Journal of Plant Sciences
2022; 10(5): 183-191
http://www.sciencepublishinggroup.com/j/jps
doi: 10.11648/j.jps.20221005.13
ISSN: 2331-0723 (Print); ISSN: 2331-0731 (Online)

Rhizobium Inoculation and Intercropping Affected on Physiological Response, and Yield Performance of Common Bean *(Phaseolus vulgaris L.)* Varieties

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To cite this article:
Shemeles Tesfaye Shumet, Hussien Mohammed Beshir, Tewodros Aylawe. *Rhizobium* Inoculation and Intercropping Affected on Physiological Response, and Yield Performance of Common Bean *(Phaseolus vulgaris L.)* Varieties. *Journal of Plant Sciences*. Vol. 10, No. 5, 2022, pp. 183-191. doi: 10.11648/j.jps.20221005.13

Received: September 19, 2022; **Accepted**: October 26, 2022; **Published**: October 30, 2022

Abstract: The field experiment was carried out at Hawassa, during the 2020 cropping season with the objectives to evaluate the impact of maize-common bean intercropping and inoculation on physiological response, nodulation and yield of common bean. The treatments consist of two common bean varieties (Hawassa Dume, Nassir), two levels of inoculation (HB-429 inoculated, un-inoculated) and three spatial arrangements (Sole Common bean, Maize one row - Common bean one row, and Maize one row - Common bean two rows). The treatments were laid out in a factorial arrangement in randomized complete block design (RCBD) with three replications and comprising twelve treatments. Data on physiological variables, nodulation, and yield of common bean were recorded. The results revealed that the main effect of spatial arrangements highly significantly ($P < 0.001$) affected the Chlorophyll A and Chlorophyll B, transpiration rate, and intercellular CO_2 concentration which were the highest on sole cropping common bean. The interaction effects of spatial arrangements and inoculation significantly ($P < 0.01$) affected the total chlorophyll contents, photosynthetic rate and chlorophyll fluorescence resulted in the maximum value on sole cropping with inoculated (HB-429). The interaction effects of spatial arrangements and inoculation significantly ($P < 0.01$) affected the number of nodules plant^{-1}, nodule dry weight plant^{-1}, number of pods plant^{-1}, number of seeds pod^{-1}, grain yield and above ground biomass yield. The highest grain yield of 2.54 t ha^{-1} was recorded from on sole cropping common bean with inoculated (HB-429) can be used at Hawassa, and areas with similar agro-ecology.

Keywords: Physiological, *Rhizobium*, Intercropping, Common Bean, Yield

1. Introduction

Common bean (*Phaseolus vulgaris L.*) is one of the most important food legume crops for direct consumption in the world [39]. Common bean - intercropping provided heavy shading as though they were a cover crop, this in turn influenced physiological response may have been responsible for the observed decreases in number of total nodules and effective nodules, yield compared to the sole cropping [30]. Land scarcity is one of the constraints facing small farmers especially in developing countries of Asia and Africa [9]. In southern Ethiopia about 30% of farmers have an average land holding of 0.5 to 1 ha and a further 40% having 0.1 to 0.5 ha [14]. Among the main abiotic factors, solar radiation is the most significant one that regulates the photosynthesis response, and consequently, the plant survival, growth and adaptation. In any habitat the light intensity varies temporally (seasonally and diurnally) and spatially. Therefore, plants develop acclimation and plasticity to cope with the varying light regimes [54].

Legume inoculation with effective inoculants can be an alternative for the sustainable improvement of soil fertility to enhance crop yields [43]. Thus, supplying N to crops and cropping systems through either inoculation or utilization of legumes is expected to increase photosynthetic processes, leaf area production, leaf area duration as well as net photosynthesis rate [3]. In this regard, N2-fixing legumes, including common ben, have an advantage over non-legumes in their ability to meet their photosynthetic N requirements from symbiotic N2-fixation and thereby fulfill the N requirement for morpho-physiological functions of plants [21]. Light intensity affects the central processes of crops such as physiology response, biochemistry and cell division in the intercropping system [51]. Physiological differences between intercrops affect the benefit from their mutual association [4].

Legume intercropping significantly affected the physiological performance and structure of the photosynthetic apparatus [52]. Crop photosynthesis varies spatially and temporally in response to environmental factors and from day to day in response to the accumulated effects of environments on canopy size and its physiological status [29]. Several researches have been done to assess photosynthetic activities of plant and their responses under different factors. Some studies have shown that plant beneficial microorganisms (*Rhizobia*) have enhanced photosynthesis because they improve plant nutrition hence increased leaf area that reflects photosynthesis [24]. In another study done by Nyoki, D & Ndakidemi, A. [36], it was reported that total leaf chlorophyll content of cowpea was significantly increased following inoculation of *Bradyrhizobium japonicum*. The same results were found in another study by [10], which showed that common bean inoculated with Rhizobia had increased leaf chlorophyll content compared with that of un-inoculated plants. However, the information about their effect on physiological response of the intercropping and *Rhizobium* inoculation common bean crops to the environment were little studied. Therefore, this study was conducted to investigate the effects of maize - common bean intercropping on physiology, nodulation, and yield performance of the common bean crops. The objectives of this study were first to evaluate the influence of maize - common bean intercropping and inoculation on gas exchange and physiological processes of common bean and second to determine the effects of common bean - maize intercropping on the nodulation and yield of common bean under *Rhizobium* inoculation.

2. Material and Methods

2.1. Description of the Study Area

The experiment was conducted in the 2020 cropping season at the experimental field of Hawassa University, Hawassa, Ethiopia. The site is located 270 km south away from the capital city Addis Ababa. Geographically the area lies at 7° 03' 05.7" N and 38° 30' 21.1" E with a mean altitude of 1694 m above sea level [35]. The soil of the experimental site was tropical Andosols [2], well drained sandy clay loam in textural classes with pH value of 7.2. The annual rainfall of 1274.5 mm with a mean minimum and maximum temperature of 13.6 and 27.8°C, respectively [35].

