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Rhizobial inoculation improves drought tolerance, biomass and grain yields of common bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* L.) at Halaba and Boricha in Southern Ethiopia

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Abstract

While pulses are staple food-legumes in Ethiopia, their productivity is low due to low soil fertility. Elite rhizobial strains that significantly increased shoot dry weight and nitrogen (N) contents of common beans and soybeans in greenhouse were selected for two-year field trials to evaluate their effect on yields of the pulses in the field. Each pulse had six treatments, namely four rhizobial inoculants, uninoculated control and synthetic N fertilizer. In the drought-affected year 2015, inoculated pulses tolerated moisture stress better than non-inoculated controls. Inoculation was conducive to higher or equivalent yields compared to synthetic N fertilizer. At Halaba, bean inoculated with strain HAMBI3562 gave the highest grain yield (1500 ± 81 kg ha⁻¹; mean $\pm$ SE) while the control yielded only 653 ± 22 kg ha⁻¹. At Boricha,

HAMBI3570 gave a grain yield ($640 \pm 35 \text{ kg ha}^{-1}$) comparable to synthetic N. When rainfall was optimal in 2016, inoculation with HAMBI3562 and HAMBI3570 gave grain yields (around 4300 kg ha^{-1}) equivalent to synthetic N. With soybean, strain HAMBI3513 produced consistently higher or comparable biomass and grain yields compared to synthetic N. In conclusion, HAMBI3562 and HAMBI3570 for beans and HAMBI3513 for soybeans can serve as inoculants for areas having similar conditions as the test areas.

Keywords: biological nitrogen fixation; *Rhizobium* strains; *Bradyrhizobium* strains; pulses; food security

Introduction

Food security is a major issue in many African countries (WFP 2018). Ethiopia, with the second largest population in Africa, about 105 million (UNDP 2018), is one of the world's food insecure countries. Ethiopian economy is fundamentally dependent on agriculture. This sector accounts for 50% of the GDP, over 80% of exports and about 80% of the labor force. Smallholder farms are the pillars of the agriculture sector, and cover about 95% of the cultivated area and 90% of the total production (Welteji 2018). However, the farming practices are characterized by low yield per unit area, mainly due to poor soil fertility of the farmlands and poor farm management practices, soil erosion, land degradation, and lack of nutrient inputs (Tesfa and Mekuriaw 2014; Fadong et al. 2018). In addition, susceptibility of the country to drought and climate change effects are becoming major challenges for food production and food security.

In Ethiopia, food legumes (pulses) are an integral part of the staple foods for the society. Common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes in Africa and Latin America. This pulse is widely cultivated for food consumption and is also the third export commodity after coffee and sesame (Katungi et al. 2010). It is largely cultivated by smallholder farmers in the Rift Valley area, and in the southeastern and southwestern parts

of the country, as a sole crop or intercropped with non-legumes, such as maize, sorghum, enset, and coffee (Katungi et al. 2010; Abebe 2017; Assefa et al. 2017). The land coverage and production of common bean in the country is increasing over time. Nevertheless, there is a huge yield gap, as its productivity in the farmers' field remains low compared to the yields obtained in the research fields. For instance, the national average yield reported in the cropping seasons of 2011/12 and 2015/16 was 1169 kg ha⁻¹ and 1485 kg ha⁻¹, respectively (Cochrane and Bekele 2018). These values were below the potential yield range 2000-3084 kg ha⁻¹ obtained at research stations (Emishaw 2007; Asfaw et al. 2008).

Soybean (*Glycine max* L.) is produced worldwide for its high food and feed values (Sinclair et al. 2014). USA, Brazil, Argentina, China, India, Paraguay, and Canada are the leading soybean producing countries in the world (<https://www.statista.com/statistics/263926/soybean-production-in-selected-countries-since-1980/>). Soybean has been introduced to Ethiopia from the 1950s (Belay 1987). The major soybean growing regions are found in the southern and western parts of the country. In these regions, the smallholder as well as large-scale farmers produce soybean mainly for cash and food, and sometimes for improvement of soil fertility by rotations with cereals. A case study in northwestern Ethiopia, Metekel Zone, shows that about 90% of soybean produced in this region is used for income generating purposes and only a small portion is used for food by the farmers (Getahun and Tefera 2017). The yield of soybean in smallholder farmers' field was on average 1633kg ha⁻¹ in 2012 and 1814 kg ha⁻¹ in 2013, which were below the corresponding yields 3175 and 2359 kg ha⁻¹ obtained in research fields in respective years (MARD 2013). According to a research conducted in farmers' field, soybean yields were found to vary from less than 1000 kg ha⁻¹ to about 6000 kg ha⁻¹ depending on soil characteristics of the farm fields (Zerihun et al. 2015).

The low yields of both common bean and soybean in Ethiopia can be attributed to lack of improved or locally adaptive varieties, poor farming systems, moisture stress, low

soil fertility, diseases and insect pests (Katungi et al. 2010; Darkwa et al. 2016). The low soil fertility of the farmlands; i.e. deficiency of organic matter and the major plant growth limiting nutrients N and phosphorus (P) in soils and lack of nutrient inputs were reported as the main factors contributing to the low yields and productivities of the pulses (Abebe 2017; Zerihun and Haile 2017).

Pulses, such as common bean and soybean make a N₂-fixing symbiotic association with soil bacteria commonly known as rhizobia. Biological nitrogen fixation (BNF) by the legumes in symbiosis with rhizobia fulfills the N requirements needed for the plant growth and consequently, increases biomass and yield of the legume crops without application of synthetic N fertilizers to the soil. In addition, litter-fall and residual decomposition of the legumes improve the N and organic C contents of the soils, which in turn benefit the sustainability of the soil fertility and enhance subsequent cereal production cultivated in rotation with legumes (Bado et al. 2013; Zerihun and Haile 2017). Thus, for yield increase, inoculation of pulses with selected superior rhizobia is feasible, instead of applying synthetic fertilizer, which is neither an alternative for poor farmers nor for sustainable food production. Common bean- and soybean-nodulating rhizobial strains were isolated in Ethiopia and characterized previously (Aserse et al. 2012a, 2012b). The first objective of this study was to test and select effective N₂-fixing rhizobia symbionts for bean and soybean, based on their symbiotic performance on host plants in greenhouse conditions. The second objective of the work was to evaluate the effect of selected rhizobial inoculants on growth, biomass and grain yield of beans and soybeans in the field conditions at Halaba and Boricha sites in southern Ethiopia for two consecutive growing seasons.

