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Research Article

Nitrogen Fixation and Yield of Common Bean Varieties in Response to Shade and Inoculation of Common Bean

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Abstract

Light is not only a primary energy source, but it is also one of the environmental factors that affect plant growth and development. Common bean *(Phaseolus vulgaris* L.*)* is commonly produced in association with maize as subordinate intercrops in the Sidama region. Under such a production system, the shade effect may limit the photosynthetic and nitrogen-fixing capacity of common beans. The objective of the current study was to assess how inoculation and shade affect the ability to fix nitrogen and yield components of common beans. Three common bean varieties (*Hawassa dume, Nassir, and Ibbado*), two shade levels (open and 25% shade) as well as two levels of inoculation (uninoculated and inoculated) were used for these purposes. The experiment was set up in factorial randomized complete block design (RCBD) in four replications. In this experiment, Rhizobium inoculation and shade significantly (*$p < 0.05$*) affected the number of pods plant^{-1} and the number of seeds pod^{-1}. The inoculated treatment produced the maximum number of pods plant^{-1} (14.02) and the lowest number of pods plant^{-1} (10.95) was obtained from uninoculated treatments. The results also showed that the inoculated Hawassa dume variety from the open treatments derived the maximum percentage of N from N_2 fixation, whereas the non-inoculated Ibbado variety from the 25% shade treatments derived the lowest percentage of N. Common bean grown on full light had significantly greater N content than shade.

More Information

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Introduction

Among the pulse crops, the common bean is the second in area of production in Ethiopia, next to the faba bean (CSA2014/2015) [1]. As high in nutrients and commercial potential, the common bean holds great promise for fighting hunger, increasing income, and improving soil fertility in Sub-Saharan Africa. The national average yield of common beans is low ranging from 1.4 tone ha^{-1}, which is far below the corresponding yield recorded at research sites 2.5-3 tones ha^{-1} using improved varieties [1]. Common bean is highly preferred by Ethiopian farmers because of their early maturing characteristics (short-season crop) that enable households to get the cash income required to purchase food when other crops have not yet matured [2]. One of the most crucial processes for ecosystems to access accessible N for all living species is biological nitrogen fixation. Even though N_2 makes up 78% of the atmosphere, the triple bond formed by two N atoms is extremely stable, and only a small number of prokaryotes can use the nitrogenase enzyme to convert N_2 into ammonia. Globally, the annual rate of natural nitrogen fixation is estimated at about 232 x 10^6 t, thus 97% depends on biological nitrogen fixation [3]. This is more than the approximately 100 x 10^6 t of chemical nitrogen fertilizer used in 2009. Common beans can utilize N_2 by forming root nodules with the rhizobia bacteria, which fix nitrogen in the soil.

The effects of light availability on N_2-fixation are less certain. Competition for light may reduce N_2-fixation by reducing growth. According to [4] grass's competition for light may have slowed down plant growth and thus reduced N_2-fixation at the plant level. However, an earlier study showed that greater competition for light [5] had a detrimental impact on the N_2-fixation of intercropped peas. Annual intercrops are becoming more common in intercropping systems, and this is leading to the development of a significant interplay between light dynamics and biogeochemical processes.

Common bean (*Phaseolus vulgaris* L.) is commonly produced in association with maize as subordinate intercrops in the Sidama region of Ethiopia. This cropping system enhances farming communities' adaptation to climate change and improves sustainable production systems. Moreover, pulses offer natural soil maintenance benefits through nitrogen-fixing, which improves yields of cereals through crop rotation, and can also result in savings for smallholder farmers from less fertilizer use [6]. Under such a production system, the shading effect may limit the photosynthetic and nitrogen-fixing capacity of common beans. Therefore, this study was designed to simulate the intercropping shading effect on the growth, development, yield component, and N_2 fixation capacity of common beans by assessing the varietal responses to shading and inoculation under field conditions.

Materials and methods

Description of the study area

The experiment was conducted at Hawassa in the Agricultural Research Station of Hawassa University, in Sidama Regional State, southern Ethiopia. The site is located at 7° 4'N latitude and 38° 31'E longitude with an elevation of 1692 m above sea level. The average rainfall of the study area is 800 to 1100 mm annually. The mean maximum and minimum temperatures were 27 °C, and 12 °C, respectively. The main rainy season extends from April to September and it is interrupted by some dry, sunshine and sometimes from May to July [7].