2.2. Source of Planting Materials

Common bean ((*P. vulgaris* L.) varieties Nassir, Hawassa Dume were obtained from Hawassa Agricultural Research Center. This variety was purposefully chosen based on its adaptation, high grain yield, acceptability by farmers and seed availability and maize hybrid variety (BH-546) was obtained from Baco Agricultural Research Center and also *Rhizobium* strain (HB-429) was purchased from Menagesha biotechnology PLC, Addis Ababa, Ethiopia.

2.3. Soil Sampling and Analysis

Before planting soil samples were taken randomly from the experimental field at 0-20 cm depth using augur. The samples were mixed well in a plastic bag, sieved and one composite representative sample was taken for analysis of physical and chemical properties (pH, total N, available P, exchangeable K, OM, C: N ratio and CEC) of the soil. The composite soil sample was sent to Debrezeit Horticoop Ethiopia (Horticulture) soil and water analysis laboratory and analysis were done following the standard procedure for each parameter. The available P content of the soil is considered to be high according to Olsen P rating as described by [20]. Soil texture analysis was performed by the Bouyoucous hydrometer method [16]. The pH of the soil was measured in water at the soil to water ratio of 1:2.5 [37]. Analysis of organic carbon content of the soil in a laboratory was determined by Walkley and wet oxidation method as described by [22]. The soil analysis result is presented in Table 1 below.

Table 1. *Physical and some chemical characteristics of experimental soils.*

Physical property				**Chemical properties**			
Soil texture (%)			**Texture class**	**pH (H_2O)**	**Total N (%)**	**Av. P (ppm)**	**OC (%)**
Silt	Clay	Sand					
28.5	32.2	40.8	Clay Loam	7.0	0.12	58	1.40

2.4. Experimental Design and Procedures

The experiment consists of two common bean varieties, one *Rhizobium* strain with control, and three spatial arrangements with that makes the total treatments 12 (2 varieties x 2 inoculation x 3 spatial arrangements). The two common bean varieties Hawassa Dume and Nassir, two levels of *Rhizobium* inoculation (HB-429) (I_1= Inoculated RS, I_2= Un-inoculated RS) and the spatial arrangements of the component crops were sole common bean, maize 1:1 common bean and maize 1:2 common bean. The experiment was arranged factorially in randomized complete block design (RCBD) with three replications.

The size of the experimental plot was 3.2m x 2.10m (6.72 m²), net area 241.9m^2, and total area 438.4m^2. The space between plots and between blocks was 80 cm and 1m respectively. Seeds of one maize hybrid (BH-546) were planted with a spacing of 80×30 cm inter and intra row spacing, respectively, in a plot consisting of four rows of maize. Seeds of common bean varieties (Nassir and Hawassa Dume) for *Rhizobium* inoculation treatment were coated with charcoal based *Rhizobium* inoculum (HB-429). For this, fresh inoculum impregnated in charcoal was purchase from Menagesha biotechnology PLC, Addis Ababa, Ethiopia, in a week time of seeding date. The charcoal base *Rhizobium* inoculum was mixed thoroughly with seeds with sticker for proper coating. Then the coated seeds were dried under shade for approximately 20 - 30 minutes and then seeded immediately. The detailed procedure is summarized in [34]. Un-inoculated common bean seeds were planted in their respective plots first then the inoculated seeds were planted to avoid contamination. The sole common bean planting was 40x10 cm inter and intra row spacing, respectively. Under maize row intercropping common bean 1:1 maize row arrangements, was planted and intercropping common bean 2:1 maize rows arrangements.

2.5. Data Collection and Measurements

2.5.1. Physiological (Gas-Exchange) Parameters

Photosynthetic rate, Transpiration rate, and CO_2 substomata were measured from three randomly selected plants leave from the central rows of each plot were used at flowering stage. Using portable infrared gas exchange analyzer LCA-4 ADC (Analytical Development Company, Hoddeson, England). The measurements were done the time between 10:00-12:00 am and 2:00-4:00 pm hours. Chlorophyll contents were extracted from randomly selected plant and well developed leaves from each treatment. To extract chlorophyll content of each common bean varieties, leaf sizes of 15 mm^2 of fresh leaves were taken and grinded with mortar and pestle using 5 ml acetone 80%. The optical density of the supernatant was determined at 645 and 663 nm using spectrophotometer. Chlorophyll a, Chlorophyll b, and total Chlorophyll, were computed using Arnon's equation [8]. The calculation was done using the equation 1, 2, and 3 described below.

$$\text{Chl a } (\mu g/ml) = 12.7\ (A_{663}) - 2.69\ (A_{645}) \quad (1)$$

$$\text{Chl b } (\mu g/ml) = 22.9\ (A_{645}) - 4.68\ (A_{663}) \quad (2)$$

$$\text{Total chlorophyll } (\mu g/ml) = \text{chl a} + \text{chl b} \quad (3)$$

Where; A = Absorbance, Chl a = Chlorophyll a, Chl b = Chlorophyll b.

Chlorophyll fluorescence was measured from three randomly selected plants leaves in each plot from each treatment using Hansatech Instruments Ltd Handy PEA Data. The measurements were done of the time between 8:00am and 9:00am hours.

2.5.2. Nodule Determination

Number of nodules was taken from five randomly selected plants at the mid-flowering stage from each plot and counted carefully to determine the average number of nodules $plant^{-1}$. Nodules dry weight after recording the fresh weight, the same nodules were oven-dried at 70°C for 48hr to determine nodule dry weight.

