Response of Faba Bean to Starter Nitrogen Dose Application and Rhizobial Inoculation in the Major Growing Areas of Arsi Zone

Wendesen Melak*, Amare Tadese Asrat Mekonen Mengistu Chemeda Almaz Admasu
Ethiopian Institute Of Agricultural Research, Kulumsa Agricultural Research Center,
Assela, Ethiopia P. O.Box 489

Abstract
Application of starter Nitrogenous fertilizer with rhizobial strains have been hypothesized to increase yield and yield parameters. Hence, to exploit productivity potential of Faba bean (Vicia faba L) a field study was conducted in Arsi zone 2015/16 and 2016/17. The test crop was uninoculated, inoculated with strain FB-1017, and supplied with six rates of Nitrogen (T1 = -Ve control (No input), T2 = 9 N kg/ha, T3 = 18 N (kg /ha), T4 = 27 - N (kg /ha), T5 = 36 - N (kg /ha), T6 = 54 - N (kg /ha)). The treatments were laid out in randomized complete block design with split plot arrangements with three replications. Application of inoculants and starter nitrogen at different levels indicated that plant height (at three locations), grain yield (at one location) and number of seeds per pod were improved significantly (p<0.05) otherwise did not improve none of yield and yield parameters in both years. In that case, though inconsistent a grain yield of more than five quintals per hectare than the negative control (uninoculated, unfertilized) was found by the highest fertilized treatment (54 kg ha-1). The results suggest that application of starter nitrogen to small holder farmers is not economical and inoculation with trustworthy and viable strains would suffice in compromise to environmental and profit margins and more studies in different locations ought to be executed for a few locations as such might not give required result.

Keywords: Arsi, Biological Nitrogen Fixation, Faba Bean, FB-1017, N starter
DOI: 10.7176/JBAH/9-5-05
Publication date: March 31st 2019

Introduction
Enhancing agricultural productivity is one of the central challenges to achieving food security and poverty reduction in Ethiopia. Considering the fact that soil fertility is one of the biggest challenges, an obvious strategy is to increase fertilizer application and promote good agronomic practices to enhance productivity (Birhan Abdulkadir et al., 2017). Among which Nitrogen is one of the most abundant elements on earth. However, it is one of the most limiting factors of growth and production of crops. Which its requirements exceed any other and rarely do soils in the tropics have enough of this nutrient to produce high sustainable yields (Otieno et al., 2007). Currently inorganic fertilizer is an immediate supply but far the most important source of fixed nitrogen derives from the activity of certain soil bacteria that absorb atmospheric N2 gas and convert it into ammonium which according to Zahran (1999) and Simon et al. (2014) approximately reduce 20 million tons of atmospheric nitrogen to ammonia which is 50% - 70% of the world Biological Nitrogen Fixation.

Integration of multipurpose, N-fixing legumes into farming systems commonly improves soil fertility and agricultural productivity through symbiotic associations between leguminous crops and Rhizobium. However, the contribution of N fixation to soil fertility varies with the types of legumes grown, the characteristics of the soils, and the availability of key micronutrients in the soil to facilitate fixation, and the frequency of growing legumes in the cropping system (Birhan Abdulkadir et al., 2017).

It is widely acknowledged that inoculation of legumes with effective rhizobia can improve yields and provide a substitute to inorganic fertilizers and different research works made in recent years revealed that inoculation of Faba Bean with R.leguminosarum increase yield by 10-50% (Asfaw Hailemariam and Angaw Tsgie, 2003; Chemining’wa et al., 2007). The technology, therefore, is good for Ethiopian soils where 85% are reported to have low levels of Nitrogen (EIAR, 2014) and several field demonstrations have confirmed that leguminous crops show remarkable growth and yield response to rhizobia inoculations in different agroecologies in Ethiopia. As a result, the use of rhizobia inoculants has shown spectacular growth in Ethiopia (EIAR, 2014).

