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RESEARCH ARTICLE

Effectiveness of *Rhizobium* Inoculation on Common Bean Productivity as Determined by Inherent Soil Fertility Status

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Abstract

Field experiments at Haramaya, Hirna, Fedis, and Babillae sites were conducted to evaluate the effectiveness of selected isolates of rhizobia on the common bean production using eight selected isolates of rhizobia with a control check and N fertilized (20 kg N ha^{-1}) treatments. The treatment was laid out in a randomized complete block design with three replications and three common bean varieties (Kufanzik, Gofta, and Dursitu).Analysis of variance revealed that inoculation, common bean varieties and their interaction significantly influenced most of the investigated yield and yield traits of common bean. Most of the tested *Rhizobium* isolates significantly increased the nodule number (NN) and nodule dry weight (NDW) as compared to the control check. Of the tested isolates, a higher number remarkably improved the remaining investigated traits in Hirna and Haramaya sites when compared to the Fedis and Babillae sites. In the Babillae site, N fertilization resulted in the highest NDW, total biomass yield (TBY), and grain yield (GY) of common bean. The GY increases due to inoculation of NSCBR-14 at Haramaya and Hirna sites, N fertilization at Babillae and NSCBR-31 at the Fedis site were 775.5, 609.7, 506.3, and 400.9 kg ha^{-1} over the uninoculated treatments of the corresponding experimental sites, respectively. The highest NN, NDW, and plant N concentration was recorded with Dursitu while the highest GY and TBY were obtained from Kufanzik. Therefore, inherent soil fertility and the prevailed environmental factors affect the effectiveness of the inoculated isolates in enhancing common bean production in the study sites.

Key words : Common bean, N fertilization, *Rhizobium*, soil fertility

Introduction

Biological nitrogen fixation (BNF) is first driven by the symbiotic plant's demand for nitrogen as a growth requirement. BNF is one of environmental friendly and sustainable sources of nitrogen (N) in the soil ecosystem and is an alternative to synthetic fertilizers (Silva and Uchida 2000).Symbiotic N_2 fixation is the major biological N_2 fixation process through mutual interaction of host plants with the endosymbionts deriving larger amount of N from atmosphere to soil. Common bean-*Rhizobium* symbiosis derives up to 50% of their N requirement from N_2fixation (Bliss 1993; Rennie and Kemp 1983; Van Kessel and Hartley 2000). When compared to the other legume species, common bean is considered as a poor nitrogen (N)-fixing legume though its promising potential to fix nitrogen has been shown in several studies (Asadi Rahmani et al. 2005; Garcia et al. 2004; Remans et al. 2008). Poor nodulation and lack of responses to inoculation in field experiments have been also frequently reported worldwide (Buttery et al. 1987; Graham 1981; Ramos and Boddey 1987). Lack of inoculation effectiveness is mainly attributed to inherent characteristics of the host plant, particularly the nodulation promiscuity (Michiels et al. 1998) and lack of sufficient supply of plant nutrients (Rodiño et al. 2011).

The numbers of resident populations, host plant activity, as well as other soil characteristics affects successful establishment of inoculant strains into the soil (Brockwell et al. 1995; Brutti et al. 1999). Low soil fertility and environmental stresses also lead to low nitrogen fixation efficiency of common bean rhizobia symbiosis (Graham 1981; Hungria and Vargas

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2000; Hungria et al. 1997). All essential mineral nutrients are absolutely essential for processes related to plant growth and development, including legume-rhizobia symbiosis (Munns 1977; Smith 1982). Optimal conditions for common beans are light loamy soils with pH between 5.5 and 7.0, rich in organic matter, and provide an adequate supply of water, nitrogen (N), and P (Singh 1999).

Soil degradation and nutrient depletion are the major causes of low soil fertility in Ethiopia (Sanchez 2002; Sanchez and Swaminathan 2005; Stoorvogel and Smaling 1998). Sanchez (2002) suggests average annual nutrient depletion rates across 37 African countries of 22 kg N ha^{-1}, 2.5 kg P ha^{-1}, and 15 kg K ha^{-1}, with the most pronounced decline in Ethiopia, Kenya, Malawi, and Rwanda due to extensive hillside cultivation (Smaling et al. 1997). It is important to emphasize that nutrient deficiencies can affect not only plants but also rhizobia soil populations (O'Hara 2001). Wielbo et al. (2015) found better symbiotic effectiveness of indigenous rhizobia populations when the status of soil fertility was medium and above. These results showed the importance of symbiosis for plant hosts beside the soil inherent fertility. On the other hand, Ronner et al. (2016) found that the response to *Rhizobium* inoculation in soybean was negatively related with the combination of soil pH, soil organic carbon, and total N, which could indicate that fields with better soil fertility had smaller responses to the treatments.

Strains of rhizobia differ widely in their ability to survive, nodulate, and fix nitrogen in soil environments (Slattery et al. 2001), thus considerable attention must be given to selecting rhizobia with specific symbiotic and competitive attributes suited to a range of soil environments. Considering the high level of adaptation by native rhizobia to local soil conditions, it is important to characterize the indigenous strains and to identify the most effective ones for use in inoculant production. Besides, an evaluation of BNF by rhizobial strains paired with advanced lines in prevailing soil conditions would be beneficial for identifying both superior strains and legume breeding lines with genetic superiority in BNF. The effect of inherent soil fertility on common bean variety-*Rhizobium* strain is not well studied. Therefore, the purpose of the present work was to characterize both endosymbiont and the host plants which performed better across soil fertility gradients. The objective of this study was to evaluate the effect of inherent soil fertility on the inoculation of selected rhizobia isolates on nodulation and productivity of improved varieties of common bean in eastern Ethiopia soils.