Source of experimental materials

Seeds of common bean varieties (Hawassa dume, Nassir, and Ibbado), were obtained from Hawassa Agricultural Research Centre. The varieties were chosen based on their high grain yield, availability, and acceptability of the varieties by farmers around the study areas. Rhizobium inoculants HB-429 were obtained from Menagesha Biotech PLC. The inoculants were brought with standard carriers and kept in the laboratory until treated to seed during planting.

Experimental design and treatments

Seeds were planted in a 2×3×2 factorial randomized complete block design with four replications. The treatments consisted of two levels of inoculation (control, and HB-429 strains) three common bean varieties (Hawassa dume, Nassir, and Ibbado), and two levels of shade (full light and 25% shade).

Cultural practices

Inoculation was done in the field before planting under shade to maintain the viability of bacterial cells. The inoculated seeds were allowed to air dry for a few minutes before planting to avoid fungal growth. Each planting hole received two seeds. To prevent cross-contamination, the uninoculated treatments were planted first and followed by inoculated treatments. Ridges were made to prevent the movement of bacteria through runoff between plots and blocks. The seeds were inoculated with peat-based carrier as per the recommended rate (10 g inoculant per kilogram of seed). Common bean seeds were planted at a spacing of 40 cm between rows and 20 cm between plants. Each experimental plot measures 2.4 m × 1 m (2.4 m^2). Weeding was carried out initially in the uninoculated plots to prevent cross-contamination. Shading was created by nets which limit the incoming solar radiation by 25%. A 25% shade was selected based on the specification on the shade net and also because of resource scarcity, it is unable to take all the shade levels. The shade level was compared with full sunlight (0% shade).

Data collection

Growth parameters

Plant height (cm): five randomly chosen plants from each plot were used to measure the height of each plant at physiological maturity.

Shoot dry weight (g): five randomly chosen plants from each plot were used to measure the above-ground biomass at the mid-flowering stage. The samples were put in perforated paper bags with labels and dried in an oven for 48 hours at 70°C. The averages were recorded as shoot dry weight $plant^{-1}$.

Yield and yield components

Number of pods per plant: Five randomly selected plants from each plot were counted to determine the number of pods per plant, and five randomly selected pods from each plot were counted to determine the number of seeds per pod at harvest.

Grain yield (kg ha^{-1}): was recorded from the two central rows after drying and threshing then the seed yield was adjusted to the moisture level of 10%. Finally, yield per plot was converted to tons $ha^{-1.}$

Harvest index (HI): was computed as the ratio of grain yield (t ha^{-1}) to total above-ground dry biomass.

N_2-fixation measurements: The N-difference method was used to determine fixed N in common bean and wheat plants. Seed samples of common bean and non-N_2-fixing reference plants (wheat) were dried and finely ground (< 0.1 mm), for analysis of total N by the Kjeldahl method. Nitrogen fixed by the ND method was calculated as by [8] method.

Quantity of N_2 fixed = (N yield fixing species/legumes) – (N yield non-fixing species/reference) (1).

Soil sampling and analysis: Twelve pre-planting soil samples were taken from the experimental site at depths ranging from 0 to 20 cm. The samples were properly mixed to produce a single representative composite sample weighing 1kg. The soil sample was air-dried and ground to pass a 2 mm sieve and analyzed for total N, available P, pH, organic carbon (OC), exchangeable cations, and physical properties at the

Horti Coop Ethiopia soil and water analysis laboratory. 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 [9]. Organic carbon was determined by the [10] method. Total N was determined using the Kjledahl method [11]. Available P was determined by the Olsen method [12] using ascorbic acid as the reducing agent. The cation exchange capacity (CEC) in cmolc (+) kg^{-1} was measured using the 1 M-neutral ammonium acetate method [13]. The soil-particle size distribution was determined using the Bouyoucos hydrometer method [14].