2.5.3. Yield Related Parameters

Number of pods $plant^{-1}$ was determined from ten plants harvested from three central rows of each plot and the average was taken as the number of pods $plant^{-1}$. Number of seeds pod^{-1} the plants harvested for pod number determination were threshed and then total seeds were divided by total pods to calculate the average seed number pod^{-1}. Above ground biomass yield (t ha^{-1}) it was measured from plants manually harvested from the central rows of each plot. The harvested plants were sun dried in an open air four days and the average total biological yield was reported in t ha^{-1}. Grain yield (t ha^{-1}) the harvested plants from central rows for biological yield determination were threshed and weighed, then converted to t ha^{-1} to determine the grain yield.

2.6. Statistical Analysis

All data collected were subjected to analysis of variance (ANOVA) appropriate to factorial experiment in an RCBD by statistical analysis system using the General Linear Model SAS version 9.0 [44]. Treatments means were compared using the least significant difference (LSD) at 5% level of significance.

3. Results and Discussion

3.1. Effect of Intercropping on Physiological Gas Exchanges

3.1.1. Chlorophyll (Chl a) and (Chl b)

The main effect of spatial arrangement showed a highly significant ($P < 0.001$) difference in chlorophyll A (Chl a) and Chlorophyll B (Chl b) (Table 2). The highest each of Chl a, (11.05 mg/g) and Chl b (4.24 µg/ml) was observed in sole common bean. Lowest was recorded at both intercropping Chl a, and Chl b (Table 2). Significantly higher chlorophyll A, (62%) and Chlorophyll B, (75%) were obtained from the sole cropping as compared to both intercropping treatments (Table 2). The increment in chlorophyll A and Chlorophyll B with the sole cropping might be due to light-harvesting maximization in the condition of a light distribution involved in the photosynthetic reaction [17]. Under the intercropping system, lowest Chl a, and Chl b, might be due to low light availability to the plant's leaf and the shading effect reduced leaf thickness. Similar studies have shown shading effects response minimizes light-harvesting in the intercropping condition through reduced chlorophyll a/b [17]. In conformity with the current result [48]. Reported, Chl a, Chl b contents decrease as the shading density increases. This is because Chl a, and Chl b are important elements as leaves pigment that affect by maize canopy than sole common bean [45].

Table 2. *Main effect of spatial arrangement on chlorophyll a/b, transpiration rate (E), and intercellular CO_2 concentration (Ci) of common bean.*

Treatments	Chlorophyll µg/ml		700nm	
	Chl a	Chl b	E mmol m^{-2} s^{-1}	Ci mmol ml^{-1}
Variety				
Nassir	8.26a	3.06a	3.16a	419.57a
Hawassa Dume	8.42a	3.12a	2.89a	420.83a
Spatial arrangement				
Sole	11.05a	4.24a	3.95a	427.89a
M1: 1CB	7.16b	2.63b	2.60b	416.23b
M1: 2CB	6.82b	2.41b	2.53b	416.47b
Inoculation				
Inoculated	8.63a	3.21a	3.06a	420.32a
Un-inoculated	8.05a	2.97a	2.99a	420.08a
LSD (0.05)	2.46	1.02	0.76	16.50
CV%	17.35	19.49	13.84	1.80

Means followed by the same letter are not significantly different at $P < 0.05$.

3.1.2. Transpiration Rate

The main effect of spatial arrangement showed a highly significant ($P < 0.001$) difference in transpiration rate (E) at 700 nm (Table 2). The highest transpiration rate (E) was observed in sole cropping common bean at 700 nm (3.95 mmol $m^{-2}s^{-1}$.). Both spatial arrangement treatments resulted in lower transpiration rate (Table 2). As compared to sole cropping, common bean the transpiration rate significantly decreased by 75.38% under both intercropping treatments at 700 nm (Table 2). The increment transpiration rate (E) under sole cropping might be due to the leaves have greater photosystem activity and better light intensity which may be resulted in a higher transpiration rate. Similar result was found by [49]. Recorded transpiration rates of 3.2, 4.1 mmol $m^{-2}s^{-1}$ in two different peanut crops at 62 days after sowing under open conditions. Similarly, [5] showed that transpiration rate (E) ranging from 2.70 to 3.29 mmol m^{-2} s^{-1} in ten different cowpea genotypes. There was a reduction of transpiration rate (E) under intercropping might be due to the shading effects of maize canopy and reduced light intensity which may resulted to a lower transpiration rate. According to [38], transpiration is a necessary evil as it is a vital unavoidable phenomenon of plants.

3.1.3. Intercellular CO_2 Concentration

The main effect of spatial arrangement showed a highly significant ($P < 0.001$) difference in intercellular CO_2 concentration (Ci) at 700 nm (Table 2). The highest intercellular CO_2 concentration was observed in sole cropping common bean (427.89 micromole mol^{-1}) at 700 nm. Whereas both spatial arrangement treatments resulted in lower intercellular CO_2 concentration (Table 2). The increased intercellular CO_2 concentration in sole cropping might be due to sun exposed leaves with greater photosystem activity, speed of electron transport, quantum yield, carboxylation efficiency, and photosynthetic capacity as compared to shaded leaves [26]. Similar result was found by [40], who reported that sole plants might show increased growth due to higher stomatal opening, leading to increased uptake of CO_2. The lower intercellular CO_2 concentration was observed under intercropping that might be shading of maize crop canopy, which reduced CO_2 availability that regulates stomatal opening and closing [31].

