

Combined use of *Rhizobium* inoculation and low phosphorus application increased plant growth, root nodulation and grain yield of common bean (*Phaseolus vulgaris*) in Ethiopia

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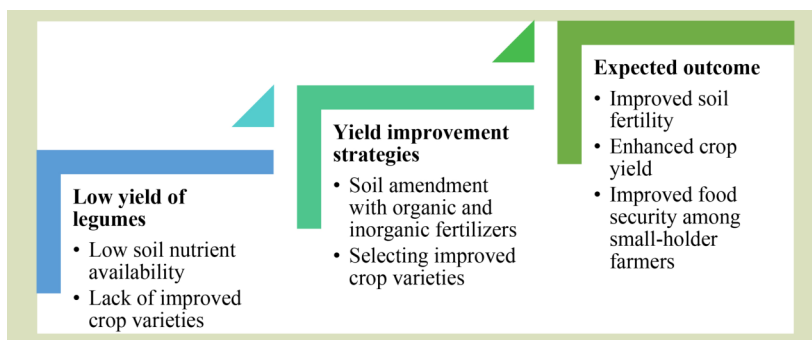
KEYWORDS

Bean cultivars, grain yield, Hawassa Dume, Ibbado, nodule dry matter, nodule number, pod number per plant, seed number per pod, shoot biomass

HIGHLIGHTS

- Two bean cultivars responded strongly to *Rhizobium* inoculation in both 2012 and 2013, relative to uninoculated control.
- Inoculating bean with strain HB-429 increased shoot biomass, nodule number, and nodule dry matter per plant.
- Rhizobial inoculation of bean increased pod number per plant, seed number per pod, and grain yield.
- Applying P to bean increased shoot biomass, nodule number, and nodule dry matter per plant.
- The combined use of *Rhizobium* inoculation and low P application is recommended for bean production in Ethiopia.

GRAPHICAL ABSTRACT



ABSTRACT

Bean (*Phaseolus vulgaris*) yields in Africa can be increased through the application of phosphorus and nitrogen fertilizers, as both nutrients are low in African soils. However, using greener technologies is preferred to mineral fertilizers for maintaining soil health. In this study, *Rhizobium* inoculation and moderate P supply (0, 10, 20, and 30 kg·ha⁻¹) to two bean cultivars were evaluated in consecutive years at Hawassa for their effects on plant growth, nodulation, and grain yield. The results showed that, relative to the uninoculated control, the two bean cultivars responded strongly to *Rhizobium* inoculation, with strain HB-429 outperforming strain GT-9 in both 2012 and 2013. Shoot biomass, nodule number and nodule dry matter per plant were increased by 9%, 40%, and 54%, respectively, in 2012, and by 20%, 39%, and 13% in 2013 with strain HB-429 inoculation. This resulted in increased pod number per plant, seed number per pod and grain yield by 56%, 51%, and 49% in 2012, and by 38%, 25%, and 69% in 2013, respectively, with strain HB-429 inoculation. Bean inoculation with GT-9 also increased grain yield by 35% and 68% in 2012 and 2013, respectively. Applying 10–30 kg·ha⁻¹ P to bean cultivars increased shoot biomass, nodule number, and nodule dry matter per plant by 7% to 39%, 23% to 59%, and 59% to 144% in 2012, respectively, and by 10% to 40%, 21% to 43%, and 12% to 35% in 2013, respectively. Relative to the zero-P

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control, adding only 10 kg·ha⁻¹ P increased pod number per plant, seed number per pod, and grain yield by 10%, 30%, and 61% in 2012, and by 11%, 11%, and 38% in 2013, respectively. The combined use of *Rhizobium* inoculation with low P application (20 kg·ha⁻¹) was found to increase bean production in Ethiopia and is thus recommended to resource-poor farmers.

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1 Introduction

Common bean (*Phaseolus vulgaris*) is one of the most important food legumes grown in developing countries including Ethiopia^[1,2]. However, grain yields are quite low due to low soil N and P, lack of improved cultivars and poor agronomic practices including zero-inputs of fertilizers and rhizobial inoculants^[3–8]. In Ethiopia, in particular, most smallholders do not use *Rhizobium* inoculants or apply mineral fertilizers at the recommended rates.

Legumes including common beans are known to establish symbiosis with soil rhizobia that convert atmospheric N₂ to NH₃ for their N nutrition^[9–13]. However, of the grain legumes, the common bean is one of the least efficient N₂-fixers contributing about 30–50 kg·ha⁻¹ N due mainly to the low effectiveness of the native soil rhizobia, as well as their low expression of the *nif* H gene when in symbiosis with some released cultivars^[14,15]. A few studies have shown that N₂ fixation can be enhanced by pre-inoculation of seeds with elite rhizobial strains, leading to greater plant growth and grain yield of common bean^[16–18], have emphasized the importance of selecting rhizobial strains suitable for common bean in low-P soils, as well as identifying P use efficient bean cultivars.

Although high P can inhibit plant growth and pollute the environment, low P availability is the single most important factor limiting the yield of common beans in Africa, especially Ethiopia. It has been reported that N₂ fixation is more affected by P deficiency in common beans than other grain legumes such as soybean, due to the adverse effects of low P on plant growth, nodule formation, and functioning^[19].

The fact that P uptake, transport, and redistribution are controlled genetically raises the possibility of breeding and/or selecting cultivars for high P use efficiency^[20]. Genotypic variation in symbiotic N₂ fixation relating to P use efficiency has been reported for common bean^[21]. Currently, all grain legumes, including common bean, grown in low-P soils in Ethiopia^[22] are known to respond positively to P supply in terms of number of pods per plant, number of seeds per pod,

100-seed weight and grain yield^[5,23–25]. However, the need to inoculate common bean with rhizobia in P-poor soils cannot be over-emphasized^[21,26,27].

This study, therefore, aimed to assess plant growth, root nodulation, and grain yield of common bean cultivars inoculated with *Rhizobium leguminosarum* bv. *phaseoli* strains HB-429 and GT-9, supplied with a range of P levels at Hawassa in Ethiopia.