Statistical analysis

The collected data was subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) of the Statistical Analysis System software [15] version 9.0. Mean separation was done using the Least Significant Difference (LSD) test at a 5% probability level.

Result and discussions

Physicochemical properties of the soil

The soil textural class of the experimental site was clay loam with average proportions of 31% clay, 28% silt, and 41% sand (Table 1). The pH value of the study soil was slightly alkaline (pH 7.4). The total nitrogen content (TN) of the study soil was 0.12%. As far as soil organic carbon (OC) is concerned, the value was 1.40%, and the cation exchange capacity (CEC) of the soil was 18 cmolc (+) kg^{-1} The study soil properties revealed that external supply of nutrients either as fertilizer or as organic inputs required for sustainable crop production.

Effects of shade and inoculation on growth parameters of common bean

Plant height: Rhizobium inoculation and shade showed significant ($p < 0.05$) influence on plant height (Table 2). Thus, the greatest plant height (43.18 cm) was recorded in the plots treated with strain HB-429. The enhanced vegetative growth brought by nitrogen fixation may be the reason for the rise in plant height after inoculation. N increased soybean plant height in the presence of Rhizobium inoculants [16]. Other authors also reported similar results from research conducted on chickpea and faba beans [17] indicating that rhizobium inoculation significantly increased plant height. Plant height was also significantly affected by shade ($p < 0.05$). Plant height was higher under 25% shade than open treatment. The reason for this may be that low light levels have a negative impact on the morphology of plants, leading to an increase in plant height and a decrease in stem diameter, which leads to lodging of plants, especially under intercropping conditions [18]. This study simulated the intercropping conditions where diffused light reached the lower-laying intercropping companion crops like common beans.

Shoot dry weight: The analysis of variance revealed that shoot dry weight was significantly affected by rhizobium inoculation and shade ($p < 0.05$) (Table 2). In comparison to the non-inoculated plot, the HB-429 strain inoculated plot resulted in a considerably larger shoot dry weight (22.95 g) $plant^{-1}$ while the non-inoculated produced (20.58 g) shoot dry weight $plant^{-1}$. The advantages of Rhizobium inoculation on beans appear to be the crop's supply of N through symbiotic N_2 fixation [19]. Similar to this finding [20] research revealed that inoculating mung beans with Rhizobium led to the largest dry matter accumulation on the plant. This is in agreement with the findings of [21] who reported seed inoculation significantly increased dry-weight biomass when compared to treatments that were not inoculated.

Shading showed a significant ($p < 0.001$) effect on shoot dry matter accumulation of common beans compared to open conditions. Thus, 25% shading gave a relatively higher shoot dry weight (24.06 g) $plant^{-1}$ that was greater than the open condition. The result pinpointed that the shading effect might reduce the harsh heat on the common bean plant. An increase in shoot dry weight with increasing shade levels could be due to an increase in vegetative growth duration and decreased root/shoot ratio under shade [22].

Effect of shade and inoculation on yield and yield components of common bean varieties

Number of pods per plant (NPPP): The number of pods per plant was significantly ($p < 0.001$) affected by Rhizobium inoculation and shade (Table 3). However, both the two-way and three-way interactions did not show a significant ($p > 0.05$) difference in number of pods per plant. The lowest number of pods per plant (10.95) was recorded from the non-inoculated treatment whereas the highest number of pods per plant (14.02) was recorded with inoculated treatment (strain

Table 1: Physicochemical properties of the soil.

Particle size distribution (%)			texture	PH	TN	OC	CEC
Clay	silt	sand	Clay loam	7.4	0.12%	1.4%	18 cmolc
31	28	41					

Table 2: Effect of shade and inoculation on plant height and shoot dry weight of common bean varieties.

Treatments	Plant height(cm)	Shoot dry weight(g)
Shade		
Open	40.22b	19.46b
25%shade	42.40a	24.06a
Inoculation		
control	39.45b	20.58b
HB-429	43.18a	22.95a
LSD	6.36	7.12
CV (%)	4.84	13.52

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

Table 3: Effect of shade and inoculation on the number of pods per plant (NPPP) and on the number of seeds per pod (NSPP).