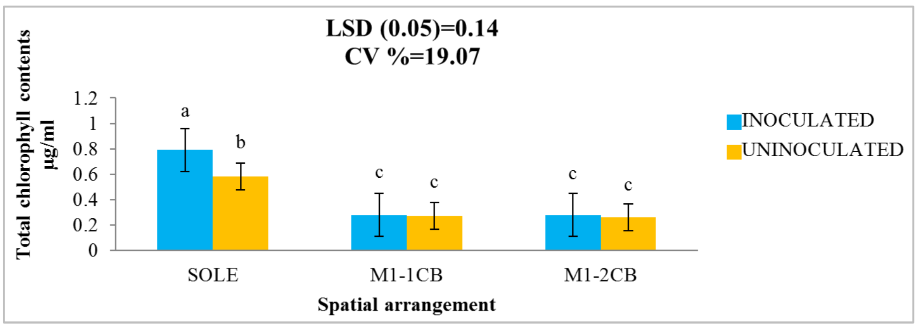

Figure 1. *Interaction effects of spatial arrangement and inoculation on total chlorophyll contents of common bean.*

3.2. Effect of Inoculation on Gas Exchange

3.2.1. Total Chlorophyll Contents

Total chlorophyll contents were significantly ($P < 0.01$) affected by the interaction effects of spatial arrangement and inoculation (Figure 1 and Table 3). The highest total chlorophyll content (0.79µg/ml) was recorded on the sole cropping with inoculated treatments. Both spatial arrangements (M1-1CB and M1-2CB) on both inoculated and un-inoculated treatments resulted in lowest total chlorophyll content (Figure 1 and Table 3). The increase in total chlorophyll content might be due to better light use efficiency and the *Rhizobium* strains (HB-429) enhanced soil fertility which contributed to leaf color change, chlorophyll content play a critical role in plant growth and greatly to the appearance of plants [23]. Similar results were found by [10] which showed that common bean inoculated with *Rhizobium* strains (HB-429) had increased leaf chlorophyll content compared with that of un-inoculated plants. It is evident from different literature that *Rhizobium* inoculation and mineral element supplementation increase the chlorophyll content of leaves, and hence improves plant biomass production [10]. The decrease of leaf chlorophyll contents under both intercropping with inoculated and un-inoculated conditions. This might be due to directly related to light shading effects of maize crop, which affect leaf chlorophyll contents. This indicates that the greenness of plant leaves in intercropped and leaf pigment lowest responds to the total chlorophyll contents. The intercropping practice has been reported by many researchers [28].

3.2.2. Photosynthetic Rate

Photosynthetic rate was significantly ($P < 0.01$) affected by the interaction effects of spatial arrangement and inoculation at 700 nm (Figure 2 and Table 3). The highest photosynthetic rate (25 µmol m^{-2} s^{-1}) was recorded on sole cropping with inoculated at 700 nm. Whereas, both intercropped with inoculated and un-inoculated resulted in lowest photosynthetic rate at 700 nm (Figure 2 and Table 3). The increase photosynthetic rate in sole cropping common bean and *Rhizobia* inoculation (HB-429) might be due to increased chlorophyll contents that is necessary in the photosynthesis *Rhizobium* also contribute for the increased leaf photosynthesis as it supplies nitrogen [55].

Similar result was recorded by [56] which indicates maximum photosynthetic rates of 23.1, 22.9 and 22.3 µmol m^{-2} s^{-1} in different bean genotypes under normal condition. Similarly, [52] reported that sole cropping and effective *Rhizobium* bacteria condition in the soil increased photosynthetic rate. The reduction of photosynthesis rate under intercropping condition might be due to the shading of main crop canopy reduced light intensity, which may result in lower photosynthesis rate. Similar finding by [50] showed that heavy shade results in reduce photosynthesis due to the decrease in Photosystem II and electro transport rate. The reductions in photosynthetic rate is reported to occur due to two main reasons; either due to decreased CO_2 diffusion into leaves, decrease in intercellular CO_2 and stomatal conductance, or due to inhibition of photosynthesis by inhibition of the leaf growth and enlargement by controlling the cell proliferation [51].

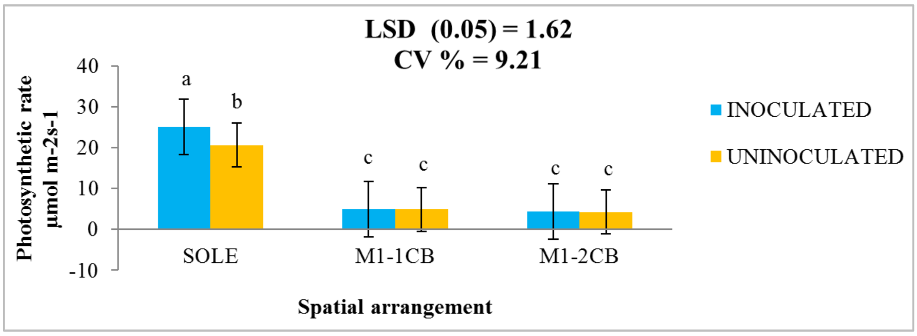

Figure 2. *Interaction effects of spatial arrangement and inoculation on photosynthetic rate µmol m $^{-2}$ s $^{-1}$ at 700 nm of common bean.*

3.2.3. Chlorophyll Fluorescence

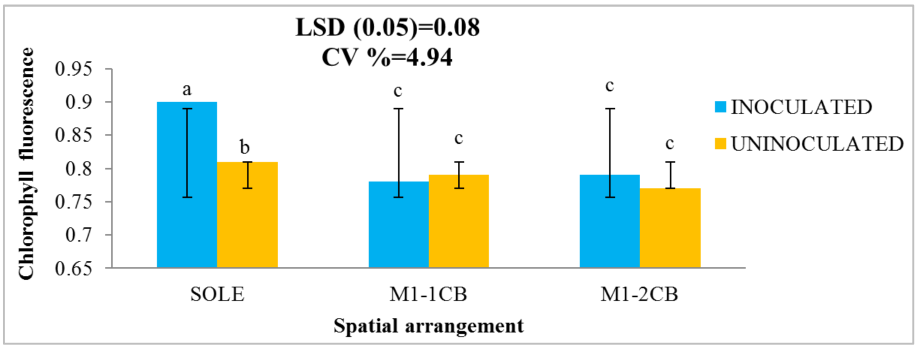

Figure 3. *Interaction effects of spatial arrangement and inoculation on chlorophyll fluorescence of common bean.*

