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Rhizobium leguminosarum bv. *viciae* sp. inoculation improves the agronomic efficiency of N of common bean (*Phaseolus vulgaris* L.)

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Abstract

Background: Due to common bean derives lower nitrogen (N) from symbiotic N_2-fixation, it requires N either from inorganic fertilizer or soil N. Field experiments were conducted at four locations to evaluate the effect of *Rhizobium leguminosarum* bv. phaseoli inoculation on agronomic efficiency of N of common bean var. Dursitu major growing areas of eastern Ethiopia.

Methods: Six levels of inorganic N (0, 20, 40, 60, 80 and 100 kg N ha^{-1}) and two inoculation treatments (uninoculated and inoculated) were factorially combined and laid out in randomized completely block design, replicated three times.

Results: AE-N, nodule number per plant (NN) and nodule dry weight per plant (NDW) decreased with N rates of application beyond 20 kg N ha^{-1}. The highest AE-Ns at Babillae, Fedis and Haramaya sites were obtained from 20 kg N ha^{-1} applied with *Rhizobium* inoculation while 40 kg N ha^{-1} supplied with *Rhizobium* inoculation at Hirna site. Regardless of experimental sites, inoculation improved AE-N. A positive relationship between AE-N and NDW was also observed in all experimental sites. Significant increase in grain yield with increasing rates of N application was also observed.

Conclusion: Hence, it can be concluded that inoculation is recommendable to increases the efficient utilization of applied Mineral N.

Keywords: Agronomic efficiency of N, Common bean, Inoculation, *Rhizobium* sp.

Background

Common bean (*Phaseolus vulgaris* L.) is the most important food legume worldwide, providing the main source of protein for more than 300 million people, supplying about 20% of the protein intake per person (CIAT 2001; Broughton et al. 2003). Despite the fact that some grain yield gain recorded over the last decades, common bean production is still very low in many regions of sub-Saharan African (SSA). Average dry bean yield in most developing countries are very much less than that of potential, indicating that common bean production could be improved by increasing yields per unit land area (Yan et al. 1995). In Ethiopia, common bean is one of the major grain legumes cultivated, with its production centred in small farmers' fields where the use of N fertilizer is limited and average yields are low, usually less than 1 ton ha^{-1} (CSA 2013). While under experimental condition, the productivity of common bean at experimental site has obtained up to 4,517 kg ha^{-1} (IAR 1997, 1998; Assefa et al. 2006). The main causes of low productivity at farmer fields are a poor technology level, utilization of low agricultural input and cropping in low fertility soils, especially with low N content (Haile 1990; Beebe et al. 2013). Low soil fertility is one of the most important yield-limiting factors in most of the bean-producing regions of Ethiopia (Tana and Fininsa 2006). Among essential plant nutrients, nitrogen (N) is the most important limiting nutrient for crop production in the tropics,

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including Ethiopia (Sanchez 1976). In Latin American, the yield losses due to N deficiency has been recorded up to 45% when compared to the N fertilized plants (Thung and Rao 1999). This indicates the need for inorganic N application and/or a search for more effective rhizobial strains to enhance the growth and grain yield of common bean.

Use of nitrogenous fertilizers by smallholder farmers to increase crop production in SSA has been limited due to unaffordable by many subsistent farmers. Beside the fact that common bean recovered usually less than 50% of the applied N fertilizer (dos Santos and Fageria 2007, 2008). These conditions have therefore necessitated an approach to crop production that emphasizes biological N_2 fixation (BNF), with programs for rhizobial strain selection with superior symbiotic performance at a low cost. However, common bean is a promiscuous legume able to form symbioses with many rhizobial species and this nature result in poor N_2 fixing plant compared to other grain legumes (Hardarson et al. 1993). N_2 fixation in common bean can be increased through inoculation with certain highly efficient strains of *Rhizobium leguminosarum* biovar *phaseoli* (Hungria et al. 2000). Hungria et al. (2003) found comparable yield of common bean at 60 kg N ha^{-1} and *Rhizobium tropici* inoculated plants. Mulas et al. (2011) observed that inoculation of effective rhizobia attenuated intrinsic soil characteristics through the production of plant growth promoting properties beside N_2 fixation (Atzorn et al. 1988; Ahemad and Khan 2011; Stajkovic et al. 2011). Studies, however, indicated that the performance of *Rhizobium* inoculation alone in the field conditions is not satisfactory (Graham 1981; Buttery et al. 1987). This indicates the need of some mineral N application even though fertilized with N owing to poor nodulation and lack of responses to inoculation under field conditions (Graham et al. 2003). Studies indicated that low rates of inorganic N application have been shown to enhance nodule formation and function but are not sufficient to achieve maximum yields (da Silva et al. 1993; Hungria et al. 2003). These authors also indicate that low levels of N fertilizer associated with the inoculation with selected rhizobial strains, can stimulate plant growth, N_2 fixation and grain yield. Under these circumstances, inoculation trials must emphasize not only the benefits of bean inoculation, but also of the combination of that practice with N fertilization, in order to achieve a decrease in mineral N input whilst still obtaining maximum yields. However, there is little information on the effect of inoculation of elite *Rhizobium* sp. on use efficiency of N by common bean. Therefore the objectives of this experiment were to evaluate the effect of inoculation of elite *Rhizobium* sp. on use efficiency of N, nodulation and productivity of common bean in four representative experimental sites of Eastern Ethiopia.

Methods

Study areas

Four major common bean growing areas of eastern Ethiopia were selected to determine the effect of *Rhizobium* on use efficiency of N, nodulation and yield of common bean. The experimental fields are located in the Hirna [N09°13.157″ and E041°06.488″ at an altitude of 1,808 m above sea level (m.a.s.l.)], Fedis (N09°06.941″ and E042°04.835″ at an altitude of 1,669 m.a.s.l.), Babillae (N09°13.234″ and E042°19.407″ at 1,669 m.a.s.l.) and Haramaya (N09°24.954″ and E042°02.037″ at an altitude of 2,020 m.a.s.l.) agricultural research centers. These experiments were conducted during 2012 cropping season.

The soils had not been inoculated before with rhizobia strain nodulating common bean. Soil samples were collected in each experimental site by taking 25 cores (diameter 2.5 cm) from the top 20 cm depth in a grid pattern covering the entire filed site after removing debris and the top 1 cm of soil. The pooled sample was thoroughly mixed and a subsample of 1 kg soil was taken. The sub-sampled soils were air-dried, crushed, and passed through a 2-mm sieve prior to physical and chemical analysis. Details of physical and chemical characteristics of the soil of experimental sites are given in Table 1.

