Ethiop. J. Agric. Sci. 26(1) 27- 48 (2015)

Inherent Soil Fertility as Affected by *Rhizobium* Inoculation and Inorganic N Application on Common Bean

Anteneh Argaw[1] and Abere Minalku[2]

[1] School of Natural Resources Management and Environmental Sciences, College of Agriculture and Environmental Sciences, Haramaya University, P.O.Box 138, Dire Dawa, Ethiopia
[2] Holleta Research Center, Ethiopian Institute of Agricultural Research, Holetta, Ethiopia

አህፅርኦት

ቦሎቄ በኢትዮጵያ በዋናነት በርካሽ ከሚገኙ የፕሮቲን ምግቦች አንዱ ነው፡፡ ነገር ግን ምርታማነቱ በኢትዮጵያ በጣም ዝቅተኛና በሄክታር ከ10 ኩንታል ያነሰ ነው፡፡ ምርታማነቱን ከሚቀንሱ ዋና ዋና ምክንያቶች ውስጥ የአፈር ለምነት በተለይ የናይትሮጅን እጥረት ነው፡፡ ስለዚህ የዚህ ምርምር ስራ ዓላማ የአፈር ለምነት፣ ከአየር ውስጥ ያለውን የናይትሮጅን ጋዝ የሚጠቅም ደቂቃ አካላትና የተለያየ መጠን ሰው ሰራሽ የናይትሮጅን ማዳበሪያ በቦሎቄ ምርታማነት ላይ ያለው ለውጥ ተጠንቷል፡፡ በዚህ መሰረት 20 ኪሎ ግራም በሄክታር በቦሎቄ ስር ላይ የሚወጣውን ዕባጭ ኖዱል አሻሽሎታል፡፡ ነገር ግን ይህ ለውጥ በአፈር ውስጥ ባለው የናይትሮጅን መጠን የተወሰነ ነው፡፡ ከዚህ በተጨማሪ ከፍተኛው የኖዲውል ቁጥርና ክብደት መጠን የተገኘውም በሀረማያ እና በሂርና የምርምር ጣቢያዎች አፈር ላይ ከተዘራ ቦሎቄ ነው፡፡ ከዚህ የምርምር ስራ በተጨማሪ ከፍተኛውን የዕፅዋት ናይትሮጅን መጠን ላሉት የተወሰዱት የምርታማነት ማሳያ መለያዎች የተገኙት በሐረማያና በሂርና አፈር ነው፡፡ ይህ ውጤት የተገኘው የተፈጥሮ የአፈር ለምነት የቦሎቄን ምርታማነትና ኖዲውልስ የሚወስን ስለሆነ ነው፡፡ በመጨረሻ ይህ የምርምር ስራ ሰው ሰራሽ ማዳበሪያ በመጠኑ ቢለያይም መጨመሩ የቦሎቄን ምርት እንደሚያሻሽል አሣይቷል፡፡ የሚያስፈልገው መጠን ግን በአፈር ውስጥ ባለው የናይትሮጅን መጠን ላይ የተወሰነ ነው፡፡ የራይዞቢያ ደቂቅ አካላት በቦሎቄ ምርት ላይ ያለው ውጤት በአፈሩ ለምነት እና የናይትሮጅን መጠን የተወሰነ አይደለም፡፡ በአጠቃላይ የራይዞቢያና የሰው ሰራሽ ማዳበሪያ መጨመር የቦሎቄ ምርትን እንደሚያሻሽል ይህ የምርምር ውጤት አሳይቷል፡፡

Abstract

The aim of this study was to evaluate the effect of soil fertility on the effectiveness of Rhizobium leguminosarum bv. phaseoli and N fertilizer on yield traits of common bean variety. The experiment was conducted in Babillae, Fedis, Haramaya and Hirna experimental sites. Six levels of N fertilizer (0, 20, 40, 60, 80, 100 kg N ha^{-1}) and two levels of inoculation were factorially arranged in a randomized complete-block design with three replications. Analysis of variance indicated a significant effect of Rhizobium leguminosarum bv. phaseoli inoculation, N rates of application, location and their interaction on yield and yield components of common bean on all soil types. The highest nodule number (NN) and nodule dry weight (NDW) values were observed at 20 kg N ha^{-1}, with the exception of Hirna location. However, the highest NN (282.00) and NDW (0.8182 g) were observed at Haramaya and Hirna sites, respectively, where the inherent soil fertility, including total N, was high. The highest plant total tissue N (PTTN) at Babillae, Fedis, Haramaya, and Hirna sites was 3.2433% at 20 kg N ha^{-1}, 4.2000% at 100 kg N ha^{-1}, 4.5567% at 40 kg N ha^{-1} and 4.8267% at 100 kg N ha^{-1}, respectively. The highest total biomass yield at Babillae, Fedis, Haramaya and Hirna sites was 3648.1 kg ha^{-1} and 4740.7 kg ha^{-1}, 6474.1 kg ha^{-} and 8638.9 kg ha^{-1}, respectively. Similarly, the highest grain yield (GY) at Babillae, Fedis, Haramaya, and Hirna sites was 2089.54, 1653.89, 2475.28 and 2441.57 kg ha^{-1}, respectively. The highest harvest index (HI) was recorded at Babillae site.

Introduction

Common bean is considered as poor-fixer of atmospheric N compared with other leguminous crops (Buttery *et al*. 1987; Fageria, 2002) and generally responds poorly to rhizobial inoculation (Graham, 1981; Buttery et al. 1987; Handarson, 1993). Therefore, common bean needs mineral N supplement to achieve a substantial grain yield increase under the existing cropping system. To this effect, common bean is often fertilized with N because of poor nodulation and the lack of response to rhizobial inoculation (Graham 1981; Buttery et al. 1987; Graham et al. 2003). Nitrogen nutrient amendment, however, can inhibit nodulation and atmospheric N_2 fixation because of the high ability of common bean to uptake N from the soil and the high sensitivity of common bean-rhizobia symbiosis to soil nitrate (Leidi and Rodríguez-Navarro 2000; Kimura et al. 2004). Low rates of inorganic N application can enhance the nodule formation and increase grain yields (Hungria *et al*. 2003) but are not sufficient to achieve maximum grain yields (Sistachs 1970; Rosas and Bliss 1986). Tsai et al. (1993) found non-significant effect of high inorganic fertilizer rates although it improved the plant total N accumulation.

Low levels of N fertilizer applied together with *Rhizobium* isolate to common bean can stimulate plant growth and N_2 fixation, and increase grain yield (Hungria *et al*. 2003). However, the information regarding the effect of *Rhizobium* inoculation and inorganic N application on nodulation and yield of common bean under different inherent soil fertility conditions is scarce in eastern Ethiopia. Hence, the objective of this experiment was to evaluate the effect of inherent soil fertility on the effectiveness of *Rhizobium leguminosarum* bv. phaseoli and N fertilizer on yield traits of common bean variety Dursitu in selected areas of eastern Ethiopia.

Materials and Methods

Description of the study areas

Field experiments were conducted in the 2012 main cropping season in in Hirna (N09°13.157″ and E041°06.488″ at an altitude of 1808 m; Fedis (N09°06.941″ and E042°04.835″ at an altitude of 1669 m; Babillae (N09°13.234″ and E042°19.407″ 1669 m; and Haramaya (N09°24.954″ and E042°02.037″ at an altitude of 2020 m. The respective soils had not been inoculated before with rhizobial strains that produce nodulation in common bean. Soil samples were taken from top soil (0-20 cm depth) before sowing to establish the baseline soil properties. Soil samples were analyzed at Haramaya University Soil Laboratory.

Sources of seeds and *Rhizobium* strain

A common bean variety 'Dursitu' was supplied by Lowland Pulses Research Program, Haramaya University, Ethiopia. The variety was selected based on its yield, maturity and adaptability to all experimental locations. The HU*PvR*-16 strain of *Rhizbium* sp. was previously evaluated under greenhouse and experimental site at Haramaya University (Anteneh Argaw, 2007; 2012). The result of Haramaya University experimental site revealed that HUPvR-16 performed better than the other tested isolates in improving the common bean production.

Inoculum preparation

Agar slope of HU*PvR*-16 culture was supplied by Soil Microbiology Research Laboratory, Haramaya University. For purification, the strain was initially cultured in yeast extract mannitol agar (YEMA) medium (10 g mannitol, 1 g yeast-extract, 1 g KH_2P0_4, 0.1 g NaCl, and 0.2 g $MgS0_4.7H_20$ per liter, pH 6.8) and incubated at 28 ^oC for five days. The pure colony of the isolate was later transferred to YEM broth medium and inoculated in a shaker incubator with gentle shaking at 120 rpm for five days. Via this procedure, the *Rhizobium* culture reached the middle or late logarithmic phase, and cell density in the culture was estimated by measuring the optical density (540 nm) using a UV-spectrophotometer. The *Rhizobium* inoculant was prepared by mixing 30 g of decomposed sterile filter-mud with 15 ml of broth cultures of HU*PvR*-16 in polyethylene bags. After incubating the inoculated filter-mud for two weeks at 28 ^oC, the count of the *Rhizobium* strain was 1 x 10^9 g^{-1} carrier material. The population of rhizobia in the inoculant was determined via duplicate plate counts.