2 Materials and methods

2.1 Site location and description

A field experiment was conducted at the research farm of Hawassa University, Hawassa, Ethiopia, over two consecutive years. The site is located at 7°3' N, 38°30' E, and 1844 m above sea level. The area received total annual rainfall of 985 and 1072 mm during the 2012 and 2013 cropping seasons, respectively. The average annual minimum and maximum temperatures are 13.4 and 19.5 °C, in 2012 and 2013, respectively (National Meteorological Agency, Hawassa).

2.2 Soil analysis

Topsoil (0–30 cm) collected from the experimental plots before the application of treatments was air-dried, sieved, and analyzed, and found to be clay-loam in texture, with a slightly acidic pH (H₂O 1:2.5), which is optimum for common bean production in Ethiopia^[28]. The soil had low organic carbon content and low to moderate total N as defined by Tadesse et al.^[29]. Extractable available P was medium in range, and the cation exchange capacity (CEC) was rated as medium to high, and satisfactory for agriculture^[30]. The exchangeable cations K, Ca, and Mg were medium to high^[31] (Table 1).

2.3 Treatments, experimental design and procedures

The treatments included two common bean cultivars (Hawassa

Table 1 Physiochemical characteristics of soil measured before planting at Hawassa experimental sites in the 2012 and 2013 cropping seasons

Year	Texture	pH	Organic C	Total N	Available P (mg·kg ⁻¹)	CEC	K	Ca	Mg
			(mg·g ⁻¹)						
2012	Clay loam	6.1	6.4	1.0	6.9	236	32	50	27
2013	Clay loam	6.1	6.4	1.4	8.0	262	47	98	27

Dume and Ibbado), seed inoculation with the *Rhizobium* (strains HB-429 and GT-9), and four P levels (0, 10, 20, and 30 kg·ha⁻¹) of P in the form of triple super phosphate in a split-split plot design with four replications. The P levels, *Rhizobium* inoculation, and bean cultivars were assigned to main-, sub-, and sub-sub treatments, respectively. The experiments were conducted in 2012 and 2013 on two adjacent fields. The crop was sown using 40 cm inter and 10 cm intra-row spacing on a gross sub-sub plot size of 2.4 m × 3.6 m (8.64 m²). P fertilizer was applied as a basal treatment at planting. Each plot had nine rows with 24 plants per row. The blocks were separated by a 1 m buffer, while the plots within a block were separated by 0.5 m.

The seeds of two common bean cultivars were obtained from the Hawassa Agricultural Research Centre, Hawassa, Ethiopia. These cultivars were chosen based on their demand, farmer interest, and seed availability. In addition, they are ranked among the top-yielding cultivars with great potential in Ethiopia. The two *Rhizobium* inoculants (HB-429 and GT-9) were obtained from the National Soil Research Laboratories, Microbiology Unit, Addis Ababa, Ethiopia and Soygro Pty Ltd., Potchefstroom, South Africa, respectively. These strains have been shown to promote growth, nodulation, and grain yield of common bean cultivars under a wide range of ecological conditions and are considered the best strains as far as the symbiotic and agronomic performance of common bean is concerned.

Seeds of the test common bean cultivars were inoculated with peat-based inoculants of *Rhizobium phaseoli* which contained an equivalent of 6.5×10^8 viable bacterial cells per gram powder and was applied according to the recommended rate of 10 g·kg⁻¹ of seed^[32]. Inoculants were applied to seeds in the shade to maintain the viability of the *Rhizobium* strains and inoculated seeds were allowed to dry for 5–7 min before planting. Two seeds were sown per hole to avoid cross-contamination; the uninoculated treatments were planted first. Soil ridges were made to prevent the possible movement of bacteria through rainwater between plots and blocks. Sown seeds were immediately covered with soil. After germination, weeding was done manually when necessary. Plant sampling

for growth, nodulation, and yield-related parameters.

Nodulation was assessed at the early pod-setting stage by carefully uprooting five plants randomly from each plot. The plants were separated into shoots, roots, and nodules. Soil adhering to the plant roots was removed by washing with tap water and detached nodules were picked by hand and washed. The nodules were counted to determine the nodule number per plant. The nodules and shoots were placed in labeled-weight paper bags and oven-dried at 70 °C for 48 h to a constant weight.

At physiological maturity, ten plants were harvested from the three central rows per plot, the number of pods per plant, number of seeds per pod and hundred seed weight were determined for each plot. After air drying to constant moisture content using a moisture meter (model HOH-EXPRESS HE 50, Jansen&Heuning, Duinkerkenstraat 11, 9723 BN Groningen, the Netherlands), grain yield was estimated per hectare using plant number per square meter.

2.4 Statistical analysis

All data on plant growth, nodulation and yield components were tested for normal distribution, before being subjected to statistical analysis using SAS software program version 9.0. A three-way ANOVA was used to compare the effect of bean cultivar, *Rhizobium* inoculation and P levels. Where treatment means differed statistically, Duncan's multiple range test was used to separate the means at $p \leq 0.05$. Pearson's correlation coefficients were also calculated to establish relationships between plant growth, symbiotic parameters, grain yield and yield components.

3 Results

3.1 Effect of *Rhizobium* inoculation and P application on plant growth and nodulation

Inoculation with the two *Rhizobium* strains markedly increased plant growth and nodulation of the bean cultivars tested

(Tables 2). Relative to uninoculated controls, shoot biomass, nodule number, and nodule dry matter per plant were increased by 9%, 40%, and 54% in 2012, and by 20%, 39%, and 13% in 2013 with strain HB-429 application (Table 2). Compared to the uninoculated control, strain GT-9 also increased plant growth by 2%, nodule number by 42%, nodule dry matter per plant by 18% in 2012; and shoot dry matter by 16%, nodule number by 34%, and nodule dry matter per plant by 13% in 2013. As a result, bean plants inoculated with strain GT-9 increased pod number per plant by 41%, seed number per pod by 47%, grain yield by 35%, and 100-seed weight by 32% in 2012, as well as raised pod number per plant by 32%, seed number per pod by 25%, and grain yield by 68% in 2013.