Material and methods

Nodulation and symbiotic efficiency test in the greenhouse

Rhizobial strains isolated from root nodules of bean and soybean plants grown in the field in Ethiopia were previously described and tested for nodulation and N₂ fixation efficiency under controlled conditions in plant growth chambers (Aserse et al. 2012a, 2012b). Based on preliminary nodulation and symbiotic performance, sixteen bean- and nine soybean-nodulating rhizobial strains were selected to assess their symbiotic effectiveness in soil culture under greenhouse conditions and to select the best strains for the field experiments (Table 1). The test was conducted at the Viikki campus greenhouse facility of the University of Helsinki, Finland. The seeds were planted in plastic pots with the dimensions of 16 cm diameter and 18 cm depth, filled with 2.5 l of potting soil containing a low N content. The soil properties were as follows: pH (H₂O) 6.5, electrical conductivity (EC) 20 mS m⁻¹, soluble N 50 mg l⁻¹, K 200 mg l⁻¹, P 60 mg l⁻¹ and bulk density 510 g l⁻¹. P and K were extracted with 0.01 M CaCl₂ – 0.002 M DTPA solution (Finnish Standards Association 2002a) and N with deionized water (Finnish Standards Association 2002b). The potting soil was purchased from a greenhouse supplies company Kekkilä Oy, Mellilä, Finland.

[Table 1 near here]

The treatments (rhizobial strains) were replicated into three pots in randomized complete design form and four seeds were planted in each pot. Ethiopian common bean variety red wolayta and soybean local cultivar 1 were used for this experiment. The seeds were sterilized with 95% alcohol followed by 3-5% sodium hypochlorite and rinsed with several changes of sterile water (Somasegaran and Hoben 1994). The strains were grown in Yeast Extract Mannitol (YEM) broth as described previously (Aserse et al. 2012a, 2012b). In the day of planting of the seeds, 1 ml suspension of the rhizobial strains (about 10⁸ l⁻¹ cells) was added on each seed, except for the control treatments that received no rhizobia inoculant. Plants were watered twice a week with tap water with an irrigation system and grown at 28 ± 2°C day and 18 °C nighttime

temperature, and a 16 h day and 8 h night cycle. The plants were harvested after 6 weeks and the plant appearance, nodule number, and nodule color data were recorded. Plant shoots (including all above ground plant parts) were oven-dried at 70 °C for 24 h, dry matter weighed, ground and analyzed for N and C concentrations using the VarioMax dry combusting instrument. The N and C contents were calculated as the product of total shoot dry matter of a plant and the respective C and N concentrations in the shoot. The SPSS statistical package was used to analysis the data and means between treatments were compared by the least significant difference (LSD) test ($p \leq 0.05$).

Field experiments

Description of experimental sites and soil properties

The field experiments were carried out at two sites in Halaba and Boricha districts in southern Ethiopia for two growing seasons in 2015 and 2016. The Halaba site is located at 07°19.04' North and 038°04.9' East with an altitude of 1650 m above sea level. The Boricha site is located at 06°54.9' North and 038°20.4' East and at the altitude of 1620 m above sea level. Based on the long-term national weather data, the annual mean rainfall of the Halaba and Boricha districts were 857 – 1085 mm and 500 – 1242 mm, respectively, and annual temperature varied from 17 to 25°C in Halaba and 18 – 29°C in Boricha district (Ganewo 2017; Tarfasa et al. 2018). Southern Ethiopia including in Halaba and Boricha has a bimodal rainfall pattern with a short rainy season that lasts from March to May, while a longer rainy main growing season takes place from July to October (Tarfasa et al. 2018). The field experiments were carried out during the main growing season (July - October) at both sites for two years. The rainfall received and its distribution in 2016 during the plant growth period was better than in 2015. There was a strong drought in 2015 due to the El Niño episode and this year the rainfall in large part of Ethiopia was below-normal during the growing season (July- September) (AKLDP 2015; Philip et al. 2018). Our field experiments were affected by the drought and moisture stress in 2015.

The major crops cultivated around Halaba and Boricha areas include maize (*Zea mays*), teff (*Eragrostis tef*), hot pepper (*Capsicum frutescence*), wheat (*Triticum* spp.) and beans (Tarfasa et al. 2018). There had not been a history of soybean and bean rhizobia inoculation before the experiment at the particular sites. Both in 2015 and 2016, bean and soybean trials at Halaba site were taking place in the fields, where wheat was grown the other year. At Boricha site, prior to the pulse trials in 2015 and 2016, the fields were planted with maize and wheat respectively.

Composite soil samples of 0–30 cm and 30–50 cm depths were collected on 8 October, 2015 and analyzed for physico-chemical characteristics mainly according to van Reeuwijk (2002). Particle size distribution was determined with a pipette method after digestion with H₂O₂ and HCl and dispersion with K₄P₂O₇. Soil pH and EC were measured in 1:2:5 soil/water (v/v) suspension. Total C and N were determined by dry combustion, while soluble P was extracted by the Olsen method (0.5 M NaHCO₃ at pH 8.5). Exchangeable Ca, Mg, K, Na and titratable acidity were extracted with 1 M ammonium acetate buffered at pH 7, and all results of cations were expressed as cmol(+) kg⁻¹. Potential cation exchange capacity (CEC_{pot}) and base saturation (BS) were calculated as $\text{Ca} + \text{Mg} + \text{K} + \text{Na} + \text{titratable acidity}$ and $\text{BS} = 100 \times (\text{Ca} + \text{Mg} + \text{K} + \text{Na}) / \text{CEC}_{\text{pot}}$, respectively.

Both experimental sites consisted of a loamy soil with practically similar percentages of clay, sand and silt (Table 2). The soils were non-saline, and they contained no sodicity. The pH (H₂O) in the topsoil was about 8.0 at Halaba and 6.4 at Boricha. Accordingly, there was no exchangeable Al, and the base saturation was high at both sites, the exchange sites being mostly occupied by Ca. The content of soil organic matter ($1.732 \times C_{\text{tot}}$) was 2.5% at Halaba and 3.1% at Boricha. Plant available P was very low at Halaba and at the borderline of low and very low at Boricha. According to the World Reference Base for Soil Resources (FAO 2014), the Halaba soil was classified as an Eutric Fluvisol (Loamic) and the Boricha soil as a Mollic Andosol (Loamic).