Results of analysis of variance showed that the interaction effects of spatial arrangement and inoculation had significantly influenced chlorophyll fluorescence (Fv/Fm) (Figure 3 and Table 3). The highest chlorophyll fluorescence (0.90) was recorded for inoculated common beans on sole cropping. Whereas, both spatial arrangements with both inoculated and un-inoculated treatments resulted in lowest chlorophyll fluorescence (Figure 3 and Table 3). The increase chlorophyll fluorescence in sole cropping with effective *Rhizobium* bacteria condition in the soil might be due to changes in leaf photosynthetic performance and higher absorbed light energy. Leaf photosynthetic rate was increased dramatically with the development of the light addition technique which could resolve chlorophyll fluorescence quenching into photochemical efficiency higher under sole cropping better light intensity [12]. Lower chlorophyll fluorescence might be due to directly related to light shading effects as this affect chlorophyll fluorescence. Similarly, [25] indicated that chlorophyll fluorescence reduced leaf photosynthetic performance as shading increases. This might be due to photochemical efficiency was lower under intercropping which reduced light intensity. Moreover, [41] reported induced chlorophyll degradation reduced photosynthesis and chlorophyll fluorescence under shading conditions.

3.3. Effect of Treatments on Nodulation of Common Bean

3.3.1. Number of Nodules Plant^{-1}

Number of nodules plant^{-1} was significantly (P<0.01) affected by the interaction effects of spatial arrangement and inoculation (Table 3). The highest number of nodules plant^{-1} (47.70) was recorded from inoculated sole cropping plot. The lowest nodule number plant^{-1} was recorded from un-inoculated intercrops (Table 3). The increased number of nodules in sole cropping and inoculated plot might be due to improved light use efficiency and N nutrition because of the inoculation in the soil. Similar results were reported by several authors [46, 30]. These results are also in line with the findings of [6] who revealed a significant improvement in nodule number with inoculation sole cropping common bean.

3.3.2. Nodule Dry Weight Plant^{-1}

The nodule dry weight was significantly (P < 0.01) affected by the interaction effects of spatial arrangement and inoculation (Table 3). The highest nodule dry weight (0.56 g plant^{-1}) was recorded by sole cropping with R*hizobium* inoculant (HB-429). Significantly lower nodule dry weight was recorded by both intercropping with inoculated and un-inoculated (Table 3). The increased nodule dry weight in sole cropping and effective R*hizobium* strains (HB-429) might be due to in the soil more nodule formation. A similar promoting effect of seed inoculation on the dry weight of nodules plant^{-1} were reported by [36]. This result was in agreement with the work of [19]. Similarly, [11] studied the effect of nodulation on soybean and stated that inoculation significantly increased nodule dry weight of legumes under sole cropping condition. Similar effects of seed inoculation on nodule dry weight were also reported by [18].

Table 3. *Interaction effects of spatial arrangement x inoculation on total chlorophyll contents (TCC), chlorophyll fluorescence (CF), photosynthetic rate, number of nodules plant^{-1} (NN), and nodule dry weight plant^{-1} (NDW) of common bean.*

Treatments		**Parameters**				
Spatial arrangement	**Inoculation**	**TCC**	**CF**	**Photosynthetic rate**	**NN**	**NDW (g)**
Sole	Inoculated	0.79a	0.90a	25.0a	47.70a	0.56a
	Un-inoculated	0.58b	0.81b	20.6b	31.61b	0.30b
M1:1CB	Inoculated	0.28c	0.72c	4.8c	28.06c	0.24c
	Un-inoculated	0.27c	0.71c	4.7c	22.56d	0.20c
M1:2CB	Inoculated	0.28c	0.72c	4.6c	27.34c	0.23c
	Un-inoculated	0.26c	0.72c	4.5c	24.81cd	0.21c
LSD.(0.05)		0.14	0.08	1.62	6.81	0.06
CV%		19.07	4.94	9.21	13.12	12.92

Means followed by the same letter are not significantly different at P <0.05.

3.4. Effect of Treatments on Yield and Yield Components of Common Bean

3.4.1. Number of Pods Plant^{-1}

Number of pods plant^{-1} was significantly (P < 0.01) influenced by the interaction, of spatial arrangement and inoculation (Table 4). The highest pods number plant^{-1} (22.51) was recorded from inoculated sole cropping common bean. Whereas, the lowest number of pods plant^{-1} was recorded on under both intercropped with inoculated and un-inoculated (Table 4). The improvement in nodule number for the inoculation treatment can be associated with enhanced N nutrition due to N_2 - fixation. This is because improved N supply improves light use efficiency and reduced abortion and abscission of flowers and pods. Similarly, [7], who also reported that number of pods per plant increased due to *Bradyrhizobium* inoculation in sole soybean. The current result is in agreement with the work of [19] who reported an increased number of pods plant^{-1} with inoculation in green gram and sole cropping soybean. Lower number of pods per plant in both intercropped with and without inoculated might be due to the shading effect of maize as the main crop caused a reduction in physiological processes. A similar finding by [13] related this reduction of photosynthesis due to the shading of associated crops to a level that the legume plants compensated by decreasing the amount of assimilate allocation to reproductive growth or grain production.

3.4.2. Number of Seeds Pod^{-1}

Interaction effects of spatial arrangement and inoculation showed a significant (P < 0.01) difference for number of seeds pod^{-1} (Table 4). Highest number of seeds pod^{-1} (5.8) was record from inoculated sole cropping. Whereas, the lower number of seeds pod^{-1} was recorded from both special arrangements either inoculated or not (Table 4). The increment in number of seeds pod^{-1} might be due to the presence of sufficient light, the photosynthetic surface area of leaves to produce assimilates and *Rhizobium* inoculation provided nitrogen that the assimilates are ultimately needed for the production of seeds per pods. These results are in line with the findings of [32] who found, seed inoculation increased the number of seeds per pod in addition to grain yield. The reduction of a number of seeds pod^{-1} might be due to the intercropping conditions that reduce net canopy photosynthesis during these periods might have reduced flower-set and number of seeds. Similar result is in agreement with the finding of [33] reported that shading effects imposed by significantly reduced the photosynthetic assimilates which indicated that low light condition is unavailable to the legume plants flower to lower seed per pods. Similar result was also recorded by [53] on sorghum-haricot bean intercropping and they found significant difference in seeds per pods.

