

Nodulation and Yield Potential of Common Bean (*Phaseolus vulgaris* L.) Varieties Under Shade in Response to Inoculation

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Abstract: Common bean (*Phaseolus vulgaris* L.) is a herbaceous annual crop which in a symbiotic relationship with specific soil bacteria, 'fixes' atmospheric nitrogen (N₂) into amino form that can be used for plant growth. Three common bean varieties were used in the field experiment to determine the effects of shade and Rhizobium inoculation on nodulation, yield, and yield components. The experimental treatments include three common bean varieties (Hawassa Dume, Nasir and Ibbado). Two levels of inoculation (inoculated and non-inoculated), and two levels of shade (25% shading and no shading). A randomized complete block design with four replications was used to setup the experiment. The outcome demonstrated that Rhizobium strain HB-429 inoculation of common bean considerably affected all parameters as compared to the non-inoculated plants in the field. The common beans 100-seed weight, grain yield, leaf area and leaf area index are influenced by interactions between inoculation, variety, and shade. The rhizobium –inoculated plots had the highest nodule number, whereas the 25% shaded treatments had the lowest nodule number on the other hand nodule dry weight was significantly affected by the main effect of Rhizobium inoculation and shade. Similar to this, the inoculated Hawassa dume variety of open treatments generated the highest grain yield, but the non-inoculated Ibbado variety of 25% shade treatments produced the lowest grain yield. The variety Hawassa dume produced the highest grain when it was treated with rhizobium strain HB-429 in the open.

Keywords: Common Bean, Nitrogen Fixation, Inoculation, Shade

1. Introduction

The annual crop common bean (*Phaseolus Vulagris* L) is a member of the Fabaceae family. Warm settings with temperatures between 18 and 24°C are optimum for its growth [1]. One of the most significant cash crops and sources of protein in many lowlands and midlands areas, it is an annual herbaceous crop with a high degree of polymorphism that grows throughout the warm seasons. It is a good source of potassium, selenium, molybdenum, thiamine, vitamin B6, and folic acid and is high in starch and dietary fiber [2]. It is used in several ways; the green, immature pods are cooked or preserved as vegetables, while the ripe seeds are prepared into nifro [3].

Like other legumes, common beans grow root nodules in association with Rhizobium bacteria in the soil. The bacteria in the nodules work as nitrogenases to fix atmospheric nitrogen into ammonia, which the bean plant uses as a nitrogen supply and minimizes the need for external fertilizers [4].

The photosynthesis rates of the common bean are impacted by shade, regardless of its source. It reduces irradiance mostly in the photosynthetically active portion of the spectrum (400 to 700 nm). One important ecological aspect that affects plant growth is the irradiance level. Both genetic and phenotypic acclimation are ways that plants react to various irradiance levels [5, 6]. When a plant is shaded by a neighbor, there are two possible reactions: shade-acclimation response and shade-avoiding response. The former

maximizes light harvesting in shade conditions by increasing specific leaf area and decreasing the chlorophyll a/b ratio, [7] while the latter does so by positioning the leaves out of the shade [8].

Studies have suggested that competition for light can reduce N_2 -fixation by reducing growth [9], and one study found that grasses may have reduced N_2 -fixation at the plant level by competing for light [10]. Reported that competition reduces an intercropped pea's ability to fix N_2 , possibly due to greater competition for light. 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.

2. Materials and Methods

2.1. Description of the Study Area

The experiment was conducted at the Research Farm of Hawassa University, which is located 273 km southwest of Addis Ababa in Sidama Regional State. The site is located at 7° 4'N latitude and 38° 31'E longitude and an altitude of 1692m above sea level. 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°C, 12°C, and 20°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 [11]. The soil textural class of the experimental site was clay loam with average proportions of 31% clay, 28% silt, and 41% sand. 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%, thus classified as medium. As far as soil organic carbon (OC) is concerned, the value was 1.40%, which was within the range of medium organic carbon content and the CEC of the study soil was 18 Cmolc (+) kg⁻¹.