Supplying P to Hawassa Dume and Ibbado significantly increased plant growth and nodulation relative to zero-P control (Table 3). The application of 10–30 kg·ha⁻¹ P increased shoot biomass, nodule number per plant and nodule dry

matter by 7% to 39%, 23% to 59%, and 59% to 144% in 2012, respectively, as well as by 10% to 40%, 21% to 43%, and 12% to 35% in 2013, respectively.

3.2 Effect of *Rhizobium* inoculation and P application on yield components and grain production

Independent of inoculation and P application, the two bean cultivars differed significantly in grain yield and yield components in 2012 and 2013 (Table 3). Hawassa Dume had 28%, 30%, and 12% greater pod number per plant, seed number per pod, and grain yield, respectively, than Ibbado in 2012. However, Ibbado scored 16% higher 100-seed weight than Hawassa Dume. In 2013, Hawassa Dume again had 62%, 34%, and 13% higher pod number per plant, seed number per pod, and grain yield, respectively, than Ibbado, which had 18%

Table 2 Plant growth and nodulation performance of common bean cultivars in response to rhizobial inoculation and P application at Hawassa during 2012 and 2013 cropping seasons

Treatments	2012			2013		
	Shoot DM	Nodule DM	Nodule number per plant	Shoot DM	Nodule DM	Nodule number per plant
	(g per plant)			(g per plant)		
Variety						
Hawassa Dume	17.57 ± 0.58a	0.50 ± 0.03a	59.25 ± 2.89a	17.26 ± 0.45a	0.52 ± 0.02a	65.07 ± 2.82a
Ibbado	16.89 ± 0.39b	0.46 ± 0.03a	56.33 ± 2.00a	16.84 ± 0.44b	0.51 ± 0.02a	64.38 ± 1.89a
<i>Rhizobium</i>						
Uninoculated	16.57 ± 0.53b	0.39 ± 0.03c	44.69 ± 2.17b	15.24 ± 0.39c	0.47 ± 0.01b	52.05 ± 2.54b
HB-429	18.08 ± 0.51a	0.60 ± 0.05a	65.25 ± 2.96a	18.35 ± 0.45a	0.56 ± 0.02a	72.52 ± 2.56a
GT-9	17.03 ± 0.75b	0.46 ± 0.02b	63.44 ± 2.54a	17.55 ± 0.63b	0.53 ± 0.02a	69.61 ± 2.31a
Phosphorus						
0	14.13 ± 0.44c	0.27 ± 0.02d	44.79 ± 2.94c	14.00 ± 0.28c	0.43 ± 0.02c	51.88 ± 2.90c
10	15.85 ± 0.61b	0.43 ± 0.02c	55.13 ± 2.62b	15.35 ± 0.40b	0.48 ± 0.02b	62.96 ± 2.86b
20	19.30 ± 0.33a	0.56 ± 0.03b	60.04 ± 3.11b	19.28 ± 0.35a	0.59 ± 0.03a	69.69 ± 2.83a
30	19.64 ± 0.65a	0.66 ± 0.04a	71.21 ± 3.17a	19.55 ± 0.52a	0.58 ± 0.01a	74.38 ± 3.19a
F-statistic						
Variety (V)	4.28*	3.10 ^{NS}	2.20 ^{NS}	5.79*	0.36 ^{NS}	0.10 ^{NS}
<i>Rhizobium</i> (R)	5.98**	34.35***	33.94***	52.96***	16.32***	33.07***
Phosphorus (P)	52.71***	56.36***	23.38***	57.29***	21.13***	19.09***
V × R	4.26*	0.86 ^{NS}	8.10**	1.06 ^{NS}	0.05 ^{NS}	3.11 ^{NS}
V × P	14.69***	1.33 ^{NS}	0.43 ^{NS}	1.60 ^{NS}	0.46 ^{NS}	1.91 ^{NS}
R × P	7.55***	5.57**	0.47 ^{NS}	6.07***	7.05***	0.23 ^{NS}
V × R × P	1.59 ^{NS}	1.02 ^{NS}	2.33 ^{NS}	1.82 ^{NS}	1.49 ^{NS}	1.92 ^{NS}

Note: Values (Mean ± SE) followed by dissimilar letters in a column are significantly different. *, **, and *** represent significant levels at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. NS, non-significant.

Table 3 Effect of *Rhizobium* inoculation and P application on yield components of common bean varieties planted at Hawassa in Ethiopia during the 2012 and 2013 cropping seasons