Materials and Methods

Description of experimental sites

Field experiments were conducted in four locations where common bean are cultivated solely and intercropped with sorghum and maize without *Rhizobium* inoculation. The experimental sites are located in Hirna (09^{o}13.157′N and 04^{o}06.488′E at an altitude of 5932ft above sea level[asl]), Fedis (09^{o}06.941′N and 042^{o}04.835′E at an altitude of 5476ft asl), Babillae (09^{o}13.234′N and 042^{o}19.407′Eat 5478ft asl) and Haramaya (09^{o}24.954′N and 042^{o}02.037′E at an altitude of 6628ft asl) agricultural research centers in the 2012 cropping season. The location of the experimental sites is indicated in Fig. 1.

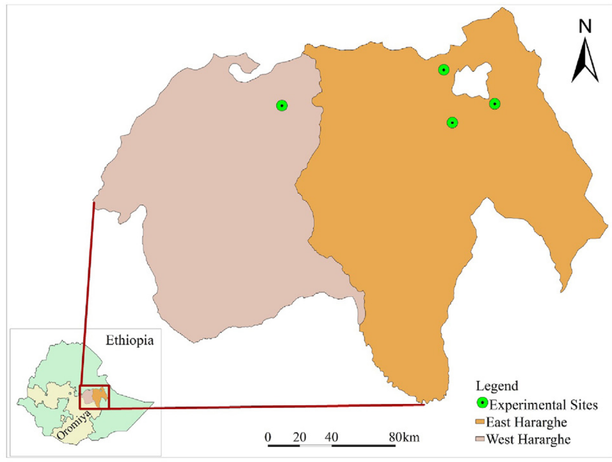

Fig. 1. The location map of experimental sites.

Composite soil sample from each experimental site before planting for soil physic-chemical were collected from the top 0-20 cm. One composite soil sample comprising 20 augering points was taken. Representative subsamples of 1 kg each were prepared for most probable number (MPN) assay stored in a refrigerator at 4^{o}C until enumerating the initial rhizobial population in the study sites. The soil physic-chemical properties were analyzed using standard procedure (Sahlemedhin and Taye 1991). The selected soil properties of all experimental sites were previously presented in Anteneh Argaw et al. (2015).

Source of the isolate and seed variety

Eight isolates of *Rhizobium* spp. (HUCBR-1, HUCBR-2, HUCBR-3, HUCBR-4, HUCBR-5, HUCBR-6, HUCBR-7, and HUCBR-8) were obtained from Biofertilizer Research and Production Project, Haramaya University (Haramaya, Ethiopia). All strains used in this study were isolated from eastern Ethiopia soils. All are characterized as superior in nodule formation and N-shoot biomass production of common bean under greenhouse conditions (Anteneh Argaw 2007).

Seeds of *Phaseolus vulgaris* varieties used in this study were obtained from the Lowland Pulse Research Program, Haramaya University. High yield varieties with different maturity groups were used. These are "Gofta", "Kufanzik", and "Dursitu" varieties that are identified as early, medium, and late-maturing varieties, respectively.

Preparation of inoculums

The pure culture of *Rhizobium* strains were obtained from the laboratory in slant culture. The bacteria were cultured in

YEM (yeast extract mannitol) agar medium. A single pure colony was transferred into YEM broth medium and kept at 28°C for 7 days on a rotary shaker at 120 rpm. Four hundred ml of liquid medium containing *Rhizobium* sp. were added to 1kg of carrier (sterile fine filer mud) and mixed thoroughly and then packed in plastic bags. Filtermud-base inoculum was incubated at 28°C for 15 days. At the time of inoculation, the number of rhizobia in the inoculum was estimated by the plate count method. One ml samples of serially diluted inoculum from 10^{-6} dilution were plated on YEM agar medium. Colonies that developed after incubation at 28 °C for 5-7 days were recorded. This test indicated that the number of rhizobia was more than 1×10^{9} g^{-1} of inocula.

Experimental layout and treatments

Field experiments were conducted in four different locations having different inherent soil fertility to evaluate effect of inherent soil fertility on the effectiveness of selected isolates of *Rhizobium* on nodulation and productivity of common bean. Thirty treatments comprised of 10 levels of *Rhizobium* inoculation (eight *Rhizobium* isolates, N fertilized at 20 kg N ha^{-1} and uninoculated check) and three common bean varieties. The treatments were laid out in a randomized complete block design with three replications.

Before sowing, 20 kg P ha^{-1} as trisuperphosphate was applied in furrows. Identical field experiments were mounted in four locations. The actual plot was ploughed thoroughly twice with a tractor and divided into sub-plots in accordance with the treatments. The net size of each experimental plot was 3×2 m^2. There were five rows per plot and the spacing was 1.5 m between blocks, 40cm between rows, and 10 cm between plants.

Common bean seeds were sterilized using 70% ethanol for 1 min and NaClO solution (0.25% as available Cl) for 3 min. The seeds were then washed carefully in sterilized deinonized water five times before sowing. Then, 20 g of the different rhizobia inoculants was added to different polyethylene bags containing 200 g of common bean seeds. A 10% (w/v) sucrose solution to increase adherence was added to each bag to enhance proper mixing and adhesion of the rhizobia carrier material to the common bean seeds. After mixing, seeds were allowed to air-dry in the shade for 15 min and sown for a maximum of 4 hrs. All standard local cultural practices were accomplished throughout the growth period. Manual weeding was done when required.

Nodulation, yield, and yield attributes

At late flowering and early pod setting stage, five plants were randomly chosen from the central three rows for nodulation and plant growth assessment. The sampled plants were then placed into plastic buckets full of water to loosen the adhering soil. Thereafter, nodules from roots were picked and nodules number and nodule dry weight per plant were recorded. Shoot dry weight was also measured after drying the samples at 70°C in an electrical oven until the weight of the samples became constant.