[Table 2 near here]

Experimental design, treatments and field management

The experiments were arranged in Randomized Complete Block Design in three replications. There were six treatments for each of bean and soybean experiments including two non-inoculated controls, i.e. treatment without N source as a negative control and plots fertilized with urea (46 kg ha⁻¹ N) as a N source. All the six treatments received Triple Superphosphate (TSP) (20 kg ha⁻¹ P) uniformly. For the bean, the rhizobial treatments were strains *Rhizobium etli* HAMBI3556 (HBR5), *Rhizobium phaseoli* HAMBI3562 (HBR10), *R. phaseoli* HAMBI3570 (HBR53) and rhizobial strain EAL428. For soybean, *Bradyrhizobium japonicum* HAMBI3524, *Bradyrhizobium elkanii* HAMBI3513 (SBR2B), *B. elkanii* HAMBI3520 (SBR8B) and rhizobial strain LF were used as inoculant treatments. Except the commercial inoculants EAL428 and LF that were used as references, all the others were characterized and stored in the HAMBI culture collections of the University of Helsinki (Aserse et al. 2012a, 2012b). *B. japonicum* strains HAMBI3524, also known by the strain code USDA136 (TAL 379) was first obtained from the NifTAL culture collection in Hawaii, USA and has been used as an inoculant strain for soybean in Ethiopia (Aserse et al. 2012a). The commercial inoculants EAL428 and LF were obtained from the local inoculant company Menagesha PLC in Ethiopia. The HAMBI strains were selected for the field experiment based on their effective N₂ fixation performance in the greenhouse experiment. The rhizobial inoculants with peat as the carrier material were prepared by the Finnish inoculant producing company Elomestari Ltd.

The experimental fields were prepared and weeded during May and June, by ploughing three times with oxen drawn implements. Local common bean variety Hawasa-Dume and soybean variety Hawasa-95 were used for the field trials. Before sowing, the seeds were soaked with tap water until the seed coat became rough and suitable for the inoculants to adhere to the seeds. Then, seeds for the plots receiving inoculants were dressed separately with each of the peat inoculants containing about 10⁸ rhizobial cells per seed under the shade

immediately prior to planting. To avoid cross-inoculation we followed all the necessary aseptic instructions as described in Somasegaran and Hoben (1994). Planting was done manually in July. The plot size, number of rows and seed spacing were the same for both bean and soybean experiments. Each plot had a 2 m × 3 m size and 5 rows. The seeds were sown at row –to– row distance of 40 cm and with 10 cm spacing between plants. The two outside rows were assigned as border rows whereas the remaining three central rows formed the net plot area. One of these was used as sampling row and the remaining rows were used for yield component estimation.

Plant sampling, harvesting and analyses

Three plants were randomly collected at mid-flowering stage from sampling rows for nodule number counting. Plant height (cm) was measured from five randomly taken plants of each net plot at 90% physiological maturity with a standard meter ruler. Number of pods per plant were counted from 20 randomly taken plants in the rows within the net plot area at harvest maturity and the means were reported as number of pods per plant. Average number of seeds per pod was inferred from the total numbers of seeds dividing by the total number of pods. All plants from the net plot area were harvested and sun-dried until a constant dry weight was attained, followed by determination of above ground biomass. After threshing and cleaning, the seed yield was adjusted to a moisture level of 10%. Hundred seed weight (g) was measured by weighing 100 randomly taken seeds from the harvested yield using an electronic weighing balance. Finally, the biomass and yield per plot was converted to hectare basis and reported as kilogram per hectare. Analysis of variance was carried out for the collected data using the SPSS statistical package with application of the F test ($p \leq 0.05$). A three-factor analysis of variance (ANOVA) was performed to evaluate the effects of treatments (synthetic N, rhizobial inoculations), locations and years and their interactions on grain yield and other yield components. Mean differences between treatments were compared by the least significant differences (LSD) test ($p \leq 0.05$).

Results

Symbiotic efficiency of rhizobial strains in the greenhouse

All the rhizobial strains listed in Table 1 nodulated their respective bean and soybean host plants and nodules were pink (except the bean inoculated with HAMBI3549), showing the effectiveness of the symbiosis. Inoculated plants looked healthy, the leaves were dark green, and plants grew better compared to the non-inoculated counterparts. The non-inoculated control plants did not produce nodules (data not shown). The symbiotic N₂ fixation effectiveness was deduced from the plant shoot dry weight (SDW) and plant N contents. The SDW and total N content of the common bean and soybean plants inoculated with the rhizobial strains increased significantly compared with the non-inoculated control treatment (Table 1). Beans inoculated with strains HAMBI3562, HAMBI3565, HAMBI3570, HAMBI3556 and HAMBI3573 performed well, providing about 19 – 18 g SDWs and 0.47 – 0.44 g N contents per plant, the increases being 9 – 10 g SDWs and about 0.3 g N per plant over the non-inoculated control. Soybean plant inoculated with strain HAMBI3524 provided the highest plant N content (0.42 g per plant). The soybeans inoculated by the strains HAMBI3520, HAMBI3524 and HAMBI3513 were among the best performing plants with regard to SDW. Considering the strains performance in terms of the plant N content and/or SDW in greenhouse tests, the bean strains (HAMBI3562, HAMBI3570, HAMBI3556) and soybean strains (HAMBI3524, HAMBI3513, HAMBI3520) were selected for field trials (Table 1).

Field trial results

The field trials were performed for two consecutive years at two sites, in Halaba and Boricha. In 2015, the fields suffered from bad drought, whereas in 2016 rainfall was normal. The mean yield components for bean and soybean field results are presented in Tables 3 and 4. Bacterial inoculations increased plant growth parameters depending on the strain. The overall mean yield components were significantly ($p \leq 0.05$) different between the experimental locations, treatments, years and/or between the interactions of the factors. The effect of year, location

and/or their interaction were highly significant and strong regarding the growth and yield parameters of the pulses, particularly for nodule numbers, biomass and grain yield (Table 3 and 4).

