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Indigenous rhizobia population influences the effectiveness of *Rhizobium* inoculation and need of inorganic N for common bean (*Phaseolus vulgaris* L.) production in eastern Ethiopia

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Abstract

Background: Supplement with inorganic N application is essential to improve the common bean production in sub-Saharan Africa. However, the influence of indigenous rhizobial population on the inorganic N requirement with *Rhizobium* inoculation to secure sustainable way of common bean production system is not well known. The effect of different rates of N application either alone or in combination with *Rhizobium* inoculation on the nodulation, yield and yield traits of common bean cultivated in soils with different rhizobial population were conducted.

Methods: Twelve treatments were produced by factorially combined six levels of N fertilizer (0, 20, 40, 60, 80 and 100 kg N ha^{-1}) and two *Rhizobium* inoculation treatments (inoculated and uninoculated). The treatments were laid out in randomized completely block design and all treatments were replicated three times.

Result: Regardless of soil types, nodule number and nodule dry weight decreased with increasing rates of N application. 20 kg N ha^{-1} both alone and in combination with *Rhizobium* inoculation resulted in the largest nodulation in all soil types. The largest nodulation were induced in soil with large rhizobial population. *Rhizobium* inoculation significantly ($P < 0.05$) improved yield and yield traits of common bean. Moreover, our result revealed that the largest values of investigated traits were observed in inoculated treatment, as compared to the corresponding N rates of uninoculated treatments. The 20, 100 and 40 kg N ha^{-1} treatments resulted in significantly greater plant total tissue N at soil types with small, medium and large rhizobial population, respectively, as compared to unfertilized control. The highest total biomass yield (TBY) and grain yield (GY) at soil types with small and medium rhizobial population were obtained by the 100 kg N ha^{-1} treatment in combination with *Rhizobium* inoculation, while 20 and 40 kg N ha^{-1} applications produced the greatest TBY and GY, respectively, in soil with large rhizobial population.

Conclusion: These results indicate that N requirement is varied based on rhizobial population and effectiveness of native rhizobia in N_2 fixation.

Keywords: Common bean (*Phaseolus vulgaris* L.), Ethiopia, Indigenous rhizobia, *Rhizobium leguminosarum* bv. Phaseoli

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Background

Common bean (*Phaseolus vulgaris* L.) is one of most important food legume cultivated on greater than 4 million ha, providing the main source of dietary protein and carbohydrate for eastern and central African peoples, and containing about 20–25 % of the protein [1]. In Ethiopia, common bean is one of the major grain legumes, with its production centered in small farmers' fields where the soil fertility is depleted. Furthermore, the use of nitrogen (N) fertilizer is limited and average yields are low, usually less than 1 ton ha^{-1} [2]. In contrast, some studies indicated that up to 4600 kg ha^{-1} seed yield of common bean were obtained from research managed experimental plots in Ethiopia [3–5]. Low soil fertility, especially low N content, is the most important limiting nutrient status for common bean production in the tropics, including Ethiopia [6, 7].

Due to high price of N mineral fertilizers, their use by subsistent farmers in sub-Saharan Africa (SSA) to increase crop production has been limited. This condition has therefore necessitated an approach to crop production that emphasizes biological N_2 fixation (BNF). However, common bean is considered as a poor nitrogen fixing plant in comparison to other grain legumes due to its promiscuous nature, i.e., it forms symbiosis with many rhizobia species [8]. On top of this, several findings revealed that common bean generally responds poorly to *Rhizobium* inoculation under field conditions [9–11]. The reason for the failure to respond inoculation is believed to be due to a high and inefficient population of native common bean nodulating rhizobia in soil [9, 12, 13].

It is indicated that N_2 fixation in common bean can be increased through highly efficient *Rhizobium* inoculation [14]. Moreover, elite *Rhizobium* isolates improved the productivity of common bean, although in soil with high rhizobial nodulating population [15–17]. Asad et al. [15] found that the efficient *Rhizobium* inoculation did not fulfill the N needs of common bean. In most cases, common bean is able to fix up to 50 kg N ha^{-1} [18], which is less than 50 % of the plant N requirement [19]. Therefore, common bean requires mineral N application to achieve substantial yields under the current cropping system in SSA. However, N mineral recommended rates >40 kg N ha^{-1} suppress nodulation and N_2 fixation [10, 20, 21]. On the other hand, the application of a small amount of fertilizer N (<30 kg N ha^{-1}) enhanced nodulation, but grain yield improvement was not satisfactory [10, 22]. Higher stimulation of plant growth, N_2 fixation and grain yield of common bean have been recorded at low levels of N fertilizer applied with *Rhizobium* inoculation [17, 23–25]. Therefore, inoculation trials must emphasize not only the benefits of common bean inoculation, but also the combination of that practice with N fertilization, in order to achieve a decrease in mineral N input, whilst still obtaining maximum yields. However, the amount of N required in conjunction with elite *Rhizobium* inoculation to get maximum yield of common bean in soil with different rhizobial population is unknown. Hence, the objective of this study was to investigate the effect of naturalized common bean rhizobia population on the N rates of applied when alone or in combination with *Rhizobium* inoculation on nodulation, yield and yield traits of common bean in major growing areas of eastern Ethiopia.

Methods

Study areas

Field experiments were conducted on four locations of Eastern Ethiopia having different indigenous rhizobia nodulating common bean in 2012 cropping season. The experimental sites were located in the Hirna [N09°13.157″ and E041°06.488″ at an altitude of 1808 m above sea level (m.a.s.l.)], Fedis (N09°06.941″ and E042°04.835″ at an altitude of 1669 m.a.s.l.) Babillae (N09°13.234″ and E042°19.407″ 1669 m.a.s.l.) and Haramaya (N09°24.954″ and E042°02.037″ at an altitude of 2020 m.a.s.l.) agricultural research centers. The soils had not been inoculated before with rhizobia isolates nodulating common bean. Before sowing, soil samples were taken from 0–20 cm depth to determine baseline soil properties. Soil samples 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.

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 its better performance in eastern Ethiopia. Strain of *Rhizobium leguminosarum* bv. Phaseoli (HU*PvR*-16) was obtained from Biofertilizer Research and Production Project, Haramaya University (Haramaya, Ethiopia). This strain was selected because it was previously found efficient while tested in this region on two improved varieties of common bean under laboratory and greenhouse conditions [26].