Shoots of the plants were later ground to pass a 0.5 cm sieve. Total N determinations were done by the Kjeldahl method of Bremner (1965). At full maturity stage, numbers of pods per $plant^{-1}$, number of seeds per pod^{-1}, plant height at harvest, and total biomass were evaluated. Grain yield was corrected for 13% moisture content after determining humidity level with a grain moisture tester.

Method of 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

The effect of Rhizobium *inoculation and varieties on nodulation of common bean*

Analysis of variance revealed that *Rhizobium* inoculation, the varieties and their interaction significantly affected the nodule number (NN) and nodule dry weight (NDW) of common bean at each experimental locations and average value of NN and NDW over locations (Table 1). However, the interaction effect of these two factors on average value of NN and NDW over locations was not significant. At the Haramaya site, almost all tested *Rhizobium* isolates, significantly increased the NN and NDW of common bean compared to the uninoculated control. The highest NN (234.00) and NDW (1.6448 g) were recorded in NSCBR-16 inoculated plants. Similarly, all inoculation treatments produced significantly higher NN and NDW than the control check at Hirna site with highest NN (248.89) and NDW (1.4966 g) with NSCBR31 inoculation. At the Babillae site, *Rhizobium* inoculations except NSCBR-57 resulted in significantly higher NN than the uninoculated control while none of the tested isolates significantly affected NDW. At the Fedis site, all tested rhizobia except NSCBR18 inoculation increased NN significantly while a significant increase in NDW was recorded with all isolate inoculations, except NSCBR-31, NSCBR-18, and NSCBR-57. The highest NN (153.33 and 204.44) and NDW (0.2086 and 1.0167 g) at Babillae and Fedis were recorded with the NSCBR-$(25)_2$ and NSCBR-59 inoculations, respectively. The average value of NN over locations was significantly enhanced by all *Rhizobium* inoculations. Inoculating NSCBR-25, NSCBR-59, NSCBR-16, and NSCBR-$(25)_2$ significantly enhanced an average NDW over locations. In all experimental locations, Dursitu produced significantly higher NN and NDW than those produced by other tested varieties while the lowest was recorded with the Kufanzik variety.

The effect of Rhizobium *inoculation and varieties on shoot dry weight*

Inoculation treatments, the varieties and their interactions significantly affected the shoot dry weight (SDW) measured

Table 1. Nodule number and nodule dry weight of common bean as influenced by Rhizobium inoculation at Babillae, Fedis, Haramaya, and Hirna sites, 2012-13 cropping season.

Inoculation	Nodule number					Nodule dry weight (g plant^{-1})				
	Haramaya	Hirna	Babillae	Fedis	Average	Haramaya	Hirna	Babillae	Fedis	Average
NSCBR-14	152.7cd	226.1a	99.9bc	143.33bc	155.5ab	1.103b	0.791e	0.116ab	0.557bc	0.642abc
NSCBR-(25)$_2$	185.3bc	222.8a	153.3a	159.8bc	180.3a	0.769cd	1.293b	0.144ab	0.527c	0.683ab
NSCBR-59	160.6cd	233.8a	91.0c	204.4a	172.4ab	0.894bc	0.922de	0.110ab	1.017a	0.736a
NSCBR-31	123.6d	248.9a	94.0c	125.6cd	148.0ab	0.368e	1.497a	0.125ab	0.293ef	0.571abc
NSCBR-16	234.0a	220.9ab	93.4c	172.0ab	180.1a	1.645a	1.106bcd	0.111ab	0.675b	0.884a
NSCBR-18	129.8d	165.3bc	113.9b	96.3de	126.3bc	0.631d	0.837e	0.168ab	0.275ef	0.480abc
NSCBR-57	181.7bc	227.0a	80.2cd	135.7c	156.1ab	0.749cd	1.217bc	0.088b	0.343de	0.599abc
NSCBR-25	215.0ab	211.4ab	93.6c	139.8bc	164.9ab	0.977bc	1.052cd	0.110ab	0.449cd	0.647ab
-Ve Control	58.8e	126.1cd	67.6de	85.7e	84.5cd	0.347e	0.557f	0.104ab	0.243ef	0.313bc
+Ve Control	45.2e	87.3d	55.4e	66.8e	63.7d	0.224e	0.330g	0.209a	0.176f	0.235c
LSD	43.0	55.9	19.7	35.4	50.4	0.250	0.201	0.117	0.126	0.407
Dursitu	216.2a	221.9a	106.3a	152.4a	174.2a	1.334a	1.154a	0.194a	0.469a	0.788a
Gofta	117.0b	217.8a	96.7b	120.4b	138.0b	0.505b	1.210a	0.115b	0.438a	0.567b
Kufanzik	112.8b	151.2b	79.7c	126.0c	117.4c	0.475b	0.516b	0.077b	0.459a	0.382c
LSD	17.3	22.4	7.9	14.2	20.4	0.100	0.081	0.047	0.051	0.165
CV (%)	18.70	18.34	13.50	17.17	33.04	20.95	13.54	58.83	17.89	55.19
					F value					
Inoculation (I)	44.07***	19.38***	39.14***	29.79***	12.83***	60.93***	65.02***	2.04*	86.39***	4.59***
Variety(V)	132.95***	36.27***	33.68***	16.76***	22.00***	273.36***	293.63***	18.62***	1.13ns	16.87***
I ×V	21.24***	9.37***	16.80***	4.52***	1.41ns	41.00***	37.24***	5.33***	18.11***	1.25ns

NS-non significant; * significant at 0.05; ***significant at 0.001; -VE-negative control (no inoculation and N application), +VE control -20 kg N ha-1; NSCBR- National soil Common Bean *Rhizobium*
Notes. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

Table 2. Shoot dry weight and number of pods per plant as influenced by *Rhizobium* inoculation at Babillae, Fedis, Haramaya, and Hirna sites, 2012-13 cropping season.