Treatments	2012				2013			
	Number of pods per plant	Number of seeds per pod	100-seed weight (g)	Grain yield (t·ha ⁻¹)	Number of pods per plant	Number of seeds per pod	100-seed weight (g)	Grain yield (t·ha ⁻¹)
Variety								
Hawassa Dume	11.52 ± 0.52a	5.15 ± 0.27a	29.89 ± 0.51b	2.20 ± 0.11a	14.94 ± 0.45a	5.90 ± 0.25a	31.68 ± 1.58b	2.22 ± 0.13a
Ibbado	9.04 ± 0.33b	3.98 ± 0.12b	34.74 ± 1.28a	1.97 ± 0.10b	9.19 ± 0.46b	4.40 ± 0.11b	37.40 ± 1.21a	1.96 ± 0.10b
<i>Rhizobium</i>								
Uninoculated	7.75 ± 0.32c	3.44 ± 0.09b	26.59 ± 0.80b	1.63 ± 0.09c	9.78 ± 0.58b	4.41 ± 0.15b	32.60 ± 2.12a	1.44 ± 0.06b
HB-429	12.06 ± 0.60a	5.19 ± 0.30a	35.18 ± 1.07a	2.42 ± 0.13a	13.47 ± 0.78a	5.53 ± 0.29a	36.15 ± 1.49a	2.43 ± 0.13a
GT-9	11.03 ± 0.49b	5.06 ± 0.26a	35.18 ± 1.23a	2.20 ± 0.12b	12.94 ± 0.73a	5.50 ± 0.29a	34.87 ± 1.68a	2.42 ± 0.13a
Phosphorus								
0	8.33 ± 0.39b	3.50 ± 0.16c	27.95 ± 1.15c	1.25 ± 0.05c	9.79 ± 0.55d	4.17 ± 0.17c	29.22 ± 1.07b	1.35 ± 0.04c
10	9.17 ± 0.51b	4.54 ± 0.28b	31.96 ± 1.43b	2.01 ± 0.10b	10.88 ± 0.73c	4.63 ± 0.23b	34.58 ± 1.71a	1.87 ± 0.11b
20	11.50 ± 0.71a	4.88 ± 0.32b	32.98 ± 1.31b	2.48 ± 0.11a	14.63 ± 0.90a	5.83 ± 0.30a	35.38 ± 2.39a	2.53 ± 0.14a
30	12.13 ± 0.71a	5.33 ± 0.34a	36.37 ± 1.44a	2.60 ± 0.12a	12.96 ± 0.93b	5.96 ± 0.35a	38.99 ± 2.37a	2.64 ± 0.17a
F-statistics								
Variety (V)	64.86***	69.18***	59.09***	16.41***	351.58***	102.32***	9.83**	23.36***
<i>Rhizobium</i> (R)	49.99***	56.67***	71.57***	62.05***	48.12***	24.73***	1.78 ^{NS}	159.73***
Phosphorus (P)	18.39***	22.32***	22.98***	101.80***	37.69***	28.16***	4.02*	126.04***
V × R	3.38*	15.97***	32.11***	4.79*	1.52 ^{NS}	10.36***	0.75 ^{NS}	3.14 ^{NS}
V × P	2.72 ^{NS}	10.88***	3.98*	0.45 ^{NS}	5.86**	14.26***	1.50 ^{NS}	4.75**
R × P	1.64 ^{NS}	2.92*	1.90 ^{NS}	3.71**	5.65***	2.50 ^{NS}	2.24 ^{NS}	13.93***
V × R × P	2.26 ^{NS}	3.12*	2.35 ^{NS}	1.42 ^{NS}	2.19 ^{NS}	1.10 ^{NS}	1.15 ^{NS}	2.10 ^{NS}

Note: Values (Mean ± SE) followed by dissimilar letters in a column are significantly different. *, **, and *** represent significant levels at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. NS, non-significant.

greater 100-seed weight than Hawassa Dume.

The increase in plant growth and nodulation with *Rhizobium* inoculation resulted in markedly greater grain yield and yield components between the two bean cultivars in 2012 and 2013 (Table 3). *Rhizobium* inoculation with strain HB-429 increased pod number per plant by 56%, seed number per pod by 51%, and grain yield by 49% in 2012, and similarly recorded increases of 38%, 25%, and 69% (in that order) in 2013 (Table 3). Also, strain HB-429 increased bean pod number per plant by 10% and grain yield by 10% over strain GT-9 in 2012, but not in 2013.

Supplying P to Hawassa Dume and Ibbado significantly increased all yield components relative to zero-P control (Table 3). Applying 10–30 kg·ha⁻¹ P increased shoot biomass, nodule number per plant, and nodule dry matter by 7% to 39%, 23% to 59%, and 59% to 144% in 2012, respectively, as well as

by 10% to 40%, 21% to 43%, and 12% to 35% in 2013, respectively. Relative to zero-P control, adding only 10 kg·ha⁻¹ P to bean plants increased the number of pods per plant, number of seeds per pod, and grain yield by 10%, 30%, and 61% in 2012, respectively, and by 11%, 11%, and 39% in 2013, respectively, indicating that P supply at a low 10 kg·ha⁻¹ can increase bean yield by 39% to 61% depending on the cropping season. Although the application of 10 kg·ha⁻¹ P at Hawassa significantly increased the number of seeds per pod, 100-seed weight, and grain yield, the highest number of seeds per pod and 100-seed weight were obtained at 30 kg·ha⁻¹ P.

3.3 Interactions

Uninoculated and inoculated plants with strain HB-429 did not differ significantly in shoot dry matter. However, the Hawassa Dume cultivar combined with strain GT-9 produced higher shoot dry matter than Ibbado (Fig. 1(a)). Regardless of the

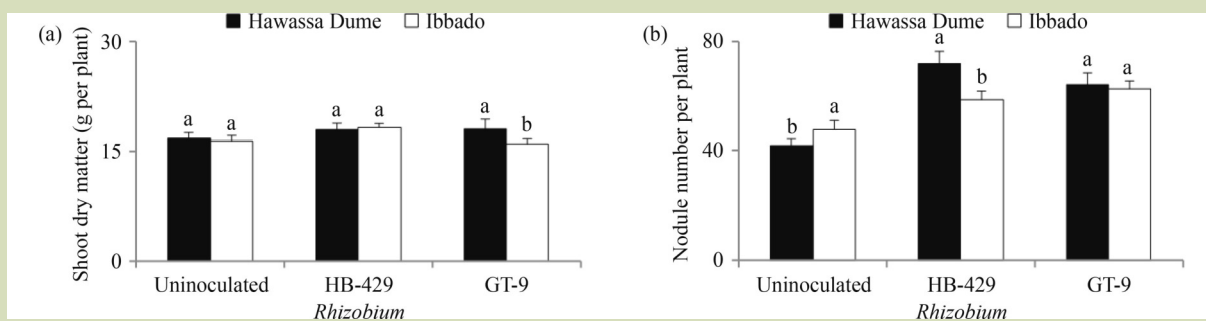


Fig. 1 Interaction of cultivar \times *Rhizobium*: (a) shoot dry matter in 2012; (b) nodule number per plant in 2012. Errors bars are SE at $p \leq 0.05$.