Faba Bean is a crop of high economic value with its edible seed serving as an important protein complement in the cereal based Ethiopian diet (Tamene Temesgen et al., 2015) and also contribute to smallholder income (FAO, 2014). Furthermore, it supplies an important added value to the crop by fixing atmospheric nitrogen in symbiosis, with root nodule bacteria known as Rhizobium leguminosarum bv. Vicine (Mutch and Young, 2004). An estimate of 240-235 amounts of N2 fixed (kilograms of N2 fixed per hectare) by Faba Bean with Faba Bean-rhizobial symbioses (Somasegaran and Hoben, 1994) thus, reducing costs by less fertilizer use and minimizing impact on the environment by natural soil maintenance (IFPRI, 2010; Alghamdi et al., 2012).

Currently rhizobial inoculants are widely used in various parts of the world. They are the solution to dwindling soil fertility, inexpensive, environment friendly, and easy to use with no side effects in most cases (Wondwosen Tena et al., 2016). In response to this, promising Faba Bean Rhizobia screening activities were carried out during...
the past ten years in the country and revealed that there is diversity in different agroecologies (Abera Mnalku et al., 2009; Alemayehu Workalemahu, 2009; Zerihun Belay and Fassil Assefa, 2011; Anteneh Argaw, 2012; Solomon Legesse and Fassil Assefa, 2014; Dereje Tsegaye et al., 2015; Getahun Negash 2015; Wendesen Melak et al., 2018). Indeed, the symbiotic interaction is initiated after an initial exchange of signals: when nitrogen present in the soil is scarce, legumes exude a series of phenolic compounds into the rhizosphere, mainly flavonoids and isoflavonoid (Clúa et al., 2018), however, Crop yield increases of 51–158% were reported in nitisols at Holleta due to the combined application of 20 kg ha⁻¹P with strain over non-inoculated ones, none of the above studies reported on the agronomic efficiency, profitability and the level of risk associated with applying starter N fertilizer on Faba bean.

However crucial is the use of organic inputs as external nutrient sources and has been advocated as a logical alternative to expensive fertilizers in Africa, application of starter Nitrogen with biological nitrogen fixing bacteria has been hypothesized to ameliorate yield and yield related parameters, but not explored. This paper reports results from a study on the effects of starter N fertilizer application and to evaluate the symbiotic potential of popular faba bean against nitrogen fixing rhizobial isolates, the essence of starter N application and to determine the starter N dose that the farmer should use and its effects on yield, agronomic efficiency, profitability, the risk associated with faba bean, in selected districts of Arsi Zone.

Material and Methods
Study sites
The study was undertaken for two years in seven districts of two potential Faba bean production weredas namely Tiyo & Limu-Bibilbo (Figure) The soils are classified as follows: at Bekoji a haplic Nitisol, and at Kulumsa an intergrade between a haplic Nitisol and a luvic Phaeozem (Amanuel Gorfu et al., 2000) where Wheat is dominantly produced with mean annual rain fall 823 mm and 1020 mm respectively of Kulumsa and Bekoji.

Experimental Design,
The Field trial were designed in split plot fashion with three replication for which two main plot factors two levels of inoculation (FB-1017 strain inoculated & uninoculated) and six levels of factorially combined inorganic N rates as sub plot factors (T1 = -Ve control (No input), T2 = 9 N kg/ha, T3 = 18 N (kg/ha), T4 = 27 - N (kg/ha), T5 = 36 - N (kg/ha), T6 = 54 - N (kg/ha)) were arranged in two way randomization.

Figure 1 Map of Study area
Sources of seeds and Rhizobium strain
Faba bean variety Dosha was supplied by Highland pulse research of Kulumsa Agricultural Research Center, Ethiopia. Variety was selected based on their yield, their maturity time and recentness of year of release. Strain of Rhizobium spp. (FB-1017) was obtained from Holeta Agricultural Research Center.

Planting and Agronomic Practices
Field experiments were carried out in the two successive years of growing seasons, 2015/2016 and 2016/2017 at seven locations in Arsi zones where Faba bean production is at potential and monoculture production system is dominant. Faba bean seeds were sown in the rate of 100 kg seeds ha⁻¹ and were cultivated in strips. Each block (4m x 37.2 m) consisted of twelve plots. Each plot area was 10.4m² and consisted of 10 rows, spaced 0.4m apart. An additional eleventh row was placed in each plot and served as a border, and was not involved in calculations. Each strip was spaced apart by 1m apart to prevent bacterial migrations. Weeds, insects, and fungal pathogens were controlled by chemical spray applications, as required, at rates according to manufacturers’ recommendations. At harvest, yield was determined by the manual mechanical harvesting of the entire plot.