2.2. Source of Experimental Material

Seeds of common bean varieties Hawassa Dume Nasir and Ibbado were obtained from Hawassa Agricultural Research Centre. The common bean varieties were chosen based on their availability and acceptability by farmers. Hawassa Dume grows as an indeterminate semi-bush and has a dark-red seed coat color. Ibbado grows as a determinate bush and has a speckled-red seed coat color and Nasir grows as an indeterminate climbing and has dark red seed coat color.

2.3. Treatments and Experimental Design

The treatments consisted of three common bean varieties (Hawassa dume, Nasir, and Ibbado) two levels of shade (open and 25% shade), and two levels of bacterial strains (inoculated and non-inoculated). The treatments were arranged in a factorial combination using a randomized complete block design (RCBD) with four replications. The Rhizobium strain HB-429 was widely used previously to enhance the symbiotic performance and yield of common beans under field conditions, and it is considered an elite

strain for common bean cultivation in Ethiopia [12]. A total of 12 treatment combinations were used in the experiment.

2.4. Experimental Procedures

The experimental plots were prepared before planting. The common bean cultivars were sown at 40cm spacing between rows and 10cm spacing between plants. The net plot size was (1 m × 2.4 m = 2.4m²). The spacing between blocks and plots was 1.5 and 1m, respectively. The seed inoculation was done just before planting under shade to maintain the viability of cells at the rate of 10g/kg of seed. Seeds were allowed to air dry for a few minutes before planting to avoid fungal growth. The uninoculated seed was planted first to avoid bacterial contamination with inoculated seeds. All plots were isolated with ridges to minimize the movement of bacteria from one plot to another. All standard local cultural practices were accomplished throughout the growth period. Manual weeding was done whenever required. To avoid cross contamination, weeding was done in the uninoculated plots first. Shading was created by nets which reduced the incoming solar radiation by 25%. The shade level was compared with full sunlight (0% shade).

2.5. Data Collection

Five plants were randomly selected from each plot at flowering and dug up carefully to expose the majority of the root mass and nodules. This destructive sample was collected from the two rows for estimate of the nodulation parameter, nodule number. After separating the nodules from the soil and roots by washing the nodules were counted. Nodules were dried at 70°C for 48 hours to determine their dry weight, which was then reported as g plant⁻¹. The plant's leaf area was determined by averaging the leaf areas of five plants. A leaf area meter (Model-LI-3100 Area meter, Li-Cor, Lincoln, USA) was used to measure it. The ratio of the crop's total leaf area to the respective ground area was used to obtain the leaf area index. The grain yield was obtained from the two central rows after drying and threshing, the grain yield was adjusted to a 10% moisture level, and the weight of 100 randomly selected seeds from the entire harvest of each plot was used to calculate the weight of one hundred seeds. The yield per plot was finally translated to t ha⁻¹.

2.6. Statistical Analysis

The data were subjected to the analysis of variance (ANOVA) using SAS software version 9.0 [13]. Where treatments differed statistically the least significant difference (LSD) was used to separate treatment means at 5% level of significance.

3. Result and Discussion

3.1. Interaction Effect of Variety, Rhizobium and Shade Level on Leaf Area and Leaf Area Index

On leaf area and leaf area index interaction effects of

variety, shade and rhizobium inoculation was showed significant difference. The largest LA (1115.5cm^2) is obtained from inoculated Hawassa dume variety with shade treatment while the lowest LA (455.6cm^2) is obtained from non-inoculated Ibbado variety from open treatment (Table 1). This may be as a result of the fact that plants that grow in shady environments invest substantially more in leaf area in order to maximize light harvesting and photosynthetic surface [5]. Another author [14] found that LA increases as a

typical bean acclimation trait to reduce irradiance.