Inoculation	Shoot dry weight (g plant^{-1})					Number of pods per plant				
	Haramaya	Hirna	Babillae	Fedis	Average	Haramaya	Hirna	Babillae	Fedis	Average
NSCBR-14	62.7cd	63.8abc	42.3ab	54.2a	55.7a	22.8abc	24.2a	12.5abc	15.8abc	18.8a
NSCBR-(25)$_2$	69.2abc	65.7abc	43.5a	51.7a	57.5a	23.4abc	23.9a	12.3abc	14.7bc	18.58a
NSCBR-59	74.8a	62.4bc	37.3abc	56.8a	57.8a	26.0a	23.4a	11.0bc	16.0abc	19.1a
NSCBR-31	59.2cd	65.2abc	30.7c	55.7a	52.7a	20.0bc	23.9a	11.3bc	13.9c	17.28a
NSCBR-16	73.9ab	63.0bc	31.4bc	57.8a	56.5a	24.7ab	22.4a	10.9c	17.0ab	18.75a
NSCBR-18	74.0ab	70.56ab	37.2ac	53.0a	58.7a	19.2c	21.2ab	12.7abc	18.4a	17.85a
NSCBR-57	63.9bcd	73.7a	40.9abc	59.1a	59.4a	24.5ab	22.3a	13.5a	16.0abc	19.06a
NSCBR-25	79.8a	64.9abc	35.4abc	56.4a	59.1a	25.3a	21.9a	13.4a	17.9a	19.6a
-Ve Control	55.8d	57.2c	34.7abc	49.1a	49.2a	20.5bc	17.9b	11.0bc	15.6abc	16.26a
+Ve Control	77.1a	68.2ab	37.4abc	53.8a	59.1a	22.9abc	22.5a	13.0ab	16.4abc	18.7a
LSD	10.9	10.3	11.4	10.0	12.9	4.8	3.3	2.0	3.0	4.1
Dursitu	65.1b	58.4b	41.9a	49.1c	53.7b	25.6a	24.2a	13.0a	16.7a	19.9a
Gofta	69.8a	76.0a	38.9a	55.3b	60.2a	22.0b	21.6b	11.3b	15.3b	17.5b
Kufanzik	72.2a	62.0b	29.5b	59.8a	55.9ab	21.2b	21.3b	12.3a	16.5a	17.8b
LSD	4.4	4.1	4.6	4.0	5.1	1.9	1.3	0.8	1.2	1.7
CV (%)	10.17	10.14	19.87	11.75	17.26	13.46	9.51	10.47	11.94	13.88
					F value					
Inoculation (I)	12.19***	4.25***	3.03**	1.96ns	1.33ns	5.16***	6.78***	5.84***	4.37***	1.20ns
Variety(V)	7.93***	58.43***	24.54***	20.77***	4.55ns	17.67***	16.99***	12.33***	4.54*	6.40**
I ×V	3.39***	12.73***	3.70***	8.88***	1.26ns	1.86*	4.64***	5.01***	1.81*	0.39ns

NS-non significant; *significant at 0.05; **significant at 0.01; ***significant at 0.001; -VE-negative control (no inoculation and N application), +VE control -20 kg N ha^{-1}; NSCBR- National soil Common Bean *Rhizobium*
Notes. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

at the late-flowering and early pod-setting stage at all experimental sites, except the effect of inoculation at the Fedis site (Table 2). All *Rhizobium* inoculation treatments, except NSCBR-14, NSCBR-31 and NSCBR-57, significantly increased SDW compared to the uninoculated control at the Haramaya site. At the Hirna site, a significant increase in SDW was recorded with NSCBR-57 and NSCBR-18. None of the inoculation treatments significantly affected the SDW at the Babillae and Fedis sites as well as average SDW across locations. The data also indicated significantly higher

Table 3. Number of seeds per pod and 100-seed weight as influenced by *Rhizobium* inoculation treatments at Babillae, Fedis, Haramaya, and Hirna sites, 2012-13 cropping season.

Inoculation	Number of seeds per pod					100 seeds weight (g)				
	Haramaya	Hirna	Babillae	Fedis	Average	Haramaya	Hirna	Babillae	Fedis	Average
NSCBR-14	5.87ab	5.88ab	5.96a	5.59a	5.82a	28.9ab	28.5ab	29.0a	30.8ab	29.3a
NSCBR-(25)$_2$	5.79ab	5.50b	6.06a	5.52ab	5.72a	28.8ab	28.2ab	27.8ab	32.0a	29.2a
NSCBR-59	5.62ab	5.52b	5.85a	4.81b	5.45a	30.9a	28.0b	27.1b	31.3ab	29.3a
NSCBR-31	5.71ab	6.12ab	5.65a	5.39ab	5.72a	29.1ab	28.3ab	27.8ab	31.0ab	29.1a
NSCBR-16	5.98a	5.86ab	5.70a	5.00ab	5.63a	30.4ab	29.3a	27.6ab	32.1a	29.9a
NSCBR-18	5.93a	5.44b	5.66a	5.26ab	5.57a	29.8ab	28.6ab	27.0b	31.2ab	29.1a
NSCBR-57	5.67ab	6.61a	5.77a	5.03ab	5.77a	29.5ab	28.4ab	27.1b	31.5ab	29.1a
NSCBR-25	5.56ab	6.03ab	5.79a	5.01ab	5.60a	29.9ab	28.6ab	27.8ab	30.7ab	29.2a
-Ve Control	5.44b	5.50b	5.50a	4.99ab	5.36a	28.4b	27.5b	26.9b	30.9ab	28.4a
+Ve Control	5.87ab	6.14ab	5.98a	5.19ab	5.79a	28.7ab	28.3ab	27.0b	30.0b	28.5a
LSD	0.46	0.76	0.99	0.75	0.47	2.54	1.10	1.48	1.81	1.96
Dursitu	6.32a	5.97a	6.41a	5.28a	5.99a	18.5c	20.2c	19.9c	20.9b	19.9b
Gofta	5.57b	5.93a	5.64b	5.25a	5.60b	34.5b	33.4a	32.0a	36.1a	34.0a
Kufanzik	5.35c	5.68a	5.33b	5.01a	5.34c	35.5a	31.5b	30.7b	36.5a	33.5a
LSD	0.18	0.3037	0.40	0.30	0.19	1.02	0.44	0.59	0.73	0.69
CV (%)	5.15	8.35	11.03	9.30	9.36	5.56	2.50	3.47	3.76	4.41
					F value					
Inoculation (I)	3.08**	5.39***	0.65ns	2.54*	2.05*	2.18*	3.37***	4.23***	2.49*	1.17ns
Variety(V)	88.73***	3.12ns	22.58***	2.92ns	32.13***	1015.33***	3029.97***	1448.70***	1729.69***	1517.66***
I ×V	3.66***	3.96***	0.74ns	1.35ns	1.36ns	0.64ns	3.27***	4.95***	2.79**	0.59ns