The population density of indigenous rhizobia nodulating common bean in the soils was determined by a plant infection technique (Vincent 1970). The seeds of common bean var. Dursitu were surface sterilized and germinated in petri dish that contained moist filter paper. One seedling was placed on pouches that contained Jensen's N-free nutrient solution. Each seedling was inoculated with 1.0 ml of soil solution 2 weeks afterwards. Nodulation was examined 21 days after inoculation. The total numbers of nodulated seedling were converted into most probable number of indigenous rhizobia nodulating common bean g^{-1} of soil.

Sources of seeds and *Rhizobium* strain

A common bean var. Dursitu was supplied by Lowland pulses research project, Haramaya University, Ethiopia. Variety was selected based on their yield, their maturity time and recentness of year of release. Strain of *Rhizbium* spp. (HU*Pv*R-16) was obtained from bio fertilizer research and production project, Haramaya University.

Inoculums preparation

Agar slope of strain of *Rhizobium* was supplied by Soil microbiology research laboratory, Haramaya University. For purification, the isolate was preliminarily cultured

Table 1 Soil analysis of experimental sites before sowing

Soil properties	Hirna soil	Babillae soil	Haramaya soil	Fedis soil
pH in H_2O	7.25	6.66	7.84	7.76
EC (mS cm^{-1})	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	9.94	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
Number of indigenous rhizobia of common bean g^{-1} soil	1.1×10^4	<10	2.8×10^3	2.5×10^2

in yeast extract mannitol agar medium (YEMA) (10 g mannitol, 1 g yeast-extract, 1 g KH_2P0_4, 0.1 g NaCl, and 0.2 g $MgS0_4{\cdot}7H_20$ per liter, pH 6.8) and incubated at 28°C for 5 days. The pure colony of the isolate was later transferred to YEM broth medium with gentle shaking at 120 rpm for 5 days. By this procedure, the *Rhizobium* culture reached the middle or late logarithmic phase, and cell density in the culture was estimated by measuring optical density (OD) using spectrophotometer at 540 nm. *Rhizobium* inoculant was prepared by mixing 30 g of sterilised decomposed filter-mud with 15 ml of broth cultures of the appropriate *Rhizobium* strain in polyethylene bags. The moisture content of the inocula was 35% (w/w). After incubating the inoculated filer-mud for 2 weeks at 28°C, the count of the *Rhizobium* was 1×10^9 g^{-1} carrier material. Populations of rhizobia in the inoculants were determined by duplicate plate counts (Vincent 1970).

Experimental design

Field trials were conducted in order to investigate the effects of dual application of inorganic N fertilizer with different rate with and without inoculations of *Rhizobium* strain on Agronomic efficiency of N of common bean. The treatments of this experiment t were obtained by factorially combined six levels of inorganic N (0, 20, 40, 60, 80 and 100 kg N ha^{-1}) and two inoculation treatments (inoculated and uninoculated). Then, the treatments were arranged in split plot with randomized complete block design (RCBD) with three replications. N rates of application were assigned as main plot treatment. *Rhizobium* treatments were applied as sub-plot treatments. N fertilizer in each level was divided into two equal parts; the first part of the N (20 kg N ha^{-1}) was applied along the furrow by hand and incorporated before planting time, and the remaining parts were used in the flowering stages.

The area was moldboard-plowed and disked before planting. The size of each main and sub plots were 3×5 m^2 and 3×2 m^2, respectively. Phosphorus (P) was applied at 20 kg P ha^{-1} as triple superphosphate uniformly before planting all plots. There were five rows per plot and the spacing was 40 cm between rows, 10 cm between plants, 1 m between sub plots and 1.5 between main plots. Disinfected seeds of common bean were planted after they were moistened with a 20% solution of sucrose and then inoculated (7 g inoculant per kg seed) with *Rhizobium.* Two seeds were sown per hill. After germination, the plants were thinned to one seedling per hill to obtain about 30 plants per row. Weeds were controlled over the growth period with hand hoeing. At late flowering and early pod setting stage (R_3-stage), five plants from the central rows of each plot were randomly chosen and harvested from central rows to record number of nodule $plant^{-1}$ (NN), nodule dry weight $plant^{-1}$ (NDW) and shoot dry weight $plant^{-1}$ (SDW). Furthermore, shoots of the plants were dried and later ground to pass a 0.5 cm sieve. Total N determinations were done by the Kjeldahl method of Bremner (1965). At physiological maturity stage on October 30, yield and yield attributed of common bean were recorded. Total dry biomass yield (TBY) and grain yield (GY) at 13% moisture content were determined. Agronomic efficiency of N (AE-N) was calculated by using the following formula (Fageria et al. 2013).

$$\mathrm{AE-N} = \mathrm{G_f} - \mathrm{G_u}/\mathrm{N_a}$$

where G_f is the grain yield of the N fertilized plot, G_u is the grain yield of the unfertilized plot, and N_a is the quantity of N applied.

Data analysis

Data were submitted to analysis of variance (SAS Institute 1999). Statistically significant differences between means were also determined by the LSD test (SAS Institute 1999).

Results and discussion

Agronomic efficiency of N

Table 2 shows a significant effect of inoculation of *Rhizobium leguminosarum* bv. *viciae* in conjunction with N rates of application on AE-N by common bean at $P < 0.05$. Regardless of the experimental sites and inoculation treatments, AE-N was significantly decreased with increasing rates of N application. Similar finding was previously reported on common bean by Fageria et al. (2014), as stated in Mitscherlich's law of diminishing yield. Reduction of AE-N could be due to the amount of grain yield of common bean increases reduced with increasing rates of N application (Rebeschini et al. 2014), consequently leading to lower AE-N at higher rate of N application. Similarly, the nutrient use efficiency usually decreased with increasing nutrient amount added (Dobermann and Cassman 2004). Salvagiotti et al. (2008) also found the largest AE-N at N rates less than 50 kg N ha^{-1}.