Treatments and experimental design

Field trials were conducted to investigate the effects of inorganic N fertilizer and *Rhizobium* inoculation on nodulation, yield and yield components of common bean in inherent soil fertility status. The main plot treatments consisted of six N fertilizer rates of 0, 20, 40, 60, 80 and 100 kg N ha^{-1}. The size of the main plots was 12 m width by 5 m length. There were three sub-plots within a main plot and the total area of each sub-plot was 3 m x 5 m. The spacing between main plots was 1.5 m. N fertilizer in each level was divided into two equal parts; the first half of the N (20 kg N ha^{-1}) was applied along the furrow by hand and incorporated before planting time, and the remainder was applied during the common bean-flowering stage. The sub-plot treatments included two levels of inoculation (inoculated and uninoculated). There were five rows per sub-plot and the spacing was 40 cm between rows, 10 cm between plants, 1 m between sub-plots. The treatments were arranged in a split-plot design with three replications.

Disinfected seeds of common bean were planted after they were moistened with a 20% sucrose solution and then inoculated (7 g inoculant per kg seed) with a

Rhizobium strain. Phosphorus (P) in the form of triple superphosphate (TSP) was applied at 20 kg P ha^{-1} to each experimental plot at planting. Inoculated seeds were hand-planted on 7 July 2012. Two seeds were sown per hill. After germination, the plots were thinned to one seedling per hill to obtain 30 plants per row. Weeds were managed with hand hoeing during the growing season.

Five plants from each sub-plot were randomly tagged at late flowering and early pod-setting stage and were harvested from the central rows to record the number of nodules per plant (NN), nodule dry weight per plant (NDW) and shoot dry weight per plant (SDW). Shoots of the plants were dried and later ground to pass through a 0.5 cm sieve. Total N determination was done via the Kjeldahl digestion method of Bremner (1965). Yield and yield attributes of common bean were recorded from three central rows at physiological maturity. Number of pods per plant (NPP) and number of seeds per plant (NSP) were counted, and plant total tissue N (PTTN), total biomass yield (TBY), 100-seed weight and grain yield (GY) at 13% moisture content were determined.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using SAS (SAS Institute 1999). Statistically significant differences between treatment means were determined; means were separated using the least significant difference (LSD) test at 5% probability level. Regression analyses GY with NN and NDW and bar graphs were produced using Microsoft Excel Work sheet version 10.

Results and Discussion

The physical and chemical analyses indicated that the experimental locations had different soil chemical and textural properties (Table 1). The organic carbon and total N in Haramaya, Hirna and Fedis sites were higher than those found in the soil samples from Babillae site. The soil pH at different experimental locations ranged from 6.66 to 7.84 and electric conductivity (EC) ranged from 0.06 to 0.2 mS cm^{-1}. According to Fageria and Santos (1998), Hirna soil had higher available P, whereas available P was lower in the soils of other experimental sites. Hirna, Haramaya and Fedis sites had optimal amounts of exchangeable Ca, Mg, K, and cation exchange capacity (CEC), while these parameters were lower in the Fedis site. Babillae soil had higher sand content than the other experimental sites. Based on these inherent soil chemical textural properties, Hirna, Haramaya and Fedis soils were grouped under fertile soils, whereas Fedis soil was regarded as infertile.

The analysis of variance indicated that N levels, inoculation, location and their interaction significantly affected NN and NDW of common bean, except inoculation effect on NN was non-significant at P <0.05 (Table 2). Beside this, Table 3 indicates a significant ($p \leq 0.05$) variation for all investigated traits, except harvest index, in different treatments; i.e., different rates of N fertilizer application with *Rhizobium* inoculated and uninoculated treatments. The treatments significantly ($p \leq 0.05$) affected NN and NDW at all four experimental locations. The number of nodules per plant and NDW were significantly reduced with increased N fertilizer rates, regardless of inoculation treatments. This finding is consistent with previous investigation of Li et al. (2009) on the same host plant. Inoculation with *Rhizobium* sp., along with an application of 20 kg N ha^{-1}, at Babillae site resulted in significantly higher (161.67) NN and NDW (0.3900 g) than did the other treatments. In addition, N rates of less than 40 kg N ha^{-1} with inoculation produced significantly higher NN and NDW than those resulting from the respective N rates of application alone (Figures 1a and 1b).

Table 1. Physical and chemical analyses of soil samples taken from the experimental sites before sowing

Soil properties	Hirna soil	Babile soil	Haramaya soil	Fedis soil
pH in H_2O	7.25	6.66	7.84	7.76
† EC (mS/cm)	0.06	0.04	0.14	0.06
Organic carbon (%)	1.65	0.56	1.96	1.32
Total nitrogen (%)	0.16	0.06	0.12	0.12
Available P (mg kg^{-1})	27.11	2.22	2.13	1.78
Ca (cmol (+) kg^{-1})	39.88	4.18	31	23.12
Mg (cmol(+) kg^{-1})	9.00	3.5	8.7	12.87
Na (cmol(+) kg^{-1})	0.14	0.15	0.33	0.12
K (cmol(+) kg^{-1})	0.80	0.34	0.14	1.09
‡CEC (cmol(+) kg^{-1})	40.03	6.59	25.98	32.22
Zn(mg kg^{-1})	0.95	0.26	0.11	0.10
B(mg kg^{-1})	0.83	ND§	0.15	0.75
NH_4-N(mg kg^{-1})	33.77	25.57	-	20.10
NO_3-N (mg kg^{-1})	33.74	27.98	-	27.75
Clay (g kg^{-1})	49	18	33	36
Silt (g kg^{-1})	39	6	18	45
Sand (g kg^{-1})	12	79	49	19
Textural class	Clay	Sandy loam	Sandy clay loam	Silty Clay loam

† EC- electric conductivity

‡ CEC- cation exchange capacity

§ND-not detected,

Table 2. Analysis of variance (mean square and F value) of block, location, nitrogen levels, inoculation and their interaction effect on the investigated traits of common bean

Sources	df†	NN†		NDW†		TBY†		GY†		HI†		PTTN†	
		MS	F value	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value
Block	2	2110.0	11.16	0.003517	1.33	268696	3.25	10445	6.27	0.00034	0.03	0.03897	0.29
Location (L)	3	62193.2	92.28***	0.787776	507.97***	122682437	539.95***	8259024	1071.83***	0.60580	57.23***	15.91908	434.94***
Nitrogen (N)	5	42230.6	223.34***	0.397674	149.88***	9221440	111.59***	977178	586.48***	0.01075	0.86ns	0.81702	6.11**
Error a	10	2.81		0.01051		58.7		8.33		0.02276		0.0746	
Inoculation (I)	1	75.1	0.14ns	0.148320	97.41***	4735235	17.68***	35498	13.56**	0.01305	1.61ns	0.50528	11.15**
Error b	12	2.75		0.00460		61.0		6.03		0.01060		0.0251	
L x N	15	4929.8	7.31***	0.090913	58.62***	650090	2.86***	75136	9.75***	0.01146	1.08ns	0.63930	17.47***
L x I	3	5469.7	8.12***	0.185868	119.85***	704426	3.10*	70355	9.13***	0.01555	1.47ns	0.37301	10.19***
N x I	5	3015.5	5.53**	0.057457	37.74***	600435	2.24**	20458	7.82**	0.00717	0.89ns	0.25173	5.56**
L x N x I	15	4342.6	6.44***	0.038076	24.55***	494752	2.18*	53575	6.95***	0.01574	1.49ns	0.22837	6.24***
Total	143												

*ns- non significant and *, **, and *** Significant at 0.05, 0.01, and 0.001 probability level, respectively.*

†df- degree of freedom, MS- mean square, NN- Nodule number, NDW- Nodule dry weight, TBY- Total biomass yield, GY- Grain yield, HI- Harvest index, PTTN- Plant total tissue N

At Fedis site, *Rhizobium* inoculation together with N application rates of 20 and 40 kg N ha^{-1} resulted in significantly higher NN than those induced at the respective rates of N application alone (Figure 1b). In contrast, inoculation plus 20, 40, 60 and 80 kg N ha^{-1} resulted in significantly lower NDW than that produced at the respective N application rates alone at Fedis site (Figure 2b). At this location, the highest (146.6) NN and NDW (0.4097 g) of all treatments were obtained from sole application of 20 kg N ha^{-1}, indicating the presence of competitive resident or indigenous rhizobia nodulating common bean.

