biotic and abiotic factors contributed to low productivity of the crop. The major biotic factor includes poor soil fertility and low existence of effective indigenous rhizoba population in the area (Carter *et al.*, 1998). Application of chemical fertilizer, particularly phosphorus is needed to improve the production of the crop (Otieno *et al.*, 2009). External seed inoculation of rhizobia is also one of another practices to increase the nitrogen fixation potential and hence the yield of the crops especially in areas where low population of effective indigenous rhizobia or due to higher competitions with non-effective ones (Tolera *et al.*, 2009). The objective of this study was, therefore, to evaluate the effectiveness of different commercial and new rhizobia strains and to evaluate the effect phosphorus on nodulation and grain yield of faba bean in the area.

II. METHODS AND MATERIAL

A. Description of the study area

This on farm experiment was conducted at Mortina Jiru district, Amhara Region, Ethiopia during the main rainy seasons of 2017 and 2018. The average annual rainfall from the nearby metrological station (Enewari) is 899.01 mm having mean minimum and maximum temperature of 21.39 and 9.09°C respectively. Vertisols are the dominant soil type in the areas. The crops widely grown in the study area include wheat, Tef, faba bean and lentil, whereas chickpea, grass pea and others have low area coverage and mostly grow on residual soil moisture at the end of the rainy season.

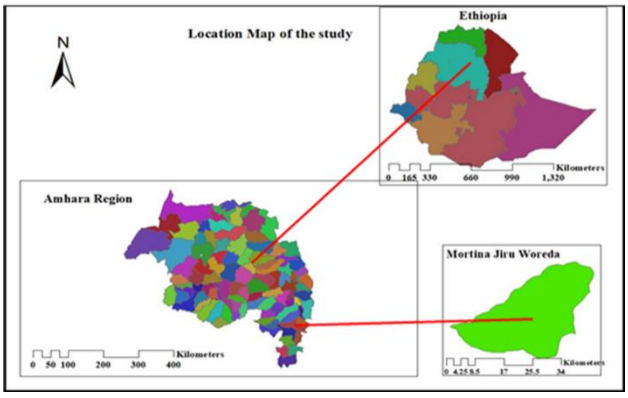

Figure 1. Location Map of the study area

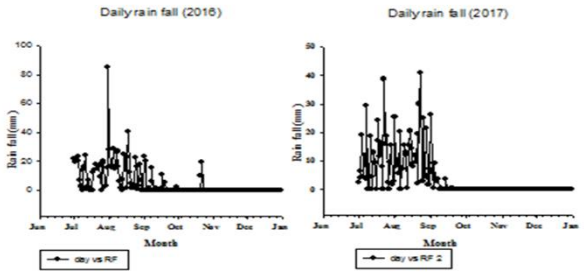

Figure 2. Daily rain fall distribution during the experiment (planting to late pod setting stage)

B. Experimental detail

Soil sample collection and processing

Before starting the experiment, initial composite soil samples were collected from the experimental plots and analyzed for texture, pH, Av.P, OC and TN. Soil particle size distribution was determined by hydrometer method (Bouyoucos, 1951). Soil pH was measured with digital pH meter potentiometerically in supernatant suspension of 1:2.5 soils to distilled water ratio (Van Reeuwijk, 1992). Organic carbon (OC) was determined by the dichromate oxidation method (Walkley and Black, 1934). Total N in the soil was measured by the micro kjeldhal method (Jackson, 1958). Available P was analyzed by Olsen method (Olsen et al., 1954) colorimetrically by the ascorbic acid- molybdate blue method (Watanabe and Olsen, 1965).

Experimental Design and Treatments

The experiment comprised of seven treatments with three rhizobia strains (EAL 110, strain 1035 and strain 1018) and combination of those strain with chemical fertilizer (10 kg P ha^{-1}). The experiment was laid out in randomized block design with three replications. The plot size was 3.6 m x 3m.

Treatments:

1. Control (No strain and fertilizer)
2. Strain 1035
3. Strain 1018
4. Strain EAL110
5. Strain 1035 + 10 kg P ha^{-1}
6. Strain 1018 + 10 kg P ha^{-1}
7. Strain EAL110 + 10 kg P ha^{-1}

Source of Rhizobial Isolates

Rhizobium strain EAL 110 was obtained from Holeta Agricultural research Center while two commercially available Rhizobium strain (1035 and 1018) was obtained from MBI (Menagesha Biotechnology Industry).

Source of Improved Seeds

The Faba bean variety "Dagem" was used as test variety. The variety was selected based on the recommendation of Debra Birhan Agricultural Research Center.