cultivars, plants inoculated with both rhizobial strains produced higher nodule numbers than the non-inoculated control (Fig. 1(b)). The highest shoot dry mass was also recorded when applying $30 \text{ kg}\cdot\text{ha}^{-1}$ P with the variety Hawassa Dume. However, the lowest value was found for the same cultivar and control (Fig. 2). The dry matter yields of shoots and nodules showed an increasing trend with the combined application of P and *Rhizobium* strains inoculated over the control (Fig. 3). Hawassa Dume produced significantly more pods per plant, seeds per pod, and much higher grain yield than cv. Ibbado (Fig. 4(a, b, d, e)), but Ibbado recorded heavier 100-seed weight with inoculation than Hawassa Dume (Fig. 4(c)), leading to a significant cultivar \times *Rhizobium* interaction. The cultivar \times phosphorus interaction showed that

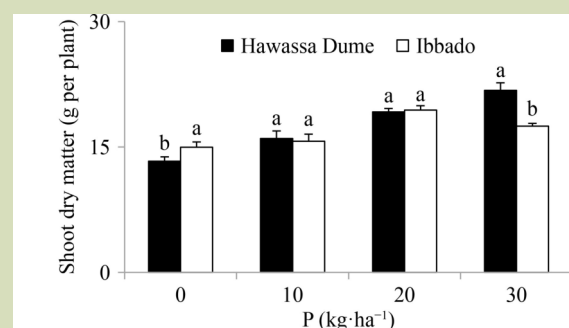


Fig. 2 Interaction of cultivar \times phosphorus ($\text{kg}\cdot\text{ha}^{-1}$) in 2012 for shoot dry matter. Errors bars are SE at $p \leq 0.05$.

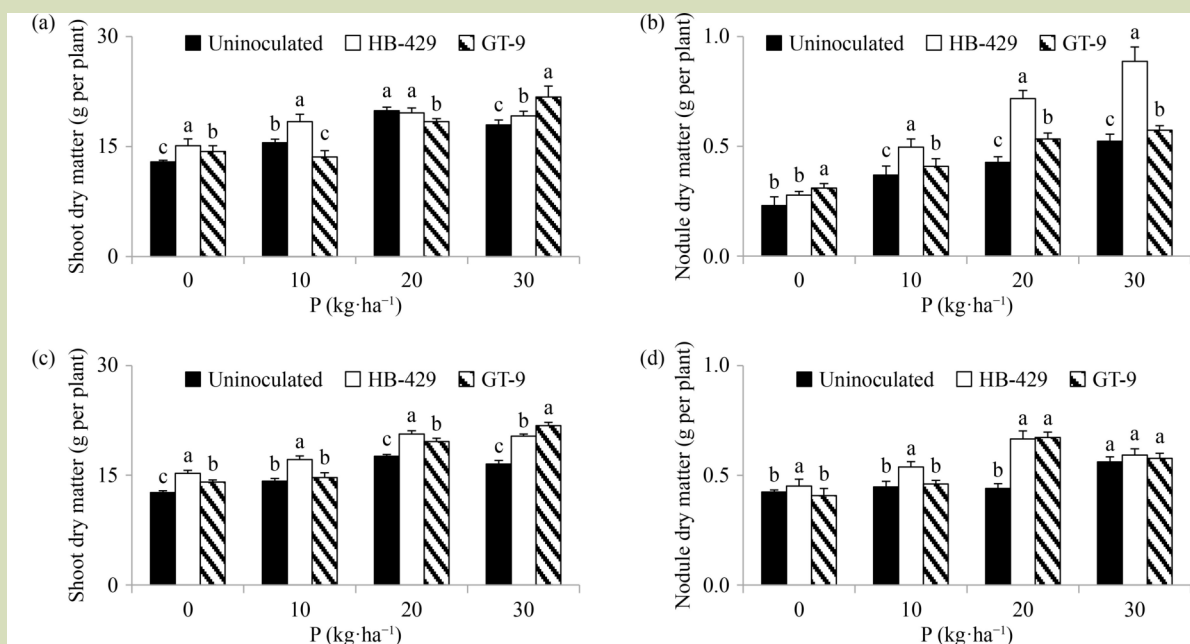


Fig. 3 Interaction of *Rhizobium* \times phosphorus ($\text{kg}\cdot\text{ha}^{-1}$) on: (a, c) Shoot dry matter in 2012 and 2013; and (b, d) Nodule dry matter in 2012 and 2013. Errors bars are SE at $p \leq 0.05$.