The sole application of 20 kg N ha^{-1} at Haramaya site resulted in significantly higher (282.00) NN than all the other treatments, but this did not significantly differ from NN produced with *Rhizobium* strain inoculation alone and together with 20 kg N ha^{-1}. However, a significant effect of inoculation was detected in the NN at Haramaya site in comparison with the uninoculated treatment without N application in the control (Figure 1c), indicating the negative effect of N application on inoculated treatments. Moreover, inoculation gave significantly higher NDW than did uninoculated treatment, regardless of N application rates (Figure 2c), indicating non-competiveness of indigenous rhizobia nodulating common bean. However, significantly higher (0.7446 g) NDW was obtained from *Rhizobium* inoculation in conjunction with 20 kg N ha^{-1} than from *Rhizobium* sp. inoculation along with application of 40 kg N ha^{-1}. The synergetic effect of low N mineral together with inoculation resulted in yield increase. Similar finding was previously confirmed by Hungria *et al*. (2003); through *Rhizobium* inoculation improved nodule occupation (Vargas *et al*. 2000), regardless of the soil N content (Tsai *et al*. 1993). It is obvious that 20 kg N ha^{-1} application stimulated plant growth and increased the formation of more nodules in common bean (Tsai et al. 1993). Furthermore, because of the presence of low inherent total N in Babillae soil, which could need supplement N fertilizer for early development of common bean until N_2 fixation starts (da Silva *et al*. 1993). Beside this, the plant growth-promoting properties of the inoculated isolate could also improve the nodulation development (Saxena *et al*. 2006).

The control treatment, i.e., plots without *Rhizobium* strain inoculation and without N application gave the highest NN (216.67) at Hirna site. This NN did not significantly differ from NN obtained with *Rhizobium* inoculation alone and 20 kg N ha^{-1} with and without *Rhizobium* inoculations. At this experimental site, inoculation with *Rhizobium* sp. significantly affected the NN in relation to the uninoculated treatment only at 20 kg N ha^{-1} treatment (Figure 1d) as previously indicated by Daba and Haile (2001).

The highest (0.8182 g) NDW at Hirna site was obtained from *Rhizobium* inoculation in conjunction with application of 20 kg N ha^{-1}, followed by *Rhizobium* inoculation alone. This could be ascribed to higher total N and available P in the Hirna soil, which could be sufficient for early growth of common bean in addition to the N derived from N_2 fixation (Ankomah *et al*. 1996), than in the other three locations. The significant effect of inoculation on NDW over the uninoculated treatments at Hirna site was detected in the control along with N application of 20 kg N ha^{-1} and 40 kg N ha^{-1}, whereas beyond 40 kg N ha^{-1}, significantly higher NDW was produced in uninoculated treatment than that produced via inoculated treatments (Figure 2d). This indicates the occurrence of high antagonistic effect of higher N application rates than indigenous rhizobial population on the effectiveness of inoculated *Rhizobium* sp. The highest nodulation, which was found at Haramaya and Hirna sites, was correlated with their higher inherent soil fertility, including high available P (Pacheco *et al*. 2012). This may lead to higher native rhizobia population as previously observed by other investigators (de Oliveira *et al*. 1998; Rys and Bonish 1981).

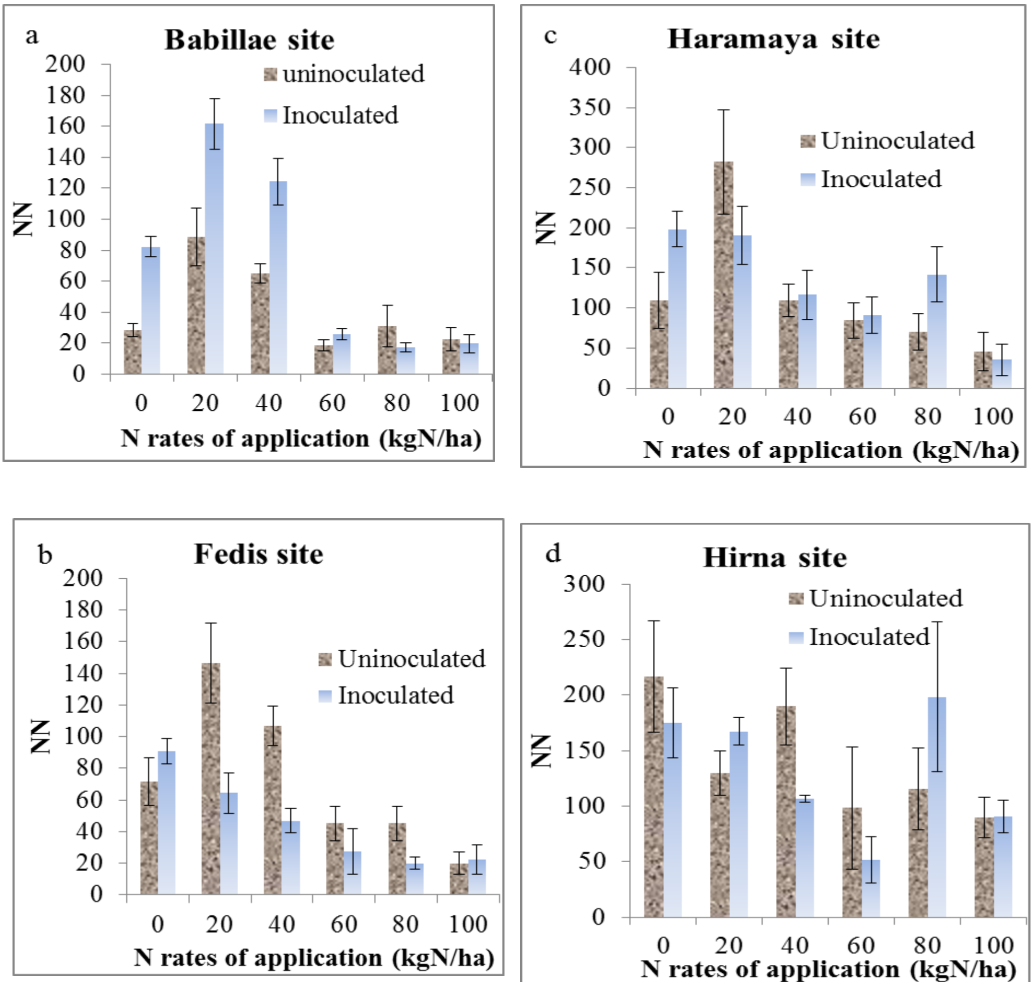

Figure 1. Effect of N application rates (kgN/ha) on nodule number per plant (NN) with inoculated and uninoculated at (a) Babillae site, (b) Fedis site, (c) Haramaya site and (d) Hirna site

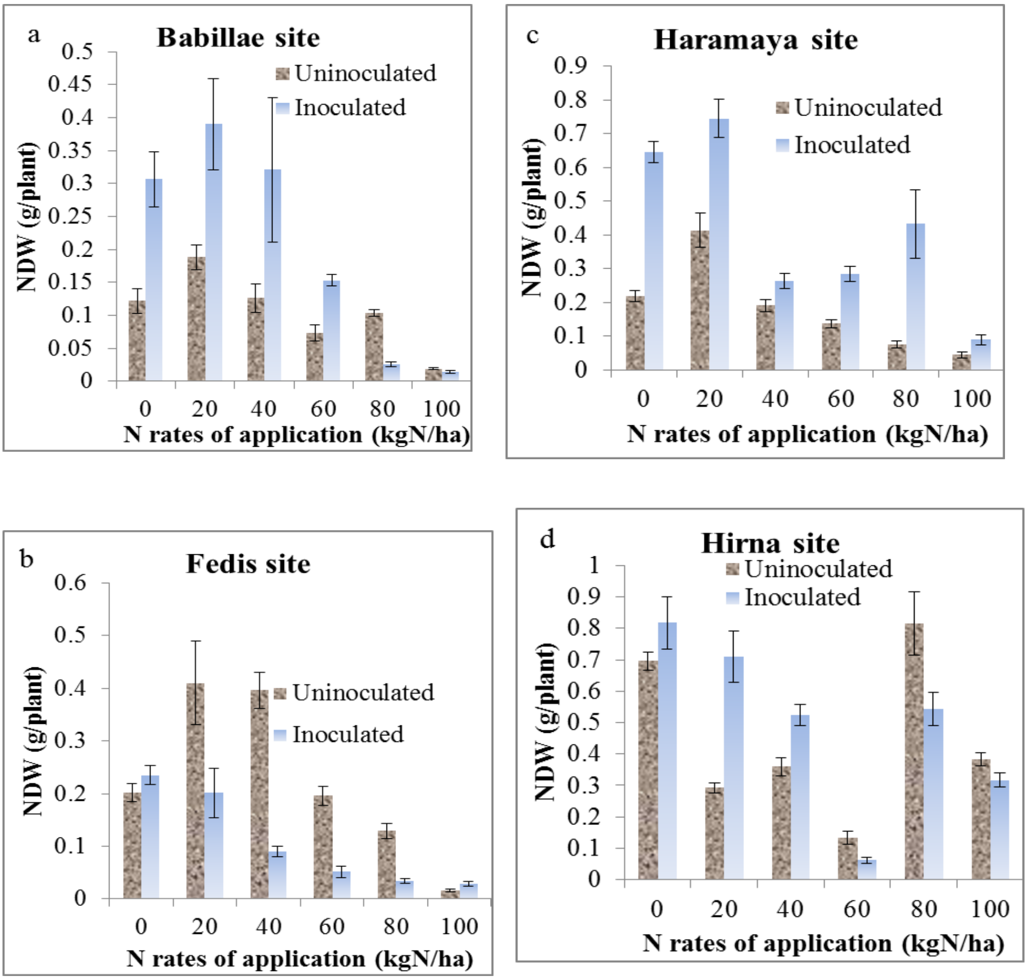

Figure 2. Effect of N application rates (kgN ha^{-1}) on nodule dry weight (NDW) (g $plant^{-1}$) with inoculated and uninoculated at (a) Babillae site, (b) Fedis site, (c) Haramaya site and (d) Hirna site