Table 4. *Interaction effects of spatial arrangement x inoculation on number of pods plant^{-1} (NPPP), number of seeds pod-^{1}, gain yield and above ground biomass yield (AGB) of common bean.*

Treatments		Parameters			
Spatial Arrangement	**Inoculation**	**No of Pod Pant^{-1}**	**Seeds No Pod^{-1}**	**Grain yield (t ha-^{1})**	**AGB (ton ha-^{1})**
Sole	Inoculated	22.51a	5.8a	2.54a	12.30a
	Un-inoculated	15.94b	4.7b	1.96b	10.60b
M1:1CB	Inoculated	9.05c	4.0c	1.35de	8.26de
	Un-inoculated	8.58c	4.0c	1.28e	7.93e
M1:2CB	Inoculated	9.51c	4.1c	1.54c	9.55c
	Un-inoculated	9.15c	4.1c	1.44cd	8.95cd
LSD. (0.05)		0.64	0.72	1.62	1.01
CV%		6.43	9.94	8.96	6.29

Means followed by the same letter are not significantly different at P <0.05.

3.4.3. Grain Yield

The analysis of variance for grain yield indicated significant ($P < 0.01$) influence of the interaction of spatial arrangement and inoculation (Table 4). Highest grain yield (2.54-ton ha^{-1}) was recorded for inoculated sole cropping, while lower grain yield was recorded when the un-inoculated with M1:1CB ratio (Table 4). Higher grain yield for sole cropping with inoculated plot might be, reduced competition to capture environmental resources (water, light, and nutrients). The results coincide with the findings of [42], who concluded that the treatments sole 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 sole cropping with *Rhizobium* inoculation. Similarly, [3] found that increased grain yield in inoculated plants may be attributed to the symbiotic relationship of *Rhizobium* (bacteria) with the roots of leguminous crops, which fix the atmospheric nitrogen into the roots. It may also be due to more nodule, and plant height, as according to the source sink relationship, more carbohydrates were produced due to more number of pods per plant and grain production. The lower grain yield from M1:1CB row arrangement might be associated with the shading effects of maize canopy and higher competition due to the extensive root system of maize. Similar result was found by [47], who indicated maize-common bean intercropping reduced seed yield by 80% common bean varieties.

3.4.4. Above Ground Biomass

Analysis of variance revealed that the above ground biomass yield was significantly ($P < 0.05$) influenced by the interaction effects of spatial arrangement and inoculation (Table 4). The highest above ground biomass (12.30-ton ha^{-1}) was recorded from inoculated sole cropping. M1-1CB arrangement with and without inoculation resulted with the lowest aboveground biomass (Table 4). The above ground biomass production in this experiment was highly responsive to the sole cropping and *Rhizobium* inoculation (HB-429). This might be due to important role sun light in an open area for better intercepted light and the *Rhizobium* inoculant (HB-429) add N which might result to higher biological yield. This finding was in agreement with [27] who reported that the highest biomass yield (kg/ha) was obtained from sole cropping faba bean. Similarly, [1] reported that above ground total biomass yield of soybean was increased up to 75% by the inoculation of different strains of *Rhizobia* as compared to un-inoculated. The reduction of above ground biomass under the intercropped might be due to the effect of shading of main crops resulted in lower aboveground biomass because of reduced plant growth. This result is in conformity with the finding reported by [15] where intercropping reduced soybean biological yield by 87% when compared with sole cropping, because of reduced plant growth and photosynthetic assimilation.

4. Conclusion

In this study inoculated sole common bean resulted in better physiological responses, nodulation formation, and economic yield than intercropping system. The physiological responses of plots were influenced by cropping systems that intercropping and un-inoculated reduced gas exchange and photosynthesis rate. The highest grain yield of 2.54 t ha^{-1} was recorded from on sole cropping common bean with *Rhizobium* inoculated (HB-429). Therefore, the observed improvement in physiological traits of inoculated and sole common bean varieties confirms the importance of *Rhizobium* inoculation to enhance the physiological performance and associated yield advantage of common bean varieties.

Acknowledgements

The authors gratefully acknowledged the financial support received from Hawassa University, in the forms of thematic project.

References

[1] Abbasi, M., Manzoor, M., & Tahir, M. (2010). Efficiency of *Rhizobium* inoculation and P fertilization in enhancing nodulation, seed yield, and phosphorus use efficiency by field grown soybean under hilly region of Rawalakot Azad Jammu and Kashmir, Pakistan. J. of Plant Nutrition, 33: 1080–1102.

[2] Abera, G., Wolde-meskel, E., & Bakken, R. (2012). Carbon and nitrogen mineralization dynamics in different soils of the tropics amended with legume residues and contrasting soil moisture contents. Biology and Fertility of Soils. 48 (1): 51-66.

[3] Ahmad, S., Habib, G., Muhammad, Y., Ullah, I., Durrani, Z., Pervaiz, U., & Rahaman, A. (2009). Effect of seed scarification, rhizobium inoculation and phosphorus fertilization on root development of barseem and soil composition. Sarhad J. Agric. 25 (3): 369-374.

[4] Akuda, M. (2001). Intercropping and population density effects on yield component, seed quality and photosynthesis of sorghum and soybean. J. Food Tech. Afri. 6 (3): 170-173.

[5] Anyia, O., & Herzog, H. (2004). Water use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. Europ. J. Agron. 20: 327-339.