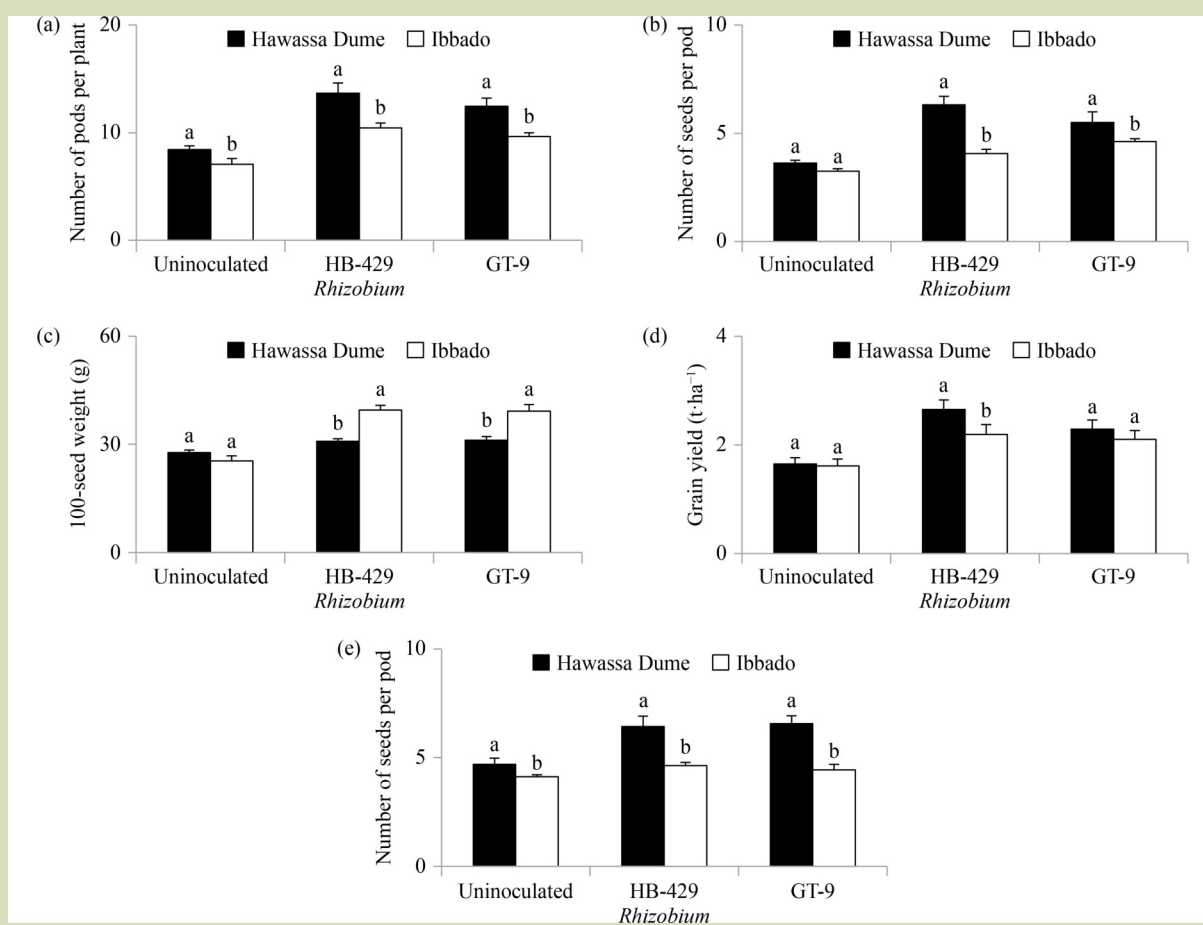


Fig. 4 Interaction of cultivar \times *Rhizobium*: (a) Number of pods per plant in 2012; (b) Number of seeds per pod in 2012; (c) 100-seed weight in 2012; (d) Grain yield in 2012; and (e) Number of seeds per pod in 2013. Errors bars are SE at $p \leq 0.05$.

applying 10, 20, and 30 kg·ha⁻¹ P increased seeds per pod in 2012, and pods per plant and seeds per pod in 2013 in Hawassa Dume than Ibbado (Fig. 5(a, c, d)). Hawassa Dume also recorded greater grain yield than Ibbado with the application of 20 and 30 kg·ha⁻¹ P (Fig. 5(e)), while Ibbado showed higher 100-seed weight at all P levels (Fig. 5(b)). *Rhizobium* \times phosphorus interaction also revealed significantly greater seeds per pod and grain yield in 2012, and pods per plant and grain yield in 2013 with *Rhizobium* inoculation compared to the uninoculated plants at zero-P level (Fig. 6). Generally, all the yield components were increased with rhizobial inoculation at all P levels compared to uninoculated control plants.

3.4 Correlation analysis

Plant biomass was correlated significantly with nodule number and nodule dry mass. Grain yield was strongly correlated with shoot dry matter, nodule dry matter, nodule number per plant, number of pods per plant, number of seeds per pod, and 100-

seed weight during both the 2012 and 2013 cropping seasons (Table 4). Similarly, the number of pods per plant correlated significantly with nodule number, nodule dry mass, shoot biomass, number of seeds per pod, and 100-seed weight (Table 4). The number of seeds per pod was also positively correlated with shoot dry matter, nodule dry matter, nodule number, and 100-seed weight (Table 4).

4 Discussion

Plant growth and N₂ fixation in legumes can be hampered by lack of nutrients, especially N and P. Low P supply is known to affect the synthesis of signal molecules that transcribe bacterial nod-genes^[33]. Phosphorus is thus needed for nodulation and N₂ fixation^[34,35]. Genotypic variations, however, do exist in the growth, nodulation, and grain yield of legumes, which are independent of endogenous and/or exogenous nutrient supply. For example, as found in the present study, greater plant