The low number of nodules per plant (NN) at Babillae (19.67), Haramaya (35.33) and Hirna (90.00) sites was obtained from combined application of 100 kg N ha^{-1} and *Rhizobium* inoculation. The lowest (20.00) NN at Fedis site was obtained with sole application of 100 kg N ha^{-1}. This indicates the negative effect of N fertilizer on nodule development at all experimental sites. However, the lowest NN produced at Hirna and Haramaya sites was higher than that produced both at Fedis and Babillae sites. The adverse effect of water stress on the survival of rhizobia at Babillae and Fedis sites could have reduced the nodulation. This adverse effect is previously observed on common bean (Mnasri *et al*. 2007; Figueiredo *et al*. 2008).

The analysis of variance indicated that TBY was significantly affected by N rates of application, inoculation, location and their interaction at P <0.05 (Table 2). In table 4, TBY was significantly ($p \leq 0.05$) affected by the treatments. *Rhizobium* inoculation in conjunction with 100 kg N ha^{-1} resulted in statistically higher TBY at Babillae site. Enhancement in nodulation and symbiotic N_2 fixation is not accompanied by high plant dry weight (Vlassak *et al*. 1996). Similarly, statistically higher TBY was obtained from inoculation in combination with 100 kg N ha^{-1} at Fedis site than from the control and *Rhizobium* inoculation alone although the soil had high total N. Tsai *et al*. (1993) also recognized the stimulatory effect of N fertilizer on N_2 fixation at high inherent soil fertility and high N application rate. It was previously reported that higher N application significantly reduced nodulation and symbiotic N_2 fixation without affecting the common bean productivity (Rai 1992).

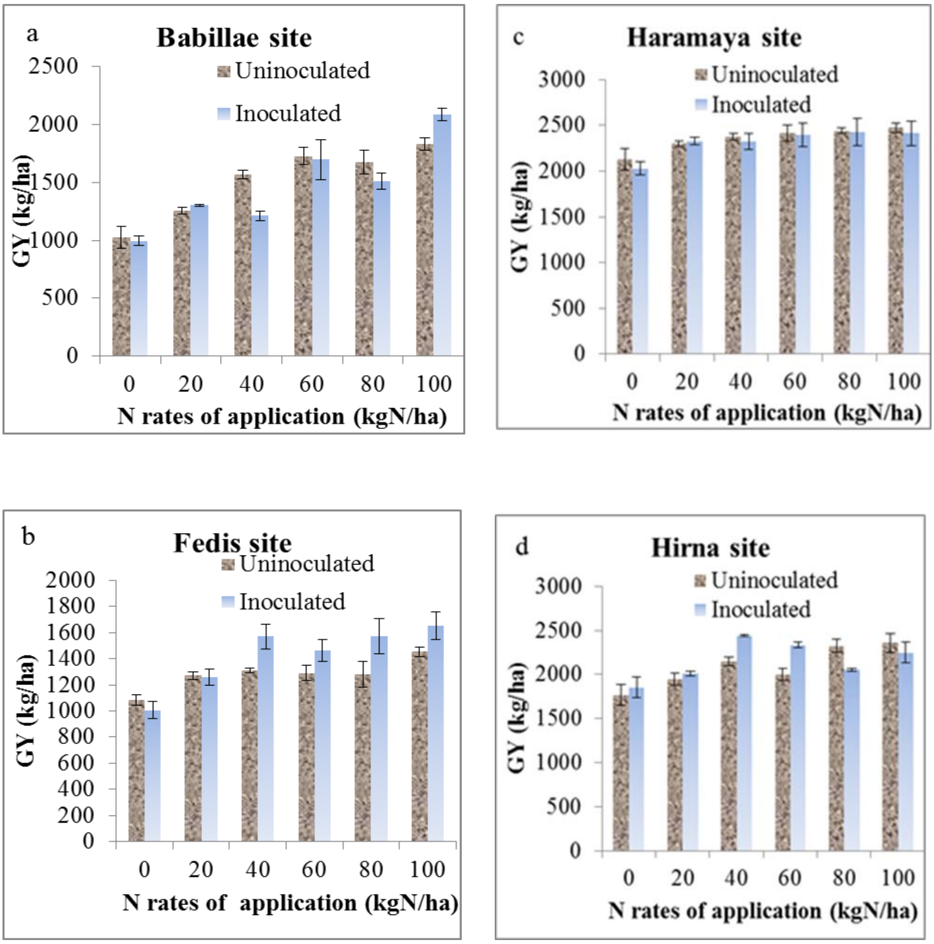

Figure 3. Effect of N application rates (kgN/ha) on grain yield (GY) (kg ha^{-1}) with inoculated and uninoculated at (a) Babillae site, (b) Fedis site, (c) Haramaya site and (d) Hirna site

Application of 100 kg N ha^{-1} alone gave the highest TBY at Haramaya. This finding is in agreement with previous investigation by Ruiz Diaz et al. (2009) on common bean. The non-significant difference in TBY was produced with increasing rates of N application with *Rhizobium* inoculation. It was previously reported that high total N in Haramaya soil caused no significant difference in TBY with increase in N fertilizer application rates (Gan *et al*. 2009). Common bean has also been cultivated for a long time at Haramaya site, which consequently leads to the presence of effective and higher number of rhizobia nodulating common bean (Vlassak *et al*. 1996). This might cause non-effectiveness of inoculated *Rhizobium* sp. (Vargas *et al*. 2000).

Significantly higher TBY was obtained from *Rhizobium* inoculation in conjunction with 20 kg N ha^{-1} than that obtained from the control or *Rhizobium* inoculation alone at Hirna site. A non-significant improvement in TBY was observed with an increase in N fertilizer application rates. The high inherent total N and high inherent soil fertility could satisfy the plant's N needs at low N fertilizer application rates, which could also favor high symbiotic N_2 fixation (Rys and Bonish 1981). The highest TBY obtained from Babillae, Fedis, Haramaya, and Hirna sites was 3648.1, 4740.7, 6474.1 and 8638.9 kg ha^{-1}, respectively. High TBY at Hirna site could relate to the better inherent soil fertility, for instance, high soil organic matter (SOM), total N, available P and CEC than at the other experimental sites as previously confirmed by Adamu *et al*. (2001) on faba bean cultivated in Ethiopia and by Tsai *et al*. (1993) on common bean.

The analysis of variance revealed that N rates of application, inoculation, location and their interaction significantly influenced the GY of common bean at $P < 0.05$ (Table 2). A significant difference in GY along with different treatments at all experimental sites was also observed at $P \leq 0.05$ (Table 4). Significantly higher GY was produced with *Rhizobium* inoculation in conjunction with 100 kg N ha^{-1} at Babillae site, even though the highest nodulation occurred at an application rate of 20 kg N ha^{-1}. This result is in agreement with the previous report by Rai (1992). Okogun and Sanginga (2003) also found that nodule formation improvement did not result in an increase in yield of soybean and enhancement in N_2 fixation. Beside this, a significant effect of inoculation over the uninoculated treatment on GY was detected at 100 kg N ha^{-1} (Figure 3a). A slight amount of N derived from symbiotic N_2 fixation at this site (Isoi and Yoshida 1991) could cause high responsiveness of common bean to N mineral application. A similar trend in response of common bean to N mineral fertilizer application up to 200 kg N ha^{-1} was previously reported by Daba and Haile (2002). It was previously recommended that an application of more than 100 kg N ha^{-1} was required to obtain more than 2000 kg ha^{-1} grain yield (da Silveria, *et al*. 2005).