The yield difference between years was associated with the difference in the moisture content of the field sites. In 2015, the rainfall distribution was very variable, sporadic and erratic during the plant growth period and at the flowering stage on our experimental sites, which resulted in moisture stress and yield reduction of the pulses. In fact the drought in 2015 caused by El Nino was a major cause of crop yield reduction in Ethiopia (AKLDP 2015; Philip et al. 2018). The drought stress of 2015 was manifested more at the Boricha than at Halaba. Thus, the mean nodule number, biomass and grain yields of the beans were two-fold or higher at Halaba compared with Borchha and the means of the main yield components of soybean were significantly higher at Halaba than at Boricha. However, there was no significant difference in the plant height, seeds per pod, pods per plant and/or hundred seed weight data of the pulses between the sites.

In 2016, when there was more moisture for the plant growth, the overall yield components of the beans were comparable between the sites. The soybean mean nodule numbers and biomass yields were higher at Halaba, but on the other hand, the grain yield and pods per plant were greater at Boricha. In that year, there was no significant difference in plant height, seeds per pod, and hundred seed weight of soybeans between the two sites (data not shown).

As the effect of treatments on the main yield components was varied depending on the sites and/or years, independent comparison of biomass and grain yields was made between treatments for each location and year (Tables 3 and 4). Generally, when the field sites were severely affected by drought, inoculation successfully helped the plants to adapt to and tolerate the moisture stress. Inoculation with at least one of the bean rhizobial strains and three of the soybean inoculants resulted in higher or equivalent biomass and grain yields compared

to the pulses receiving synthetic N fertilizer. The lowest bean and soybean biomass and grain yields were recorded from plots that received neither synthetic N fertilizer nor the rhizobial inoculants, known as “non-inoculated control” hereafter (Tables 3 and 4). The non-inoculated control yields of the beans were substantially affected by the different water supplies, grain yields being only 140 – 650 kg ha⁻¹ in 2015 and as much as 2800 – 3600 kg ha⁻¹ in 2016.

At Halaba, under the drought stress in 2015, the bean inoculated with HAMBI3562 delivered the highest biomass yield (3013 ± 355 kg ha⁻¹) as well as grain yield (1500 ± 81 kg ha⁻¹). At Borchia, the biomass and grain yields obtained from bean inoculated with strain HAMBI3570 and bean receiving synthetic N were comparable, with strain HAMBI3570 giving 1240 ± 788 kg ha⁻¹ biomass and 640 ± 35 kg ha⁻¹ grain yields. Increase of grain yield, compared to the non-inoculated bean without synthetic N fertilization was 500 and 870 kg ha⁻¹ for HAMBI3570 and HAMBI3562, respectively.

In 2016, at Halaba site, generally the biomass and/or grain yields of inoculated beans were comparable to the bean receiving synthetic N, but higher compared to the control plot. The biomass and grain yields obtained from bean inoculated with strain HAMBI3562 were 7022 ± 130 kg ha⁻¹ and 4360 ± 81 kg ha⁻¹, respectively. Inoculation with the HAMBI strains increased bean grain yields by 500 – 1800 kg ha⁻¹ over the control. At Boricha, bean biomass and/or grain yields due to inoculation were also statistically at the same level as the plot receiving synthetic N. Similarly, as the year 2015, bean inoculated with HAMBI3570 performed well in 2016, showing 6750 kg ha⁻¹ and 4250 ± 289 kg ha⁻¹ biomass and grain yields, respectively, which in terms of grain yield was 680 kg ha⁻¹ more than in the non-inoculated control.

[Table 3 near here]

Depending on the years and/or the experimental sites, soybeans inoculated with rhizobial strains produced higher or equivalent biomass and grain yield compared to the plots receiving synthetic N. At the Halaba site in the dry year 2015, soybean inoculated with strain HAMBI3513 showed

the highest biomass ($2600 \pm 57 \text{ kg ha}^{-1}$), followed by HAMBI3520 and soybean receiving synthetic N. The rhizobial inoculants HAMBI3513, HAMBI520 and the commercial inoculant LF were conducive to about 200 kg ha^{-1} higher soybean grain yields (617 ± 16 to $643 \pm 142 \text{ kg ha}^{-1}$) over the non-inoculated control. The grain yield obtained from plot receiving synthetic N was $613 \pm 81 \text{ kg ha}^{-1}$. In that year at Boricha, soybean inoculated with strains HAMBI513, HAMBI3524 or the commercial inoculant LF showed significantly higher biomass (1260 ± 17 to $1397 \pm 26 \text{ kg ha}^{-1}$) and grain yields (427 ± 20 to $507 \pm 23 \text{ kg ha}^{-1}$) compared to the other treatments, the grain yields of these treatments being $100 - 180 \text{ kg ha}^{-1}$ higher than in the control.

At Halaba when the moisture condition was optimal in 2016, the strains HAMBI3513, HAMBI3524 and commercial inoculant LF gave higher biomass (13426 ± 197 to $13951 \pm 41 \text{ kg ha}^{-1}$) and grain yields (4525 ± 241 to $4966 \pm 47 \text{ kg ha}^{-1}$) than the other treatments, the grain yields of these plots being $1430 - 1870 \text{ kg ha}^{-1}$ higher than in the non-inoculated control. The biomass and grain yields obtained from soybean receiving synthetic N were $12738 \pm 487 \text{ kg ha}^{-1}$ and $3383 \pm 494 \text{ kg ha}^{-1}$ respectively. In the second year at Boricha, soybeans inoculated with strains HAMBI3513, HAMBI3520 and HAMBI3524 provided higher biomass (12222 ± 147 to $12778 \pm 111 \text{ kg ha}^{-1}$) and grain yield (5667 ± 117 to $6611 \pm 56 \text{ kg ha}^{-1}$, the increase being $2220 - 3160 \text{ kg ha}^{-1}$ over the control), followed by soybean receiving synthetic N fertilizer (Table 4).

[Table 4 near here]

Discussion

Pulses are staple foods and important sources of protein in Ethiopia (Asfaw et al. 2009; Katungi et al. 2010). Pulse legumes are also used in cropping systems to improve soil fertility for subsequent crops (Bado et al. 2013; Zerihun and Haile 2017). Inoculation of pulses with

superior N₂-fixing rhizobia enhances food production as well as helps to reduce environmental impacts caused by synthetic N-fertilizer.

