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Growth, symbiotic and yield response of N-fertilized and *Rhizobium* inoculated common bean (*Phaseolus vulgaris* L.)

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Common bean (*Phaseolus vulgaris* L.) is one of the most important and widely cultivated pulse crops in most developing countries. However, its cultivation is globally constrained mainly by low soil fertility and lack of improved agronomic practices. A field experiment was conducted at Hawassa University College of Agriculture, Hawassa Southern Ethiopia to determine the effect of N fertilization and *Rhizobium* phaseoli strain HB-429 inoculation on growth, nodulation, yield and yield components of common bean variety Hawassa Dume. The experiment was laid out as a randomized complete block design with three replications. Results showed significant increase in growth, nodulation, yield and yield components in plants inoculated with *Rhizobium* strain HB-429 over the control. Thus, from the results of this study, it can be concluded that *Rhizobium* inoculation with strain HB-429 is the best performing treatment to be recommended for profitable grain yield of common bean at the Hawassa and other similar areas.

Key words: Common bean, grain yield, nitrogen, nodulation, *Rhizobium* inoculation.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a major grain legume grown and consumed in sub Saharan Africa, including Ethiopia. It is the most important food legume, fodder and cover crop (Gidago et al., 2012). It matures early, has wider ecological adaptation and broad range of local genetic diversity (Fikru, 2007). Nutritionally, common bean grains are rich in protein, carbohydrates, oil, fiber and sucrose (Gebre-Egziabher et al., 2014; Tekle et al., 2014). Its green leaves and young pods also contain high levels of essential nutrients (USAID, 2012; Rocha-Guzman et al., 2007). Thus, inclusion of common bean in the daily diet has several health benefits such as reduction of cholesterol level (Rosa et al., 1998), reduction of coronary heart diseases, favourable effects against cancer (Oomah et al., 2005), decreasing diabetes and obesity and high antioxidant capacity (Mitchell et al., 2009).

Common bean has a potential of 5 t/ha grain yield (Graham and Ranalli, 1997). However, in most African countries average yield of this crop is often less than 1.0

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t/ha. For example, average yield ranges were: 0.14 to 0.77 t/ha in Kenya (Kapkiyai et al., 1998), 0.6 to 0.8 t/ha in Uganda (Kalyebara, 2008), and 0.5 to 0.8 t/ha in Ethiopia (EPPA, 2004). This implies that farm yield of common bean in Africa ranges far below its potential. This situation has been attributed to low soil fertility, poor agronomic practices, and biotic and abiotic stress during the growth of the plant (Polania et al., 2016). To alleviate these constraints and exploit the genetic yield potential of common bean more effort is needed among others, assessing them under different agronomic practices. As a result the yield potential of common bean has been raised and the risk associated with the production of crop has been minimized, leading to increased grain yield per unit area, thus improving food security among small holder farmers.

Inoculation with effective *Rhizobium* strains substantially increases the nitrogen fixing potential and yields of legumes, including common bean. However, farmers have a wrong notion that common bean, being a legume crop, does not need any nutrition and usually grow it on marginal land without applying any fertilizer. This seems to be an important reason for its low seed yield in Ethiopia. This constraint could be alleviated through seed and/or soil inoculation with the proper *Rhizobium* bacteria before or at planting to facilitate N-fixation (Ndakidemi et al., 2006). Therefore, to increase the productivity of the farmers, it is crucial to increase the awareness of farmers towards the utilization of improved agronomic practices that increase their production and accelerate food security through proper implementation. To this end, a field study was initiated with the objective of assessing growth, symbiotic and yield response of N-fertilized and *Rhizobium* inoculated common bean.

MATERIALS AND METHODS

Description of experimental site

The experiment was conducted in Hawassa University, College of Agriculture which is located at 273 km south west of capital Addis Ababa in South Nation Nationalities and People Regional State. The site is located 7° 4'N latitude and 38° 31'E longitude and an altitude of 1969 m. The average rainfall of the area is 800 to 1100 mm annually. The average annual maximum, minimum and mean temperature of the area is 27, 12, and 20°C, respectively. The main rainy season extends from April to September and it is interrupted by some dry sun shine and sometimes from May to July (NMA, 2015).

Source of planting material and experimental design

Seeds of common bean (*P. vulgaris* L.) variety Hawassa Dume were obtained from Hawassa Agricultural Research Centre. This variety was purposefully chosen based on its adaptation, high grain yield, acceptability by farmers and seed availability. Seeds were planted at the beginning of May 2016 in a complete randomized block design with three treatments and three replicates. Treatments were inoculation of bean with HB-429, addition of 23 kg N/ha, and an untreated control.