Statistical Analysis
The ANOVA model was used according to Jones and Nachtshheim (2009) which is given by

\[ Y_{ijk} = \mu. + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_{k(i)} + \epsilon_{ijk} \]

Where \( \mu. \) is constant; a whole-plot treatment effects, are constants subject to \( \Sigma \alpha_i = 0; \beta_j \); the b split-plot treatment effects, are constants subject to \( \Sigma \beta_j = 0; (\alpha\beta)_{ij} \); the ab interaction effects, are constants subject to \( \Sigma (\alpha\beta)_{ij} = 0 \) for all j and \( \Sigma (\alpha\beta)_{ij} = 0 \) for all i; \( \gamma_{k(i)} \), the nw = ac whole-plot errors, are independent \( N(0, \sigma^2) \); \( \epsilon_{ijk} \) independent are \( N(0, \sigma^2) \); i = 1,...,a, j = 1,...,b, k = 1,...,c. and Tukey Honest Significant Difference (HSD) test was used to separate treatment means at a probability level of 0.05 (SAS, 2009; R, 2018)

Yield and Yield Components
yield and yield attributes of faba bean were recorded. Plant height (PH), Pods per plant (PPP), seeds per pod (SPP), Total grain yield (GY) per hectare adjusted to 10% moisture content, biological yield (BY) per hectare and harvest index (HI) content were determined.
Results and Discussions

Rhizobial inoculation with starter-N treatments had no significant effect on all of parameters studied (Table and Table) the two years growing season but plant height at Bekoji where unfertilized plots performed significantly (p<0.05) less than other treatments during the first year. This result did not agree to previous studies, that goes in line with the general notion that biological nitrogen fixation is affected by inorganic nitrogen. Starter nitrogen caused decline in grain yield that was explained could suppress nodulation and hence yield related parameters, but it was also noted that a moderate dose of starter-N demonstrated to stimulate seedling growth and subsequent nitrogen fixation (Chemining’wa et al., 2007). Inorganic Nitrogen is required by legume plants during the ‘nitrogen hunger period’ for their nodule development, shoot and root growth before the onset of N2-fixation. The success of legume grain crops is dependent on their capacity to form effective nitrogen-fixing symbioses with root-nodule bacteria (Youseif et al., 2017). However, many soils may do not have adequate amounts of native rhizobia in terms of number, quality, or effectiveness to enhance biological nitrogen fixation.

The increments in seed yields in most of the N-fertilized plots and/or inoculated plots, in relation to the uninoculated non-N fertilized plots controls indicate that, in those soils, nitrogen is not a limiting factor, and that crop yields could be strongly improved by means of competitive and viable strains inoculation but fertilization might not help. However, we found that response to inoculation with the best rhizobia strains was greater comparably better than the full N fertilization even though statistically not different. Our results showed that faba bean inoculation could effectively reduce the need of applied inorganic N-fertilizers while achieving higher grain yield, but in this particular case even the main plot factor, that inoculating did not have improved difference than un inoculated control while a study reported in Youseif et al. (2017) from which effective inoculation improved grain yield by 35%-48% and faba bean yield and yield components could be significantly improved through the combined use of Rhizobium/Agrobacterium inoculations and starter N application (48kg N ha−1) under low fertility sandy soil conditions but according to Chemining’wa et al. (2007) even Rhizobia inoculation failed to improve yield. The mean grain yield in the two years varied 3.69 to 4.34 ton ha−1 in 2015/16 and 2.80 to 5.40 ton ha−1 in 2016/17 which actually is far better than the national average 1.91 and 2.05 ton ha−1 (CSA, 2016; CSA, 2017), that can be ascribed to the relatively low precipitation and higher temperature (Figure) during floral initiation when nodule initiation is maximum

Table 1 Effects of Starter nitrogen dose on yield and yield traits of faba bean (2015/16)

Table 2 Effects of Starter nitrogen dose on yield and yield traits of faba bean (2016/17)