Inoculums preparation

Agar slope of HU*PvR*-16 strain was obtained from Soil Microbiology Research Laboratory, Haramaya University, Ethiopia. For purification, this isolate was preliminarily cultured in YEMA medium (10 g mannitol, 1 g yeast-extract, 1 g KH_2P0_4, 0.1 g NaCl, and 0.2 g $MgSO_4{\cdot}7H_2O$ 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

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)	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

medium and incubated at 28 °C for 5 days with gentle shaking at 120 rpm in shaker incubator. By this procedure, cell density in the culture was estimated by measuring optical density (540 nm) to determine whether the *Rhizobium* culture reached the middle or late logarithmic phase. *Rhizobium* inoculant was prepared by mixing 30 g of sterilized decomposed filter-mud with 15 ml of broth culture containing HU*PvR*-16 strain in polyethylene bags. After incubating the inoculated filter-mud for 2 weeks at 28 °C, the count of the *Rhizobium* was reached 1×10^9 g^{-1} of inoculant. Populations of rhizobia in the inoculants were determined by duplicate plate counts (Vincent, 1970).

Enumeration of indigenous rhizobia nodulating common bean

The initial indigenous rhizobia population was determined by the plant infection technique, using inoculation of serially diluted soil on germinated common bean seedling for nodulation assessment following the method of Brockwell et al. [27]. This experiment was conducted under controlled condition in growth chamber. The most probable number (MPN) was calculated from the most likely number, using the MPN tables of Vincent [28]. The rhizobial population that nodulated common bean in all study sites are indicated in Table 1.

Experimental design

Field trials on three soil types which had different rhizobial population nodulating common bean were conducted in order to investigate the effect of indigenous rhizobial population on the effect of N rates when applied alone or in combination with *Rhizobium* inoculation. The treatment effects were evaluated via determination of nodulation, yield and yield traits of common bean. The experimental design was a split plot in randomized complete block design (RCBD) with three replications. Main plot treatments consisted of six levels of inorganic N: 0, 20, 40, 60, 80 and 100 kg N ha^{-1}. Two *Rhizobium* inoculations (inoculated and uninoculated) were assigned as subplot treatments. Nitrogen fertilizer in each level was divided into two equal parts: (1) the first part of the N (20 kg N ha^{-1}) was applied along the furrow by hand and incorporated before planting time, and (2) the remaining parts were applied at flowering stages (R_3-stage).

The area was moldboard-plowed and disked before planting. The sizes of the main and subplot were 3×5 m^2 and 3×2 m^2, respectively. There were five rows per subplot and the spacing was 40 cm between rows, 10 cm between plants, 1 m between subplots and 1.5 between main plots. Disinfected seeds of common bean were sown after they were moistened with a 20 % solution of sucrose and then inoculated (7 g inoculant per kg seed) with *Rhizobium.* Inoculated seeds were hand planted on July 7, 2012. Phosphorus (P) was uniformly applied at planting at rate of 20 kg P ha^{-1} as triple superphosphate. 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 central rows were randomly chosen and harvested to record number of nodule plant^{-1} (NN), nodule dry weight plant^{-1} (NDW) and shoot dry weight plant^{-1} (SDW). 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 [29]. At physiological maturity stage on October 30, 2012, yield and yield traits of common bean were recorded. Number of pods plant^{-1} (NPP), number of seeds plant^{-1} (NSP), 100 seed weight, total biomass yield (TBY) and grain yield (at 13 % moisture content) (GY) were determined.

Data analysis

Data were submitted to analysis of variance using SAS version 9.1. Statistically significant differences between means were also determined by the LSD test. The bar graphs were constructed using Microsoft excel version 10.

Result and discussion

Nodulation

The soil samples from four experimental sites were aseptically collected to enumerate the rhizobia population that nodulated common bean by using the plant infection method. The result of this experiment revealed that the rhizobia of common bean varied from <10 to 10^5 g^{-1} of soil. Based on this rhizobial population, the experimental sites were grouped into three soil types. Accordingly, Babillae soil had <100 rhizobia of common bean g^{-1} soil. Rhizobia population >1000 g^{-1} of soil was found in Haramaya and Hirna soils. While the rhizobial population in Fedis soil was between 100 and 1000 g^{-1} soil. Therefore, Babillae, Fedis, and Haramaya and Hirna soils were categorized into low, medium and high rhizobia containing soil types, as it has been previously described by Howieson and Ballard [30]. None of common bean cultivating history in Babillae and Fedis sites could be attributed to lower rhizobial population nodulating common bean [31]. Continuous cultivation of the host plant could increase rhizobial population at Haramaya and Hirna soils [32].

Different rates of N application either alone or in combination with *Rhizobium leguminosarum* bv. Phaseoli inoculation significantly ($P < 0.05$) affected common bean nodulation in all study sites (Table 2). In all soil types, the NN and NDW were significantly decreased with increasing rates of N application either alone or in combination with inoculation. Other authors have found similar trends of nodulation along N rates with different

Table 2 Nodulation status and shoot dry weight of common bean var. Dursitu along different rates of N application with and without inoculation of *Rhizobium leguminosarum* bv. *Phaseoli* at selected areas of eastern Ethiopia

Treatments	NN			NDW			SDW		
	Soil type 1	Soil type 2	Soil type 3	Soil type 1	Soil type 2	Soil type 3	Soil type 1	Soil type 2	Soil type 3
Control	28.33d	71.67bcd	163.00abcd	0.1217cd	0.1920bc	0.4407b	36.23cd	31.37e	49.92b
20 kg N ha^{-1}	88.67c	146.67a	206.00a	0.1880b	0.4097a	0.3399bc	35.30cd	38.03cde	58.13ab
40 kg N ha^{-1}	65.00c	106.67b	149.67abcde	0.1263cd	0.3963a	0.2299bc	41.17bc	48.57bcd	60.77ab
60 kg N ha^{-1}	18.67d	45.00def	91.33cde	0.0437d	0.1727bc	0.1300c	49.97ab	53.23abc	63.60ab
80 kg N ha^{-1}	31.00d	45.00def	93.00cde	0.1033cd	0.1610bcd	0.1621c	47.53ab	55.70ab	63.67ab
100 kg N ha^{-1}	22.33d	20.00f	68.00e	0.0193d	0.0160e	0.2127bc	44.83abc	56.33ab	76.47a
Rhizobium sp.	82.33c	90.67bc	186.67ab	0.3063ab	0.2533b	0.7184a	30.77d	39.00cde	59.07ab
Rhizobium sp. + 20 kg N ha^{-1}	161.67a	64.33cde	178.67abc	0.3900a	0.2010bc	0.7111a	42.63bc	36.40de	70.50ab
Rhizobium sp. + 40 kg N ha^{-1}	124.33b	46.67def	111.50bcde	0.3207ab	0.0897cde	0.3337bc	52.93a	64.90a	78.03a
Rhizobium sp. + 60 kg N ha^{-1}	26.00d	27.33ef	71.50de	0.1530cd	0.0516de	0.1596c	47.47ab	52.83abc	75.68a
Rhizobium sp. + 80 kg N ha^{-1}	17.33d	20.00f	170.00abc	0.0287d	0.0340e	0.3238bc	50.77ab	51.70abcd	76.38a
Rhizobium sp. + 100 kg N ha^{-1}	19.67d	22.33f	63.17e	0.0210d	0.0279e	0.2124bc	43.93abc	51.97abcd	66.92ab
Mean	57.11	58.86	129.38	0.1518	0.1671	0.3312	43.63	48.35	66.59
F value	66.17***	29.26***	7.05***	21.55***	33.36***	12.78***	11.46***	9.80***	3.71**
LSD	30.06	37.15	93.59	0.1389	0.119	0.27	10.22	16.20	22.32
CV (%)	17.88	21.44	36.85	31.07	24.19	41.54	7.95	11.38	17.07