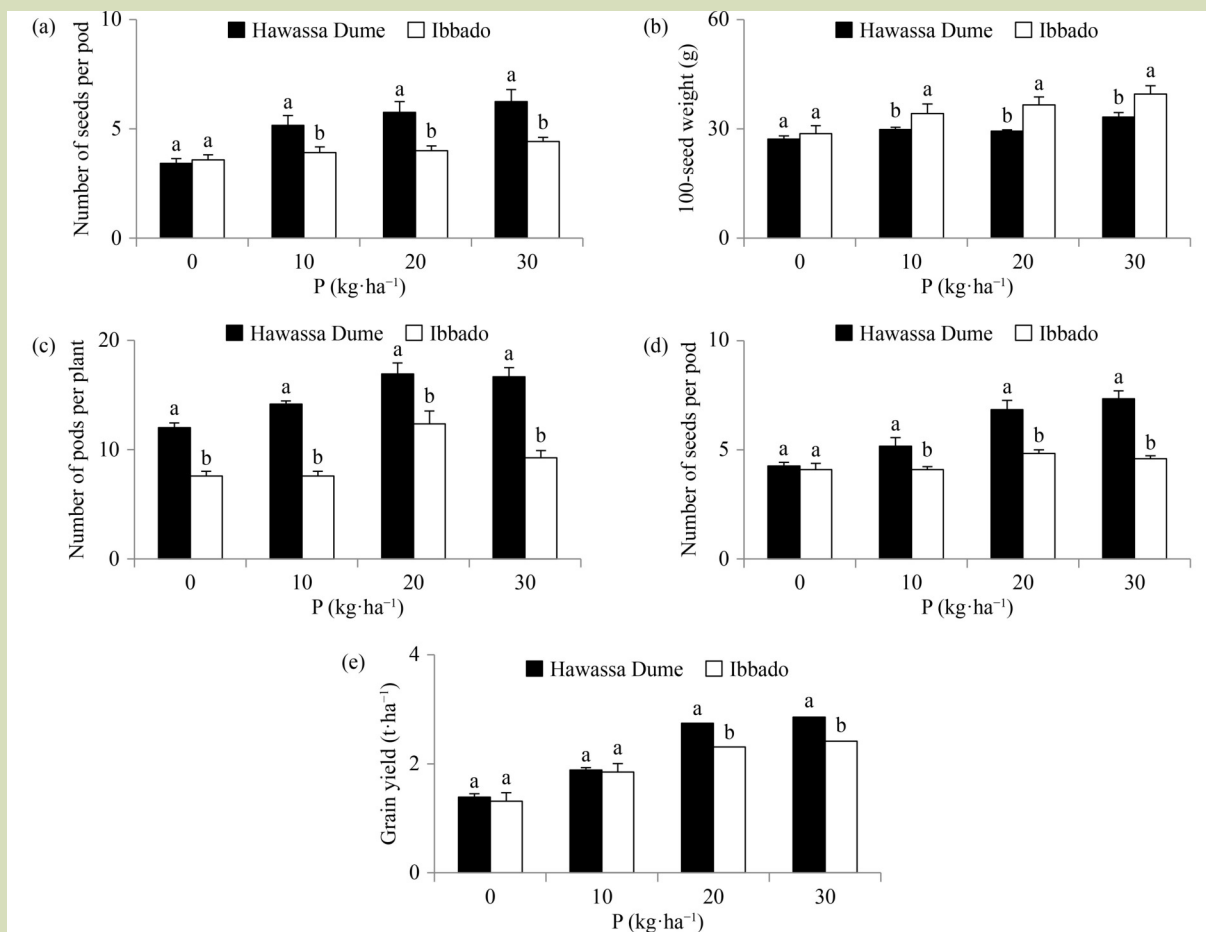


Fig. 5 Interaction of cultivar × phosphorus (kg·ha⁻¹): (a) Number of seeds per pod in 2012; (b) 100-seed weight in 2012; (c) Number of pods per plant in 2013; (d) Number of seeds per pod in 2013; and (e) Grain yield in 2013. Errors bars are SE at $p \leq 0.05$.

growth, grain yield, and other yield components were observed in Hawassa Dume than Ibbado during the two cropping seasons, despite their similarity in nodulation performance (Table 2). Hawassa Dume was more high-yielding as it produced much greater number of pods per plant and number of seeds per pod, resulting in higher grain yield when compared to Ibbado during the two years of experimentation (Table 3). The greater grain yield by cv. Hawassa Dume was also due to its ability to produce more and longer pods, as well as higher seed number per pod, which together enhanced its economic yield and profitability as a crop. These differences between the two bean cultivars tested can be attributed to genetic variation [18,26,36,37], although environmental and climatic factors prevailing at the site of experimentation could have also caused differences in plant growth response [14,19,38,39].

In the present study, *Rhizobium* inoculation increased plant growth, root nodulation (Table 2), and grain yield in the two

common bean cultivars (Table 3), these findings are consistent with those obtained for the same species [40,41]. Although strain HB-429 induced greater nodule dry matter and shoot biomass than strain GT-9 in 2012, root nodulation was similar for the two strains in 2013. The greater shoot growth induced by strain HB-429 in 2012 led to 10% increase in pod number per bean plant, and hence 10% increase in grain yield than strain GT-9.

The nodulation data in the present study revealed the presence of nodules on the roots of uninoculated field plants, which suggests the presence of native soil rhizobia capable of nodulating common bean in Ethiopia. However, these nodules were smaller in size, numerous in number, distributed throughout the root system, and were ineffective as evidenced by the white/green interior coloration. Inoculation of common bean should therefore be an important routine agronomic practice in Ethiopia if farmers are to obtain economic yields. The strong response of bean to *Rhizobium* inoculation in the present study supports the argument by Havlin et al. [28] that

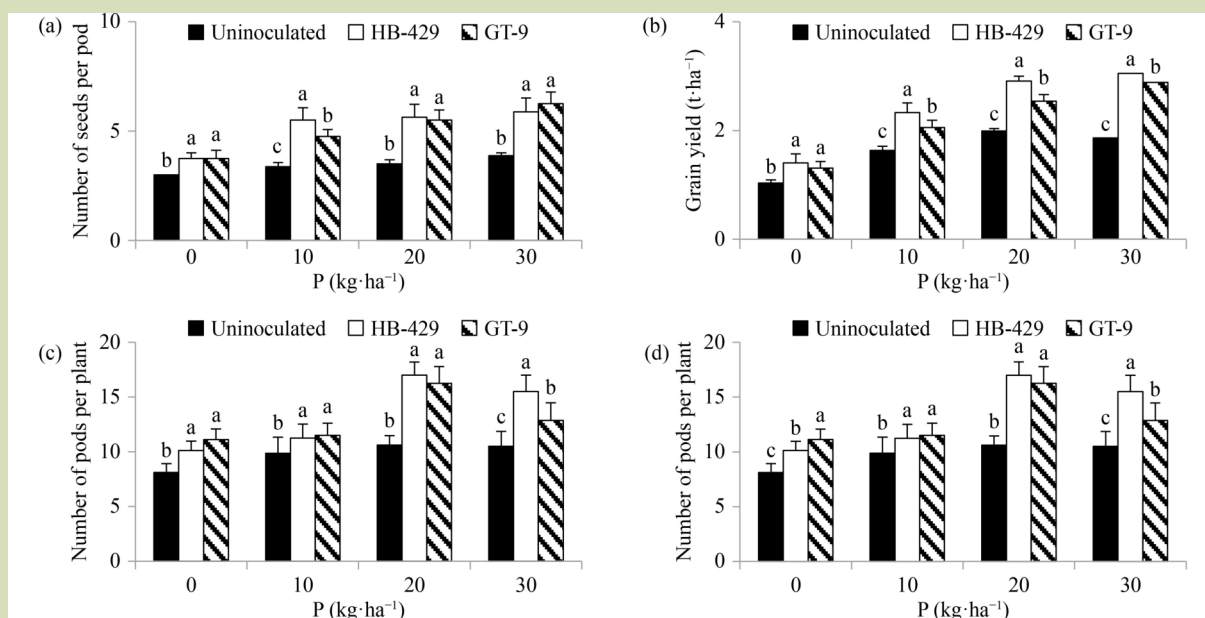


Fig. 6 Interaction of *Rhizobium* × phosphorus (kg·ha⁻¹) on (a) number of seeds per pod in 2012; (b) grain yield in 2012; (c) number of pods per plant in 2013; and (d) grain yield in 2013. Errors bars are SE at $p \leq 0.05$.