Means followed by the same letter within a column are not significantly different at the P=0.05 level using LSD test (PH=plant height, PPP=number of pods per plant, SPP=number of seeds per pod, GY=grain yield, BY=Biomass yield, HI=harvest index)
Conclusions and recommendations
Application of starter nitrogen with faba bean had not had any apparent effect on yield and yield components. The reason could be attributed to poor inoculant viability, adequate soil mineral, or highly competitive indigenous strains, besides to that Legumes have a high internal phosphorous requirement for their symbiotic nitrogen fixation, in addition to the nodule formation, deficiency of phosphorous in legumes also markedly affects the development of effective nodules and the nodule leghæamoglobin content which directly affect productivity. It is therefore suggested that the status of available phosphorous in soils ought to be taken through as in the soils of the experimental field may be beneficial to nodule nitrogen fixation through the prevention of the decrease of the phosphorous concentration in the plants at the later growth stage. Hence For better verification of this investigation it is also suggested to repeat the study in other carefully evaluated in prevalent N depleted in small holder farmers with other cultivars and bio-fertilizers and even the inoculants of different strains of rhizobia that was formed from indigenous soils of Arsi.

Acknowledgments
We Would like to Thank EIAR for funding this research and Kulumsa Agricultural Research Center Technical assistants whom their precious years were always valuable.

References
Abere Mnalku, Heluf Gebrekidan and Fasil Assefa (2009). Simbiotic effectivness and characterization of rhizobium strains of faba bean (Vicia faba L.) collected from Eastern and Western Hararghe highlands of Ethiopia. *Ethiopian Journal of Natural Resources, 11*(2):223-244.

Alemayehu Workalemahu (2009). The Effect of Indigenous Root-Nodulating Bacteria on Nodulation and Growth of Faba Bean (Vicia faba L) in the Low-Input Agricultural Systems of Tigray Highlands, Northern Ethiopia. *Momona Ethiopian Journal Of Science, 1*(2):30-43.

Alghamdi, S. S., Ammar, M. H., Siddique, K. H. M., Migdadi, H. M. and Paull, J. G. (2012). Faba bean genomics: current status and future prospects: A Review. *Euphytica* 186::609–624.

Amanuel Gorfu, Kühne, R. F., Tanner, D. G. and Vlek, P. L. G. (2000). Biological nitrogen fixation in faba bean (Vicia faba L.) in the Ethiopian highlands as affected by P fertilization and inoculation. *Biogy and Fertility of Soils, 32*(5):353-359.

Anteneh Argaw (2012). Characterization of symbiotic effectivness of rhizobia nodulating faba bean(Vicia faba L.) isolated from central Ethiopia. *Research Journal of microbiology:1*-17.

Asfaw Hailemariam and Angaw Tsgie (2003). BNF Research on food legumes in Ethiopia. In: Ali, K., Gemechu Keneni, Seid, A., Malhotra, R., Beniwal, S., Makkouk, K. and Halia, M. H. (eds.) Food and forage legumes of Ethiopia:Progress and prospects,proceedings of the workshop on food and forage legumes,22-26 September 2003,Addis Ababa ,Ethiopia.: Ethiopian Institute of Agricultural Research(EIAR) and International Center for Agricultural Research in Dry land Areas (ICARDA),Aleppo,Syria.

Birhan Abdulkadir, Sofiya Kassa, Temesgen Desalegn, Kassu Tadesse, Miheatreab Haileselassie, Girma Fana, Tolea Abera, Tilahun Amede and Tibebe, D. (2017). Crop response to fertilizer application in Ethiopia; a review.1-28.

Chemining’wa, G. N., Theuri, S. W. M. and Muthomi, J. W. (2007). Effect of Rhizobia Inoculation and Starter-N on Nodulation, Shoot Biomass and Yield of Grain Legumes. *Asian Journal of Plant Sciences, 10*(3):363-387.

Clúa, J., Roda, C., Zanetti, M. E. and Blanco, F. A. (2018). Compatibility between Legumes and Rhizobia for the Establishment of a Successful Nitrogen-Fixing Symbiosis. 10.3390/genes9030125.

CSA (2016). Report on area and production of major crops. The Federal Democratic Republic Of Ethiopia Central Statistical Agency(CSA).

CSA (2017). Report on area and production of major crops. Statistical bulletin ed.: The Federal Democratic Republic Of Ethiopia Central Statistical Agency(CSA).