Similarly the largest LAI (2.78cm^2) is obtained from inoculated Hawassa dume variety from the shade treatment while the lowest LAI (1.13cm^2) is obtained from non-inoculated Ibbado variety from open treatment (Table 1). Increases in shade have resulted in higher LAI; this is because plants that thrive in shaded environments invest considerably more on their leaf area to boost light harvesting and photosynthetic surface [5].

Table 1. Interaction effect of variety, rhizobium and shade level on leaf area and leaf area index.

Variety	Shade level	Inoculation	Leaf area (cm^2)	Leaf area index
Hawassa Dume	open	Inoculated	657.6cde	1.64cde
		Uninoculated	611.0de	1.52de
	25%	Inoculated	1115.5a	2.78a
		Uninoculated	751.9cd	1.87bcd
Nasir	open	Inoculated	656.2cde	1.64cde
		Uninoculated	573.5ef	1.43ef
	25%	Inoculated	844.1b	2.11b
		Uninoculated	717.9bcde	1.79bcde
Ibbado	Open	Inoculated	616.8de	1.54de
		Uninoculated	455.6f	1.13f
	25%	Inoculated	784.5bc	1.96bc
		Uninoculated	665.2cde	1.66cde
CV (%)			14.4	14.42
LSD			338.3	0.84

CV = Coefficient of variation, Means in the same column followed by the same letter(s) are not significantly different at 5% probability level.

3.2. Effect of Variety, Shade and Rhizobium Inoculation on Nodule Number (NN) and Nodule Dry Weight (NDW)

Rhizobium inoculation and shade significantly affects ($P < 0.001$) the number of nodules produced per plant. However, there was no significant difference in the number of nodules per plant between the two-way and three-way interactions. The strain HB-429 recorded the highest nodules number (43.17) whereas the lowest nodules number per plant (35.98) was reported with non-inoculated treatment. The average number of nodules per plant was 39.57. (Table 2). The fact that there are more nodules after rhizobium inoculation may be related to how well the introduced rhizobia out compete the local bacteria that live in the soil. These outcomes are consistent with those of [15].

Number of nodules were significantly reduced by shade; un-shaded plants had more nodules (40.92) than plants with 25% shade (38.23). (Table 3). The reduced root and consequently nodule number can be linked to variations in nodule quantity per plant caused by shade treatments. Plants grown in the full light had a larger nodule mass than those grown in shade. Both a decrease in nodule number and a decrease in average nodule weight contributed to the reduction in nodule mass [16]. Both rhizobium inoculation and shade significantly ($P < 0.001$) impacted nodule dry weight. However, there was no marked change in the nodule dry weight between the two-way and three-way interactions. Nodule dry weight was highest in the inoculated condition (0.51g plant^{-1}), whereas it was lowest in the non-inoculated

treatment (0.37g) (Table 2). Both [17] and [18] on soybean revealed similar effects of seed inoculation on nodule dry weight, indicating that inoculation considerably increases nodule dry weight of legumes under field conditions. High nodule dry weight frequently indicates a need for boosting N_2 -fixation in legumes rather than nodule number, according to [19]. Full-light-grown plants had more nodules than plants grown in 25% shade. The reduced root and consequently nodule output can be linked to variations in nodule quantity per plant caused by shade treatments. Plants grown in full light had a larger nodule mass than those grown in shade. Both a decrease in nodule number and a decrease in average nodule weight contributed to the reduction in nodule mass [16].

Table 2. Effect of variety, shade and rhizobium inoculation on nodule number (NN) and nodule dry weight (NDW).

Treatments	Nodule №	Nodule dry weight (g)
Variety		
Hawassa dume	42a	0.47a
Nasir	40a	0.45ab
IbbadoShade	36b	0.41b
Open	41a	0.48a
25%Inoculation	38b	0.40b
Inoculated	43a	0.51a
Uninoculated	31b	0.37b
CV (%)	10.35	16.76
LSD	12.76	0.23

CV = Coefficient of variation, ns; non-significant. Means in the same column followed by the same letter(s) are not significantly different at 5% probability level.