In all experimental sites, the *Rhizobium* inoculated treatment recorded higher AE-N than those obtained corresponding rates of N without inoculation (Figures 1, 2). Similarly, Fagaria et al. reported higher N use efficiency in 50 mgN treated with *Rhizobium* inoculation as compared to that obtained from 200 mgN treatment). On top of fixing N from atmosphere, many species of rhizobia can secrete growth hormone and thereby improving root growth (Antoun et al. 1998; Canbolat et al. 2006; Remans et al. 2008; Ahemad and Khan 2012) and nutrients uptake. This could also be due to increases the plant access to soil nutrients and, improving P availability through solubilizing unavailable P (Zaidi et al. 2009) and thus enhancing the N use efficiency of common bean. In addition, Camerini et al. (2008) found that inoculation of *Rhizobium* sp. enhanced the Indol-acetic acid (IAA) biosynthetic pathway thereby produced 60 times more IAA in its nodule than the wild counterpart. Kumar et al. (2011) found that plant growth promoting rhizobia improved grain yield of fenugreek by 36%.

The present study also indicated the positive effect of *Rhizobium* inoculation on AE-N at low rates of N application (i.e., less than 40 kg N ha^{-1}) (Figure 3). Figure 2 also displays that the rates of N beyond 20 kg N ha^{-1} applied with *Rhizobium* inoculation displayed the non-significant effect on AE-N. Several studies indicated 20 kg N ha^{-1} N application is the starter dose which

Table 2 Agronomic efficiency of N of common bean var. Dursitu obtained from selected areas of eastern Ethiopia with and without *Rhizobium leguminosarum*. bv. *vicieae* inoculation

Treatments	Agronomic efficiency of N (%)							
	Babillae		Fedis		Haramaya		Hirna	
	Non inoculated	Inoculated	Non inoculated	Inoculated	Non inoculated	Inoculated	Non inoculated	Inoculated
20 kg N ha^{-1}	6.81b	15.29a	9.40a	19.15a	8.70a	24.20a	8.84a	7.81b
40 kg N ha^{-1}	11.26a	5.38c	5.63b	17.35a	6.32ab	11.90b	9.49a	14.70a
60 kg N ha^{-1}	10.16a	11.69ab	3.46bc	9.77b	4.87b	9.21bc	3.83b	8.02b
80 kg N ha^{-1}	6.95b	6.44c	2.48c	8.70b	3.93b	7.26bc	6.93ab	2.52c
100 kg N ha^{-1}	7.12b	10.93b	3.72bc	7.78b	3.50b	5.69c	5.90ab	3.92c
Mean	8.46	9.95	4.94	12.55	5.47	11.65	7.00	7.39
F-value	10.91**	23.39***	22.63***	19.66***	9.96**	45.72***	4.90*	96.66***
LSD	2.95	3.90	2.69	5.55	3.11	5.09	4.80	2.24
CV (%)	12.98	14.60	20.24	16.46	21.17	16.24	25.50	11.29

Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

NS non significant.

* Significant at 0.05; ** significant at 0.01; *** significant at 0.001.

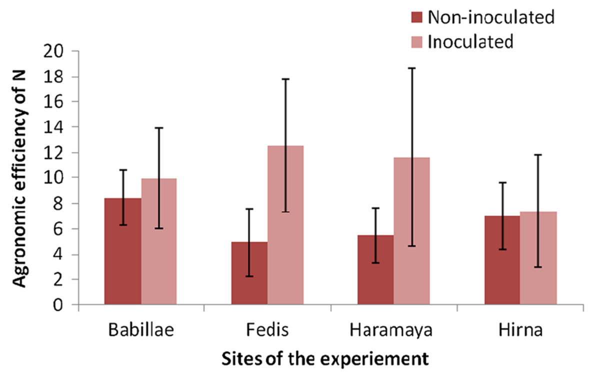

Figure 1 Agronomic efficiency of N of common bean with and without *Rhizobium leguminosarum* bv. *vicieae* cultivated in selected areas of eastern Ethiopia.

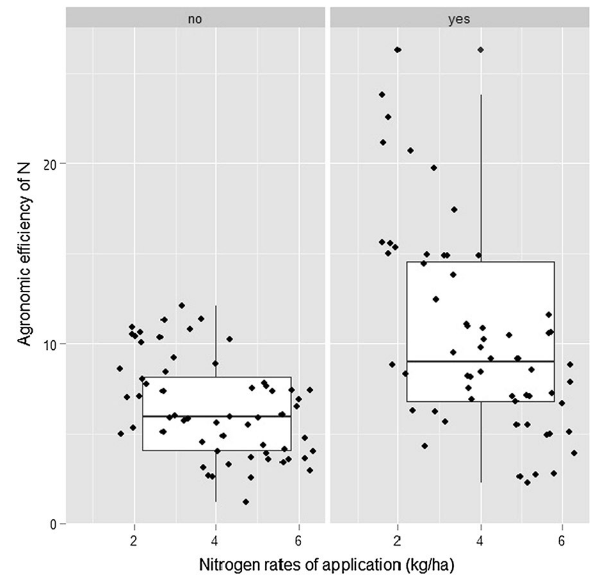

Figure 3 Agronomic efficiency of N of common bean with and without *Rhizobium leguminosarum* bv. phaseoli cultivated in selected areas of eastern Ethiopia.

enhances productivity and nodulation of common bean (Tsai et al. 1993b; Daba and Haile 2000; Hungria et al. 2003).

At Babillae site, the highest AE-N (10.16 and 11.6 kg kg^{-1}) from inoculated and uninoculated treatments was obtained at 40 and 20 kg N ha^{-1}, respectively. At Fedis site, 20 kg N ha^{-1} resulted in the highest AE-N (9.4 and 19.15 kg kg^{-1}) along uninoculated and inoculated treatments, respectively. Similarly at Haramaya site, 20 kg N ha^{-1} application gave the highest AE-N (8.7 and 24.2 kg kg^{-1}) along uninoculated and inoculated treatments, respectively. At Hirna site, 40 kg N ha^{-1} gave the highest AE-N (9.49 and 14.70 kg kg^{-1}) among uninoculated and inoculated treatments, respectively. This indicates the need of inoculation to boost grain yield at Fedis site. This also indicates the need of lower rate of N when inoculated common bean with effective *Rhizobium* sp. for the highest AE-N. All study locations, the AE-N obtained from uninoculated treatment were ranged from 3.9 to 11.8 kg kg^{-1} which was previously reported for common bean by Fageria et al. (2013). They found the range of AE-N between 3.9 and 11.8 kg kg^{-1}.