NS-non significant; *significant at 0.05; **significant at 0.01; ***significant at 0.001; -VE-negative control (no inoculation and N application), +VE control -20 kg N ha^{-1}; NSCBR- National soil Common Bean *Rhizobium*
Notes. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

average SDW over locations with the Gofta than Dursitu varieties.

The effect of Rhizobium *inoculation and varieties on number of pods per plant*

Analysis of variance revealed that inoculation treatments, the varieties, and their interaction significantly affected the number of pods per plants (NPP) in all experimental sites (Table 2). At the Haramaya site, NSCBR-59 and NSCBR-25 inoculations resulted in a significant increase in the NPP compared to the uninoculated control. All inoculation treatments except NSCBR-18, significantly enhanced NPP at the Hirna site. At the Babillae site, the NSCBR-57 and NSCBR-25 inoculations had a significantly higher NPP than the uninoculated control. None of the tested isolates had significantly improved the NPP at the Fedis site. At all experimental sites, N-fertilized plants produced statistically similar NPP with the highest NPP obtained due to inoculation. Beside this, the data showed significantly higher NPP with the Dursitu variety than other tested varieties.

The effect of Rhizobium *inoculation and varieties on number of seeds per pod*

Though a significant difference in number of seeds per pod was observed due to inoculation treatments at the Babillae, Fedis, and Hirna sites whilenone of *Rhizobium* inoculations had improved this trait over the control check at the Haramaya site (Table 3). A slight reduction in NSP due to N fertilization was recorded at all experimental sites. The results also revealed the non-significant differences in average NSP over locations by inoculation. Among the tested varieties, Dursitu produced a significantly higher NSP than the remaining tested varieties.

The effect of Rhizobium *inoculation and varieties on 100-seed weight*

The effect of inoculation treatments, the varieties and their interaction was significant on 100-seed weight in all experimental sites, except the effect of interaction at the Haramaya site (Table 3). Inoculating NSCBR-59, NSCBR-16, and NSCBR-14 significantly increased 100-seed weight at Haramaya, Hirna, and Babillae sites, respectively. However, the non-significant effect of inoculation on 100-seed weight was found at the Fedis site. The highest average 100-seed weight across treatments was noted with Gofta followed by the Kufanzik variety.

Table 4. Total biomass yield and grain yield of common bean as influenced by *Rhizobium* inoculation treatments at Babillae, Fedis, Haramaya, and Hirna sites, 2012-13 cropping season.

Inoculation	Total biomass yield (kg ha^{-1})					Grain yield (kg ha^{-1})				
	Haramaya	Hirna	Babillae	Fedis	Average	Haramaya	Hirna	Babillae	Fedis	Average
NSCBR-14	6015.4ab	6938.3ab	2053.4cd	4068.5cd	4768.9a	3286.3a	2951.4a	1131.1c	1694.9a-e	2265.9a
NSCBR-(25)$_2$	5777.5bc	5948.1de	2437.0bc	4925.9a	4772.1a	2913.1bc	2647.8cd	1432.9ab	1885.6ab	2219.8a
NSCBR-59	5905.9abc	7246.9a	2290.1bcd	4408.6bc	4962.9a	2924.8bc	2892.8ab	1443.1ab	1763.2a-d	2256.0a
NSCBR-31	5342.0c	6603.7abc	2227.2bcd	4309.9cd	4620.7a	2739.0cde	2712.2bc	1259.9bc	1921.5a	2158.1a
NSCBR-16	5876.2abc	6463.0bc	2209.9bcd	4769.1ab	4829.6a	2901.5bcd	2704.6bc	1266.5bc	1584.9b-e	2114.4a
NSCBR-18	5321.0c	6708.6ab	2080.2cd	4437.0bc	4636.7a	2635.7de	2756.2abc	1232.9bc	1812.6abc	2109.3a
NSCBR-57	6503.7a	5690.1e	2036.7d	4204.3cd	4608.7a	2964.0bc	2381.9e	1241.7bc	1435.9e	2005.9a
NSCBR-25	5821.0bc	6018.5cde	2480.9b	4116.0cd	4609.1a	2858.8bcd	2430.0de	1431.1ab	1456.8de	2044.2a
-Ve Control	5438.6bc	5596.9e	1913.0d	3968.5d	4229.2a	2510.7e	2341.6e	1167.5c	1520.6cde	1885.1a
+Ve Control	5992.3ab	6892.0ab	3061.3a	4308.0cd	5063.4a	3045.8ab	2892.8ab	1637.4a	1809.4abc	2346.3a
LSD	631.51	643.86	386.57	377	1320.8	275.64	231.99	260.42	322.49	561.97
Dursitu	5578.2b	6252.8b	1774.66b	3773.33c	4344.73b	2167.1c	2078.5c	1000.3b	1256.8c	1625.7c
Gofta	5974.0a	6453.1ab	2472.78a	4245.00b	4786.23ab	3107.9b	2677.0b	1473.7a	1604.0b	2215.6b
Kufanzik	5845.9a	6525.9a	2589.44a	5036.48a	4999.45a	3358.9a	3257.8a	1499.3a	2204.8a	2580.2a
LSD	253.01	257.96	154.88	151.04	534.68	110.43	92.95	104.34	129.2	227.49
CV (%)	7.03	6.48	10.95	5.59	9.76	6.18	5.61	12.70	12.33	11.81
					F value					
Inoculation (I)	7.02***	16.71***	15.54***	13.88***	0.61ns	13.24***	19.51***	7.78***	6.58***	1.23ns
Variety(V)	7.36**	3.47*	93.49***	206.28***	4.32*	373.91***	464.91***	83.77***	159.17***	49.70***
I ×V	6.86***	3.35***	10.04***	6.25***	0.26ns	4.28***	5.91***	11.19***	4.08***	0.24ns