Dereje Tsegaye, Fasil Assefa, Heluf Gebrekidan and Gemechu Keneni, W. (2015). Nutritional, eco-physiological and symbiotic characteristics of rhizobia nodulating faba bean (Vicia faba L.) collected from acidic soils of Ethiopia. *African Journal of Environmental Science and Technology, 9*(7):646-654.

EIAR (2014). Rhizobia-based bio-fertilizer:Guidelines for smallholder farmers. EIAR.

FAO (2014). Country fact sheet on food and agriculture policy trends :Ethiopia, p.1.

Getahun Negash , T. (2015). Symbiotic and phenotypic characteristics of indigenous rhizobia nodulating faba bean (Vicia faba L.) growing in some parts of Wello, Northern Ethiopia. MSc thesis Hawassa University.111 p.

IFPRI (2010). Pulses Value Chain in Ethiopia Constraints and Opportunities For Enhancing Exports, p.44.

Jones, B. and Nachttsheim, C. J. (2009). Split-Plot Designs:What, Why, and How. *Journal of Quality Technology, 10*1080/00224065.2009.11917790.
Mutch, L. A. and Young, J. P. W. (2004). Diversity and specificity of Rhizobium leguminosarum biovar viciae on wild and cultivated legumes. *Molecular Ecology*, 13:2435–2444.

Otieno, P. E., W. Muthomi, J., Chemining’wa, G. N. and H. Ndertu, J. (2007). Effect of rhizobia inoculation, farmyard manure and nitrogen fertilizer on growth, nodulation and yield of selected food grain legumes. *Proceedings of African Crop Science* 2007, Egypt, 305-312.

R (2018). R Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/. 3.5.1 ed.

SAS (2009). SAS/STAT 9.2® User’s Guide, Cary, NC, USA, SAS Institute Inc., 7886.p.

Simon, Z., Mtei, K., Amare Gessesse and Ndakidemi, P. A. (2014). Isolation and Characterization of Nitrogen Fixing Rhizobia from Cultivated and Uncultivated Soils of Northern Tanzania. *American Journal of Plant Sciences*, 5:4050-4067.

Solomon Legesse and Fassil Assefa (2014). Symbiotic And Phenotypic Characteristics Of Rhizobia Nodulating Faba Bean (Vicia Faba) From Tahtay Koraro, Northwestern Zone Of Tigray Regional State, Ethiopia *IJTEE*, 2(11):1-9.

Somasegaran, P. and Hoben, H. J. (1994). Handbook for Rhizobia:Methods in Legume-Rhizobium Technology, USA, Springer-Verlag.455.p.

Tamene Temesgen, Gemicheu Keneni, Tadese Seferaa and Mussa Jarso (2015). Yield stability and relationships among stability parameters in faba bean (Vicia faba L.) genotypes. *The Crop Journal*, 3:258 – 268.

Wendesen Melak, Tulu Degefu, Endalkachew Welde-Meskel and Solomon Yilma (2018). Morphophysiological and Symbiotic Characteristics of Rhizobia Nodulating Faba Bean (Vicia faba L.) from Bale, Ethiopia. *Journal of Biology, Agriculture and Healthcare*, 8(15):51-61.

Wondwosen Tena, Endalkachew Wolde-Meskel and Fran Walley (2016). Symbiotic Efficiency of Native and Exotic Rhizobium Strains Nodulating Lentil (Lens culinaris Medik.) in Soils of Southern Ethiopia. *Agronomy*, 6(11):10.

Yousef, S. H., El-Megeed, F. H. A. and Saleh, S. A. (2017). Improvement of Faba Bean Yield Using Rhizobium/Agrobacterium Inoculant in Low-Fertility Sandy Soil. *Agronomy*, 7(2):1-12.

Zahran, H. H. (1999). Rhizobium-Legume Symbiosis and Nitrogen Fixation under Severe Conditions and in an Arid Climate. *Microbiology and molecular biology reviews*, 63(4):968–989.

Zerihun Belay and Fassil Assefa (2011). Symbiotic and phenotypic diversity of Rhizobium leguminosarum bv. viciae from Northern Gondar, Ethiopia. *African Journal of Biotechnology*, 10(21):4372-4379.