In this work, the growth, and nodulation, SDW and total N contents of the pulses inoculated with rhizobial strains in the greenhouse were increased significantly over non-inoculated control plants, confirming the establishment of effective N₂-fixing symbiosis between the rhizobia and their host legume pulses. Subsequently, three effective N₂-fixing rhizobial strains were tested in field conditions with bean and soybean plants. In the field, the growth of the inoculated pulses increased significantly compared with the non-inoculated controls. Inoculation gave equal to or higher biomass and yields than those of pulses receiving synthetic N fertilizer depending on the rhizobia strains and field sites, in accordance with previous findings (Solomon et al. 2012; Argaw and Muleta 2017).

The drought in 2015 caused major crop yield reduction in a large part of Ethiopia (AKLDP 2015; Philip et al. 2018). Likewise, in the first year of the field study, the plant growth was affected by drought stress. As a result, the mean nodule number, the biomass and yields of the pulses were significantly lower compared to the corresponding results in the second year, which was normal. According to Lodeiro et al. (2000), inoculated beans showed better tolerance and survival status as well as delayed leaf senescence during water deficits than bean provided with sufficient levels of synthetic N fertilizer. A finding in Egypt by Yanni et al. (2016) indicated that the symbiotic performance and productivity of beans inoculated with stress tolerant native rhizobia improved in moisture stressed field. In our study, beans and soybeans inoculated with elite rhizobial strains generally showed better tolerance to drought than plants receiving synthetic N, which was expressed as delayed leaf senescence of the plants in the field. Access of synthetic N fertilizer by the legumes and the supply of nutrients via mineralization could be decreased due to the lack of enough moisture during drought. Consequently, plant growth and production would be affected negatively (Sardans et al. 2008). On other hand, the

drought tolerance of pulses that obtain their N source via BNF can be enhanced by rhizobia inoculation.

The higher nodule number obtained due to inoculation, regardless of the sites and years, over the non-inoculated controls and plots receiving synthetic N fertilizer, suggests that the introduced inoculants were more competent than the background soil rhizobia to make nodules in symbiosis with the host legumes. On the other hand, this result also suggests that the population of rhizobia nodulating pulses occur in lower numbers in the field sites.

Previous reports (Asad et al. 2004; Fatima et al. 2007; Assefa et al. 2017) indicate that seeds per pod, pods per plant, plant height, and hundred seed weight of beans increased as a result of rhizobia inoculation. According to other studies (Fageria and Santos 2008; Mulas et al. 2015), these parameters were varied more depending on bean varieties and/or environmental factors rather than by inoculation and synthetic N application. In our study, overall the parameters were improved by rhizobial inoculation over the non-inoculated control or varied between treatments depending on locations and years or by their interaction (Table 3 and 4).

A study in Ethiopia by Samago et al. (2017) described a success story of rhizobia inoculation, by which symbiotic performances and grain yield of beans improved. According to Abebe (2017), the yield and yield components of beans were significantly increased due to rhizobia inoculation. In our study, inoculation in general improved nodule numbers, and biomass and grain yields of the pulse compared to the non-inoculated controls. However, there was a disparity in biomass and grain yields of inoculated pulse depending on the strains and the field sites (Table 3). This could be attributed to the differences in adaptability between strains to different local conditions. The Boricha site is characterized by lower soil pH (6.4) compared to the conditions at Halaba site (pH 8). Strain HAMBI3570 seems to be better adapted to the slightly acidic soils of the Boricha site, whereas strain HAMBI3562 performed better at the Halaba site with the slightly alkaline condition (Table 2). Both in 2015 and 2016, beans inoculated with strains HAMBI3562 and HAMBI3570 at Halaba and Boricha respectively gave

the highest or equivalent biomass and grain yields compared to the other inoculants and synthetic N fertilizer. Thus, our result supports the need for location specific rhizobial inoculation, as was previously suggested by Argaw and Muleta (2017), based on their research conducted in eastern Ethiopia.

Soybean cultivation is relatively new in Ethiopia. Consequently, rhizobia nodulating soybean are not commonly isolated from the soils of Ethiopia, except in a few successful studies (Aserse et al. 2012a; Muleta et al. 2017). Therefore, exotic and commercially available soybean-nodulating strains were tested in the field as inoculants and compared with native strains (Merkeb et al. 2016; Muleta et al. 2017). According to these studies, soybean yields were variable depending on the experimental locations. For example, significant increases in soybean yield components because of inoculation with strain TAL379 was reported by Solomon et al. (2012). On the contrary, in another investigation, soybean yield could not be improved by inoculation with this inoculant (Yoseph and Worku 2014). In our study, depending on the sites or growing seasons soybeans inoculated with the reference strains HAMBI3524 (obtained as TAL379), the commercial inoculant LF and the native HAMBI520, generally produced comparable or higher biomass and grain yields than soybean that received N fertilizer. However, inoculation with Ethiopian native strain HAMBI3513 gave consistently higher or comparable soybean biomass and grain yields than the plots received synthetic N fertilizer regardless of the locations and the test years.

Conclusion

Rhizobial inoculation improved plant drought tolerance, growth, and biomass and grain yield of beans as well as soybeans. This suggests that the rhizobial strains are adapted to the local environmental conditions to form effective N₂-fixing symbiosis with the respective hosts. The results also show the benefit of rhizobia inoculation as an alternative to synthetic N application for mitigating the soil fertility problems, contributing to the opportunities for smallholder farmers to sustainably produce nutritious and protein-rich food. Even though soybean biomass

and grain yields increased by inoculation with both the native and reference exotic rhizobial strains, Ethiopian strain HAMBI3513 was the most consistent. Therefore, the local strain HAMBI3513 for soybeans and Ethiopian strains HAMBI3562 and HAMBI3570 for beans, could be selected as inoculants for areas having similar soil properties and climatic conditions as the sites used in our study.

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Disclosure statement

No conflict of interest.