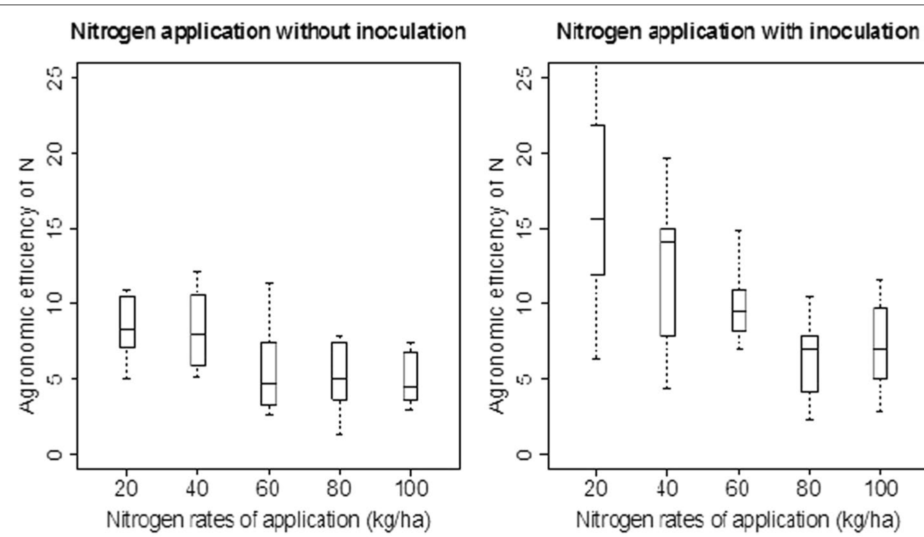

Figure 2 Agronomic efficiency of N of common bean with and without *Rhizobium leguminosarum* bv. phaseoli cultivated in selected areas of eastern Ethiopia.

The highest AE-N of common bean at Babillae, Fedis and Haramaya were 15.29, 19.15 and 24.20 kg kg^{-1}, respectively, all obtained from *Rhizobium* inoculation in conjunction with 20 kg N ha^{-1} application. The highest AE-N (14.70) from Hirna site was recorded from *Rhizobium* inoculation with 40 kg N ha^{-1} application. The lowest AE-N observed at Babillae and Hirna were 6.44 and 2.52 kg kg^{-1}, respectively, from combined application of inoculation and 80 kg N ha^{-1}. The lowest AE-N (2.48 and 3.50 kg kg^{-1}) at 80 and 100 kg N ha^{-1}, were obtained from Fedis and Haramaya sites, respectively.

Agronomic efficiency of N and nodulation

The regression analysis indicated significant and polynomial association between AE-N and increasing rates of N application solely and in combination with *Rhizobium* inoculation. Concave upward ($R^2 = 0.372$ at $P \leq 0.05$) and downward ($R^2 = 0.440$ at $P \leq 0.05$) associations between the AE-N and N rates of application at Babillae site were observed in inoculated and uninoculated treatment, respectively (Figure 4a). This indicates the need of inoculation with low N application to increase AE-N at this experimental site. Negative and polynomial association between the AE-N and N rates of application were observed at Fedis site, with coefficient of determination of $R^2 = 0.819$ and $R^2 = 0.898$ in *Rhizobium* inoculated and uninoculated treatments, respectively, at $P < 0.05$ (Figure 4b). In Haramaya site, negative and polynomial association between AE-N and N rates of application with higher coefficient of determination ($R^2 = 0.906$) was observed in *Rhizobium* inoculated treatment (Figure 4c). Negative polynomial and linear association AE-N and N rates of application at Hirna site were observed in *Rhizobium* inoculated and uninoculated treatments, respectively. (Figure 4d). This may indicate the native rhizobia could have been competent and effective N_2 fixation than the inoculated *Rhizobium* isolate.

It is also noted that the positive association between AE-N and NDW was observed in all experimental sites irrespective of inoculation treatments. Both inoculation treatments, the non-significant association between AE-N and NDW was observed at Babillae site (Figure 5a). At Fedis site, positive and significant association between AE-N and NDW was observed, with coefficient of determination of $R^2 = 0.812$ and $R^2 = 0.504$ in inoculated and uninoculated treatments, respectively (Figure 5b). Concave downward polynomial association was observed in *Rhizobium* inoculated treatments, whereas in uninoculated treatment exhibited concave upward polynomial association. At Haramaya site, significant and polynomial association between AE-N and NDW with coefficient of determination of $R^2 = 0.701$ and $R^2 = 0.742$ were observed in *Rhizobium* inoculated and uninoculated treatment, respectively (Figure 5c). These associations were concave upward and downward in inoculated and uninoculated treatments, respectively. This indicates that the highest AE-N was recorded at lower NDW value with inoculated treatment than uninoculated treatment. This could be due to the highly effective and competitiveness of inoculated isolate than those present in the indigenous soils as previously confirmed by Willams and Phillips (1983) and; Asad et al. (1991). Significant and concave downward association between AE-N and NDW in Hirna site was observed only in uninoculated treatments, but the association in inoculated treatment was insignificant (Figure 5d).

Nodulation

A significant variation in NN and NDW along increasing rates of N application alone and in combination with *Rhizobium* inoculation was observed (Tables 3, 4).

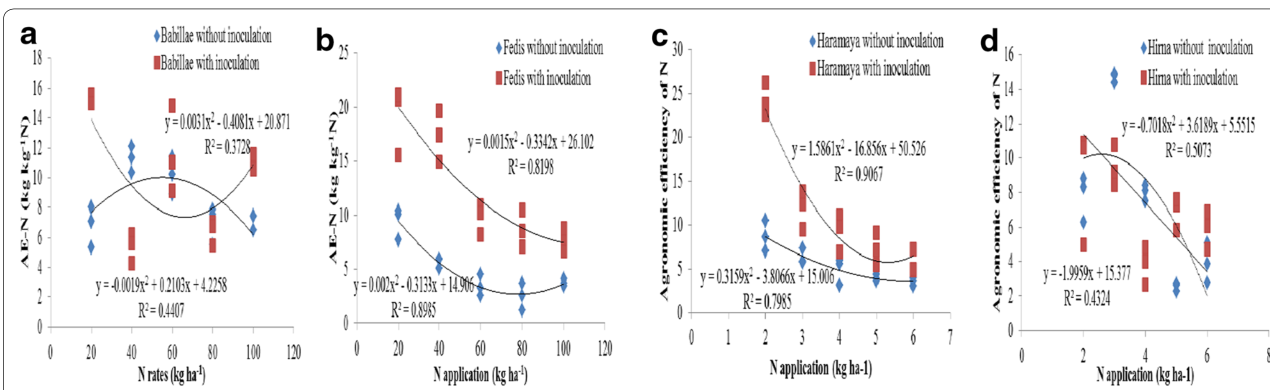

Figure 4 Regression of agronomic efficiency of N (AE-N) of common bean var. Dursitu on N rates of application at **a** Babillae, **b** Fedis, **c** Haramaya and **d** Hirna experimental sites.