Treatments	NPPP	NSPP
Open	13.53a	4.73a
25%shade	11.44b	4.28b
Inoculation		
control	10.95b	4.12b
HB-429	14.02a	4.89a
LSD	5.23	1.31
CV (%)	10.65	8.16

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

HB-429). The higher pod number with applied inoculants may be attributed to higher assimilate accumulation and improved development brought on by symbiotic N_2 fixation.

The present result was consistent with [23,24] who found that *Bradyrhizobium japonicum* inoculation in soybean enhanced the number of pods per plant. Shade considerably (p < 0.05) affects the number of pods per plant. The open treatments produced the maximum number of pods per plant (13.53) and the lowest number of pods per plant (11.44) was recorded from 25% shade treatments. This indicates that shading had a significant negative effect on seed yield, yield-related parameters, and pod number. The reduced photosynthetic rate and biomass accumulation may be responsible for this [25].

Number of seeds per pod (NSPP): The analysis of variance revealed that the number of seeds per pod was significantly (p < 0.001) affected by Rhizobium inoculation and shade, but was not by the main effect of variety (Table 3). The result demonstrates that Rhizobium inoculation was critical to common bean generative growth, which led to a significant increase in the number of seeds per pod. The considerable difference in the number of seeds per pod between the inoculated and non-inoculated was consistent with [26] which showed that seed inoculation enhanced grain yield in addition to the number of seeds per pod. The results showed that shade had a significant (p < 0.05) impact on the number of seeds per pod. The 25% shaded treatment produced the fewest seeds per pod (4.28) whereas the open treatments produced the most (4.89). This indicates that shade significantly decreased the number of seeds per pod. This may be because of the lower photosynthetic rate and biomass accumulation. [25].

Interaction effect of variety, shade, and Rhizobium inoculation on grain yield and harvest index of common bean

Grain yield: The analysis of variance revealed a significant (p ≤ 0.05) difference due to the interaction effects of variety, shade, and rhizobium inoculation on grain yield (Table 4). The largest grain yield (4.03t ha^{-1}) was obtained from inoculated Hawassa dume variety from the open treatment while the lowest grain yield (1.68 t ha^{-1}) was obtained from the non-inoculated Ibbado variety from the shaded treatment.

The considerable increase in grain yield following inoculation with Rhizobium stain HB-429 may be attributable to the increased N in the soil that is available for plant roots to absorb through fixed N_2. The findings agree with [27] who came to the conclusion that treatments with Rhizobium inoculation generated higher grain production than those of non-inoculated. This may be because of increased seed and pod production brought on by Rhizobium inoculation. Similar to this [28] observed that Rhizobium inoculation had a similar increasing effect on soybean grain production. Shading significantly reduced soybean production and yield components [29,30] which are comparable to our results. Similar to our findings, previous studies have found that soybean plants' seed yields were much lower in shading as compared to normal conditions [31].

Harvest index: The results showed that the harvest index was significantly (p ≤ 0.001) affected by the interaction effect of variety, shade, and rhizobium inoculation (Table 4). The highest harvest index (42%) was recorded from the variety Hawassa dume inoculated under open conditions, while the lowest harvest index (21%) was recorded from the variety Ibbado non-inoculated under shade conditions. This might result in a longer period of vegetative growth and a lower root/shoot ratio under shade, which allocated more nutrients to shoot growth than to root growth [22]. Due to the inoculation of the HB-429 strain, the highest harvest index (HI) was attained. This may be due to the higher partitioning of dry matter into grain. Similar results were reported by [32].

Percentage of N derived from biological nitrogen fixation

The results showed that the biological N2 fixation of common bean was significantly different in response to variety, shading, and inoculation (Table 5). The greatest N was

Table 4: Interaction effect of variety, shade, and Rhizobium inoculation on grain yield and harvest index.

Variety	Shade level	Inoculation	Grain yield(t/ha^{-1})	Harvest index
Hawassa dume	Open	inoculated	4.03a	0.42a
		uninoculated	2.15g	0.28d
	25%	inoculated	2.95d	0.34b
		uninoculated	1.92h	0.25e
Nasir	Open	inoculated	3.55b	0.42a
		uninoculated	1.98h	0.27de
	25%	inoculated	2.67e	0.33b
		uninoculated	1.81i	0.23f
Ibbado	Open	inoculated	3.16c	0.35b
		uninoculated	1.96h	0.26de
	25%	inoculated	2.36f	0.29c
		Uninoculated	1.68j	0.21g
CV (%)			2.71	3.32
LSD			0.96	0.13

Means in the same column followed by the same letter(s) are not significantly different at a 5% probability level.