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548

549 Table 1. Shoot dry weight (SDW), and C and N contents (means of three replicates $\pm$ SE) of
550 inoculated common bean and soybean plants grown in the greenhouse

HAMBI code	Species	SDW(g)/ plant	N(g)/ plant	C(g)/ plant
Common bean plant				
HAMBI3562*	<i>Rhizobium phaseoli</i> HBR10	19.3 $\pm$ 1a	0.47 $\pm$ 0.01a	7.41 $\pm$ 0.4a
HAMBI3570*	<i>R. phaseoli</i> HBR53	17.9 $\pm$ 2a	0.46 $\pm$ 0.01a	6.66 $\pm$ 0.8a
HAMBI3556*	<i>R. etli</i> HBR5	18.3 $\pm$ 1a	0.45 $\pm$ 0.01a	7.00 $\pm$ 0.4a
HAMBI3573	<i>R. phaseoli</i> HBR79	17.90 $\pm$ 0.2a	0.45 $\pm$ 0.02a	6.84 $\pm$ 0.1a
HAMBI3565	<i>R. leguminosarum</i> HBR42	19.0 $\pm$ 0.4a	0.44 $\pm$ 0.01a	7.25 $\pm$ 0.2a
HAMBI3542	<i>R. phaseoli</i> HBR1	16.2 $\pm$ 1ab	0.42 $\pm$ 0.02a	6.26 $\pm$ 0.3a
HAMBI3547	<i>R. phaseoli</i> HRB18	15.5 $\pm$ 1.1b	0.36 $\pm$ 0.03ab	5.93 $\pm$ 0.4a
HAMBI3560	<i>R. etli</i> HBR24	15.2 $\pm$ 1.6b	0.33 $\pm$ 0.04b	5.73 $\pm$ 0.6a
HAMBI3557	<i>R. leucaenae</i> HBR12	14.1 $\pm$ 1b	0.33 $\pm$ 0.02b	5.31 $\pm$ 0.3ab
HAMBI3559	<i>R. etli</i> HBR23	18.1 $\pm$ 1.4a	0.31 $\pm$ 0.03b	6.87 $\pm$ 0.8a
HAMBI3563	<i>R. etli</i> HBR31	15.4 $\pm$ 1.4b	0.27 $\pm$ 0.02bc	5.77 $\pm$ 0.5ab
HAMBI3543	<i>R. etli</i> HBR3	13.7 $\pm$ 0.8bc	0.26 $\pm$ 0.02bc	5.11 $\pm$ 0.3ab
HAMBI3550	<i>R. etli</i> HBR26	15.6 $\pm$ 1.4b	0.23 $\pm$ 0.02c	5.89 $\pm$ 0.5a
HAMBI3558	<i>R. leguminosarum</i> HBR22	14.2 $\pm$ 0.8b	0.22 $\pm$ 0.01c	5.35 $\pm$ 0.3ab
HAMBI3553	<i>R. etli</i> HBR19	10.6 $\pm$ 1.1c	0.19 $\pm$ 0.02c	3.95 $\pm$ 0.4b
Non-inoculated control beans		9.0 $\pm$ 1.2cd	0.18 $\pm$ 0.02cd	3.48 $\pm$ 0.4b
HAMBI3549	<i>R. giardinii</i> HBR21	8.3 $\pm$ 1cd	0.11 $\pm$ 0.01cd	3.02 $\pm$ 0.4b
p-values	Treatments	0.00	0.00	0.00
Variance		13.05	0.014	1.985
Soybean plant				
HAMBI3524*	<i>Bradyrhizobium japonicum</i> TAL379	16.7 $\pm$ 0.6a	0.42 $\pm$ 0.02a	6.82 $\pm$ 0.2a
HAMBI3513*	<i>B. elkanii</i> SBR2B	17.2 $\pm$ 0.2a	0.36 $\pm$ 0.01b	6.97 $\pm$ 0.1a
HAMBI3520*	<i>B. elkanii</i> SBR8B	17.5 $\pm$ 0.2a	0.35 $\pm$ 0.01b	7.10 $\pm$ 0.1a
HAMBI3516	<i>Bradyrhizobium</i> sp. SBR6	15.5 $\pm$ 1.3ab	0.34 $\pm$ 0.03b	6.26 $\pm$ 0.6ab
HAMBI3515	<i>B. elkanii</i> SBR5B	16.1 $\pm$ 0.2ab	0.33 $\pm$ 0.01b	6.51 $\pm$ 0.1ab
HAMBI3517	<i>B. elkanii</i> SBR7A	16.3 $\pm$ 0.6ab	0.32 $\pm$ 0.01b	6.58 $\pm$ 0.2ab
HAMBI3525	<i>B. genosp.</i> SA2 strain SBR1B	16.2 $\pm$ 0.6ab	0.25 $\pm$ 0.01c	6.15 $\pm$ 0.2ab
HAMBI3518	<i>B. elkanii</i> SBR7B	16.1 $\pm$ 0.6ab	0.16 $\pm$ 0.01d	5.46 $\pm$ 0.2b
Non-inoculated control soybeans		14.5 $\pm$ 0.3b	0.14 $\pm$ 0.00d	5.28 $\pm$ 0.1b
p-values	Treatments	0.05	0.02	0.06
Variance		1.51	0.01	0.26

*Top three strains selected for field trials.

Means with the same letter in the same column show not significant difference between treatments at $p \leq 0.05$ by the least significant differences (LSD) test.

Table 2. Soil properties of the experimental sites

	Clay	Silt	Sand	Soil type	C tot	N tot	pH (H ₂ O)	EC	Ca	K	Mg	Na	TA	CEC _{pot}	BS	P Olsen
	%	%	%		%	%		$\mu\text{S cm}^{-1}$				$\text{cmol}_c \text{ kg}^{-1}$			%	mg kg^{-1}
Halaba soybean topsoil	23	44	33	Loam	1.5	0.12	7.9	219	17.9	2.5	2.2	0.4	0	23	100	1.5
Halaba soybean subsoil					1.3	0.09	7.9	257	22.2	2.5	2.8	2.0	0	29.5	100	
Halaba common bean topsoil	21	45	34	Loam	1.4	0.10	8.1	217	17.8	2.5	2.3	0.2	0	22.7	100	2.0
Halaba common bean subsoil					1.1	0.07	7.7	137	12.2	3.0	3.3	0.8	0.9	20.2	96	
Borincha topsoil	26	45	29	Loam	1.8	0.13	6.4	126	13.0	1.1	1.8	0.1	3.0	19.0	84	5.5
Borincha subsoil					1.1	0.07	6.9	69	10.2	0.8	2.4	0.3	2.1	15.8	87	

EC: Electrical conductivity of soil extract (1:2.5), TA: Titratable acidity, BS: Base saturation

Table 3. Effect of rhizobia inoculation on biomass yield and yield parameters (means of three replicates $\pm$ SE) of common beans in the field conditions.