Table 3. Interaction effect of variety, shade and Rhizobium inoculation on grain yield and 100 seed weight of common bean.

Variety	Shade level	Inoculation	Grain yield (t/ha ⁻¹)	100seed weight (g)
Hawassa dume	Open	inoculated	4.03a	29.12cd
		uninoculated	2.15g	27.5cde
	25%	inoculated	2.95d	28.25cd
		uninoculated	1.92h	25def
Nasir	Open	inoculated	3.55b	24ef
		uninoculated	1.98h	21.75f
	25%	inoculated	2.67e	22.25ef
		uninoculated	1.81i	20.5f
Ibbado	Open	inoculated	3.16c	46.5a
		uninoculated	1.96h	36.25b
	25%	inoculated	2.36f	40.75b
		uninoculated	1.68j	30.5c
		CV (%)	2.71	12.79
		LSD	0.96	9.77

CV = Coefficient of variation,. Means in the same column followed by the same letter(s) are not significantly different at 5% probability level

3.3. Interaction Effect of Variety, Shade and Rhizobium Inoculation on Grain Yield and 100 Seed Weight of Common Bean

Interaction effects of variety, shade and rhizobium inoculation showed significant difference on grain yield and 100 seed weight. However, the two way interaction was not showed significant difference to grain yield and 100 seed weight. The largest grain yield 4.03tha⁻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 non-inoculated Ibbado variety from shaded treatment (Table 3). The considerable increase in grain production following inoculation with Rhizobium stain HB-429 may be attributable to the increased N in the soil that is available for plant roots to assimilate through fixed N₂. The outcomes are consistent with those of [15], who came to the conclusion that Rhizobium inoculation treatments produced better grain yields than non-inoculated treatments. This could possibly be because of increased seed and pod production brought on by Rhizobium inoculation. Rhizobium inoculation has also been shown to increase soybean grain yield, according to [20]. Similar to our findings, others have observed that the seed yield of soybean plants greatly declined in shade conditions as compared to open conditions [23]. It has been found that shading conditions significantly affected the soybean production and yield components [21, 22].

Seed weight was significantly ($P < 0.05$) affected by interaction effect of variety, Rhizobium inoculation and shade (Table 3). However, the interaction of shade with inoculation were not showed significant difference to 100 seed weight. In comparison to other treatments, common bean inoculation with HB-429 enhanced the weight of one hundred seeds. The findings imply that Rhizobium inoculation may have helped to boost the effectiveness of main nutrient usage, which in turn increased grain weight and production. [24] also noted that effective rhizobium inoculation of seeds at an early growth stage enhanced root nodulation and increased grain weight. [25] found a 91%

increase in hundred seed weight by inoculation, which is inconsistent with this result. The genetic makeup of the cultivars may be the second most significant cause of variation in hundred seed weight.

4. Conclusion

The short growing cycle and adaptability to various cropping methods of the common bean make it suitable for food security. However the impacts of shade on nodulation of common bean were studied little. The mean value of nodule numbers varied between 38.23 to 40.92 for shade and 35.98 and 43.17 for rhizobium inoculation. The rhizobium inoculation was attributed for the maximum nodule number. The mean value of grain yield varied between 4.03ton ha⁻¹ to 1.68ton ha⁻¹. The highest grain yield was recorded from inoculated Hawassa dume variety of open field treatments while the lowest grain yield was recorded from non-inoculated Ibbado variety of 25% shade treatments. The outcome of this experiment therefore showed that inoculation of rhizobial strain HB-429 combined with Hawassa dume in open improves number of nodule, nodule dry weight and grain yield, compared to 25% shade and non-inoculated treatments. This implies that variety Hawassa dume inoculated with rhizobium strain HB-429 in open generated the highest seed yield.

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