[6] Argaw, A. (2012). Evaluation of Co-inoculation of *Bradyrhizobium* japonicum and phosphate solubilizing pseudomonas Spp effect on soybean (*Glycine max L.*) in Assosa area Journal of Agricultural Science Technology, (14): 213-224.

[7] Argaw, A. (2014). Symbiotic effectiveness of inoculation with *Bradyrhizobium* isolates on soybean (*Glycine max L.*) genotypes with different maturities. Springe Plus, 3: 753.

[8] Arnon, I. (1949). Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol; 24: 1-15.

[9] Awal, MAH., Koshi., & Ikeda, T. (2006). Radiation interception and use by maize/peanut intercrop canopy. Agricultural and Forest Meteorology. 139: 74-83.

[10] Bambara, S., & Ndakidemi, A. (2009). Effects of *Rhizobium* inoculation, lime and molybdenum on photosynthesis and chlorophyll content of *Phaseolus vulgaris* L. Afr. J. Microbiol. Res. 3 (11): 791- 798.

[11] Bhuiyan, MMH., Rahman, MM., Afroze, F., Sutradhar, GNC., & Bhuiyan, MSI. (2008). Effect of Phosphorus, Molybdenum and *Rhizobium* Inoculation on Growth and Nodulation of Mung bean. J. Soil Nat. 2 (2): 25-30.

[12] Bradbury, M., & Baker, NR. (1984). A quantitative determination of photochemical and non-photochemical quenching during the slow phase of the chlorophyll fluorescence induction curve of bean leaves. Biochimicaet Biophysica Acta 765, 275–281.

[13] Carruthers, K., Prithiviraj, B., Cloutier, DFeQ., & Martin, RC. (2000). Intercropping of corn with Soybean, Lupine and forages: Silage yield and quality. European Journal of Agronomy 12: 103-115.

[14] Central Statistical Agency (CSA). (2010). Statistical bulletin, Agricultural sample survey for 2010, Volume I. Report on area and production of major crops, private peasant holdings, Meher season. Addis Ababa, Ethiopia.

[15] Chui, N. (2003). Influence of spatial arrangements of maize on performance of an associated soybean intercrop, Fields Crop Research 8: 187-198.

[16] Day, P. (1965). Hydrometer method of particle size analysis. Methods of soil analysis. Agronomy, (9): 562-566.

[17] Evans, J., Mcneill, AM., Unkovich, MJ., Fettell, NA., & Heenan, DP. (2001). Net nitrogen balances for cool-season grain legume intercropping and contributions to wheat nitrogen uptake: a review. Aus. J. Exp. Agric. 41: 347-359.

[18] Getahun, A., & Abady, S. (2016). Effect of Maize (Zea may L.) on Common Bean (*Phaseolus vulgaris* L.) yield and its components in Maize-Bean intercropping. International Journal of Science and Research 5: 126-133.

[19] Ghosh, PK., Manna, MC., Bandyopadhyay, KK., Ajay, AK., Tripathi, RH., Wanjari, KM., Hati, AK., Misra, CL., Acharya, A., & Subba, R. (2006). Inter specific Interaction and Nutrient Use in Soybean/Sorghum Intercropping System. Bioresour. Technol. 98: 25–30.

[20] Havlin, JL., Beaton, JD., Tisdale, SL., & Nelson, WL. (1999). Soil fertility and fertilizers: An introduction to nutrient management. 6thed. New Jersey. Pp. 300–357.

[21] Hikosaka, K., Terashima, I. (1995). A model of the acclimation of photosynthesis in the leaves of C3 plants to sun and shade with respect to nitrogen use. Plant Cell Environ. 18, 605–618.

[22] Jackson, ML. (1958). Soil Chemical Analysis Practice Hall of India. New Delhi.

[23] Judkins, WP., & Wander, IW. (1950). Correlation between leaf color, leaf nitrogen content, and growth of apple, peach, and grape plants Plant Physiol; 25 (1): 78.

[24] Kaschuk, G., Kuyper, TW., Leffelaar, PA., Hungria, M., & Giller, KE. (2009). Are the rates of photosynthesis stimulated by the carbon sink strength of *Rhizobial* and arbuscular mycorrhizal symbioses, Soil Biol Biochem; 41 (6): 1233-1244.

[25] Khan, AG., Azim, A., Nadeem, MA., & Ayaz, M. (2000). The effect of formaldehyde treatment of solvent and mechanical extracted cottonseed meal on the performance, digestibility and nitrogen balance in lambs. Asian-Aust. J. Anim. Sci., 13 (6): 785-790.

[26] Larcher, W. (1995). Physiological Plant Ecology, 3rd ed.; Springer: Berlin, Germany; p. 506.

[27] Legesse, H., Gobeze, L., & Tenaw, W. (2015). Effect of barley (*Hordeum vulgare L.*) / faba bean (*Vicia fabae L.*) intercropping on productivity and land use efficiency in highlands of Southern Ethiopia. Journal of Biology, Agriculture and Healthcare; Vol. 5, No. 14.

[28] Lemlem, A. (2013). The effect of intercropping maize with cowpea and lablab on crop yield, Herald Journal of Agriculture and Food Science Research; 2 (5): 156– 170.

[29] Loomis, S., & Connor, J. (1992). Crop Ecology: Productivity and Management in Agricultural Systems. Cambridge University Press, Cambridge. pp. 104-128.

[30] Maphosa, M. (2015). Growth, nodulation and yield response of promiscuous and non-promiscuous soybean cultivars to inoculation in different soil types under glasshouse and field conditions. MSc Thesis.

[31] Martinis, S., Kuenzer, C., & Twele, A. (2015). Flood Studies Using Synthetic Aperture Radar Data. Remote Sensing of Water Resources, Disasters and Urban Studies; Taylor and Francis: London, UK.