Method of Seed Inoculation

Seed inoculation was performed before sowing using the procedure developed by Fatima et al. (2007). To ensure the sticking of the applied inoculant to the seeds, the required quantity of seed was suspended in 1:1 ratio in 10% sugar solution. The inoculant was gently mixed with dry seeds at the rate of 10 g per kg of seed. Inoculation was done just before sowing under shade to maintain the viability of cells and allow to air dry for a few minutes and then the inoculated seeds were sown at recommended rate and spacing to the respective plots. To avoid contamination, plots with un-inoculated seeds were planted first followed by the inoculated ones.

Data Collection

Data Collected at Late Flowering Stage

Sampling for nodulation was performed by excavating the roots of plants randomly from two rows next to boarder rows of each plot at the mid flowering stage of the crop. The plants from each plot were used to record number of effective nodule, nodule size and nodule volume.

Data Collected at Early Pod Setting Stage and after harvesting

At early pod setting and after harvesting plant height, number of pod per plant, seed yield, straw yield and 1000 seed weight was determined

Other agronomic Management

Disease and pest control: (Redomil) and (Caratin) to control faba bean gall disease (kormed) and boll worm.

Data analysis

The collected data were subjected to analyses of variance (ANOVA) on the selected parameters using SAS 9.1 statistical software. Where ever the treatment effects were significant, mean separation were made using the least significance (LSD) test at 5% level of probability (Gomez, 1984).

III. RESULTS AND DISCUSSION

A. Selected Physico-chemical Properties of the Soils of the Study Sites

Texture

Soil texture is one of the inherent soil properties less affected by management and which determines nutrient status, organic matter content, air circulation and water holding capacity of a given soil. Based on the soil analysis made, the soil texture of the entire sites was clay. This soil is characterized by high water holding capacity and CEC.

pH of Soil

The results of the selected soil physical and chemical properties are presented in Table1. The pH of the experimental soils ranged from 6.4 to 6.9. According to Tekalign (1991) all sites are rated as neutral.

Organic Matter

According to Tekalign (1991), the entire site had low OM content (Table 1) and ranged from 0.55 to 1.38. This is because of continuous cultivation without returning residue to the soil.

Total N

Nitrogen (N) is the fourth plant nutrient taken up by plants in greatest quantity next to C, O and H, but it is one of the most deficient elements in the tropics for crop production (Mesfin, 1998). It has been observed in Table 1, that total N in the study sites varied from 0.06% to 0.14% with a mean value of 0.25%. Based on Tekalign (1991), total nitrogen content of all sites was low (Table 1). This indicates external source of N is mandatory for plant growth.

Extractable P

Olsen extractable P content of the soil in the experimental sites ranged from 3.96 to 12.66 mg kg-1 with a mean value of 9.47 mg kg-1 (Table 1). According to Landon (1991), the available P was rated as low for sites.

Table 1. Selected Physico-chemical properties of the site

Parameters	Before planting				
	Sites				
	1+	2+	3+	4*	5*
pH (1:2.5 H_2O)	6.6	6.61	6.4	6.65	6.9
Organic Carbon (%)	0.7	0.8	0.54	0.55	0.32
Organic matter (%)	1.2	1.38	0.93	0.95	0.55
Total N (%)	0.08	0.06	0.14	0.12	0.09
Av. P (mg kg^{-1})	3.9	10.4	8.30	12.7	12.1
Sand (%)	10	10	12	10	6
Silt (%)	30	26	18	12	20
Clay (%)	60	64	70	78	74
Textural class	Clay	Clay	Clay	Clay	Clay

NB; +=indicates farmers field on which the experiment was conducted in 2017, indicates farmers field on which the experiment was conducted in 2018

B. Nodule position

Nodule position is one of the most important parameters in assessing the performance of nodules in accordance with their ability to fix atmospheric nitrogen. In Table 2, the nodule position which was obtained from both years was not significantly influenced by the treatments.

C. Number of effective nodule

Many authors have reported that legume nodules having dark pink or red colors due to presence of leghemoglobin are an indication for effectiveness of the rhizobial strains used, which is well correlated with nitrogen fixation (Adjei and Chambeiss, 2002; Butler and Evers, 2004). In Table 2, number of effective nodule was not significantly influenced by the treatment. Moreover, the number of effective nodule observed in the inoculated and un-inoculated plot was comparable to each other indicating the non-effectiveness of inoculated rhizobia over the native rhizobia (Table 2).

Table 2. Noulation parameters of faba bean as affected by different strain of Rhizobium and inorganic P fertilizer

Treatment	NP		ENN	
	2017	**2018**	**2017**	**2018**
Control	2.7	2.7	8.6	8.6
St.1035	2.6	2.6	12.4	12.4
St.1018	2.5	2.5	10.1	10.1
St. EAL 110	2.4	2.4	10.8	10.8
St.1035+ 10 kg P	2.6	2.6	10.5	10.5
St.1018+ 10 kg P	2.4	2.4	11.9	11.9
St. EAL 110+ 10 kg P	2.5	2.5	12.2	12.2
CV(%)	**12.56**	**12.56**	**22.61**	**22.61**
$LSD_{0.05}$	**ns**	**ns**	**ns**	**ns**

Note: Means with the same letter are not significantly different at P>0.05 level of probability following LSD, st.= strain, NP= Nodule Position, ENN=effective Nodule Number

D. Nodule Volume per Plant

In Table 3, the nodule volume which was obtained from both years was not significantly influenced by either sole application of different strains of Rhizobium or combination of different Rhizobium with inorganic fertilizer.