NS-non significant; *significant at 0.05; **significant at 0.01; ***significant at 0.001; -VE-negative control (no inoculation and N application), +VE control -20 kg N ha^{-1}; NSCBR- National soil Common Bean *Rhizobium*
Notes. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

The effect of Rhizobium *inoculation and varieties on total biomass yield*

Analysis of variance revealed that inoculation treatments, the varieties and their interaction significantly affected total biomass yield (TBY) in all experimental sites (Table 4). However, the main effect of inoculation treatments and its interaction with varieties had no significant influence on the average TBY over locations. In general, tested isolates of rhizobia performed differently in improving TBY in four experimental sites. For instance, inoculating NSCBR-57 significantly enhanced the TBY at the Haramaya site while all inoculated isolates except NSCBR-(25)$_2$, NSCBR-57, and NSCBR-25 resulted in a significant increase in the TBY at the Hirna site. Isolates NSCBR-(25)$_2$ and NSCBR-25 inoculation significantly improved the TBY at the Babillae site. A significant increase in TBY at the Fedis site was recorded withNSCBR-25, NSCBR-57, NSCBR-16, and NSCBR-18. At all experimental sites excluding Fedis, N fertilized plants produced a statistically similar TBY with those top performing inoculation treatments. The lowest TBY was produced in the uninoculated control. Though there was no significant effect of inoculation on average TBY over locations, the highest value was recorded with N fertilization.

The effect of Rhizobium *inoculation and varieties on grain yield*

The effect of inoculation treatments, the varieties and their interaction was significant on grain yield in all experimental sites (Table 4). However, the main effect of inoculation and its interaction effect with varieties had no significant effect on average GY over locations. All inoculation treatments excluding NSCBR-57 and NSCBR-25 resulted in a significant increase and the GY at the Hirna site. Inoculation of NSCBR-(25)$_2$, NSCBR-59, and NSCBR-25 improved significantly the GY at Babillae while the highest GY at Fedis were obtained from N fertilization, NSCBR-(25)$_2$, and NSCBR-31.

The highest GY (3286.27 and 2951.36 kg ha^{-1}) at the Haramaya and Hirna sites, respectively, was noted with NSCBR-14 treatment. Inorganic N application and NSCBR-31 inoculation gave the highest GY (1637.35 and 1921.45 kg ha^{-1}) at the Babillae and Fedis sites, respectively. The overall effect of *Rhizobium* inoculation did not improve the average GY over locations significantly but the highest value was produced with the N-fertilized treatment. As compared to the other varieties, the Kufanzik variety showed the highest GY across treatments.

Table 5. Total plant N accumulation of common bean as influenced by *Rhizobium* inoculation treatments at Babillae, Fedis, Haramaya, and Hirna sites, 2012-13 cropping season.

Inoculation	Total Plant N accumulation (%)				
	Haramaya	Hirna	Babillae	Fedis	Average
NSCBR-14	2.750d	3.919a	2.473c	2.727e	2.967a
NSCBR-(25)2	2.743d	3.837ab	2.791ab	3.257ab	3.157a
NSCBR-59	3.048b	3.544c	2.386c	3.058a-d	3.009a
NSCBR-31	2.936bcd	3.481c	2.621bc	3.078a-d	3.029a
NSCBR-16	3.377a	3.664bc	2.997a	3.129abc	3.292a
NSCBR-18	2.909bcd	3.466c	3.044a	3.203abc	3.156a
NSCBR-57	3.003bc	3.881ab	2.849ab	3.036bcd	3.192a
NSCBR-25	3.093b	3.667bc	2.838ab	3.011cd	3.152a
-Ve Control	2.797cd	3.671bc	2.896ab	2.846de	3.052a
+Ve Control	3.083b	3.866ab	2.623bc	3.291a	3.216a
LSD	0.236	0.235	0.288	0.241	0.378
Dursitu	3.626a	3.995a	2.854a	3.564a	3.510a
Gofta	2.793b	3.415c	2.821a	2.928b	2.989b
Kufanzik	2.503c	3.689b	2.580b	2.698c	2.868b
LSD	0.095	0.094	0.1154	0.0966	0.1529
CV (%)	5.13	4.10	6.76	5.08	5.19
			F value		
Inoculation (I)	14.41***	11.19***	12.42***	11.45***	1.52ns
Variety(V)	438.03***	110.05***	19.43***	248.55***	55.13***
I ×V	13.74***	12.69***	6.25***	8.64***	1.14ns

NS- non significant; ***significant at 0.001; -VE-negative control (no inoculation and N application), +VE control -20 kg N ha^{-1}; NSCBR- National soil Common Bean *Rhizobium*
Notes. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

The effect of Rhizobium *inoculation and varieties on total plant N accumulation*