Table 4 Correlation (r) between plant growth, nodulation, yield and yield components of common bean cultivars grown at Hawassa in Ethiopia during the 2012 and 2013 cropping seasons

Parameters	Significance			
	2012		2013	
	r	p value	r	p value
Grain yield vs. shoot dry matter	0.61	***	0.79	***
Grain yield vs. nodule dry matter	0.74	***	0.60	***
Grain yield vs. nodule number per plant	0.69	***	0.68	***
Grain yield vs. number of pods	0.68	***	0.63	***
Grain yield vs. number of seeds	0.68	***	0.67	***
Grain yield vs. 100-seed weight	0.46	***	0.25	**
Number of pods vs. shoot dry matter	0.50	***	0.53	***
Number of pods vs. nodule dry matter	0.63	***	0.49	***
Number of pods vs. nodule number	0.62	***	0.44	***
Number of pods vs. number of seeds	0.74	***	0.74	***
Number of pods vs. 100-seed weight	0.32	**	0.05	NS
Number of seeds vs. shoot dry matter	0.47	***	0.59	***
Number of seeds vs. nodule dry matter	0.56	***	0.42	***
Number of seeds vs. nodule number	0.62	***	0.54	***
Number of seeds vs. 100-seed weight	0.25	*	0.10	NS

Note: $n = 96$; *, **, and *** represent significant levels at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. NS, non-significant.

farmers should use rhizobial inoculants as N-source to increase common bean production in Ethiopia, and also confirms earlier findings on legume response to rhizobial inoculation^[18,41–44].

In Ethiopia, P nutrition is a major constraint limiting bean production, as low P is functionally known to adversely affect the synthesis of signal molecules that transcribe bacterial nod-genes during nodule formation^[33]. As found in the present study (Table 2) and in other studies^[34,35], P is needed for increased legume nodulation and N₂ fixation, a process that provides N for driving photosynthesis, the source of de novo photosynthate that conversely drives N₂ fixation. P nutrition, N₂ fixation and photosynthesis are therefore biochemically and physiologically interlinked in their support of plant growth and reproduction.

As expected, in the present study, plant-available P concentration in the rhizosphere of common bean plants rose markedly with P application (10, 20, and 30 kg·ha⁻¹ P), but also unexpectedly with *Rhizobium* inoculation (data not shown). The latter suggests that strains HB-429 and GT-9 are P-solubilizing rhizobia capable of making soil organic P available for uptake by the bean plants^[45], thereby increasing the supply of N from symbiosis and P from *Rhizobium* inoculation to the bean plants when compared to the uninoculated, zero-P control. Elsewhere, P application of common bean was found to increase shoot biomass and root nodulation^[22,46], results similar to those obtained in the present study when 10, 20, or 30 kg·ha⁻¹ P were supplied to the two bean cultivars, Hawassa Dume and Ibbado (Table 2). Functionally, P is known to affect the synthesis of signal molecules that transcribe bacterial nod-genes during nodule formation^[33]. Phosphorus is therefore needed for increased legume nodulation and N₂ fixation^[34], a process that provides N for driving photosynthesis, the source of de novo photosynthate that conversely also drives N₂ fixation. Thus, P nutrition, N₂ fixation, and photosynthesis are biochemically and physiologically interlinked in their support of plant growth and reproduction.

Also, added P is known to promote early root growth and lateral fibrous root development^[47] which enable plants to absorb more water and mineral nutrients for increased growth, measured as greater dry matter yield. The application of 10, 20,

and 30 kg·ha⁻¹ P to bean plants resulted in 61%, 98%, and 108% increase in grain yield in 2012, respectively, and 38%, 87%, and 96% increase in 2013, respectively. There were thus minimal differences in grain yield between 20 and 30 kg·ha⁻¹ P, which supports the recommended rate of 20 kg·ha⁻¹ P for optimum grain yield of common bean in Ethiopia^[22]. This should be of interest to resource-poor Ethiopian farmers who cannot afford expensive mineral fertilizers in large quantities. The growth achieved by the test cultivars with the supplementation of 20 kg·ha⁻¹ P is consistent with the finding by Turuko and Mohammed^[22] for common bean in Ethiopia. Stewart et al.^[48] also found that P supplementation improved legume crop yields.

Although the yield components of the two bean cultivars seemed genetically predetermined, in the present study there were interlinking biological relationships within each cultivar as evidenced by the significant and positive correlations found between grain yield, plant growth, root nodulation and yield components such as number of pods, number of seeds, and 100-seed weight produced per plant (Table 4). The observed relationship between test parameters have been found in other studies^[49–52], and the direct link between grain production and some yield components could be a useful tool for improving common bean yield through breeding programs^[36].

5 Conclusions

In summary, bean inoculation with *R. leguminosarum* *bv.* *phaseoli* alone or in combination with P application, increased grain yield and other yield components of the two common bean cultivars tested at Hawassa during two cropping seasons. The large yield increases from rhizobial inoculation when combined with the supply of 10 or 20 kg·ha⁻¹ P to the bean plants (Fig. 6(b, d)) support the recommendation of these agronomic inputs for use by resource-poor farmers in Ethiopia. The use of rhizobial inoculants can replace expensive chemical N fertilizers, which are inaccessible to smallholders in Ethiopia and are also known to cause global warming. Also, the present study has shown the superior yield performance of Hawassa Dume when compared to Ibbado. Therefore, Hawassa Dume could be recommended for adoption by farmers in combination with the *Rhizobium* inoculant strain HB-429 and P at 20 kg·ha⁻¹ P in Ethiopia.

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Compliance with ethics guidelines

Tarekegn Y. Samago and Felix D. Dakora declare that they have no conflicts of interest or financial conflicts to disclose. All applicable institutional and national guidelines for the care and use of animals were followed.

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