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, *NN* nodule number, *NDW* nodule dry weight, *SDW* shoot dry weight

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

legume pulses [21, 33, 34]. In soil with low rhizobial population, *Rhizobium* inoculation alone produced significantly higher NN and NDW than the uninoculated treatments. In contrast, the data indicated the non-significant increases of NN and NDW due to inoculation in soil types with medium and high rhizobial population. Similarly, the effect of inoculation on nodulation of common bean varied due to different indigenous rhizobial population, as previously observed by Asad et al. [15]. In soil having low rhizobial population, *Rhizobium* inoculated with 20 and 40 kg N ha^{-1} resulted in significantly higher NN than the remaining N treatments. Moreover, *Rhizobium* inoculated with 20 kg N ha^{-1} produced the highest NDW. The overall effect of inoculation on NN and NDW in this soil type was higher than for the corresponding N rates of applied without inoculation (Fig. 1a, b), thus showing the competitive advantage of inoculated *Rhizobium* over the indigenous rhizobial population. Similarly, low rate of N with inoculation in soil with low indigenous rhizobial population resulted in an enhancement of nodulation [24]. In contrast, N application as low as 15 kg N ha^{-1} and applied at sowing, suppressed nodule dry weight of common bean [25]. Furthermore, N applied at planting had beneficial effect on nodulation at late growth stage [35].

The soil type with medium rhizobial population responded to the 20 kg N ha^{-1} with significantly higher NN and NDW than the other treatments, followed in the order by that obtained from 40 kg N ha^{-1}. On top of this, it was also observed a slight reduction of NN and NDW due to inoculation as compared to the uninoculated treatment (Fig. 1a, b). Similar result was previously observed by Msumali and Kipe-Nolt, [36], when indigenous rhizobia may have been more potent than inoculated *Rhizobium* strain. On the other hand, for soil having high rhizobial population, the 20 kg N ha^{-1} treatment alone gave the highest NN, although without significant difference from that obtained with either *Rhizobium* inoculation alone or 40 kg N ha^{-1} alone or inoculation applied with 20 kg N ha^{-1}. Moreover, the highest NDW were obtained from *Rhizobium* inoculation applied either alone or in combination with 20 kg N ha^{-1}. In general, it was observed a slight increment of NN and a remarkable improvement of NDW due to inoculation, as compared to the uninoculated treatments with corresponding rates of N (Fig. 1a, b). Similar to this finding, a significant improvement of nodulation due to inoculation in soil with >1000 rhizobial population was observed by Hungria et al. [17]. Inoculation together with a 20 kg N ha^{-1} treatment resulted in the highest NDW at soil with low and high rhizobial population. While the 20 kg N ha^{-1} treatment alone gave the highest NDW in soil with medium rhizobial population. The highest NN produced in soil with low, medium and high rhizobial population were 161.67, 146.67 and 206.00, respectively. These various NN in different soil types could be attributed to different number of rhizobia present in these three soil types. Similarly, Patrick and Lowther [37] found that the size of rhizobial population affects nodulation. Previous work confirmed that the largest the soil native rhizobial population, the greater is the nodulation [38]. The lowest NN and NDW produced in soil having relatively lower rhizobial population was previously observed by Chemining'wa and Vessey [21].

Shoot dry weight

The experimental treatments (different rates of N application solely and in combination with *Rhizobium* inoculation) resulted in significant variation of SDW (Table 2). In all soil types, a significant improvement of SDW was observed with increasing rates of N applied either alone or in combination with *Rhizobium* inoculation. In soil with low and medium rhizobial population, *Rhizobium* inoculation together with the 40 kg N ha^{-1} treatment, in which the highest nodulation was produced, resulted in significantly higher SDW than those produced at N rates lower than 40 kg N ha^{-1} in both inoculation treatments. This indicates the importance of higher nodulation for SDW production of common bean. In contrast, the high N rate application decreased nodulation, but an increase of above ground biomass production was observed [21]. In soil having high rhizobial population, the non-significant difference in SDW among N treatments, excluding the control, was observed. This implies that the sole N reserves in soil with lower N rate of application could have been sufficient for maximum shoot biomass production of common bean at R_3 stage. The highest SDW obtained from soils having low, medium and high rhizobial population were 52.93, 64.90 and 78.03 g, respectively. All these shoot biomass were obtained from *Rhizobium* inoculated with 40 kg N ha^{-1}. It has been shown that number of native rhizobia had a detrimental impact on productivity of above ground dry biomass [39]. Denton et al. [40] found increased shoot biomass production with increased *Rhizobium* inoculation rate. In all soil types, the lowest SDW were produced for the control treatments (uninoculated and unfertilized). This suggests that the N is the major limiting nutrient for common bean production in all study sites.