Table 3. Nodulation status of common bean var. Dursitu along different N application rates with and without inoculation with *Rhizobiumleguminosarum*bv. *Phaseoli*

Treatments	NN†				NDW† (g plant^{-1})			
	Babillae	Fedis	Haramaya	Hirna	Babillae	Fedis	Haramaya	Hirna
Control	28.33d	71.67bcd	109.33bcd	216.67a	0.1217cd	0.2020bc	0.2183de	0.6630ab
20 kgN ha^{-1}	88.67c	146.67a	282.00a	130.00bcde	0.1880bc	04097a	0.3873bc	0.2926cd
40 kgN ha^{-1}	65.00c	106.67b	109.33bcd	190.00ab	0.1263cd	0.3963a	0.1669def	0.3262cd
60 kgN ha^{-1}	18.67d	45.00def	84.33cd	128.33bcde	0.0737cd	0.1960bc	0.1259def	0.1341d
80 kgN ha^{-1}	31.00d	45.00def	70.33cd	94.67de	0.1033cd	0.1289cd	0.0757ef	0.1636d
100 kgN ha^{-1}	22.33d	20.00f	46.00d	90.00e	0.0191d	0.0160e	0.0418e	0.1502d
HU*PvR*-16††	82.33c	90.67bc	198.33ab	175.00abc	0.3063ab	0.2349b	0.6185a	0.8182a
HU*PvR*-16. + 20 kgN ha^{-1}	161.67a	64.33cde	190.00ab	167.33abcd	0.3900a	0.2010bc	0.7446a	0.6776a
HU*PvR*-16 + 40 kgN ha^{-1}	124.33b	46.67def	141.67bc	106.67cde	0.3207a	0.0897de	0.4334b	0.4601bc
HU*PvR*-16 + 60 kgN ha^{-1}	26.00d	27.33ef	116.33bcd	173.33abc	0.1530c	0.0516de	0.2676cd	0.4052c
HU*PvR*-16 + 80 kgN ha^{-1}	17.33d	20.00f	91.33cd	108.33cde	0.0253d	0.0340e	0.2064def	0.2766cd
HU*PvR*-16 + 100 kgN ha^{-1}	19.67d	22.33f	35.33d	91.00de	0.0143d	0.0279e	0.0827ef	0.3421cd
Mean	57.11	58.86	122.86	139.28	0.1535	0.1657	0.2807	0.3925
LSD (0.05)	30.06	37.15	94.17	76.39	0.1201	0.0892	0.1652	0.2152
CV (%)	17.88	21.44	26.03	18.63	26.58	18.28	19.98	18.62

*** *Significant at 0.001 probability level.*

†NN- Nodule number per plant, NDW- Nodule dry weight.

††HUPvR-16- Haramaya University Phaseolus vulgaris Rhizobium-16

Statistically higher GY was produced from *Rhizobium* inoculation in combination with 100 kg N ha^{-1} application at Fedis site than that from the control and *Rhizobium* inoculation alone. Furthermore, significantly higher GY was produced with *Rhizobium* inoculation than with the uninoculated treatment at and beyond application rates of 40 kg N ha^{-1} (Figure 3b). There was 571.76 kg difference between the highest GY and that obtained from the control treatment at this particular experimental site. A significant increase in GY was recorded with increasing rates of N application in both inoculation treatments at Babillae and Fedis experimental sites. Moreover, irrespective of N rates of application, inoculation of *Rhizobium* produced higher GY than uninoculated treatment at Babillae and Fedis experimental sites. This indicated the remarkable effect of *Rhizobium* inoculation even though Fedis soil has higher total N. In contrast, Hungria *et al*. (2003) found that inoculation improved only the common bean yield in soil having low N content, which increased the yield from 63 to 290 kg ha^{-1} over the uninoculated treatments. In another study, however, more than 20 kg N ha^{-1} application substantially reduced the number of nodules and did not increase the bean yield any further (da Silva *et al*. 1993).

At Haramaya site, excluding the control and *Rhizobium* inoculation alone treatments, increasing rates of N application did not significantly improve the GY of common bean. However, the highest GY was obtained at 100 kg N ha^{-1} application, even though highest nodulation and plant tissue N accumulation was obtained at 20 kg N ha^{-1} N application. Beside this, inoculation did not improve the GY over the uninoculated treatment along with the respective N application rates (Figure 3c). This indicates that resident or indigenous rhizobia, in this experimental site, could be more effective than inoculated *Rhizobium* isolate. This could due to the cultivation of common bean as a main crop in the experimental area, which leads to higher native rhizobia nodulating common bean (Pryor and Crush 2006). This condition might have reduced the nodule occupation by the effective inoculated isolate. A similar finding was reported on common bean by Vargas et al. (2000). In contrast to this observation, Fesenko et al. (1995) found a direct and significant correlation between seed yield and plant tissue N accumulation.

Significantly higher GY was obtained at *Rhizobium* inoculation in integration with 40 kg N ha^{-1} at Hirna site than the control. Beside this, the significant improvement in GY with *Rhizobium* inoculation in relation to uninoculated treatments was also obtained at 40 and 60 kg N ha^{-1} (Figure 3d). The better performance of inoculated *Rhizobium* in soil with high inherent P fertility was previously confirmed by Ankomah et al. (1996). This also indicates the need for *Rhizobium* inoculation to boost common bean GY. A similar effect was reported in a previous experiment performed on common bean (Hungria *et al*. 2000). Hungria *et al*. (2003) also found that *Rhizobium* inoculation along with 15 kg N ha^{-1} application at planting and 30 kg N ha^{-1} at early flowering stage gave the highest yield in common bean. They also reported 310 kg ha^{-1} GY improvement, which was lower than the 590.9 kg ha^{-1} GY enhancement obtained at Hirna site. At this site, the GY of common bean increased with increasing rates of N application alone. When N was applied in conjunction with *Rhizobium* inoculation, a slight reduction in GY was recorded in N rates beyond 40 kg N ha^{-1}. This could be attributable to the fact that the high native soil fertility enhanced the effectiveness of *Rhizobium* inoculation at this site and satisfied the need for N nutrient at 40 Kg N ha^{-1} to obtain the highest GY in common bean at Hirna site. A similar finding was also reported by Tsai *et al*. (1993), who found that N application improved the plant N accumulation, whereas *Rhizobium* inoculation with N application improved common bean grain yield.

The highest GY obtained from Babillae, Fedis, Haramaya, and Hirna sites was 2089.54, 1653.89, 2475.28 and 2441.57 kg ha^{-1}, respectively. The highest GY recorded at Haramaya, followed by the grain yield from Hirna site, could be associated with higher native soil fertility (Adamu *et al*. 2001), besides the presence of an environmental condition conducive for common bean production

(Fageria and Santos, 2008). This GY produced here is higher than that reported from eastern Ethiopia (Fininsa and Tesso 2006), in which the highest yield of common bean genotype 'Ikinnimba' obtained was 1830 kg ha^{-1}. The grain yields obtained at Haramaya and Hirna sites were comparable with that produced by 224 kg N ha^{-1}-treated plants (Buttery *et al*. 1987).

The regression analysis indicated negative and polynomial upward association between GY and NN, with a higher coefficient of determination at Fedis site (R^2=0.494 at p $\leq$0.05) that that at Babillae site (R^2=0.411 at p$\leq$0.05) (Figure 4a). Similarly, the association between GY and NDW was negative and polynomial upward, with a higher coefficient of determination at Fedis site (R^2=0.564 at p$\leq$0.05) than that at Babile site (R^2=0.513 at p$\leq$0.05) (Figure 4b). The lowest grain yields at these sites were obtained at 100 and 0.3000 g of NN and NDW, respectively. Above these nodulation values, the GY increased with increase in NN and NDW. This indicates the importance of inoculation for higher GY at Babillae and Fedis experimental sites. In contrast, Tajini *et al*. (2008) found that the common bean productivity decreased with an increase in NN beyond 80. Common bean has not been continuously cultivated in these two experimental locations, leading to lower native rhizobia in common bean and thus inoculation could perform better (Thies *et al*. 1991). In the case of Haramaya and Hirna sites, the association between GY with NN and NDW was significant and inversely related but the association between GY and NDW at Hirna site was non-significant. The figure 4a also indicated the GY in common bean decreased with an increase in NN at Hirna site, whereas the lowest GY was obtained at 200 nodules per plant at Haramaya site. Above this NN, the GY increased with an increase in NN. This indicates the importance of N application at Haramaya site to boost the common bean production even though the nodulation and PTTN was drastically reduced (Buttery *et al*. 1987).

The analysis of variance indicated that the non-significant effect of *Rhizobium* inoculation, N rates of application and their interaction on the harvest index of common bean, except the location effect (Table 2). The research data also revealed that there was no significant difference in harvest index (HI) among the experimental sites (Table 5). Although not statistically significant, the HI value calculated for Babillae site was higher than those obtained from the other experimental sites.