Table 3. Nodule volume as affected by Rhizobium inoculation, S and Zn fertilizer rates

Treatment	NV	
	2017	**2018**
Control	0.92	0.79
St.1035	0.92	0.88
St.1018	0.92	1.00
St. EAL 110	0.93	0.81
St.1035+ 10 kg P	0.93	0.98
St.1018+ 10 kg P	0.92	0.88
St. EAL 110+ 10 kg P	0.95	0.85
CV(%)	**2.58**	**12.58**
$LSD_{0.05}$	**ns**	**ns**

Note: Means with the same letter are not significantly different at P>0.05 level of probability following LSD, st.= strain, NV= Nodule Volume

E. Plant height

Plant height which was obtained from both years was significantly affected by the treatment. In 2017, significantly the highest plant height (86.4 cm) was observed from combined application of EAL 110 strain with 10 kg P ha^{-1} (Table 4). The lowest plant height (78.4 cm) was observed with inoculation of the seed of faba bean with strain 1018. In 2017 the highest plant height was observed with sole application of strain 1035. But this treatment was statically as par with sole application of strain 1018, combined application of strain 1035 with 10 kg P ha^{-1} and combined application of strain 1018 with 10 kg P ha^{-1}. The lowest plant height (72.5 cm) was observed from the control. Similar result also concluded by Sameh et al (2017).

F. Number of pod per plant

Number of pod per plant also responded for the treatment. In 2017, significantly the highest number if pod per plant (21.4) was observed with combined application of strain EAL 110. But this treatment is statically as par with the control (Table 4). The lowest number of pod per plant was observed with inoculation of the seed of faba bean with strain 1018. In 2018, significantly the highest number of pod per plant (26.8) was observed with combined application of EAL 110 with 10 kg P (Table 4).

Table 4. Growth and yield related parameters of faba bean as affected by different strain of Rhizobium and inorganic P fertilizer

Treatment	PH		NPPP	
	2017	2018	2017	2018
Control	80.9^{ab}	72.5^{c}	21.3^{a}	20.6^{b}
St.1035	79.3^{b}	80.0^{a}	20.5^{ab}	22.5^{b}
St.1018	78.4^{b}	76.3^{a}	16.1^{e}	22.1^{b}
St. EAL 110	80.4^{ab}	77.7^{a}	16.9^{de}	22.9^{b}
St.1035+ 10 kg P	84.0^{ab}	76.9^{a}	18.1^{cd}	22.8^{b}
St.1018+ 10 kg P	84.3^{ab}	76.0^{a}	19.1^{bc}	24.2^{b}
St. EAL 110+ 10 kg P	86.4^{a}	74.6^{c}	21.4^{a}	26.8^{a}
CV(%)	**5.31**	**4.66**	**10.91**	**17.33**
LSD$_{0.05}$	*	*	*	*

Note: Means with the same letter are not significantly different at P>0.05 level of probability following LSD, st.= strain, PH=Plant height (cm), NPPP= number of pod per plant

G. Thousand Seed weight

The analysis of variance revealed that thousand seed weight was significantly responded for the treatment only in 2018. The highest hundred seed weight (370.1 g) was observed with combined application of EAL 110 strain and 10 kg P ha^{-1}. This treatment combination was found statically as par with sole application of the three strain and combined application of strain 1035 with 10 kg P ha^{-1} (Table 5). While the lowest thousand seed weight (332.3 g) was observed with combined application of 1018 with 10 kg P ha^{-1} (Table 5).

Table 5. Nodule dry weight as affected by Rhizobium inoculation, S and Zn fertilizer rates

Treatment	TSW	
	2017	2018
Control	331.1	347.6^{b}
St.1035	329.6	368.8^{a}
St.1018	332.8	361.3^{a}
St. EAL 110	341.2	366.4^{a}
St.1035+ 10 kg P	360.9	356.5^{a}
St.1018+ 10 kg P	340.7	332.3^{b}
St. EAL 110+ 10 kg P	341.7	370.1^{a}
CV(%)	**14.06**	**5.36**
LSD$_{0.05}$	**ns**	*

Note: Means with the same letter are not significantly different at P>0.05 level of probability following LSD, st.= strain, TSW= Thousand seed weight