Number of pods per plant

The treatments of this experiment affected significantly ($P < 0.05$) the NPP in all soil types (Table 3). In soil having low rhizobial population, 20 kg N ha^{-1} alone gave the highest NPP. Inoculation with 60 kg N ha^{-1} resulted in significantly higher NPP than those produced

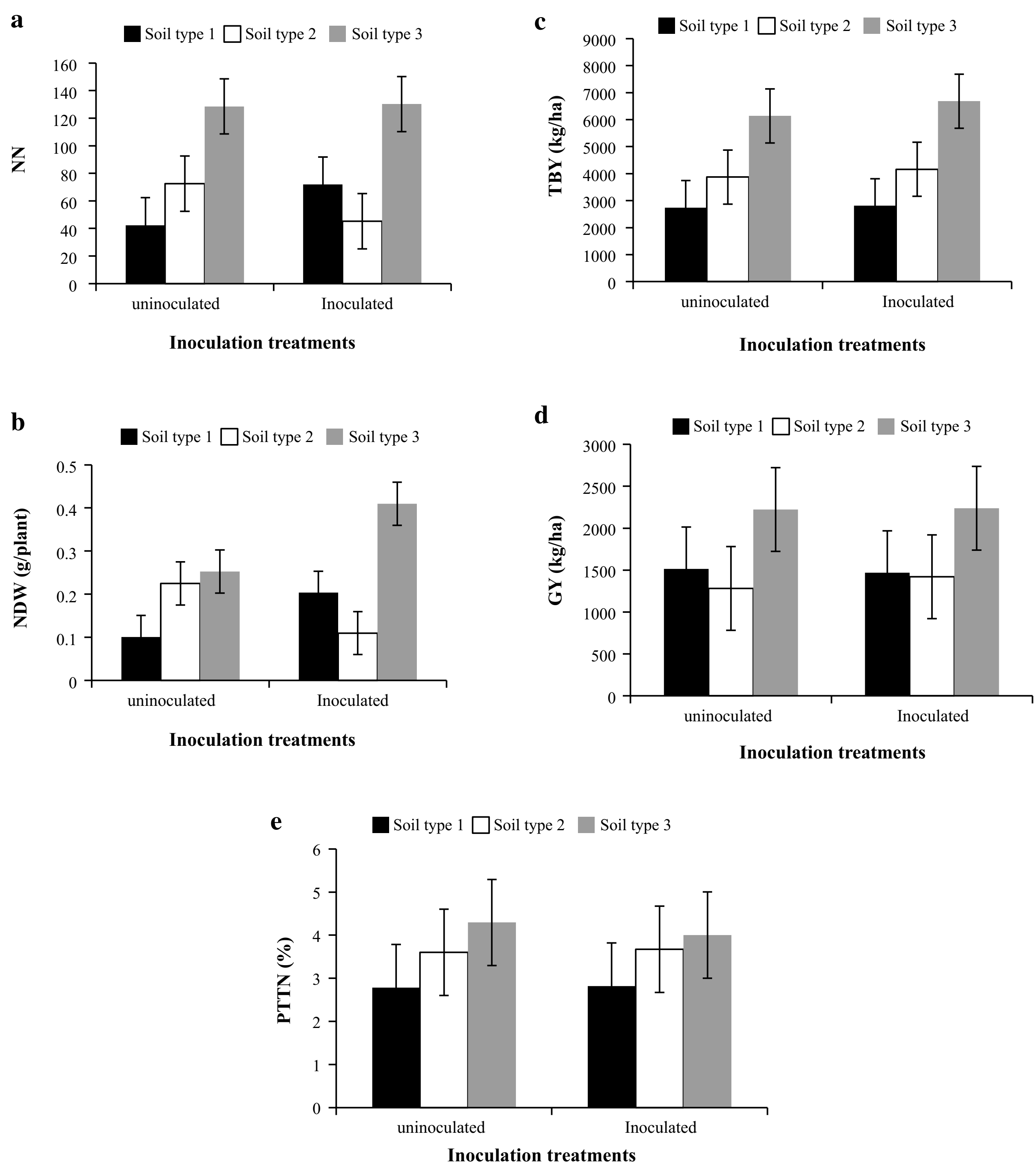

Fig. 1 The effect of indigenous rhizobial population on the effectiveness of inoculation on **a** nodule number (NN), **b** nodule dry weight (NDW), **c** grain yield (GY), **d** total biomass yield (TBY), **e** plant total tissue N (PTTN). Soil type 1—soil having <100 rhizobial population, soil type 2—soil having rhizobial population between 100 and 1000 and soil type 3—soil having rhizobial population >1000

for the control and inoculation alone, in soils having medium and high rhizobial population. Similarly, a significant improvement of NPP ranging from 20.2 at the control to 24.15 at N treated plants was obtained from faba bean [41]. The highest NPP produced in soils having low, medium and high were 18.63, 24.99 and 31.05,

Table 3 Number of pods per plant, number of seeds per pod and 100 seed weight of common bean var. Dursitu along different rates of N application with and without inoculation of *Rhizobium leguminosarum* bv. *Phaseoli* at selected areas of eastern Ethiopia

Treatments	NPP			NSP			100 seed weight		
	Soil type 1	Soil type 2	Soil type 3	Soil type 1	Soil type 2	Soil type 3	Soil type 1	Soil type 2	Soil type 3
Control	12.55bc	10.33d	20.89c	4.61b	5.40bc	5.82b	19.77c	20.90cd	19.33a
20 kg N ha^{-1}	18.63d	18.66ab	20.83c	6.78a	6.07abc	6.62ab	21.30ab	22.80abc	19.18a
40 kg N ha^{-1}	17.11ab	23.22ab	23.00bc	6.55a	6.30ab	6.70ab	21.37ab	22.07bc	18.92a
60 kg N ha^{-1}	16.00abc	18.00abc	25.22abc	6.44a	6.97a	6.92ab	21.17abc	22.13abc	19.42a
80 kg N ha^{-1}	14.89abc	22.00ab	25.83abc	6.99a	6.40ab	7.13a	21.20abc	22.90ab	19.42a
100 kg N ha^{-1}	16.44ab	22.22ab	25.78abc	6.66a	6.63a	6.70ab	21.57a	22.90ab	19.02a
Rhizobium sp.	10.78c	11.22 cd	23.66bc	5.33ab	5.50bc	6.50ab	20.00bc	19.90d	19.28a
Rhizobium sp. + 20 kg N ha^{-1}	13.66abc	16.44bcd	29.33ab	6.89a	5.20c	6.65ab	21.07abc	22.57abc	18.87a
Rhizobium sp. + 40 kg N ha^{-1}	16.00abc	22.00ab	29.11ab	6.66a	6.30ab	6.85ab	21.40ab	23.03ab	18.97a
Rhizobium sp. + 60 kg N ha^{-1}	16.22abc	24.99a	31.05a	6.22ab	6.30ab	6.35ab	22.17a	22.20abc	18.83a
Rhizobium sp. + 80 kg N ha^{-1}	16.44ab	22.11ab	28.89ab	6.99a	6.40ab	6.72ab	21.47ab	21.37bcd	18.87a
Rhizobium sp. + 100 kg N ha^{-1}	15.22abc	22.22ab	27.05abc	6.89a	6.00abc	6.38ab	20.97abc	24.07a	19.10a
Mean	15.33	19.45	25.89	6.42	6.12	6.61	21.12	22.24	19.10
F value	3.93**	11.06***	5.86***	4.68***	6.87***	2.11*	5.09***	8.41***	0.21 ns
LSD	5.48	7.20	6.66	1.72	1.02	1.10	1.49	1.94	2.30
CV (%)	12.13	12.58	13.10	9.11	5.64	8.48	2.38	2.96	6.12