Table 4. Total biomass yield and grain yield of common bean var. Dursitu along different N application rates with and without inoculation with *Rhizobiumleguminosarum* bv. *phaseoli*

Treatments	TBY† (Kg ha^{-1})				GY† (Kg ha^{-1})			
	Babillae	**Fedis**	**Haramaya**	**Hirna**	**Babillae**	**Fedis**	**Haramaya**	**Hirna**
Control	2055.6ef	2077.8c	4819.8c	5015.3d	1025.65f	1082.13cd	2124.81bc	1767.29f
20 kgN ha^{-1}	2277.8def	4018.5ab	4970.1bc	6248.8bcd	1254.35e	1270.19bc	2298.89ab	1944.06def
40 kgN ha^{-1}	26.31.5cd	4333.3a	5732. 4abc	6970.4abcd	1568.52c	1307.13bc	2377.78ab	2146.76bcd
60 kgN ha^{-1}	3131.5abc	4055.6a	6388.9ab	6574.1bcd	1727.63bc	1289.44bc	2416.94a	1997.13de
80 kgN ha^{-1}	3082.4bc	4388.9a	6452.8ab	6990.7abcd	1674.26bc	1280.46bc	2439.35a	2321.85ab
100 kgN ha^{-1}	3260.8ab	4374.1a	6474.1a	7011.6abcd	1830.09b	1454.26ab	2475.28a	2357.59ab
HU*PvR*-16††	1787.0f	3133.3b	5260.1abc	5685.2cd	996.39f	1005.37d	2029.91c	1853.43ef
HU*PvR*-16. + 20 kgN ha^{-1}	2148.1def	4087.0a	6172.2abc	8638.9a	1302.22de	1258.70bc	2328.80ab	2009.54de
HU*PvR*-16 + 40 kgN ha^{-1}	2498.1de	4249.2a	6225.0abc	7111.1abc	1211.48ef	1569.72a	2320.74ab	2441.57a
HU*PvR*-16 + 60 kgN ha^{-1}	3375.4ab	4277.8a	6406.9ab	7296.3abc	1697.59bc	1461.85ab	2397.50a	2334.63ab
HU*PvR*-16 + 80 kgN ha^{-1}	3421.4ab	4462.2a	6435.2ab	6537.0bcd	1511.57cd	1571.57a	2425.28a	2055.40cde
HU*PvR*-16 + 100 kgN ha^{-1}	3648.1a	4740.7a	6444.4ab	8000.0ab	2089.54a	1653.89a	2413.80a	2245.00abc
Mean	2775.48	4016.54	5981.82	6839.95	1490.78	1350.39	2337.42	2122.86
LSD (0.05)	557.86	913.75	1484.4	2052.2	226.81	226.53	266.34	227.06
CV (%)	6.82	7.73	8.43	10.19	5.17	5.70	3.87	3.63

**** Significant at 0.001 probability level.*

†*TBY- Total biomass yield, GY- Grain yield*

††*HUPvR-16- Haramaya University Phaseolus vulgaris Rhizobium-16*

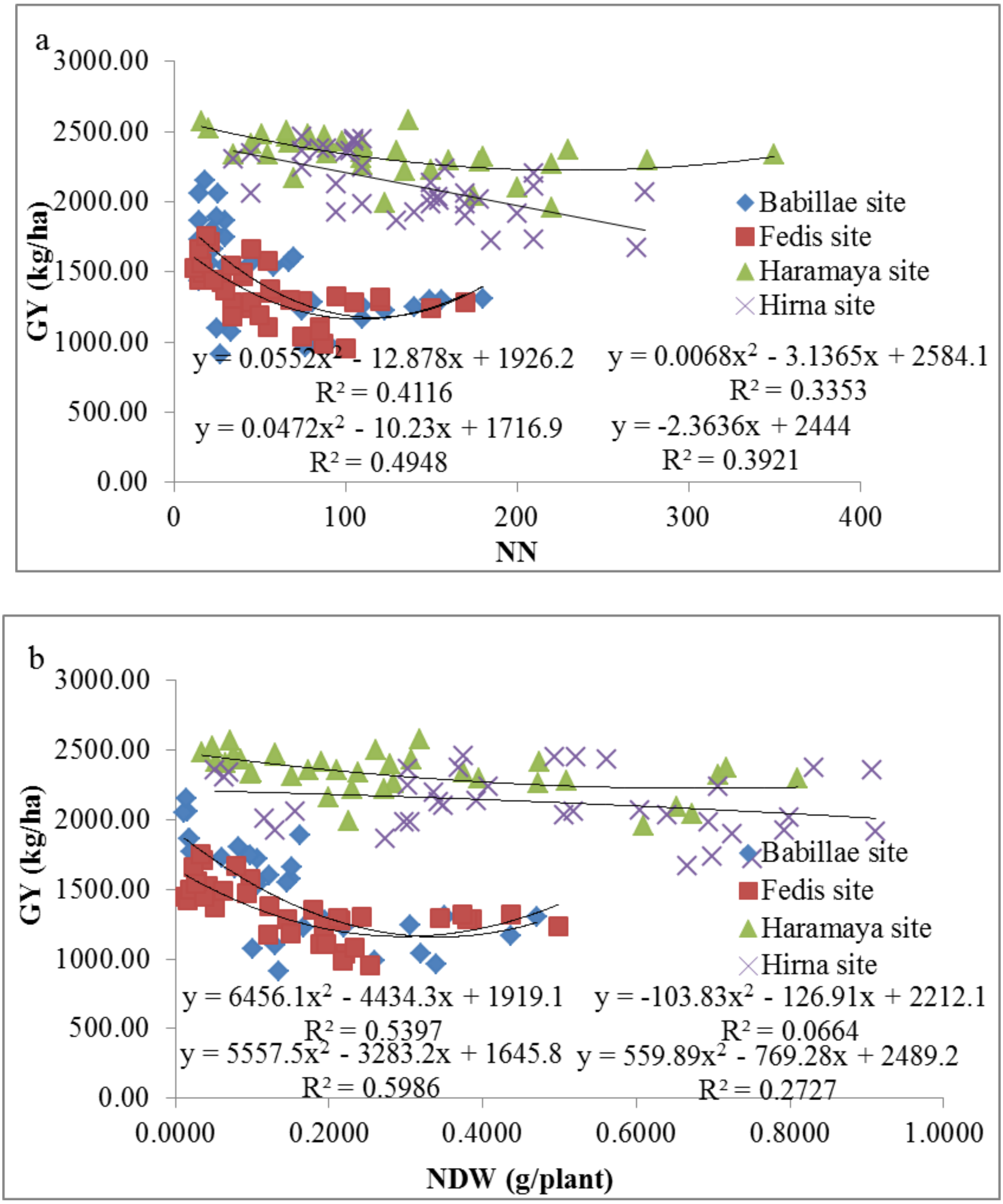

Figure 4. Regression of grain yield (GY) of common bean var. Dursitu on (a) nodule number per plant (NN) and (b) nodule dry weight (NDW) (g plant^{-1}) over different representative locations in eastern Ethiopia

Table 5. Harvest index and plant total tissue N of common bean var. Dursitu along different rates of N application with and without inoculation of *Rhizobiumleguminosarum*bv. *phaseoli*

Treatments	HI†				PTTN† (%)			
	Babillae	Fedis	Haramaya	Hirna	Babillae	Fedis	Haramaya	Hirna
Control	0.5449a	0.3574a	0.4077a	0.3553a	2.3800de	2.5200c	4.2100ab	3.9867d
20 kgN ha^{-1}	05549a	0.3479a	0.3927a	0.3092a	3.2433a	3.5533b	4.4367a	4.6233ab
40 kgN ha^{-1}	05645a	0.3290a	0.3375a	0.2590a	2.8533abcd	3.7800ab	4.5567a	4.7500a
60 kgN ha^{-1}	05400a	0.3499a	0.3314a	0.2486a	2.5567bcde	3.6933ab	3.7967abc	4.3567abcd
80 kgN ha^{-1}	0.5496a	0.3359a	0.4085a	0.2800a	2.5167cde	3.8600ab	4.0933ab	4.4867abc
100 kgN ha^{-1}	0.5489a	0.3099a	0.3957a	0.2967a	3.1567abc	4.2000a	3.4133bc	4.8267a
HU*PvR*-16††	0.5529a	0.3070a	0.3536a	0.3272a	2.1600e	3.3833b	4.0533abc	4.0500cd
HU*PvR*-16. + 20 kgN ha^{-1}	0.8076a	0.2973a	0.3733a	0.2933a	3.1800ab	3.3633b	4.3667a	4.0067d
HU*PvR*-16 + 40 kgN ha^{-1}	0.4371a	0.3458a	0.4048a	0.3230a	2.6567abcde	3.6800ab	3.9067abc	4.2533bcd
HU*PvR*-16 + 60 kgN ha^{-1}	0.5591a	0.3581a	0.3813a	0.2977a	2.7000abcde	3.8200ab	3.4133bc	4.5933ab
HU*PvR*-16 + 80 kgN ha^{-1}	0.8416a	0.3395a	0.3569a	0.3018a	3.2000ab	3.9200ab	3.2300c	4.6633ab
HU*PvR*-16 + 100 kgN ha^{-1}	0.5669a	0.4268a	0.2747a	0.2849a	3.0133abcd	3.8767ab	3.4800bc	4.0367cd
Mean	0.5890	0.3421	0.3682	0.2981	2.8014	3.6375	3.9130	4.3861
LSD (0.05)	-	-	-	-	0.6467	0.5621	0.8332	0.4799
CV (%)	29.26	13.29	21.86	17.64	7.84	5.25	7.23	3.72d

NS- non significant; *** Significant at 0.001 probability level.