Year	Site	Treatment	Biomass yield (kg ha ⁻¹)	Grain yield (kg ha ⁻¹)	Nodule number	Plant height (cm)	Seed/pod	Pod/plant	Hundred seed weight (g)
2015	BORICHA	EAL428	760 ± 23c	476 ± 58c	14 ± 3a	39 ± 2a	4 ± 0.3a	10 ± 0.3b	24 ± 3a
		HAMBI3562	870 ± 358bc	537 ± 3bc	17 ± 3a	39 ± 2a	4 ± 0.3a	14 ± 3a	26 ± 2a
		HAMBI3570	1240 ± 788a	640 ± 35ab	14 ± 2a	38 ± 3a	4 ± 1a	9 ± 1b	24 ± 2a
		HAMBI3556	927 ± 15bc	510 ± 29bc	13 ± 2a	37 ± 1a	5 ± 0.3a	15 ± 0.2a	26 ± 1a
		NF	1137 ± 199ab	683 ± 72a	8 ± 1b	35 ± 5a	4 ± 1a	10 ± 3b	25 ± 2a
		NoNF	340 ± 0d	140 ± 0d	8 ± 2b	32 ± 2a	4 ± 1a	8 ± 2b	19 ± 1b
	HALABA	EAL428	1587 ± 143bc	810 ± 69cd	36 ± 11b	37 ± 1a	4 ± 0.3a	7 ± 1a	24 ± 1a
		HAMBI3562	3013 ± 355a	1500 ± 81a	61 ± 21ab	35 ± 3a	4 ± 0.3a	9 ± 2a	26 ± 1a
		HAMBI3570	1910 ± 46bc	1013 ± 32bc	80 ± 1a	35 ± 2a	4 ± 0.1a	10 ± 2a	24 ± 1a
		HAMBI3556	1546 ± 9bc	803 ± 113cd	59 ± 5ab	36 ± 5a	3 ± 0.1a	9 ± 1a	26 ± 2a
		NF	2020 ± 69b	1117 ± 61b	27 ± 2b	39 ± 3a	4 ± 0.3a	12 ± 1a	25 ± 0a
		NoNF	1423 ± 87c	653 ± 22d	23 ± 3b	29 ± 1b	3 ± 0.4a	8 ± 2a	20 ± 2b
2016	BORICHA	EAL428	6523 ± 416ab	3483 ± 665b	29 ± 5ab	38 ± 2a	5 ± 1a	17 ± 0.3a	24 ± 1a
		HAMBI3562	6517 ± 546ab	4026 ± 507a	42 ± 4a	54 ± 3a	5 ± 1.5a	19 ± 0.2a	24 ± 1a
		HAMBI3570	6750 ± 0a	4250 ± 289a	23 ± 5ab	34 ± 3a	7 ± 1a	16 ± 1a	24 ± 1a
		HAMBI3556	6292 ± 109ab	4080 ± 72a	28 ± 9ab	35 ± 1a	5 ± 2a	18 ± 0.3a	24 ± 3a
		NF	6917 ± 546a	4333 ± 464a	16 ± 4b	35 ± 3a	4 ± 1a	23 ± 0.4a	26 ± 0a
		NoNF	5093 ± 535c	3570 ± 101b	13 ± 3b	28 ± 3b	4 ± 2a	18 ± 0.2a	23 ± 2a
	HALABA	EAL428	6273 ± 1040ab	4141 ± 1000a	31 ± 6ab	47 ± 9ab	5 ± 0.2a	11 ± 3.2a	24 ± 1a
		HAMBI3562	7022 ± 130a	4360 ± 81a	41 ± 4a	54 ± 11a	5 ± 1.1a	10 ± 9a	25 ± 2a
		HAMBI3570	5210 ± 644b	3296 ± 637ab	26 ± 7b	34 ± 2b	6 ± 2a	9 ± 4a	24 ± 1a
		HAMBI3556	6050 ± 635ab	4608 ± 160a	37 ± 14ab	35 ± 3b	5 ± 1a	10 ± 3a	25 ± 1a
		NF	7175 ± 812a	5064 ± 1569a	22 ± 3b	45 ± 1ab	4 ± 1a	13 ± 13a	24 ± 0a
		NoNF	5243 ± 1007b	2793 ± 660b	18 ± 3b	37 ± 5b	5 ± 2a	10 ± 2.4a	23 ± 1a
p-values	Location	0.08	0.26	0*	0.06	0*	0*	0.98	
	Treatment	0.03*	0.36	0*	0.01*	0*	0.01*	0.01*	
	year	0*	0*	0.31	0.15	0*	0*	0.15	
	Location × Treatment	0.36	0.84	0.11	0.58	0.35	0.02*	0.8	
	Year × Location	0.01*	0.49	0*	0.01*	0*	0*	0.17	
	Year × Treatment	0.63	0.85	0.1	0.47	0*	0.11	0.2	
	Year × Location × Treatment	0.94	0.87	0.09	0.45	0.67	0.02*	0.77	
	Variance	7127704.813	3760474.637	414.089	58.443	37.698	545.668		

* The mean difference is significant at $p \leq 0.05$ level. Means with the same letter show not significant difference between treatments at $p \leq 0.05$, when it is calculated for each site and for each year separately and the different letters indicate presence of significant differences.

SE, standard error; NF, nitrogen fertilizer; NoNF, no nitrogen fertilizer and rhizobia application, non-inoculated control.

Table 4. Effect of rhizobia inoculation on biomass, yield and yield parameters (means of three replicates $\pm$ SE) of soybean in the field conditions.