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, *NPP* number of pods per plant, *NSP* number of seeds per pod

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

respectively, confirming the positive effect of indigenous rhizobial population on NPP.

Number of seeds per pod

The data revealed significant variation of NSP due to the treatments (Table 3). In all soil types, NSP increased with increasing rates of N application solely and in combination with *Rhizobium* inoculation. Previous study reported that N nutrition increased the seeds per pod [42, 43]. In soil having low rhizobial population, significantly higher NSP (6.99) was recorded at 80 kg N ha^{-1} and inoculation in conjunction with 80 kg N ha^{-1} as compared to the control. In soils having medium rhizobial population, 60 kg N ha^{-1} resulted in significantly higher NSP than those produced at N rates below 20 kg N ha^{-1} in both inoculation treatments. 80 kg N ha^{-1} resulted in the highest NSP in soil having high rhizobial population. Non-significant differences in NSP obtained from different N rates, excluding control were observed for both inoculation treatments, in soils with low and high rhizobial population.

100 seed weight

The 100 seed weight of common bean was significantly ($P < 0.05$) varied due to the treatments, except those observed in soil having high rhizobial population, in which this trait exhibited a non-significant difference (Table 3). The non-significant difference in common bean seed size was previously observed by Mulas et al. [44]. Similar to this, 100 seed weight of soybean was not improved by either inoculation or N fertilizer application [45]. In soil having low rhizobial population, significantly higher 100 seed weight was obtained from 100 kg N ha^{-1} and *Rhizobium* inoculated with 60 kg N ha^{-1}, as compared to the control treatment. The sole 100 kg N ha^{-1} addition and in combination with *Rhizobium* inoculation gave significantly higher 100 seed weight compared to the control, in soil having medium rhizobial population. This result is in agreement with that obtained previously by El Hardi and Elsheikh [46] who found that inoculation and N application significantly improved 100 seed weight of chickpea over the control. N application increased 100 seed weight by 7.4 % over those produced for the uninoculated treatment [41].

Total biomass yield

The significant variation of TBY due to treatments was observed at $P \leq 0.05$ (Table 4). In all soil types, *Rhizobium* inoculated with 100 kg N ha^{-1} resulted in significantly higher TBY than those produced in the control treatment and inoculation alone. This result supports the findings of Mulas et al. [44] that inoculation and

Table 4 Total biomass yield, grain yield and total plant tissue N of common bean var. Dursitu along different rates of N application with and without inoculation of *Rhizobium leguminosarum* bv. *Phaseoli* at selected areas of eastern Ethiopia

Treatments	TBY			GY			PTTN		
	Soil type 1	Soil type 2	Soil type 3	Soil type 1	Soil type 2	Soil type 3	Soil type 1	Soil type 2	Soil type 3
Control	2055.6ef	2077.8c	4917.6c	1025.65f	1082.13cd	1946.05bc	2.3800de	2.5200c	4.0983ab
20 kg N ha^{-1}	2277.8def	4018.5ab	5609.5bc	1254.35e	1270.19bc	2121.48abc	3.2433a	3.5533b	4.5300ab
40 kg N ha^{-1}	2631.5cd	4333.3a	6351.4abc	1568.52c	1307.13bc	2262.27ab	2.8533abcd	3.7800ab	4.6533a
60 kg N ha^{-1}	3131.5abc	4055.6a	6481.5abc	1727.63bc	1289.44bc	2207.04abc	2.5567bcde	3.6933ab	4.0767ab
80 kg N ha^{-1}	3082.4bc	4388.9a	6721.8ab	1674.26bc	1280.46bc	2380.60a	2.5167cde	3.8600ab	4.2900ab
100 kg N ha^{-1}	3260.8ab	4374.1a	6742.8ab	1830.09b	1454.26ab	2416.44a	3.1567abc	4.2000a	4.1200ab
Rhizobium sp.	1787.0f	3133.3b	5472.6bc	996.39f	1005.37d	1941.67c	2.1600e	3.3833b	4.0517ab
Rhizobium sp. + 20 kg N ha^{-1}	2148.1def	4087.0a	7405.6a	1302.22de	1258.70bc	2169.17abc	3.1800ab	3.3633b	4.1867ab
Rhizobium sp. + 40 kg N ha^{-1}	2498.1de	4249.2a	6668.1ab	1211.48ef	1569.72a	2381.16a	2.6567abcde	3.6800ab	4.0800ab
Rhizobium sp. + 60 kg N ha^{-1}	3375.4ab	4277.8a	6851.6ab	1697.59bc	1461.85ab	2366.06a	2.7000abcde	3.8200ab	4.0033ab
Rhizobium sp. + 80 kg N ha^{-1}	3421.4ab	4462.2a	6486.1abc	1511.57cd	1571.57a	2240.34abc	3.2000ab	3.9200ab	3.9467ab
Rhizobium sp. + 100 kg N ha^{-1}	3648.1a	4740.7a	7222.2a	2089.54a	1653.89a	2329.40a	3.0133abcd	3.8767ab	3.7583b
Mean	2776.48	4016.54	6410.89	1490.78	1350.39	2230.14	2.8014	3.6375	4.1496
F value	32.12***	16.30***	4.95***	57.11***	19.75***	5.99***	8.10***	14.64***	1.73 ns
LSD	557.86	913.75	15,380.6	226.81	226.53	318.67	0.6467	0.5621	0.8943
CV (%)	6.82	7.73	12.56	5.17	5.70	7.28	7.84	5.25	10.98

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, *TBY* total biomass yield, GY grain yield, *PTTN* plant total tissue N