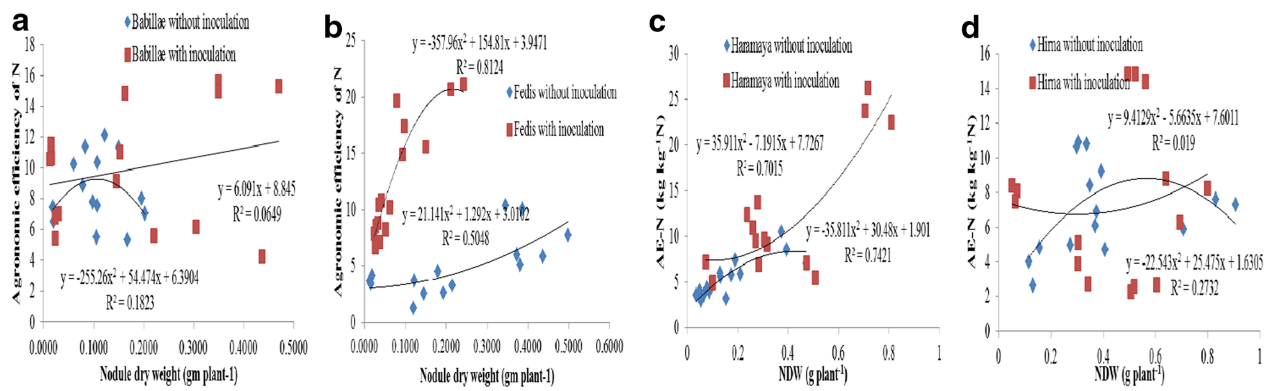

Figure 5 Regression of agronomic efficiency of N (AE-N) of common bean var. Dursitu on nodule dry weight (NDW) at **a** Babillae, **b** Fedis, **c** Haramaya, and **d** Hirna experimental sites.

Table 3 Nodule number per plant of common bean var. Dursitu obtained from selected areas of eastern Ethiopia with and without *Rhizobium leguminosarum*. bv. *vicieae* inoculation

Treatments	Nodule number per plant							
	Babillae		Fedis		Haramaya		Hirna	
	Non inoculated	Inoculated	Non inoculated	Inoculated	Non inoculated	Inoculated	Non inoculated	Inoculated
Control	28.33b	82.33c	71.67bc	90.67a	109.33b	198.33a	216.67a	175.00a
20 kg N ha^{-1}	88.67a	161.67a	146.67a	64.33ab	282.00a	190.00ab	130.00bc	167.33ab
40 kg N ha^{-1}	65.00a	124.33b	106..67ab	46.67bc	109.33b	141.67abc	190.00ab	173.33a
60 kg N ha^{-1}	18.67b	26.00d	45.00 cd	27.33c	84.33b	116.33bc	128.33bc	106.67c
80 kg N ha^{-1}	31.00b	17.33d	45.00 cd	20.00c	70.33b	91.33 cd	94.67c	108.33bc
100 kg N ha^{-1}	22.33b	19.67d	20.00d	22.33c	46.00b	35.33d	90.00c	91.00c
Mean	42.33	71.89	72.50	45.22	116.89	128.83	141.61	136.94
F-value	21.57***	113.76***	29.92***	23.69***	17.24***	14.17***	9.02***	9.59***
LSD	28.73	27.26	40.62	27.30	96.56	77.88	81.10	59.59
CV (%)	24.74	13.83	20.43	22.10	30.12	22.04	20.88	15.87

Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

NS non significant.

* Significant at 0.05; ** significant at 0.01; *** significant at 0.001.

Regardless of inoculation treatments, NN and NDW were decreased with increasing rates of N fertilizer application in all experimental sites. Similarly Brockwell et al. (1989) demonstrated that nodulation was diminished at high N application irrespective of rates of inoculation. Rebeschini et al. (2014) observed reduction of nodule number and dry weight with increasing rates of N application. Beside this, several findings also indicated reduction of nodule formation and development by inorganic N application (Chemining'wa et al. 2004; Gentile et al. 2006; Otieno et al. 2009; Shamseldin and Moawad 2010). This reduction of nodulation could arise from reducing the flavonoids and isoflavonoids by the plant roots into the soils (Waterer and Vessey 1993).

At Babile site, statistically higher NN and NDW was produced at 20 kg N ha^{-1} alone and in combination with inoculation of *Rhizobium*. This study, however indicated the non-significant difference of NDW obtained from 20 kg N ha^{-1} and the control treatment (without N application). The highest NN produced at Babile site was 161.67 at 20 kg N ha^{-1} with *Rhizobium* inoculation. At Fedis site, significantly higher NN and NDW were observed at 20 and 40 kg N ha^{-1} applications along uninoculated treatment. Along *Rhizobium* inoculation treatment, the control treatment induced the highest NN and NDW. Likewise, at Haramaya site, 20 kg N ha^{-1} application without inoculation resulted in significantly higher NN and NDW than the other treatments. Along

Table 4 Nodule dry weight of common bean var. Dursitu obtained from selected areas of eastern Ethiopia with and without *Rhizobium leguminosarum*. bv. *vicieae* inoculation

Treatments	Nodule dry weight (g/plant)							
	Babillae		Fedis		Haramaya		Hirna	
	Non inoculated	Inoculated	Non inoculated	Inoculated	Non inoculated	Inoculated	Non inoculated	Inoculated
Control	0.1217ab	0.3063ab	0.1920b	0.2533a	0.2183b	0.6185ab	0.6630a	0.8182a
20 kg N ha^{-1}	0.1880a	0.3900a	0.4097a	0.2010a	0.3873a	0.7446a	0.2926b	0.6776ab
40 kg N ha^{-1}	0.1263ab	0.3207a	0.3963a	0.0897b	0.1669bc	0.4334bc	0.3262b	0.4610bc
60 kg N ha^{-1}	0.0437bc	0.1530bc	0.1727b	0.0516b	0.1259bcd	0.2676 cd	0.1341c	0.4052c
80 kg N ha^{-1}	0.1033abc	0.0287c	0.1610b	0.0340b	0.0757 cd	0.2064d	0.1636c	0.2766c
100 kg N ha^{-1}	0.0193c	0.0210c	0.0160c	0.0279b	0.0418d	0.0827d	0.1502c	0.3421c
Mean	0.1004	0.2033	0.2246	0.1096	0.1693	0.3922	0.2883	0.4968
F-value	8.53***	23.96***	31.90***	24.54***	29.82***	40.79***	93.16***	13.93***
LSD	0.0992	0.1537	0.1276	0.0911	0.1078	0.1890	0.0985	0.2658
CV (%)	36.03	27.58	20.71	30.32	23.22	17.57	12.46	19.51

Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

NS non significant.