The total number of plots was 9 on each plot, there were 4 rows. In each plot, seeds were planted (two seeds per hole) with 10 cm between plants and 40 cm between row spacing. A total number of plants per experiment were 360 and 40 plants on each plot. Before sowing, seeds were pre-inoculated with a peat-based *Rhizobium* inoculant strain HB-429 in the shade and inoculated seeds were allowed to air dry for few minutes before planting. This *Rhizobium phaseoli* strain HB-429 was previously proven to enhance the symbiotic performance and yield of common bean under field condition, and it is considered an elite strain for common bean cultivation in Ethiopia (Tarekegn, 2012). After sowing, seeds were covered with soil to avoid desiccation. For the N-treatment, urea (23 kg N/ha) was applied by hand to designated plots at planting and 4 weeks after sowing. Weeds within plots were removed manually two weeks after seedling emergency and three weeks later. To avoid cross contamination, weeding was done in the un-inoculated plots first. Field management like watering, cultivation, weeding and others were carried out as recommendation.

Soil sampling and analysis

Before planting, soil samples were randomly taken from the experimental field at a depth of 0 to 30 cm using an auger and the samples were mixed thoroughly to produce one representative composite sample of 1 kg. The soil sample was air-dried and ground to pass 2 and 0.5 mm (for total N) sieves and analyzed for total N, available P, pH, organic carbon (OC), exchangeable cations and physical properties at Hawassa University College of Agriculture Soil Laboratory. Soil analysis was made as per the standard laboratory procedure (Sahlemedhin and Taye, 2000). The soil pH was measured in the supernatant suspension of a 1: 2.5 soil to water ratio using a standard glass electrode pH meter (Rhoades, 1982). The Walkley and Black (1934) method was used to determine the organic carbon (%). Total N was determined using Kjledahl method as described by Bremner and Mulvaney (1982). Available P (mg/kg) was determined by Olsen et al. (1954) method using ascorbic acid as the reducing agent. The cation exchange capacity (CEC) in cmol (+) kg^{-1} was measured using 1 M-neutral ammonium acetate method (Jackson, 1967). The soil-particle size distribution was determined using the Bouyoucos hydrometer method (Bouyoucos, 1962).

Data collection

Plant height

Five plants from the central rows of each plot were randomly selected for measuring plant height. Then the average values of these plants were recorded as plant height of the crop.

Shoot dry matter

Shoots dry matter was determined at early pod setting stage from plants that were sampled for plant height and nodulation. The plant samples were placed in labeled perforated paper bags and oven dried at 70°C for 48 h until a constant shoot dry matter.

Nodule number/plant:

Nodulation assessment was undertaken at mid (50%) flowering stage by carefully uprooting five plants randomly from each plot. The plants were separated into shoot and roots. The adhering soil was carefully washed from the roots over a metal sieve. The nodules from each plant were picked and spread on the sieve to

Table 1. Effect of N fertilization and *Rhizobium* inoculation on plant height, shoot dry weight, nodule number and nodule dry weight of common bean.

Treatments	Plant height (cm)	Shoot dry weight (g)	Nodule number	Nodule dry weight (g)
Control	37.5[b]	20.3[b]	36.6[c]	0.37[b]
Strain HB-429	41.2[a]	31.3[a]	47.6[a]	0.60[a]
23 kg N/ha	40.8[a]	26.1[a]	41.0[b]	0.46[b]
Significance level	*	*	**	*
CV (%)	2.9	9.7	4.6	11.0
LSD	2.7	5.7	4.4	0.1

Means with the same letter(s) within a column are not significantly different at $p< 0.05$.

drain water from their surface. Nodules were counted and their average was taken for plots as nodule number/plant. Then after, the nodules were oven-dried at 70°C for 48 h for nodule dry weight determination.

Yield and yield components:

At harvesting time for the determination of yield and yield components such as number of pods/plant, number of seeds/pod, hundred seed weight and grain yield, ten randomly picked plants were used. Seed weight was determined by randomly taking 100 seeds of the ten sample plants and weighing it with sensitive balance after oven drying to constant weight.

Statistical analysis

The obtained data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) of the Statistical Analysis System (SAS, 2002) version 9.0. Mean separation was done using Least Significant Difference (LSD) test at 5% probability level. Uninoculated plants and plants grown in non-N amended soil served as control.