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

inorganic N application enhanced the common bean production in all soil type regardless of the indigenous rhizobial population. Significant enhancement of above ground biomass production by 22 % due to *Rhizobium* inoculated with inorganic N over the uninoculated treatment was also observed by Chemining'wa and Vessey [21]. In soil having medium rhizobial population, it was observed a non-significant difference in TBY produced at 20 kg N ha^{-1} and beyond rates of N, with both inoculation treatments. This could have confirmed the presence of effective common bean–rhizobia symbiosis at low inorganic N [47] and thus satisfy the N need for boost the biomass production. The control treatment, inoculation alone, and 20 kg N ha^{-1} alone gave significantly lower TBY than those produced in other treatments in soil having high rhizobial population. In soil having low rhizobial population, statistically lower TBY was produced for control treatment, and for those with 20 and 40 kg N ha^{-1} either alone or in combination with *Rhizobium* inoculation, as compared to those produced in other treatments. This may indicate that the native rhizobia in this site are capable but not effective to fix N_2 to satisfy the N requirement of common bean. The presence of higher rhizobial population is not an indicator of the symbiotic effectiveness between rhizobia and N derived from the atmosphere [31]. In all soil types, the present study revealed that inoculation slightly increased the TBY as compared to those obtained from the corresponding N treatments without *Rhizobium* inoculation (Fig. 1c). The highest TBY produced in soils having low, medium and high rhizobial population were 3648.1, 4740.7 and 7222.2 kg ha^{-1}, respectively. The highest biomass in soil having high rhizobial population was previously confirmed by Furseth et al. [48]. These authors found that yield of soybean positively correlated with soil indigenous rhizobial population across environments.

Grain yield

The GY of common bean revealed significant variation due to treatments at $P \leq 0.05$ (Table 4). In soil having low rhizobial population, increasing rates of N application increased GY production, though the highest nodulation and PTTN were recorded at 20 kg N ha^{-1}. The 100 kg N ha^{-1} addition in conjunction with inoculation also gave significantly higher GY than those produced in other treatments. Similarly, inhibition effect of higher N application on nodulation and nitrogenase enzyme without affecting grain yield was previously determined by Rai [23]. Our result may be confirm that the previous findings, although N mineral reduced the nodulation and N_2 fixation and yields of common bean can be improved by increasing N availability [49]. da Silveira et al. [47] also found that common bean responded well up to 200 kg N ha^{-1}. Asad et al. [15] observed that nodulation improvement due to inoculation had not significantly enhanced plant biomass production. This present study also confirms that the common bean–rhizobia symbiosis was not satisfactory to fulfill the N needs as it has been previously observed by Fesonko et al. [50]. Contrary to this, Denton et al. [40] showed that improvement of shoot N increased the grain yield of faba bean by 1 Mg ha^{-1} in soil having low rhizobial population. This indicates that biological N_2 fixation alone is not a sufficient N requirement of common bean to get the local attainable yield in the prevailing environmental condition.

Inorganic N at 100 kg N ha^{-1} with *Rhizobium* inoculation gave the highest GY at soil having medium rhizobial population as it has been observed in PTTN. This may indicate that N derived from indigenous and inoculated rhizobia alone did not satisfy the N requirement of common bean. Voisin et al. [51] observed that high N requirement at the seed setting stage was supported by both N_2 fixation and mineral N supply. Although N fertilizer enhanced the plant N uptake, the common bean production did not improve [25]. The present study indicates a non-significant difference in GY obtained from inoculation when either coupled to 40, 60, 80 and 100 kg N ha^{-1} treatment, or sole application of 100 kg N ha^{-1} addition. This may indicate the inoculated *Rhizobium* satisfies the N requirement of common bean with relatively low inorganic N application.

In soil having high rhizobial population, the 40 kg N ha^{-1} applied in conjunction with *Rhizobium* resulted in significantly higher GY than those produced at the control and inoculation alone. Similarly, a previous finding recommended N application to maximize common bean yield in soil having high indigenous rhizobial population [31]. In this soil type, it was recorded a non-significant difference in GY at different N rates of application, excluding control. Besides this, treatment with low N rate applied together with both inoculation treatments, produced statistically similar GY with those found at higher N rates application. The synergetic effect between low rates of N application and *Rhizobium* inoculation on common bean production in soil having rhizobial population >1000 was previously confirmed by Hungria et al. [17]. These authors found the highest seed yield from treatments with *Rhizobium* inoculated together with 15 kg N ha^{-1} applied at planting and further 15 kg N ha^{-1} addition at early flowering stages. Due to the fact that biological N_2 fixation is not active at early stage of common bean, a starter dose of N application is required to enhance plant growth and eventually improve the grain yield production [35, 52, 53]. Nitrogen application in addition to starter N reduced nodulation and failed to increase the common bean yield [24]. Vargas et al. [31] found that increasing rates of N decreased the number of nodules from inoculated cells, whereas it increased that by indigenous rhizobia.

The overall effect of inoculation on GY was slightly decreased in soil having low rhizobial population but slightly improved in soil having medium and high rhizobial population, as compared to that obtained at corresponding rates of N application without inoculation (Fig. 1d). This result supports the finding of Hungria et al. [54] who showed that inoculation increased the yield of common bean as compared to the control treatment. A previous study also reported that an inoculation response was observed in soil having rhizobial population between 300 and 1000 g^{-1} soil [55, 56]. The highest GY produced for soil types having low, medium and high rhizobial population were 2089.54, 1653.89 and 2381.16 kg ha^{-1}, respectively. These GY are comparable with those previously produced at 100 kg N ha^{-1} N, for in which 2000 kg ha^{-1} was reported [47]. At least 29 % faba bean yield advantage was provided for soil having higher rhizobial population over that yield obtained from low rhizobial population, as determined by Sorwle and Mytton [41].

Plant total tissue N

The PTTN was significantly varied due to different treatments at $P \leq 0.05$ (Table 4). In soil having low and high rhizobial population, N application beyond 20 kg N ha^{-1} with both inoculation treatments resulted in a slight decrease of PTTN. 20 kg N ha^{-1} application resulted in the highest PTTN in soil having low rhizobial population. The lower PTTN at higher N application could be attributed to the negative effect of inorganic N on fixed N through the inhibition of the nitrogenase activity [20, 57].