* Significant at 0.05; ** significant at 0.01; *** significant at 0.001.

Rhizobium inoculated, the control treatment produced the highest NN, although this NN was statistically similar with those NN produced at 20 and 40 kg N ha^{-1}. At Haramaya site, 20 kg N ha^{-1} produced significantly higher NDW than those treatment of N application greater than 20 kg N ha^{-1}. These results indicate that the inoculated isolate could fulfill the N needs of the plant in addition to the native soil total N at Fedis and Haramaya site as previously observed by Mnasri et al. (2007). The result obtained in these three sites agreed with previously reported by Daba and Haile (2000) who found that starter N with *Rhizobium* inoculation recommended to improve nodulation and yield of common bean in eastern Ethiopia. The application of a small amount of fertilizer N (15–30 kg N ha^{-1}) enhances nodulation of different legume crops (da Silva et al. 1993; Tsai et al. 1993b; Hungria et al. 2003). The better performance of common bean in nodulation in inoculated treatments might be related with plant growth promoting activities of inoculated isolate, beside N_2 fixation (Atzorn et al. 1988). They also found that plant growth hormones biosynthesis genes are closely linked to the genes for nodulation and N fixation. Wani et al. (2007) found that plant growth promoting rhizobia improved the nodule number by 23%.

The highest NN at Fedis and Haramaya were 146.67 and 282.00, respectively, at 20 kg N ha^{-1} application. Combined application of 20 kg N ha^{-1} and *Rhizobium* inoculation produced the higher NDW at Babillae and Haramaya, indicating the needs of *Rhizobium* inoculation and starter N to improve the nodule formation and development at these sites. Sole application of 20 kg N ha^{-1} resulted in the highest NDW at Fedis site, implying that this site could have higher native rhizobia nodulating common bean, thus inhibiting the effectiveness of inoculated isolate (Giller et al. 1998).

Regardless of the inoculation treatments, the highest NN and NDW at Hirna site were observed at the control treatments. The highest NN (216.67) at Hirna site was obtained from the control treatments with no inoculation of *Rhizobium* sp. This may indicate that soil had sufficient and effective number of rhizobia nodulating common bean (Theuri et al. 2006) and sufficient inherent soil N until the plant start fix N. Similar finding was reported by Chemining'wa et al. (2007) who found reduction in nodule number and nodule dry of common bean when added starter N fertilizer and improvement of these traits when inoculated *Rhizobium* alone.

Total biomass yield

TBY of common bean exhibited significant differences along increasing rates of N application alone and in combination with *Rhizobium* inoculation, excluding TBY at Haramaya site along inoculation treatment (Table 5). Generally, TBY was significantly improved with increasing rates of N application solely and in combination with *Rhizobium* inoculation. Similar trends of TBY improvement was previously observed by Herridge and Rose (2000) who found that enhancement of shoot biomass has been achieved through increasing N availability from N_2 fixation. Fageria et al. (2006) found that N application improves the leaf growth, leaf area duration, and photosynthetic rate per unit leaf area, consequently improving

Table 5 Total biomass yield of common bean var. Dursitu obtained from selected areas of eastern Ethiopia with and without *Rhizobium leguminosarum*. bv. *vicieae* inoculation

Treatments	Total biomass yield (kg ha^{-1})							
	Babillae		Fedis		Haramaya		Hirna	
	Non inoculated	Inoculated	Non inoculated	Inoculated	Non inoculated	Inoculated	Non inoculated	Inoculated
Control	2,055.6c	1,787.0c	2,077.0b	3,133.3b	4,819.8b	5,260.1a	5,051.3b	5,685.2b
20 kg N ha^{-1}	2,277.8bc	2,148.1bc	4,018.5a	4,087.0a	4,970.1b	6,172.2a	6,248.8a	8,638.9a
40 kg N ha^{-1}	2,631.5b	2,498.1b	4,333.3a	4,249.2a	5,732.4ab	6,225.0a	6,970.4a	7,111.1ab
60 kg N ha^{-1}	3,131.5a	3,375.4a	4,055.6a	4,277.8a	6,388.9a	6,406.9a	6,574.1a	7,296.3ab
80 kg N ha^{-1}	3,082.4a	3,421.4a	4,388.9a	4,462.2a	6,452.8a	6,435.2a	6,990.7a	6,537.0ab
100 kg N ha^{-1}	3,260.8a	3,648.1a	4,374.1a	4,740.7a	6,474.1a	6,444.4a	7,011.6a	8,000.0ab
Mean	2,739.92	2,813.05	3,874.7	4,158.4	5,806.3	6,157.3	6,468.5	7,211.4
F-value	30.29***	37.74***	22.99***	10.28***	6.47**	2.56 ^{ns}	10.33***	4.10*
LSD	429.05	596.71	886.73	814.16	1417.2	1347.5	1142.8	2450.2
CV (%)	5.71	7.73	8.34	7.14	8.90	7.98	6.44	12.39

Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

NS non significant.

* Significant at 0.05; ** significant at 0.01; *** significant at 0.001.

the total biomass of the crop. In all experimental sites, 100 kg N ha^{-1} gave significantly higher TBY, compared to the control treatments regardless of inoculation treatments, indicating the need of N application for common bean production. In both inoculation treatments, 40 kg N ha^{-1} and above rates of N application produced statistically similar TBY at Babillae site. At Fedis site, statistically similar TBY were also observed in all N treatments excluding the control, in both inoculation treatments. Rates of N application above 40 kg N ha^{-1} resulted in statistically similar TBY along uninoculated treatment at Haramaya site. While along inoculated treatment, TBY exhibited the non-significant difference with increasing rates of N application, excluding the unfertilized treatment. This may show the presence of high number of rhizobia nodulating common bean and higher total inorganic N in this soil (Thies et al. 1991; Tsai et al. 1993a; Gan et al. 2009). This might causes ineffectiveness of inoculated *Rhizobium* sp. (Li et al. 2009) and less response to N application (Mulvaney et al. 2001). Regardless of N rates of application, all N rates of application gave significantly higher TBY than the control in both *Rhizobium* inoculation treatments at Hirna site.