†HI- Harvest index,

††HUPvR-16- Haramaya University Phaseolus vulgaris Rhizobium-16

This could be attributed to the semi-arid nature of Babillae site that triggered the plants to induce flowering and pod setting earlier; this might have led to higher HI at Babillae site due to the shorter vegetative stage. Generally, the HI obtained from this experiment is comparable with the previously reported values on various common bean genotypes (Snyder and Carlson 1984).

The analysis of variance indicated that a significant effect of N rates of application, inoculation, location and their interaction on the PTTN of common bean at $P < 0.05$ (Table 2). A significant ($p \leq 0.05$) difference in PTTN was recorded among different treatments (Table 5). The PTTN decreased with an increase in N application rates in both inoculation treatments at Babillae and Fedis experimental sites. This slight reduction in PTTN could be attributed to the inhibitory effect of higher N fertilization on nitrogenase enzyme (Tsai *et al*. 1993), consequently reducing the symbiotic N_2 fixation (Sanginga et al. 1996; Voisin *et al.* 2002). N_2 fixation improvement resulting from increased nodulation in common bean was previously confirmed by Asad *et al*. (1991). Nitrogen fertilizer application at 20 kg N ha^{-1} alone resulted in significantly higher PTTN at Babillae site. The highest PTTN was obtained at 40 kg N ha^{-1} alone at Haramaya site. A similar trend of nodulation and plant N accumulation was previously confirmed by Mothapo *et al.* (2013).

At Hirna and Fedis experimental sites, the highest PTTN was obtained from 100 kg N ha^{-1} alone, even though the highest nodulation was observed at low N application rates. A similar trend of plant N accumulation with increased mineral N application was previously reported on soybean by Tewari *et al*. (2007). Thuita et al. (2012) found that nodule improvement did not enhance N_2 fixation. Increase in plant N accumulation by mineral N application rather than inoculation was previously investigated in common bean by Asad *et al*. (1991). The highest (4.8267%) PTTN was obtained at Hirna site, followed by 4.5567% from Haramaya site. High native soil fertility probably increases the efficient utilization of soil N and effective symbiotic N_2 fixation (Sanginga *et al*. 1991; Ankomah et al. 1996).

Conclusions

In general, this investigation indicated that the *Rhizobium leguminosarum* bv. *viciae* inoculation and N application rates had various effects on nodulation, yield and yield components of common bean cultivated in different inherent soil fertility conditions. All investigated traits of common bean were higher in the high fertility soils than in the low fertility soils. The need for starter N to increase nodulation varied depending on the total soil N and inherent soil fertility. The inherent soil fertility affected the responsiveness of the common bean to inorganic N application. The native soil fertility, including the total soil N, also had a remarkable effect on the need for N for maximum common bean production. The effectiveness of *Rhizobium* inoculation varied regardless of the inherent soil fertility and soil total N. This result could be related to the prevailing weather conditions in the study sites. Further researches may be needed to investigate the effect of environmental conditions on the effectiveness of inoculation with *Rhizobium* species across different locations with various soil fertility statuses.

References

Adamu A, F Assefa, A Haile Mariam, and E Bekele. 2001. Studies of *Rhizobium* inoculation and fertilizer treatment on growth and production of faba bean (*Vicia faba*) in some 'yield depleted' and 'yield sustained' regions of Semien Shewa. *Ethiopia J. Scie.* 24(2): 197-211.

Ankomah AB, F Zapata, G Hardarson, and SKA Danso. 1996. Yield, nodulation, and N_2 fixation by cowpea cultivars at different phosphorus levels. *Biol. Fertil. Soils* 22:10-15.

Anteneh Argaw. 2007. *Symbiotic and phenotypic characterization of rhizobia nodulating common bean (Phaseolus vulgaris L.) from Eastern Ethiopia*. MSC thesis, Addis Ababa University, Addis Ababa, Ethiopia.

Anteneh Argaw, Bulti Tesso, Ayele Akuma. 2012. Assessment and Evaluation of Need of Inoculation of Effective Rhizobia Isolates on Common Bean (*Phaseolus vulgaris* L.) in Ethiopia Soils. In: *29th Annual Research and Extension Review Proceedings*. Edited by Mengistu Urge, Nigussie Dechassa, Mengistu Ketema, Mulugeta Yitayih, Getachew Animut. March 2012, Haramaya University, DireDawa, Ethiopia; pp. 60-62.

Asad S, KA Malik, and FY Hafeez. 1991. Competition between inoculated and indigenous *Rhizobium/Bradyrhizobium* spp. strains for nodulation of grain and fodder legumes in Pakistan. *Biol. Fertil. Soils* 12:107-111.

Buttery BR, SJ Park and WI Findlay. 1987. Growth and yield of white bean (*Phaseolus vulgaris* L.) in response to nitrogen, phosphorus and potassium fertilizer and to inoculation with *Rhizobium*. *Can. J. Plant Sci.* 67:425-432.

da Silva PM., SM Tsai, and R Bonetti. 1993. Response to inoculation and N fertilization for increased yield and biological nitrogen fixation of common bean (*Phaseolus vulgaris* L.). *Plant Soil* 152:123-130.

da Silveira PM, AJBP Braz, HJ Kliemann *et al*. 2005. Nitrogen fertilization of common bean grown under no-tillage system after several cover crops. *Pesqui. Agropecu. Bras.* 40:377–381.

Daba S and M Haile. 2002. Effects of rhizobial inoculant and nitrogen fertilizer on yield and nodulation of common bean under intercropped conditions. *J. Plant Nutr.* 25:1443–1455.

de Oliveira, WS, LW Meinhardt, A Sessitsch and SM Tsai. 1998. Analysis of *Phaseolus-Rhizobium* interactions in a subsistence farming system. *Plant soil* 204:107-115.

Fageria NK. 2002. Nutrient management for sustainability dry bean production in the tropics. *Commun. Soil Sci. Plan.* 33:1537–1575.

Fageria NK. 2014. Growth, Nutrient Uptake, and Use Efficiency in Dry Bean in Tropical Upland Soil. *J. Plant Nutr.* 37:2085-2093.

Fageria NK. and AB Santos. 2008. Yield Physiology of Dry Bean. *J. Plant Nutr.* 31:983-1004.

Fageria, NK., AB dos Santos and A Moreira. 2010. Yield, Nutrient Uptake, and Changes in Soil Chemical Properties as Influenced by Liming and Iron Application in Common Bean in a No-Tillage System. *Commun. Soil Sci. Plan.* 41:1740-1749.

Fageria NK and AB Santos. 1998. Phosphorus fertilization for bean crop in lowland soil. *Braz. J. Agric. Environ. Eng.* 2:119-246.

Fesenko AN, NA Provorov, IE Orlova, VP Orlov and BV Simarov. 1995. Selection of *Rhizobium leguminosarum* by. *viceae* strains for inoculation of *Pisum sativum* L. cultivars: Analysis of symbiotic efficiency and nodulation competitiveness. *Plant Soil* 172:189-198.

Figueiredo MVB, HA Burity, CR Martinez and CP Chanway. 2008. Alleviation of drought stress in the common bean (*Phaseolus vulgaris* L.) by co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici*. *Appl. Soil Ecol.* 40:182–188.

Fininsa C and B Tesso. 2006. Breeding food legumes for eastern Ethiopia. In *Food and forage legumes of Ethiopia: Progress and Prospects*. Edited by K. Ali, S. Ahmed, S. Beniwal, G. Kenneni, R. S. Malhotra, K. Makkouk, and M. H. Halila. Proceedings of the Workshop on food and forage legumes 22-26 September 2003, Addis Ababa, Ethiopia. Sponser: EIAR and ICARDA. International Center for Agriculture Research in the Dry Areas (ICARDA), Aleppo, Syria, pp. 124-130.

Gan YT, TD Warkentin, CL McDonald, RP Zentner and AVandenberg. 2009. Seed Yield and Yield Stability of Chickpea in Response to Cropping Systems and Soil Fertility in Northern Latitudes. *Agron. J.* 101:1113-1122.