In soil having medium rhizobial population, progressively larger rates of N application increased the PTTN, though the highest NN and NDW was produced at 40 kg N ha^{-1}. The 100 kg N ha^{-1} treatment gave significantly higher PTTN than those obtained from either the control treatment or that with 20 kg N ha^{-1} applied alone or in combination with *Rhizobium* inoculation. This could be due to low effectiveness of indigenous rhizobia of common bean in N_2 fixation. Asad et al. [15] found the significant improvement of plant total N accumulation of common bean was due to N application rather than to inoculation. This was attributed to the fact that common bean rarely derives more than 50 % of its N from symbiotic N_2 fixation [58].

In soil type having high rhizobial population, 40 kg N ha^{-1} alone resulted in significantly higher PTTN than that obtained from inoculation with the 100 kg N ha^{-1} addition. Shutsrirung et al. [59] demonstrated that inoculation may not be effective in soil having high effective native rhizobial population. In top of this, PTTN was also decreased with increasing rates of N application following both inoculation treatments. This indicates that the native rhizobia in this site might have been more effective in N_2 fixation than the inoculated stain. Furthermore, inoculation slightly increased the PTTN in soil having low and medium rhizobial population whereas it showed a decreasing trend in soil with high rhizobial population, in comparison to PTTN found for corresponding N rates without inoculation (Fig. 1e). The highest PTTN was observed at low, medium and high rhizobial population and reached 3.24, 4.20 and 4.65 %, respectively. The highest PTTN could be attributed to a higher nodulation induced by the high rhizobial population and, thus, an enhanced N_2 fixation [38, 48]. Due to synergetic effect of a starter N application on symbiotic N_2 fixation, the highest accumulated N in plants was recorded for the 20 kg N ha^{-1} application in soil having high rhizobial population [24].

Correlation analysis

The significant correlation among investigated traits of common bean was observed in all soil types at $P < 0.05$ (Tables 5, 6, 7). The amount of NN produced at soil with low rhizobial nodulating population inversely correlated with TBY ($r = -0.6754$; $P \leq 0.05$) and with GY ($r = -0.5629$; $P \leq 0.05$) (Table 5). In this soil type, a negative and strong correlation between NDW with TBY ($r = -0.7563$; $P \leq 0.01$), GY ($r = -0.6953$; $P \leq 0.05$) was observed. NN had an inverse and strong correlation with both SDW ($r = -0.5958$; $P \leq 0.05$) and GY ($r = -0.6090$; $P \leq 0.05$) in soil with medium rhizobial population (Table 6). Conversely, NDW and GY revealed an inverse and significant correlation ($r = -0.6356$; $P \leq 0.05$). In soil with high rhizobial population, the correlation analyses between the NN and both TBY ($r = 0.5599$; $P \leq 0.05$) and GY ($r = 0.7538$; $P \leq 0.01$) were positive (Table 7), whereas NDW had an inverse and significant correlation with GY ($r = -0.6917$; $P \leq 0.05$). In general, this finding indicates that N mineral fertilizer rather than symbiotic N_2 fixation had a determinant effect on common bean yield. This result supports the results of the other authors [60], in which NDW and N fixation showed negative correlation with yield related parameters. La Favre and Eaglesham [61] also observed an inverse and significant relationship between the starting N application and both NN and NDW. In contrast, Aggarwal [62], who conducted experiment in Malawi, found positive and significant correlations between NN and GY for common bean under inorganic N treatment. Mothapo et al. [38] also reported that plant biomass production correlated positively with NDW when inoculation alone was applied.

In all soil types, also SDW of common bean had a significant correlation with NPP, NSP, 100 seed weight, TBY and GY. However, NPP and NSP had the non-significant association with SDW with low and high rhizobial population (Tables 5, 6, 7). In particular, in soil with low rhizobial population, all listed growth traits had positive and significant correlation with SDW at $P \leq 0.05$ (Table 5). SDW had also a positive and significant ($P \leq 0.05$) correlation with 100 seed weight ($r = 0.5087$) and TBY ($r = 0.6996$), and even highly significant ($P \leq 0.01$) with NPP ($r = 0.7719$), NSP ($r = 0.7751$) and GY ($r = 0.7270$). In the same soil, a positive and strong correlation was also observed between SDW and either NPP ($r = 0.8726$; $P \leq 0.001$), or TBY ($r = 7378$; $P \leq 0.01$) or GY ($r = 0.7496$; $P \leq 0.01$). Highly significant relationships among SDW, shoot N content and seed yield are previously shown by Pereira and Bliss [63].

Table 5 Correlation among the investigated traits of common bean treated different rates of N with and without inoculation in soil having rhizobial population nodulating common bean <100 g^{-1} soil

Traits	NN	NDW	SDW	NPP	NSP	100 SW	TBY	GY	HI
NN									
NDW	0.93***								
SDW	−0.19 ns	−0.28 ns							
NPP	−0.15 ns	−0.39 ns	0.44 ns						
NSP	0.11 ns	−0.14 ns	0.59*	0.66*					
100 SW	−0.08 ns	−0.21 ns	0.67*	0.78**	0.74**				
TBY	−0.68*	−0.76**	0.69*	0.50*	0.56*	0.65*			
GY	−0.56*	−0.70*	0.52*	0.46 ns	0.59*	0.58*	0.91***		
HI	0.13 ns	−0.04 ns	0.08 ns	−0.05 ns	0.30 ns	0.08 ns	0.09 ns	−0.00 ns	
PTTN	0.13 ns	−0.18 ns	0.23 ns	0.66*	0.72*	0.56*	0.36 ns	0.38 ns	0.52*

Ns non-significant, *NN-Nodule* number per plant, *NDW* nodule dry weight per plant (g $plant^{-1}$), *SDW* shoot dry weight (g $plant^{-1}$), *NPP* number of pods per plant, *NSP* number of seeds per pod, *100 SW* 100 seed weight (g), *TBY* total biomass yield (kg ha^{-1}), *GY* grain yield (kg ha^{-1}), *HI* harvest index

* Significant at 0.05; ** highly significant at 0.01; *** very highly significant at 0.001

Table 6 Correlation among the investigated traits of common bean treated different rates of N with and without inoculation in soil having rhizobial population nodulating common bean between 100 and 1000 g^{-1} soil