Table 5: Effect of inoculation, shade, and variety on amount of N fixed (kg ha^{-1}) at Hawassa, Sidama region.

Variety	Shade level	Inoculation	% N fixed	N content (kg ha^{-1})
Hawassa dume	Open	inoculated	1.67	0.06
		uninoculated	1.48	0.03
	25%	Inoculated uninoculated	1.42 1.32	0.04 0.02
	Open	inoculated	1.57	0.05
		uninoculated	1.47	0.03
Nasir				
	25%	inoculated	1.38	0.04
		uninoculated	1.26	0.02
	Open	Inoculated	1.56	0.05
		uninoculated	1.45	0.03
Ibbado				
	25%	inoculated	1.34	0.03
		Uninoculated	1.1	0.02

The % N fixed was calculated from seed.

fixed when Hawassa Dume (60 kg ha^{-1}) was inoculated with strain HB-429 under open conditions, in contrast, the lowest N was fixed with Ibbado (20 kg ha^{-1})in non-inoculated condition grown under 25% shade. Hawassa Dume was symbiotically more effective than Ibbado, similarly, it contributed much more symbiotic N and therefore produced significantly greater gain yield when compared to the Ibbado variety. The increased plant growth as a result of Rhizobium inoculation also resulted in greater grain yield. This was evidenced in this study by the significantly positive correlation between the percent N derived from fixation and bean grain yield. Rhizobium-inoculated common bean plants accumulated significant amounts of symbiotic N when compared to non-inoculated ones. This improved N nutrition from enhanced N supply via N_2 fixation by the introduced strains resulted in greater photosynthate production for higher grain yield. These results are consistent with the findings of [33] which showed that bean inoculation with an effective strain increased N nutrition and grain yield. These findings are also in agreement with those of [34,35] who reported a stimulatory effect of rhizobium inoculation on the growth and symbiotic performances of common beans. On the other hand, similar results have been previously reported with lentils and peas [36]. This implies that improving nodulation through the use of compatible strains is important to enhance the total N in plant tissue. Variation in plant N accumulation among varieties could be due to the presence of variability in biological nitrogen fixation (BNF) capacity among common bean varieties [37,38]. Similarly, previous studies found that plant N concentration in different pulse crops is influenced by the host plant cultivar as well as by the Rhizobium strain [37,39]. On the other hand, some studies have found that light limitation can decrease the N_2-fixation capabilities of legumes [5].

The limitation of this study was that it did not include other shade levels example 50%, 75%, and other bacterial strains and the benefit is in the possibility of intercrop variety Hawasssa Dume with other crops like maize. Finally, the future prospect of the study was to accomplish a study on the other shade levels and other types of bacterial strains to give full information to farmers in order to use their limited resources wisely by practicing intercropping.

Conclusion

As common to most pulse crops, common beans have the ability to form a symbiotic relationship with soil bacteria capable of trapping nitrogen gas (N_2) from the atmosphere and converting it into ammonia, which can be used by the plant for growth, development, and seed production. The results of the study revealed that plant height was significantly affected by shade and rhizobium inoculation. The number of pods per plant, number of seeds per pod, and shoot dry weight, were significantly affected by the main effect of Rhizobium inoculation and shade. On the other hand, the interaction effect of variety, shade, and rhizobium inoculation showed significant differences in grain yield, harvest index, and nitrogen fixation. In summary, the results indicated that inoculation of rhizobia strain HB-429 combined with variety Hawassa dume in open conditions improves shoot dry weight, number of pods per plant, number of seeds per pod, grain yield, amount of N fixed as compared to 25% shade and non-inoculated treatments. Therefore, we recommend a variety of Hawassa dume with rhizobium strain HB-429 to be widely produced under open conditions for its higher seed yield and greater N fixation.

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