Year	Site	Treatment	Biomass Yield (kg ha ⁻¹)	Grain Yield (kg ha ⁻¹)	Nodule number	Plant height (cm)	Seed/ pod	Pod/ plant	Hundred seeds weight (g)
2015	BORICHA	LF	1397 $\pm$ 26a	487 $\pm$ 38ab	5 $\pm$ 1bc	34 $\pm$ 5a	2 $\pm$ 0.2a	27 $\pm$ 10b	12 $\pm$ 1a
		HAMBI3513	1260 $\pm$ 17a	427 $\pm$ 20ab	11 $\pm$ 1a	34 $\pm$ 7a	3 $\pm$ 0.2a	27 $\pm$ 11b	15 $\pm$ 4a
		HAMBI3520	853 $\pm$ 66c	347 $\pm$ 18c	13 $\pm$ 5a	30 $\pm$ 2a	2 $\pm$ 0.1a	18 $\pm$ 5b	15 $\pm$ 2a
		HAMBI3524	1393 $\pm$ 43a	507 $\pm$ 23a	6 $\pm$ 0bc	38 $\pm$ 6a	2 $\pm$ 0.2a	34 $\pm$ 5a	13 $\pm$ 1a
		NF	1090 $\pm$ 55b	370 $\pm$ 10c	1 $\pm$ 0c	35 $\pm$ 3a	2 $\pm$ 0.1a	23 $\pm$ 9b	18 $\pm$ 5a
		NoNF	860 $\pm$ 40c	323 $\pm$ 3c	1 $\pm$ 0c	31 $\pm$ 4a	2 $\pm$ 0.2a	27 $\pm$ 4b	13 $\pm$ 2a
	HALABA	LF	1767 $\pm$ 88b	630 $\pm$ 139a	18 $\pm$ 8bc	32 $\pm$ 1a	2 $\pm$ 0a	28 $\pm$ 6a	16 $\pm$ 0.2a
		HAMBI3513	2600 $\pm$ 57a	617 $\pm$ 16a	75 $\pm$ 4a	32 $\pm$ 1a	3 $\pm$ 0.1a	37 $\pm$ 3a	15 $\pm$ 1a
		HAMBI3520	2100 $\pm$ 208ab	643 $\pm$ 142a	42 $\pm$ 7ab	38 $\pm$ 3a	2 $\pm$ 1a	48 $\pm$ 5a	17 $\pm$ 1a
		HAMBI3524	1777 $\pm$ 412b	533 $\pm$ 110ab	15 $\pm$ 5bc	38 $\pm$ 1a	2 $\pm$ 0.2a	46 $\pm$ 6a	16 $\pm$ 1a
		NF	1933 $\pm$ 145ab	613 $\pm$ 81a	6 $\pm$ 3c	34 $\pm$ 4a	2 $\pm$ 0.1a	43 $\pm$ 12a	17 $\pm$ 1a
		NoNF	1373 $\pm$ 27b	417 $\pm$ 34b	2 $\pm$ 0c	32 $\pm$ 2a	2 $\pm$ 0.2a	40 $\pm$ 6a	15 $\pm$ 0.4a
2016	BORICHA	LF	7778 $\pm$ 55b	3611 $\pm$ 73b	8 $\pm$ 2bc	42 $\pm$ 2a	2 $\pm$ 1a	81 $\pm$ 18a	12 $\pm$ 1a
		HAMBI3513	12222 $\pm$ 147a	6222 $\pm$ 62a	32 $\pm$ 8a	40 $\pm$ 4a	3 $\pm$ 1a	67 $\pm$ 13abc	15 $\pm$ 3a
		HAMBI3520	12778 $\pm$ 111a	6611 $\pm$ 56a	37 $\pm$ 4a	38 $\pm$ 7a	2 $\pm$ 0.3a	92 $\pm$ 2a	15 $\pm$ 2a
		HAMBI3524	12222 $\pm$ 241a	5667 $\pm$ 117ab	25 $\pm$ 6ab	40 $\pm$ 1a	3 $\pm$ 0.1a	60 $\pm$ 2bc	13 $\pm$ 1a
		NF	9999 $\pm$ 96ab	5278 $\pm$ 100ab	1 $\pm$ 0c	41 $\pm$ 2a	2 $\pm$ 0.3a	71 $\pm$ 10ab	18 $\pm$ 5a
		NoNF	8056 $\pm$ 73b	3444 $\pm$ 55b	1 $\pm$ 1c	38 $\pm$ 4a	3 $\pm$ 1a	48 $\pm$ 7c	13 $\pm$ 1a
	HALABA	LF	13426 $\pm$ 197a	4525 $\pm$ 241a	52 $\pm$ 18ab	43 $\pm$ 4a	3 $\pm$ 0.2a	14 $\pm$ 1a	13 $\pm$ 0.2a
		HAMBI3513	13951 $\pm$ 41a	4966 $\pm$ 47a	86 $\pm$ 26a	38 $\pm$ 1a	3 $\pm$ 0.3a	16 $\pm$ 2a	14 $\pm$ 1a
		HAMBI3520	12680 $\pm$ 113ab	3094 $\pm$ 563b	56 $\pm$ 7ab	41 $\pm$ 3a	3 $\pm$ 0.2a	17 $\pm$ 2a	13 $\pm$ 0.4a
		HAMBI3524	13782 $\pm$ 54a	4574 $\pm$ 78a	84 $\pm$ 17a	38 $\pm$ 2a	3 $\pm$ 0.3a	15 $\pm$ 1a	15 $\pm$ 1a
		NF	12738 $\pm$ 487ab	3383 $\pm$ 494b	1 $\pm$ 0c	42 $\pm$ 1a	3 $\pm$ 0.1a	16 $\pm$ 2a	15 $\pm$ 0.3a
		NoNF	10450 $\pm$ 1582b	3094 $\pm$ 281b	1 $\pm$ 0c	41 $\pm$ 2a	3 $\pm$ 0.2a	19 $\pm$ 3a	13 $\pm$ 1a
p-values	Location		0.00*	0.00*	0.00*	0.51	0.01*	0.00*	0.69
	Treatment		0.002*	0.03*	0.00*	0.82	0.18	0.55	0.07
	year		0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.08
	Location $\times$ Treatment		0.199	0.04*	0.01*	0.61	0.73	0.29	0.2
	Year $\times$ Location		0.01*	0.00*	0.35	0.98	0.45	0.00*	0.08
	Year $\times$ Treatment		0.04*	0.07	0.11	0.56	0.85	0.12	0.99
	Year $\times$ Location $\times$ Treatment		0.05*	0.02*	0.4	0.94	0.62	0.09	0.99
	Variance		30255325.13	5389561.33	1065.26	35.43	0.57	593.5	9.16

*The mean difference is significant at $p \leq 0.05$ level. Means with the same letter show not significant difference between treatments at $p \leq 0.05$, when it is calculated for each site and for each year separately and the different letters indicate presence of significant differences.

SE, standard error; NF, nitrogen fertilizer; NoNF, no nitrogen fertilizer and rhizobia application, non-inoculated control.