Traits	NN	NDW	SDW	NPP	NSP	SW	TBY	GY	HI
NN									
NDW	0.96***								
SDW	−0.60*	−0.55*							
NPP	−0.40 ns	−0.34 ns	0.77**						
NSP	−0.35 ns	−0.26 ns	0.78**	0.66*					
SW	−0.26 ns	−0.26 ns	0.51*	0.66*	0.34 ns				
TBY	−0.36 ns	−0.29 ns	0.70*	0.88***	0.57*	0.71**			
GY	−0.61*	−0.64*	0.73**	0.81**	0.49 ns	0.69*	0.77**		
HI	−0.25 ns	−0.29 ns	0.19 ns	0.26 ns	0.16 ns	0.46 ns	0.17 ns	0.50*	
PTTN	−0.42 ns	−0.36 ns	0.77**	0.84**	0.72**	0.52*	0.91***	0.69*	0.03 ns

ns non-significant, *NN-Nodule* number per plant, *NDW* nodule dry weight per plant (g plant^{-1}), *SDW* shoot dry weight (g plant^{-1}), *NPP* number of pods per plant, *NSP* number of seeds per pod, *100 SW* 100 seed weight (g), *TBY* total biomass yield (kg ha^{-1}), GY grain yield (kg ha^{-1}), *HI* harvest index

* Significant at 0.05; ** highly significant at 0.01; *** very highly significant at 0.001

Table 7 Correlation among the investigated traits of common bean treated different rates of N with and without inoculation in soil having rhizobial population nodulating common bean >1000 g^{-1} soil

Traits	NN	NDW	SDW	NPP	NSP	SW	TBY	GY	HI
NN									
NDW	0.72**								
SDW	0.45 ns	−0.23 ns							
NPP	0.45 ns	−0.10 ns	0.87***						
NSP	0.18 ns	−0.29 ns	0.41 ns	0.27 ns					
SW	0.00 ns	−0.07 ns	−0.68*	−0.62*	0.05 ns				
TBY	0.56*	−0.27 ns	0.74**	0.79**	0.49 ns	−0.50 ns			
GY	0.75**	−0.69*	0.75**	0.61*	0.55*	−0.39 ns	0.77**		
HI	0.33 ns	0.34 ns	0.08 ns	−0.15 ns	−0.28 ns	0.06 ns	−0.49 ns	−0.25 ns	
PTTN	0.44 ns	−0.02 ns	0.38 ns	−0.53*	0.27 ns	0.03 ns	−0.26 ns	−0.09 ns	0.11 ns

Ns non-significant, *NN-Nodule* number per plant, *NDW* nodule dry weight per plant (g plant^{-1}), *SDW* shoot dry weight (g plant^{-1}), *NPP* number of pods per plant, *NSP* number of seeds per pod, *100 SW* 100 seed weight (g), *TBY* total biomass yield (kg ha^{-1}), *GY* grain yield (kg ha^{-1}), *HI* harvest index

* Significant at 0.05; ** highly significant at 0.01; *** very highly significant at 0.001

In sites with soil having a low rhizobial population, GY of common bean correlated positively with SDW ($r = 0.5152$; $P \leq 0.05$), NSP ($r = 0.5907$; $P \leq 0.05$), 100 seed weight ($r = 0.5791$; $P \leq 0.05$) and TBY ($r = 0.9121$; $P \leq 0.001$) (Table 5). Similarly, strong and positive correlation was observed between GY with SDW ($r = 0.7271$; $P \leq 0.01$), NPP ($r = 0.8098$; $P \leq 0.01$), 100 seed weight ($r = 0.6939$; $P \leq 0.05$) and TBY ($r = 0.7713$; $P \leq 0.01$) in experimental soil with medium rhizobial population (Table 6). In soil showing a high rhizobial population, we noted a positive correlation between GY and SDW ($r = 0.7496$; $P \leq 0.01$), NPP ($r = 0.6067$; $P \leq 0.050$), NSP ($r = 0.5474$; $P \leq 0.05$) and TBY ($r = 0.7712$; $P \leq 0.01$) (Table 7). Similarly, Bayuelo-Jiménez et al. [64] had previously indicated a positive and significant correlation among yield and yield components of common bean.

The correlation analysis indicated a positive relationship between PTTN and NPP ($r = 0.6644$; $P \leq 0.05$), NSP ($r = 0.7195$; $P \leq 0.05$) and 100 seed weight ($r = 0.5562$; $P < 0.05$) in experimental sites showing soil with low rhizobial population (Table 5). In those with medium rhizobial population, a positive and strong correlation between PTTN and SDW ($r = 0.7665$; $P \leq 0.01$), NPP ($r = 8386$; $P \leq 0.01$), NSP ($r = 0.7154$; $P \leq 0.01$), 100 seed weight ($r = 0.5206$; $P \leq 0.05$), TBY ($r = 0.9114$; $P \leq 0.001$) and GY ($r = 0.6941$; $P \leq 0.05$) (Table 6). These results agree with the previous studies, which reported the significant correlation between plant N accumulation with seed yield, seed weight and total biomass [45, 50]. Ruiz-Díez et al. [45] also demonstrated that plant N accumulation was the most suitable trait for the selection of highly effective and highly competitive *Rhizobium* isolates. In contrast, in soil with high rhizobial population, PTTN was only significantly but inversely correlated with NPP ($r = -0.5296$; $P \leq 0.05$), thus, indicating the negative impact of mineral N treatment on soil productivity, despite the plant N accumulation was noted (Table 7). The present study also indicated a non-significant relationship between PTTN and both NN and NDW in all soil types. Similar result was previously observed by da Silva et al. [24] who reported that the correlation between fixed N and nodulation was decreased with increasing rates of N application. In contrast to this, Tsia et al. [25] found a positive and significant relationship between both NN and NDW and N in shoots at 45 days after emergency.

Conclusion

The results of this study indicate the significant effect of N treatments on the nodulation, yield and yield traits of common bean in the major growing areas of eastern Ethiopia. Our experiments indicated the need of inoculation of *Rhizobium* beside that of a starting N dose in soil having <100 rhizobial population g^{-1} of soil. An N requirement to reach the highest yield of common bean was not affected by the native rhizobial population. The effect of *Rhizobium* inoculation on nodulation of common bean appears to depend on the type of soil. Our data also indicated that the amount of mineral N required for maximum seed yield is dependent on the original soil rhizobial population. Moreover, the indigenous rhizobial population that nodulates common bean also affects the bean production. Further research on effectiveness of the combination between indigenous rhizobia and inoculated rhizobial population would be recommended.

Authors' contributions

Both of us participated equally starting from the development of the research idea, writing proposal and competing research grant and development of this manuscript. But I participated more in the management and collection of data from the field experiment, which is why I am the first author of this manuscript. Both authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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