Graham PH. 1992. Stress tolerance in *Rhizobium* and *Bradyrhizobium* and nodulation under adverse soil conditions. *Can J Microbiol* 38: 675-684.

Graham PH. 1981. Some problems of nodulation and symbiotic nitrogen fixation in *Phaseolus vulgaris* L.: a review. *Field Crops Res.* 4:93–112.

Graham PH, JC Rosas, CE de Jensen, EPeralta, BTlusty, JAcosta-Gallegos, PAA Pereira. 2003. Addressing edaphic constraints to bean production: the Bean/Cowpea CRSP project in perspective. *Field Crops Res.* 82:179–192

Haile M. 1990. Preliminary studies of biological nitrogen fixation by Haricot bean on two soil types in Hararghe, Ethiopia. In *Proceeding second regional workshop on bean research in eastern Africa*, March 5-8, 1990, Nairobi, Kenya, pp. 97-109.

Hardarson G. 1993. Methods for enhancing symbiotic nitrogen fixation. *Plant Soil* 152:1–17.

Hungria M, DS Andrade, LMO Chueire, A Probanza, FJ Guttierrez-Manero, and M. Megias. 2000. Isolation and characterization of new efficient and competitive bean (*Phaseolus vulgaris* L.) rhizobia from Brazil. *Soil Biol. Biochem.* 32:1515 1528.

Hungria M., RJ Campo and IC Mendes. 2003. Benefits of inoculation of the common bean (*Phaseolus vulgaris*) crop with efficient and competitive *Rhizobium tropici* strains. *Biol. Fertil. Soils* 39:88–93.

Isoi T, and S Yoshida. 1991. Low nitrogen fixation of common bean (*Phaseolus vulagaris* L.). *Soil Sci. Plant Nutr.* 37:559-563.

Kimura DS, K Schmidtke, R Tajima, K Yoshida, H Nakashima and R Rauber. 2004. Seasonal N uptake and N fixation by common and adzuki bean at various spacing. *Plant Soil* 258:91-101.

Leidi EO and DN Rodriguez Navarro. 2000. Nitrogen and phosphorus availability limit N fixation in bean. *New Phytol.* 147:337–346.

Li BY, DM Zhou, L Cang, HL Zhang, XH Fan and SW Qin. 2007. Soil micronutrient availability to crops as affected by long-term inorganic and organic fertilizer applications. *Soil Till. Res.* 96:166–173.

Li YY, CB Yu, X Cheng, CJ Li, JH Sun, FS Zhang, H Lambers and L Li. 2009. Intercropping alleviates the inhibitory effect of N fertilization on nodulation and symbiotic N fixation of faba bean. *Plant Soil* 323:295–308.

Mnasri B, ME Aouani and R Mhamdi. 2007. Nodulation and growth of common bean (*Phaseolus vulgaris*) under water deficiency. *Soil Biol. Biochem.* 39:1744–1750.

Mothapo NV, JM Grossman, T Sooksa-nguan, J Maul, SL Bräuer and W Shi. 2013. Cropping history affects nodulation and symbiotic efficiency of distinct hairy vetch (*Vicia villosa* Roth.) genotypes with resident soil rhizobia. *Biol. Fertil. Soils* 49:871–879.

Okogun JA, and N Sanginga. 2003. Can introduced and indigenous rhizobial strains compete for nodule formation by promiscuous soybean in the moist savanna agroecological zone of Nigeria?. *Biol. Fertil. Soils* 38:26–31.

Pryor HN and JR Crush. 2006. Elevated populations of effective rhizobia in the rhizoplane of white clover growing in pasture. *New Zeal. J. Agr. Res.* 49:85–87

Rai R. 1992. Effect of nitrogen levels and *Rhizobium* strains on symbiotic N_2 fixation and grain yield of *Phaseolus vulgaris* L. genotypes in normal and saline-sodic soils. *Biol. Fertil. Soils* 14:293-299.

Rosas JC and FA Bliss. 1986 Host plant traits associated with estimates of nodulation and nitrogen fixation in common bean. *Hort. Sci.* 21:287–289.

Ruiz-Díez B, S Fajardo and M Fernández-Pascual. 2012. Selection of Rhizobia from Agronomic Legumes Grown in Semiarid Soils to be employed as Bioinoculants. *Agron. J.* 104:550-559.

Rys GJ and PM Bonish. 1981. Effectiveness of *Rhizobium trifolii* populations associated with *Trifolium* species in Taranaki. *New Zeal. J. Exp. Agr.* 9:329-335.

Sanginga N, GD Bowen and SKA Danso. 1991. Intra-specific variation in growth and P accumulation of *Leucaena leucocephala* and *Gliricidia sepium* as influenced by soil phosphate status. *Plant Soil* 133:201-208.

Sanginga N, LE Wirkom, A Okogun, IO. Akobundu, RJ Carsky and G Tian. 1996. Nodulation and estimation of symbiotic nitrogen fixation by herbaceous and shrub legumes in Guinea savanna in Nigeria. *Biol. Fertil. Soils* 23:441-448.

Saxena AK., SK. Rathi and KVBR Tilak. 1996. Selection and evaluation of nitrate-tolerant strains of *Rhizobium leguminosarum* biovar *viceae* specific to the lentil. *Biol. Fertil. Soils* 22:126-130.

Sistachs E. 1970 Efectos de la fertilización nitrogenada y la inoculación en el rendimento y contenido de nitrogeno del frijol negro (*Phaseolus vulgaris*). *Rev. Cubana Ci. Agrie.* 4:233–237.

Snyder FW and GE Carlson. 1984. Selecting for partitioning of photosynthetic products in crops. *Adv. Agron.* 37: 47–72.

Tajini F, J Drevon, L Lamouchi, ME Aouani and M Trabelsi. 2008. Response of common bean lines to inoculation: comparison between the *Rhizobium tropici* CIAT899 and the native *Rhizobium etli* 12a3 and their persistence in Tunisian soils. *World J. Microbiol. Biotechnol.* 24:407–417.

Tesso B and Y. Moges. 2012. Common Bean Research Programme. In *Proceedings of the 29th Annual Research and Extension Review Workshop, 2011/2012*. Edited by M. Urge, N. Dechassa, M. Ketema, M. Yitayih, and G. Animut. editors. Haramaya University Office of Research Affairs, Haramaya, Ethiopia, pp. 16-20.

Pryor, H. N., and J. R. Crush. 2006. Elevated populations of effective rhizobia in the rhizoplane of white clover growing in pasture. *New Zeal. J. Agr. Res.* 49:85-87.

Tewari K., T Sato, M Abiko, N Ohtake, K Sueyoshi, Y Takahashi, Y Nagumo, T Tutida, and T Ohyama. 2007. Analysis of the nitrogen nutrition of soybean plants with deep placement of coated urea and lime nitrogen. *Soil Sci. Plant Nutr.* 53:772–781.

Thies JE, PW Singleton and B Bohlool. 1991. Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field grown legumes. *Appl. Environ. Microb.* 57:19–28.

Thuita M, P Pypers, L Herrmann, RJ Okalebo, C Othieno, E Muema and D Lesueur. 2012. Commercial rhizobial inoculants significantly enhance growth and nitrogen fixation of a promiscuous soybean variety in Kenyan soils. *Biol. Fertil. Soils* 48:87–96.

Thung M and IM Rao. 1999. Integrated management of abiotic stresses. In *Common bean improvement in the twenty-first century*. Edited by S. P., Singh. Kluwer, Dordrecht, pp 331–370.

Tsai SM., PM da Silva, WL Cabezas and R Bonetti. 1993. Variability in nitrogen fixation of common bean (*Phaseolus vulgaris* L.) intercropped with maize. *Plant Soil* 152:93-101.

Tsai SM, R Bonetti, SM Agbala and R Rossetto. 1993. Minimizing the effect of mineral nitrogen on biological nitrogen fixation in common bean by increasing nutrient levels. *Plant Soil* 152: 131–138.

Vargas MAT, IC Mendes and M Hungria. 2000. Response of field-grown bean (*Phaseolus vulgaris* L.) to *Rhizobium* inoculation and nitrogen fertilization in two Cerrados soils. *Biol. Fertil. Soils* 32:228–233.

Vlassak K., J Vanderleyden and A Franeo. 1996. Competition and persistence of *Rhizobium tropici* and *Rhizobium etli* in tropical soil during successive bean (*Phaseolus vulgaris* L.) cultures. *Biol. Fertil. Soils* 21:61-68.

Voisin A, C Salon, NG Munier-Jolain and B Ney. 2002. Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (*Pisum sativum* L.). *Plant Soil* 243:31–42.

Wortmann C, R Kirgby, C Eledu et al. 1998. Atlas of common bean (*Phaseolus vulgaris* L.) production in Africa. CIAT, Cali