The highest TBY (3,648.1 kg ha^{-1}) at Babillae was produced at 100 kg N ha^{-1} applied with *Rhizobium* inoculation. This TBY was 387.3 kg ha^{-1} over that obtained from 100 kg N ha^{-1} alone. Similarly, at 100 kg N ha^{-1} supplied with *Rhizobium* sp. inoculation had the highest TBY (4,740.7 kg ha^{-1}) at Fedis site. At Haramaya site, however, the highest TBY (6,474.1 kg ha^{-1}) was obtained from 100 kg N ha^{-1} applied alone, indicating the presence of competitive and/or effective rhizobia in Haramaya soil. While at Hirna site, 100 kg N ha^{-1} with *Rhizobium* inoculation had the highest TBY (8,000.0 kg ha^{-1}) which was 988.4 kg ha^{-1} over that obtained from 100 kg N ha^{-1} alone. This indicates that common bean cultivated at this site highly responded to *Rhizobium* inoculation in comparison to uninoculated treatment, although the soil had higher soil N and native rhizobia nodulating common bean. The lowest TBY at Babillae, Fedis, Haramaya and Hirna sites were 1,787.0, 2,077.0, 48.19.8 and 5,051.3 kg ha^{-1} respectively. The control treatment without inoculation gave the lowest TBY at Fedis, Haramaya and Hirna sites. While at Babillae site, the control treatment with inoculation resulted in the lowest TBY, indicating the non-responsiveness of common bean for inoculation without starter N.

Grain yield

It is notice that there was a significant effect of N rates of application solely and in combination with *Rhizobium* inoculation on GY of common bean at $P \leq 0.05$ (Table 6). Generally, GY of common bean was significantly increased with increasing rates of N application solely and along with *Rhizobium* inoculation. Similar response of common bean for N fertilizer was previously observed by Vargas et al. (2000). This shows that the N obtained from biological N fixation and the experimental soil was inadequate to minimize the yield gaps and increase the common bean production. This finding is in line with previous report of Küçük (2011) who found that significant improvement of grain yield

Table 6 Grain yield of common bean var. Dursitu obtained from selected areas of eastern Ethiopia with and without *Rhizobium leguminosarum*. bv. *vicieae* inoculation

Treatments	Grain yield (kg ha^{-1})							
	Babillae		Fedis		Haramaya		Hirna	
	Non inoculated	Inoculated	Non inoculated	Inoculated	Non inoculated	Inoculated	Non inoculated	Inoculated
Control	1,025.65d	996.39e	1,082.13c	1,005.37c	2,124.81b	2,029.91b	1,767.29c	1,853.43e
20 kg N ha^{-1}	1,254.35c	1,302.22 cd	1,270.19b	1,258.70c	2,298.89ab	2,328.80a	1,944.06bc	2,009.54de
40 kg N ha^{-1}	1,568.52b	1,211.48de	1,307.13ab	1,569.72a	2,377.78a	2,320.74ab	2,146.76ab	2,441.57a
60 kg N ha^{-1}	1,727.63ab	1,697.59b	1,289.44b	1,461.85ab	2,416.94a	2,397.50a	1,997.13bc	2,334.63ab
80 kg N ha^{-1}	1,674.26ab	1,511.57bc	1,280.46b	1,571.57a	2,439.35a	2,425.28a	2,321.85a	2,055.40 cd
100 kg N ha^{-1}	1,830.09a	2,089.54a	1,454.26a	1,653.89a	2,475.28a	2,425.28a	2,357.59a	2,245.00bc
Mean	1,513.42	1,468.13	1,280.60	1,420.19	2,355.51	2,319.33	2,089.12	2,156.59
F-value	57.24***	66.38***	14.49***	20.15***	10.27***	5.71**	22.12***	30.56***
LSD	194.55	226.8	148.12	259.09	189.89	295.06	231.02	190.03
CV (%)	4.68	5.63	4.22	6.65	2.94	4.64	4.03	3.22

Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

NS non significant.

* Significant at 0.05; ** significant at 0.01; *** significant at 0.001.

of common bean was observed at 40 kg N ha^{-1} alone and in combination with *Rhizobium* inoculation. *Rhizobium* inoculated with 100 kg N ha^{-1} gave significantly higher GY than the other treatments at Babillae site. This indicates the synergetic effect of inoculation to utilize efficiently the applied inorganic N fertilizer. Along uninoculated treatment, at Fedis site, 100 kg N ha^{-1} resulted in significantly higher GY compared to the other N treatments. While among *Rhizobium* inoculation treatments, 100 kg N ha^{-1} gave the highest GY, but had no significant difference in GY N rated between 40 and 100 kg N ha^{-1}. This indicates the need of low rates of N application to boost the grain yield of common bean when applied with inoculation as compared to N fertilizer alone.

N rated between 20 and 100 kg N ha^{-1} was exhibited non-significant effect on GY at Haramaya sites. In both inoculation treatments, the result indicated increases in GY with increasing rates of N application, although N rates of application reduced NN and NDW. Similar finding was observed on soybean by Osborne and Riedell (2011). At Hirna site, a significant increase in GY with increasing rates of N application without inoculation was observed. While along *Rhizobium* inoculation treatment, a significant improvement of GY was observed up to 40 kg N ha^{-1} but the rates above 40 kg N ha^{-1} exhibited a significant reduction of GY. The presence of competitive and efficient indigenous rhizobia (Thies et al. 1991) and high soil total N (Gan et al. 2009) could be the causes of need of low N application when applied in conjuction with inoculation.