RESULTS AND DISCUSSION

Soil physico-chemical properties of the study area

Analysis of the top soil (0 to 30 cm) before the application of treatments revealed that the soil at the experimental site is clay-loam in texture, slightly acidic in reaction with a pH value of 6.1, which is in the optimum range for common bean production (Havlin et al., 1999). The soil has low total N (1.0 mg/g) and organic carbon content (6.1 mg/g) as per the limit set by Tekalign (1991). Extractable available P (6.90 mg/kg) was in the range of medium, and the cation exchange capacity (CEC) was 23.6 cmol/kg and could be rated as medium and satisfactory for agricultural crops with the use of agronomic practices (Landon, 1991). The exchangeable cations K 3.5 cmol/kg, Ca 5.8 cmol/kg and Mg 2.9 cmol/kg were within the range of medium to high (FAO, 2006).

Effect of N fertilization and *Rhizobium* inoculation on plant growth and nodulation

Plant height

N fertilization and *Rhizobium* inoculation had significant ($P\leq 0.05$) effect on plant height (Table 1). The shortest plant height (37.5 cm) was recorded from the control, which was significantly lower to other treatments. Both the *Rhizobium* inoculated and 23 kg N/ha applied plants performed similarly on plant height. The reasons for increase in plant height under inoculation and N fertilizer could be due to the increased vegetative growth with applied N and nitrogen fixation. This result is in line with the report of Kubota et al. (2008) who stated that plant height of soybean was increase with N in the presence of *Rhizobium* inoculants. Other authors also reported similar results from researches conducted on chickpea and faba bean, El-Wakeil and El-Sabai (2007) indicating that *Rhizobium* inoculation significantly increased plant height.

Shoot dry weight

N fertilization and *Rhizobium* inoculation had significant effect on shoot dry matter accumulation of common bean compared to the control (Table 1). Inoculation with strain HB-429 gave relatively higher shoot dry weight that was greater by 102.3% over the control. However, statistically significant variation on shoot dry weight was not detected between the strains HB-429 and 23 kg N/ha. The observed benefits on bean by *Rhizobium* inoculation seem to be to the supply of N to the crop through symbiotic N_2-fixation (Togay et al., 2008). Similar to this result, the research outcomes of Bhuiyan et al. (2008) showed that the highest dry matter accumulation on mung bean was obtained from inoculation with *Rhizobium*. Sharma et al. (2000) reported the significant effect of seed inoculation on dry weight biomass compared to the control treatments.

Table 2. Effect of N fertilization and *Rhizobium* inoculation on yield and yield components of common bean.

Treatments	Number of pods/plant	Number of seeds/pod	Hundred seed weight (g)	Grain yield (t/ha)
Control	9.7[c]	4.4[b]	31.5[b]	1.4[b]
Strain HB-429	14.2[a]	7.7[a]	42.7[a]	2.5[a]
23 kg N/ha	11.8[b]	6.7[a]	40.5[a]	2.4[a]
Significance level	**	*	**	*
CV (%)	5.9	14.2	3.8	16.7
LSD	1.6	2.0	3.3	0.8

Means with the same letter(s) within a column are not significantly different at p< 0.05.

Nodule number/plant

Nodule number/plant was significantly (P<0.01) affected by N fertilization and *Rhizobium* inoculation (Table 1). The maximum mean nodule number (47.6) was recorded from the strain HB-429 and, zero application of treatments result the minimum nodule number per plant, which was markedly lower than the effect of other treatments. The increased nodule number with *Rhizobium* inoculation could be associated with the efficiency of introduced rhizobia, to compete with indigenous bacteria dwelling in the soil. These results are in line with the findings of Sajid et al. (2011) who revealed that the *Rhizobium* inoculation significantly enhanced nodule number. Similarly, application of N fertilizer at a rate of 23 kg N/ha increased nodule number/plant when compared to the control. However, Kessel and Hartley (2000) observed a significant decrease in nodulation of several varieties of common beans following the application of 40 kg N/ha. The lower native total N (0.10 mg/g) contents observed on the surface soil of the experimental field may have positively affected crop response and increment in common bean nodule number under application of mineral N fertilizer. This is further supported by Omoregie and Okpefa (1999), who noted that when initial levels of available soil nitrogen were low, a period of nitrogen hunger can reduce nodulation.

Nodule dry weight/plant

The effects of N fertilization and *Rhizobium* inoculation on the nodule dry weight of common bean were found to be statistical significant (Table 1). Strain HB-429 had the highest nodule dry weight (0.60 g) while the control and 23 kg N/ha treatments had the lower nodule dry weight (0.37 and 0.46 g), respectively. Similar effects of seed inoculation on nodule dry weight have also been reported by Dereje (2007) and Bhuiyan et al. (2008) on soybean who stated that inoculation significantly increase nodule dry weight of legumes under field condition. According to Fatima et al. (2006) high nodule dry weight can be generally a prerequisite for increasing N_2-fixation in legumes rather than number of nodules.