[32] Muhammad, A. (2002). Production efficiency of chickpea as affected by inoculation, phosphorus levels and intercropping. PhD. (Agriculture) Thesis, University of Agricultural Sciences, Faisalabad. pp. 48-79.

[33] Muneer, S., Kim, E., Park, J., & Lee, J. (2014). Influence of green, red and blue light emitting diodes on multiprotein complex proteins and photosynthetic activity under different light intensities in lettuce leaves (Lactuca sativa L.). International Journal of Molecular Sciences, 15, 4657–4670.

[34] N2Africa. (2014). Better soybean through good agricultural practice. Africa Soil Health. Consortium. http://africasoilhealth.cabi.org/wpcms/wpcontent/uploads/2014/09/292-N2Africa-Ethiopia-common-beans-booklet.pdf

[35] NMA (National Meteorology Agency). (2020). Annual Meteorology data report, Hawassa, Ethiopia.

[36] Nyoki, D., & Ndakidemi, A. (2014). Effects of phosphorus and *Bradyrhizobium* japonicum on growth and chlorophyll content of cowpea (*Vigna unguiculata* (L) Walp). Am. J. Agric. 4: 1120-1136.

[37] Page, L. (1982). Methods of soil analysis. Part II. Chemical and Microbiological Properties.

[38] Pandey, N., & Sinha, K. (1996). Plant Physiology (3rd revised ed.). Vikas publishing house PVT LTD, New Delhi, India.

[39] Porch, T. (2013). Use of Wild Relatives and Closely Related Species to Adapt Common Bean to Climate Change, Agronomy, 3 (2), pp. 433–61.

[40] Roriz, M., Carvalho, SMP., & Vasconcelos, MW. (2014). High relative air humidity influences mineral accumulation and growth in iron deficient soybean plants. Front. Plant Sci: 5-726.

[41] Sairam, RK., Kumutha, D., Ezhilmathi, K., Deshmukh, PS., & Srivastava, GC. (2008). Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi-110012, India. BIOLOGIA PLANTARUM 52 (3): 401-412.

[42] Sajid, MA., Rab, F., Wahid, SNM., Shah, I., Jan, MA., Khan, SA., Hussain, MA., & Iqbal, Z. (2011). Influence of rhizobium inoculation on growth and yield of groundnut cultivars. Sarhad J. Agric. 27 (4): 573-576.

[43] Samago, TY., Anniye, EW., Dakora, FD. (2018). Grain yield of common bean (Phaseolus vulgaris L.) Varieties is markedly increased by rhizobial inoculation and phosphorus application in Ethiopia. Symbiosis 75, 245–255.

[44] SAS Institute Inc. (2002). SAS User's Guide, Statistics Version 9.0 ed. SAS Institute, Cary, NC, USA.

[45] Su, B., Song, Y., Song, C., Cui, L., Yong, T., & Yang, W. (2014). Growth and photosynthetic responses of soybean seedlings to maize shading in relay intercropping system in southwest china Photosynthetic 52: 332-340.

[46] Tarekegn, Y., & Walelign, W. (2014). Effect of NP Fertilizer Rate and *Bradyrhizobium* inoculation on Nodulation, N-Uptake and Crude Protein Content of Soybean (Glycine Max L.) at Jinka, Southern Ethiopia. Journal of Biology, Agriculture and Healthcare, 4 (6): 49-54.

[47] Walelign, W. (2008). Evaluation of common bean (*Phaseolus vulgaris* L.) genotypes of diverse growth habit under sole and intercropping with maize (*Zea mays* L.) in Southern Ethiopia. J. Agron. (7) 4: 306-313.

[48] Wittmann, C., Aschan, G., & Pfanz, H. (2001). Leaf and twig photosynthesis of young beech (Fagus sylvatica) and aspen (Populus tremula) trees grown under different light regime. Basic & Applied Ecology; 2: 145-154.

[49] Wright, C., & Bell, J. (1992). Plant population studies on peanut (*Arachis hypogaea* L.) in subtropical Australia Growth and water use during a terminal drought stress Aust. J. Exp. Agri. 32 (2): 197-203.

[50] Wu, Y., Gong, W., Wang, Y., Yong, T., Yang, F., Liu, W., Wu, X., Du, J., Shu, K., & Liu, J. (2018). Leaf area and photosynthesis of newly emerged trifoliolate leaves are regulated by mature leaves in soybean. – J. plant res. 131 (4): 671-680.

[51] Yang, F., Fan, Y., Wu, X., Cheng, Y., Liu, Q., Feng, L., Chen, J., Wang, Z., Wang, X., & Yong, T. (2018b). Auxin-to-gibberellin ratio as a signal for light intensity and quality in regulating soybean growth and matter partitioning. Fron. Plant Sci. 9: 56.

[52] Yao, X., Li, C., Li, S., Zhu, Q., Zhang, H., Wang, H., Yu, C., Martin, SKS., & Xie, F. (2017). Effect of shade on leaf photosynthetic capacity light-intercepting, electron transfer and energy distribution of soybeans. Plant Growth Regul. 83: 409-416.

[53] Yesuf, M. (2003). Effects of planting arrangement and population densities of haricot bean on productivity of sorghum/haricot bean additive mixture. M.Sc. Thesis, Alemaya University. Pp. 57.

[54] Zhang, S., Ma, K., & Chen, L. (2003). Response of photosynthetic plasticity of *Paeonia suffruticosa* to changed light environments. *Environ. Exper. Bot;* 49: 121-133.

[55] Zhou, XJ., Liang, Y., Chen, H., Shen, SH., & Jing, YX. (2006). Effects of *Rhizobia* inoculation and nitrogen fertilization on photosynthetic physiology of soybean Photosynthetic; 44: 530-535.

[56] Zlatev, S., & Yordanov, T. (2004). Effects of soil drought on photosynthesis and chlorophyll fluorescence in bean plants Bulg. J. Plant Physiol; 30: 3-18.