Total plant N accumulation (TPNA) in all experimental sites was significantly affected by *Rhizobium* inoculation, the varieties and their interaction at $P<0.05$ (Table 5). However, the average TPNA over locations was not affected significantly by the main effect of inoculation and its interaction with varieties. Inoculating NSCBR-59, NSCBR-16, and NSCBR-25 increased TPNA significantly at the Haramaya site. At the Hirna site, only NSCBR-14 had significantly superior TPNA when compared to the control. The non-significant effect of inoculation on TPNA at the Babillia site was observed. At the Fedis site, inoculating NSCBR-$(25)_2$, NSCBR-16, and NSCBR-18 resulted in significantly increased TPNA over other treatments. The highest TPNA (3.377and 3.919%) at the Haramaya and Fedis sites, respectively, were recorded at N fertilizer-treated plants while 2.997 and 3.291% at the Babillae and Fedis sites were recorded with NSCBR-18 and N-fertilized treatments, respectively. The result also revealed that the Dursitu variety accumulated significantly the highest plant N concentration than Gofta and Kufanzik varieties.

Discussion

Inherent soil fertility is a major factor affecting the effectiveness of legume host-*Rhizobium* symbiosis and the productivity of the host plant. To evaluate the effect of selected soil properties on symbiotic N_2 fixation by common bean, this experiment was initiated to determine the effect of inherent soil fertility on the effectiveness of elite *Rhizobium* inoculation on nodulation, yield, and yield traits of different common bean varieties at a major growing area in eastern Ethiopia. Inoculation of all *Rhizobium* isolates at Haramaya, Hirna, and Babillae sites significantly increased NN and NDW. A previous study indicated similar findings on common bean in Tunisian soil (Tajini et al. 2008). Regardless of inoculation treatments, thelowest NN and NDW were recorded on Babillae soil, probably due to low inherent soil fertility that prevailed in study site (De Oliveira et al. 1998).

The data showed the non-significant effect of NSCBR-18 on NN and NSCBR-31, NSCBR-18, and NSCBR-57 on NDW at the Fedis site. This might be related with low competitiveness of these isolates against indigenous rhizobia of common bean (Buttery et al. 1992; Rosas et al. 1998).). In addition, water stress and other related constraints in this study site could also reduce the competitiveness of inoculated isolates and consequently nodulation formation (Kurdali et al. 2002; Miller et al. 2003). Low P availability in the Fedis soil could also reduce the biomass and number of nodules (Mandri et al. 2012).

The present study revealed that inoculating NSCBR-16, NSCBR-31, NSCBR-$(25)_{2,}$ and NSCBR-59 were recorded the highest nodulation at the Haramaya, Hirna, Babillae, and Fedis sites, respectively. This difference performance of isolates could be related with the presence of different inherent soil fertility and competitiveness of indigenous rhizobia population against inoculated isolates (Gan et al. 2009a; Zeng et al. 2007). We also found the highest NDW at the Babillae site due to N fertilization. This increase in nodulation could be attributed to low SOM and total N in the Babillae site which created conducive conditions for a positive response to inorganic N application and thus improving the early plant growth and photosynthetic products (Gan et al. 2009b; Walley et al. 2005). This could consequently improve nodule formation and symbiotic N_2 fixation (Sundstrom et al. 1983). Bakhoum et al. (2016) found that nodulation tended to be higher in soils poor in nutrients especially N compared to fertile soils. Beside this, some common bean varieties may be superior in nodulation and N_2 fixation in the presence of N (Park and Buttery 1989). Gates and Müller (1979) also found that mineral N can stimulate soybean nodulation in the presence of high levels of P and S. Tsai et al. (1993) and Russo and Perkins-Veazie (1992) also confirmed similar finding for common bean and showed a synergistic effect on nodulation and nitrogen fixation, under high soil fertility conditions with amendments of P, K, and S plus micronutrients.

The current result displayed the highest NN and NDW in the Hirna site followed by the Haramaya site in most of inoculation treatments, including the control check while the lowest was recorded in Babillae soil. This indicates the presence of a relatively higher rhizobia population at Haramaya and Hirna soils when compared to the other study sites (Furseth et al. 2012) with good environmental conditions. The nodulation failure and poor nitrogen fixation has been reported due to the adverse effects of water stress on rhizobial survival in the soil (Hungria and Vargas 2000; Kyei-Boahen et al. 2005).In all study sites, Dursitu produced significantly higher NN and NDW than those produced in other tested varieties which was probably due to the presence of a difference in nodule dry mass production among common bean varieties (Nleya et al. 2009). Besides, this could be due to the presence of compatible indigenous rhizobia for Dursitu in the study site.

Though most tested isolated improved the nodulation, the effect of inoculation on SDW, NPP, NSP, and 100-seed weight was non-significant at the Fedis sites. Likewise, inoculation did not significantly (P<0.05) increase the SDW and NSP at the Babillae site. This finding is in line with Karasu et al. (2011) who found that seed number per pod of common bean did not affect by *Rhizobium* inoculation, N application, and varieties. However, these traits significantly improved due to some inoculation treatments at the Haramaya and Hirna sites. This different response to *Rhizobium* inoculation could be due to the presence of various amounts of essential nutrients and water availability in different study sites. Ssali (1988) found that common bean production was significantly increased where inoculation was applied in P-rich soil and adequate soil moisture while Graham (1992) demonstrated that P deficiency negatively affected rhizobia survival and nodulation process in legumes. High SOM in Haramaya and Hirna soils is relevant for the survival of inoculated isolates in soils (Ferreira et al. 2000; García-Orenes et al. 2016). Other soil properties including calcium concentration, total exchangeable bases, percent base saturation, and altitude exhibited positive correlation with *Rhizobium* effectiveness (Rys and Bonish 1981). The presence of these good soil conditions increased nodulation and N derived from symbiotic N_2 fixation (Mete et al. 2015; Zwietenet al. 2015).