The highest GY (2,089.54 kg ha^{-1}) at Babillae site was obtained from 100 kg N ha^{-1} applied with *Rhizobium* inoculation. This GY was 259.45 kg ha^{-1} over those obtained from 100 kg N ha^{-1} alone. Similarly, at Fedis site, the highest GY (1,653.89 kg ha^{-1}) was produced from 100 kg N ha^{-1} applied with *Rhizobium* inoculation. Similar finding on chickpea has been previously reported by Namvar et al. (2011) who found that inoculation together with N application gave better yield of chickpea than those obtained from N applied without inoculation. The highest GY (2,475.28 kg ha^{-1}) at Haramaya site was obtained from sole application of 100 kg N ha^{-1}. Similarly, improvement of common bean seed yield has been previously observed by Soratto et al. (2004) and Pelegrin et al. (2009). They found that the highest GY of common bean was obtained at 130 and 182 kg N ha^{-1} of N application in different tillage practices. *Rhizobium* inoculation applied with 40 kg N ha^{-1} gave the highest GY (2,441.57 kg ha^{-1}) at Hirna site. Similarly, Hungria et al. (2003) found the highest common bean production at low rates of N applied with *Rhizobium* inoculation. On the other hand, the lowest GY at Babillae, Fedis and Haramaya were 996.39, 1,005.37 and 2,029.91 kg ha^{-1} obtained from *Rhizobium* inoculated without N application. This indicates that *Rhizobium* inoculation without N application in these experimental sites is insufficient to increase common bean yield even though, it was improved significantly the NN and NDW. However, the lowest GY (1,767.29 kg ha^{-1}) at Hirna site was produced at the control treatment without inoculation implies that the native rhizobia are not effective in N_2 fixation.

Plant total tissue N

In Table 7 indicates a significant effect of N rates of application solely and along with *Rhizobium* inoculation on PTTN at $P \leq 0.05$. At Babillae site, 20 kg N ha^{-1} gave significantly higher PTTN, compared to the other N rates of application along uninoculated treatments. While along *Rhizobium* inoculated treatment, 80 kg N ha^{-1} had the highest PTTN, although this PTTN had no significant difference with those PTTN obtained from other N treatments. The highest PTTN at Fedis site along uninoculated treatment was recorded at 100 kg N ha^{-1}. While along inoculated treatments, the PTTN exhibited the non-significant difference along increasing rates of N application. This implies that the presence of higher soil N causes the ineffectiveness of inoculated *Rhizobium* sp., despite the fact that inoculation improved nodulation. At Haramaya site, decreases in PTTN with increasing rates of N application were recorded regardless of inoculation treatments. This may indicate the presence of competitive and effective indigenous rhizobia nodulating common bean. in this site, the highest PTTN were obtained from 40 kg N ha^{-1} alone and 20 kg N ha^{-1} applied with inoculation. On the other hand, low PTTN at high N application could be related with the inhibitory effect of N application on nodulation and N_2 fixation of common bean in this soil, as has already been indicated by Herridge and Brockwell (1988). Decrease in plant tissue N of *Onobrychis viciifolia* was also observed with increasing rates of N application beyond 40 kg N ha^{-1} (Tufenkci et al. 2006). Excessive application of N had a detrimental effect, possibly through reducing nitrogenase activities (Tsai et al. 1993b; Saxena et al. 1996) and thus reducing the symbiotic N_2 fixation (Voisin et al. 2002). Sanginga et al. (1988) observed that N fertilizer depressed N fixation by 56%. At Hirna site, slight increase in PTTN with increasing rates of N application was observed. Accordingly, the highest PTTN was obtained at 100 kg N ha^{-1} alone and 80 kg N ha^{-1} conjunction with inoculation. Enhancement of PTTN with increasing N application in high P containing soil has been previously reported by Ankomah et al. (1996).

Conclusion

The result of this experiment indicated that inoculation of elite isolate of *Rhizobium leguminosarum* bv. Phaseoli improves the use efficiency of N by common bean in all experimental locations. Similarly the AE-N of common bean showed direct and positive relationship with nodule dry eight common beans, indicating the importance of nodulation and the consecutive output of this biological process on to improve efficient utilization of applied mineral N fertilizer. Accordingly, the starter amount of N fertilizer is recommended for all experimental sites to get the highest AE-N of common bean. Beside this, the soil inherent soil fertility status and indigenous rhizobia nodulating common bean affects effectiveness of sole application of N fertilizer and in combination with *Rhizobium* inoculation on the productivity of common bean. *Rhizobium* inoculation reduced the need of exogenous N application to get maximum common bean yield in the study sites.

Table 7 Plant total tissue N of common bean var. Dursitu obtained from selected areas of eastern Ethiopia with and without *Rhizobium leguminosarum*. bv. *vicieae* inoculation

Treatments	Plant total tissue N (%)							
	Babillae		Fedis		Haramaya		Hirna	
	Non inoculated	Inoculated	Non inoculated	Inoculated	Non inoculated	Inoculated	Non inoculated	Inoculated
Control	2.3800c	2.1600b	2.5200c	3.3833a	4.2100ab	4.0533ab	3.9867b	4.0500b
20 kg N ha^{-1}	3.2433a	3.1800a	3.5533b	3.3633a	4.4367a	4.3667a	4.6233a	4.0067b
40 kg N ha^{-1}	2.8533abc	2.6567ab	3.7800ab	3.6800a	4.5567a	3.9067ab	4.7500a	4.2533ab
60 kg N ha^{-1}	2.5567bc	2.7000ab	3.6933b	3.8200a	3.7967ab	3.4133bc	4.3567ab	4.5933a
80 kg N ha^{-1}	2.5167c	3.2000a	3.8600ab	3.9200a	4.0933ab	3.2300c	4.4867a	4.6633a
100 kg N ha^{-1}	3.1567ab	3.0133a	4.2000a	3.8767a	3.4133b	3.4800bc	4.8267a	4.0367b
Mean	2.7844	2.8183	3.6011	3.6739	4.0844	3.7417	4.5050	4.2672
F-value	7.18**	11.01***	40.88***	3.73*	5.16**	10.19***	9.54***	10.95***
LSD	0.6348	0.5683	0.4251	0.6064	0.8849	0.6496	0.4709	0.4219
CV (%)	8.13	7.35	4.30	6.02	7.90	6.33	3.18	3.60

Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

NS non significant.

* Significant at 0.05; ** significant at 0.01; *** significant at 0.001.

Authors' contributions
Both of us participated starting from the development of the research idea, writing proposal and competing research grant and development of this manuscripts. But, mine was more participated in the management and collection of data from the field experiment that is way; I am the first author of this manuscript. Both authors read and approved the final manuscript.

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Compliance with ethical guidelines

Competing interests
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