H. Seed yield

Seed yield exhibited a significant response to the treatment in both years and mean value combined over years (Table 6). In 2017, significantly the highest seed yield (2537 kg ha^{-1}) was observed with combined application of EAL 110 with 10 kg P ha^{-1} (Table 6). The lowest seed yield (1931.6 kg ha^{-1}) was observed with the control plot (Table 6). But this treatment combination was found statically as par with sole application of strain 1018. In 2018, significantly the highest seed yield was observed with inoculation of the seed of faba bean with strain EAL 110 alone. But it also statically as par with sole application of strain 1018 and combined application of those three stain with 10 kg P ha-1 (Table 6). Combined over year, the highest mean seed yield which was obtained from combined application of EAL 110 with 10 kg P ha^{-1} which increase seed yield of faba bean by 20 % (425.1 kg ha^{-1}) over the lowest seed yield (2082.4 kg ha^{-1}) observed from the control plot (Table 6). The result also indicated that sole application of strain 1035, 1018 and EAL 110 resulted in a yield advantage of 5, 14 and 14% respectively compared with the un-inoculated and unfertilized control plot. Moreover, the combination of those strain with 10 kg P ha^{-1} resulted in a grain yield advantage of 8, 19 and 20% respectively compared with the control (Table 6). In line with this study Wassie et al (2008) reported that inoculation of the seed of faba bean with EAL 110, EAL 120 and chemical fertilizer increased grain yield by 61%, 68% and 80%, respectively, over the control. Similar result also concluded by Pearce et al. (2007), Negash (2000)

Amanuel et al (2000), Sameh et al (2017), Evans (2005), Carter et al (1994). But Abebe and Tolera reported that introduction of new Rhizobium strain to Gedo highlands did not significantly increase grain yield.

Table 6. Seed yield of faba bean as affected by different strain of Rhizobium and inorganic P fertilizer

Treatment	Seed yield		
	2017	**2018**	**Mean**
Control	1931.6^{c}	2233.1^{ab}	2082.4^{b}
St.1035	2117.9^{bc}	1964.8^{b}	2191.4^{ab}
St.1018	1974.4^{c}	2523.4^{a}	2248.9^{ab}
St. EAL 110	2223.2^{bc}	2541.7^{a}	2382.5^{ab}
St.1035+ 10 kg P	2434.1^{b}	2539.3^{a}	2486.7^{a}
St.1018+ 10 kg P	2262.4^{bc}	2501.3^{a}	2381.9^{ab}
St. EAL 110+ 10 kg P	2537.0^{a}	2479.2^{a}	2508.1^{a}
CV(%)	**7.74**	**13.67**	**14.83**
LSD$_{0.05}$	**	*	**

Note: Means with the same letter are not significantly different at P>0.05 level of probability following LSD, st.= strain

I. Straw Yield

Straw yield which was obtained from both years and mean value combined over locations was significantly influenced by the treatment (Table 7). Combined over year, significantly the highest straw yield was observed with combined application of EAL 110 with 10 kg P ha^{-1} which would increase straw yield.

Table 7. Seed yield of faba bean as affected by different strain of Rhizobium and inorganic P fertilizer

Treatment	Straw yield		
	2017	**2018**	**Mean**
Control	1706.15^{c}	1917.2^{bc}	1811.7^{c}
St.1035	1909.32^{b}	2103.6^{bc}	2006.5^{b}
St.1018	1773.45^{c}	2001.0^{b}	1887.2^{bc}
St. EAL 110	1985.82^{ab}	2149.0^{ab}	2067.4^{ab}
St.1035+ 10 kg P	1967.89^{ab}	2134.4^{ab}	2051.1^{ab}
St.1018+ 10 kg P	1921.81^{ab}	2175.0^{ab}	2048.4^{ab}
St. EAL 110+ 10 kg P	2000.39^{a}	2190.6^{ab}	2095.5^{a}
CV(%)	**8.76**	**13.49**	**14.24**
LSD$_{0.05}$	**	*	**

IV. CONCLUSION AND RECOMMENDATION

N2 fixation by leguminous crops is a relatively low-cost alternative to N fertilizer for small-holder farmers in developing countries. N2 fixation in faba bean (Vicia faba L.) is affected by P fertilization and inoculation. The present study was conducted with the objectives of the analysis of variance also showed that, Seed yield exhibited a significant response to the treatment. In 2017, significantly the highest seed yield (2537 kg ha^{-1}) was observed with combined application of EAL 110 with 50 kg ha^{-1}. The lowest seed yield (1931.6 kg ha^{-1}) was observed from the control plot In 2018, significantly the highest seed yield was observed with inoculation of the seed of faba bean with strain EAL 110 alone. Hence, Rhizobium inoculation with strain EAL with 10 kg P ha^{-1} could be recommended for faba bean production at the experimental locations in Mortina Jiru district

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