Effect of N fertilization and Rhizobium inoculation on yield and yield components of common bean

Number of pod/plant

The analysis of variance revealed the number of pods/plant was significantly affected by N fertilization and *Rhizobium* inoculation (Table 2). The lowest number of pods/plant (9.7) was recorded from the control which was significantly lower to both *Rhizobium* strain HB-429 inoculated and N fertilizer applied treatments. The increased pod number with applied inoculants and N fertilizer could be associated with enhanced growth and higher assimilate accumulation which resulted from better N nourishment due to symbiotic N_2-fixation and applied N. The result is in agreement with the work of Malik et al. (2006) and Dereje (2007) who conclude that increased number of pods per plant with *Bradyrhizobium japonicum* inoculation in soybean.

Number of seeds/pod

The analysis of variance revealed that number of seeds/pod was significantly affected by N fertilization and *Rhizobium* inoculation (Table 2). The lowest number of seeds/pod (4.4) was recorded from the control which was significantly lower than both *Rhizobium* inoculation and N application. The magnitude of increase in number of seeds/pod over the control and application of 23 kg N/ha due to inoculation of strain HB-429 was 175 and 115%, respectively. The result shows that N fertilization and *Rhizobium* inoculation played an important role in common bean generative growth and therefore made a marked increase in the number of seeds/pod. The significant difference on number of seeds/pod among inoculation/N fertilizer and the control was in line with the finding of Muhammad (2002) who found that, seed inoculation increased the number of seeds/pod in addition to grain yield.

Hundred seed weight

Significant differences (P<0.01) were observed among N fertilization and *Rhizobium* inoculation for hundred seed weight (Table 2). Inoculation with strain HB-429 gave relatively heavier seed weight that was greater by 136% over the control. However, statistically significant variation in hundred seed weight was not detected between strain HB-429 and 23 kg N/ha. This result is in line with the finding of Ali et al. (2004) that states inoculation brought a significant effect on hundred seed weight of chickpea.

Grain yield

As observed in all yield parameters in the experiment, grain yield was also highly affected by N fertilization and *Rhizobium* inoculation (Table 2). The highest grain yield (2.5 t/ha) was recorded from plants inoculated with *Rhizobium* strain HB-429 and it was followed by 23 kg N/ha, which did not vary significantly between each other but significantly higher than the control. The lowest grain yield (1.4 t/ha) was recorded from the control and was significantly lower than the rest of the treatments. Yield increments of 179 and 171% were obtained from strains HB-429 and 23 kg N/ha, respectively as compared to the control. The significant increase in grain yield in response to *Rhizobium* stain HB-429 inoculation might be attributed to the increased availability of N in the soil for uptake by plant roots, through fixed N_2. The results coincide with the findings of Sajid et al. (2011), who concluded that the treatments with *Rhizobium* inoculation gave higher grain yield than those without inoculation. It may also be due to more number of pods and seeds due to *Rhizobium* inoculation and applied N. A similar increasing effect of *Rhizobium* inoculation on grain yield of soybean has also been reported by Abbasi et al. (2008). On the other hand, the increases in grain yield with N application support the finding of Nebret and Nigussie (2012) who reported that common bean crop supplied with 23 kg N/ha resulted in significantly more grain yield than the control.

Conclusion

Application of N fertilizer and *Rhizobium* inoculation resulted in significant improvement on plant height, shoot dry weight, nodule number and nodule dry weight. In this study, inoculation of common bean with strain HB-429 increased nodule number and nodule dry weight by 130 and 162% as compared to the control treatment. Thus, indicating the effectiveness of this strain in N_2-fixation. Similarly, for yield and yield components N fertilizer and *Rhizobium* inoculation showed significant effects on all parameters and strain HB-429 appeared to be more productive than all other treatments, though in some parameters it was not significantly different with 23 kg N/ha. For instance, inoculation of common bean with strain HB-429 increased number of pods/plant, number of seeds/pod and hundred seed weight by 146.3, 175.0 and 135.6% over the control, respectively. The significant differences in these test parameters had contributed to the superior grain yield performance of strain HB-429 (2.5 t/ha) compared to the control (1.4 t/ha). Generally, the result of this study indicated that treatments under fertilizer and *Rhizobium* inoculation gave the highest growth and yield related parameters of common bean plant. Thus, based on the finding, using effective *Rhizobium* strain was advisable to achieve optimum common bean yield under Hawassa condition.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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