The result revealed none of the tested isolates significantly affected SDW, NPP, NSP, and 100-seed weight compared to the N-fertilized plot in all experimental sites. This suggests that the N derived from inoculated bacteria might not higher than N derived from indigenous rhizobia in the supplement of inorganic N. Previous work indicated that the effectiveness of strains was masked when soil contained competent and effective indigenous rhizobia (Ansari and Rao 2014; Batista et al. 2015). Conversely, Mulas et al. (2015) found that number of seeds per pod and 100-seed weight of common bean were remarkably affected either by *Rhizobium* inoculation or N fertilizer application.

The highest SDW, NPP, and NSP was obtained from the Gofta variety at all experimental locations except Fedis in which Kufanizik produced the highest SDW and NPP.A similar finding was previously reported by Remans et al. (2008). This could be due to the environmental conditions that influence interactions and consequent efficacy of the bean variety-*Rhizobium* association (Buttery et al. 1997; Graham and Vance 2000; Rennie and Kemp 1983).This difference could also be due to the fact that genetic differences in bean performance in low fertile soil are well documented under tropical field conditions (Salinas 1978; Singh et al. 1989; Thung 1990; Yan et al. 1995). Moreover, different performances of common bean varieties could be related to the presence of variety-specific indigenous rhizobia in different locations (Yadegari et al. 2010).

In contrast to the nodulation results, significantly higher TBY and GY in all locations were obtained from the Kufanzik variety than from the Dursitu variety. This result concurs with the observations made by Fageria and Santos (2008). They demonstrated that some traits of common bean were genetically controled beside being affected by environmental and soil management. As has been observed in nodulation (NN and NDW) traits, Dursitu produced significantly higher total plant N accumulation than other varieties. This indicates the need for nodulation and symbiotic N_2 fixation to increase the total N accumulation in plant tissue. Jensen (1996) found that nodulation directly correlated with N_2 fixation and N derived from symbiotic N_2 fixation. On top of this, the genetic variability of common bean varieties has determined the amount of N_2 fixation and other related traits (Bliss 1993).

Several studies have sought to identify efficient and competitive strains of rhizobia to cope the nitrogen requirements of common bean (Asadi-Rahmani et al. 2011; Giongo et al. 2007; Shamseldin et al. 2005).The present study showed that all *Rhizobium* inoculation except NSCBR-31 and NSCBR-16 at the Haramaya site and NSCBR-57 and NSCBR-25 at the Hirna site significantly increased the GY production of common bean. This indicates that these isolates may be more effective in N_2 fixation and competent against the native rhizobia nodulating common bean. The observed benefits due to inoculation seem to be because conducive soil fertility prevailed in these study sites, creating a conducive environment for inoculated *Rhizobium* and thereby increased their effectiveness in N_2 fixation (Zwieten et al. 2015).

At the Babillae and Fedis sites, most of inoculated isolates did not improve remarkably the GY of common bean. This was probably due to the unconducive environmental conditions prevailing in these study sites (Hungria and Vargas 2000). This could also be because of less competitiveness of introduced isolates against native rhizobia (Vieira1994) as a result of unfavorable environmental conditions that prevailed in these experimental sites. However, the best yield of common bean results when symbiotic strains and all the different steps of the symbiosis formation are resistant to environmental conditions and nutrient deficiencies (Zahra et al. 1984).

The result here also indicates the non-significant differences in GY and TBY between inoculation treatments and N fertilization in all experimental locations. This indicates that inorganic N (20 kg N ha^{-1}) application with native rhizobia can improve the productivity of common bean without inoculation. However, N fertilization at the Fedis site significantly reduced TBY when compared to some inoculated treatments. This is probably related with ineffective indigenous rhizobia and imbalanced nutrient supply by the soil at the Fedis site (Lynd and Ansman1989). Low plant density as a result of N application might be also the cause of a reduction in crop production (Gan et al. 2009b).

The result of the present study also revealed that significantly higher TBY and GY was produced at the Haramaya and Hirna sites than at the Fedis and Babillae sites with all treatments except NSCBR-$(25)_2$ and NSCBR-18 treatments. This difference could be the fact that the inherent soil fertility at the Haramaya and Hirna sites are better than at the Fedis and Babillae sites (Dakora and Keya1997; De Oliveira et al. 1998; Ssali 1988) and thus enhances the effectiveness of *Rhizobium* and crop production (Peoples et al. 2013).These results are in accordance with the work of Fageria et al. (2010) and Ronner et al. (2016). They found that better soil chemical fertilities had a significant positive association with grain yield of common bean and soybean. Fageria (2014) also found that N, P, K, and Cu were relatively more important in improving grain yield of dry bean. Ndakidemi et al. (2006) also found that relative to uninoculated and unfertilized plots, grain yields of common bean were markedly increased by 60-78% from inoculation alone, and 82-95% from inoculation + 26 kg Pha^{-1}.Due to high P and SOM, all inoculation treatments including the control produced the highest common bean production (Raposeiras et al. 2006).However*, Rhizobium tropici* strain CIAT-899 inoculation significantly increased grain yield of common bean even under poor soil conditions (Hungria et al. 2000; Vargas and Graham 1989).

Conclusion

In general, inoculation improved the nodulation of common bean in all study sites. Also this inoculation enhanced the productivity of common bean in all study sites, though none of the inoculated isolates consistently performed in all experimental sites. The production and productivity of common bean is dependent on the common bean variety due to variation of performance of common bean varieties in different environmental sites. The inherent soil fertility affects effectiveness inoculated *Rhizobium* and the productivity of common bean. Therefore, improving inherent soil fertility is essential to enhance the performance of *Rhizobium* inoculation and productivity of crop in general, and legume pulses in particular. Our results clearly show the importance of promoting inoculant use in African agriculture, especially for resource-limited farmers who cannot afford expensive chemical fertilizers.

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