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Bradyrhizobium japonicum Inoculation and Phosphorus Supplementation on Growth and Chlorophyll Accumulation in Soybean (*Glycine max* L.)

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ABSTRACT

A field and glasshouse experiment was conducted to study the effect of *B. japonicum* inoculation and phosphorus supplementation on growth and leaf chlorophyll content in soybean. The treatments consisted of *B. japonicum* inoculation (with & without), phosphorus supplementation at the levels of 0, 20, 40 and 80 kgP·ha^{-1}. Both treatments were replicated four times in a split plot design. The following parameters were measured: plant height (cm), number leaves per plant, number of days to 50% flowering, number of days to 50% pod formation, stem girth and leaf area (LA). Results showed that inoculation with *B. japonicum* significantly increased plant height, number of leaves, leaf chlorophyll content, stem girth, leaf area (LA) and leaf area index (LAI). However, Phosphorus supplementation had significant effects in some parameters measured. The use of effective strain of *B. japonicum* and phosphorus supplementation was an efficient way of enhancing the growth of soybean.

Keywords: Days to 50% Flowering; Days to 50% Pod Formation; Legumes; Plant Height; Leaf Area (LA); Leaf Area Index (LAI); Number of Leaves per Plant; Stem Girth

1. Introduction

Nitrogen is a crucial element in the production of both leguminous and non-leguminous crops and has constructive impacts on growth of legumes [1,2]. It is a major constituent of chlorophyll, the most essential pigment needed for photosynthesis and amino acids. It is also found in other biomolecules such as ATP and nucleic acids [3]. Nitrogen is a factor in many biological compounds that plays a major role in photosynthetic activity. It is part of the enzymes associated with chlorophyll synthesis which reflect relative crop N status present in plants [4]. N is a building block of proteins and is highly needed for all enzymatic reactions in a plant [5]. It is a major part of the chlorophyll molecules and plays a necessary role in photosynthesis and also is a major component of several vitamins [6]. Nitrogen supply has significant effect on leaf growth because it increases the leaf area of plants and consequently it influences photosynthesis function [7]. Furthermore, in legumes and other leafy vegetables, N improves the quality and quantity of dry matter and protein [8]. However, green colour in the leaf is vanished due to Nitrogen deficiency and this may cause the decrease in leaf area and intensity of photosynthesis. This deficiency is also associated with symptoms of yellowing, dropping of leaves, poor growth, delayed flowering and fruiting [9]. Through this, it constitutes one of the major yield limiting factors for crop production. Inoculation with appropriate strain(s) of *B. japonicum* may be an effective way of increasing growth and leaf chlorophyll content in legumes.

Phosphorus is a fundamental component of the substances that are the building blocks of genes and chromosomes [8]. It is an essential part of the process of carrying the genetic code from one generation to the next, giving the blueprint for all characteristic of plant growth and development [10]. Plants need phosphorus for growth throughout their life cycle, especially during the early stages of growth and development. The primary

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role of phosphorus compounds in plants is to store and transfer energy produced by photosynthesis to be used for growth and reproduction [11]. Sufficient phosphorus is also required to enhance different plant organs growth, promote nodulation and early maturity in legumes [12]. Studies by Shahid, Saleem, Khan and Anjum, 2009 [13] indicated that, increased phosphorus application enhanced plant height significantly. Apart from growth, Gangasuresh, Muthuselvi, Muthulakshmi, Muthumari and Maniammal, 2010 [14] noted that, phosphorus is a crucial element in legume crop production which plays an important role for many characteristics such as sugar and starch utilization, photosynthesis, cell division and organization and nodule formation. Phosphorus is required in large quantities in young cells particularly shoots tips where metabolism is high and cell division is rapid. Insufficient levels of phosphorus may hinder plant growth; lower the chlorophyll accumulation which limits photosynthesis [15]. Furthermore, studies involving different types of crops have revealed that when phosphorus is limited, the most prominent effects are a reduction in leaf expansion, leaf surface area and the number of leaves [16]. Commonly, inadequate phosphorus slows the processes of carbohydrate utilization, development of a dark green leaf color or plants leaves developing a purple color [17]. Low levels of P and N found in most tropical soils, together with inadequate compatible rhizobial strain to a particular legume plants may result into poor plant growth including less chlorophyll formation and photosynthesis. Therefore, this study was conducted with the aim of assessing the effects of *B. japonicum* inoculation and phosphorus (P) supplementation on growth and chlorophyll accumulation in soybean grown in farmers' fields in northern Tanzania.

2. Materials and Methods

2.1. Narrative of Site Location

The glasshouse and field experiments were conducted at Northern zone of Tanzania of which, trials were laid down under irrigation and rain fed respectively. The glasshouse trial was conducted at Selian Agricultural Research Institute (SARI) from March, 2013 to May, 2013 under irrigation in glasshouse at Arusha-Tanzania. SARI is located at Latitude 3˚21′50.08″S and Longitude 36˚38′06.29″E. Field experiments was also conducted during long rain season in Tanzania Coffee Research Institute (TaCRI) located in Kilimanjaro region in the northern part of Tanzania. TaCRI receives the mean annual rainfall of 1200 mm with relative humidity of about 94% and elevation of 1268 masl. The mean maximum and minimum temperatures are 21.7˚C and 13.6˚C respectively.

2.2. Experimental Design

The glasshouse and field experimental treatments consisted of 2 levels of Rhizobia (with and without Rhizobia), 4 levels of P-supplementation (0, 20, 40 and 80 $kgP \cdot ha^{-1}$). The experimental design was a split plot with 4 replications per treatment. The soybean seeds were seeded at a spacing of 50 cm by 20 cm. Plot size measured at 4 m by 3 m. The plant population density was 200,000 plants per hectare. The *Bradyrhizobium japonicum* strain USDA 110 (Batch number 23011302, S) were obtained from *MEA* Company Nairobi-Kenya, sold under license from University of Nairobi. Soybean seeds (Soya 2 Variety) were obtained from the breeder based at Uyole Agricultural Research Institute, Mbeya, Tanzania. Land was cleared and all the necessary practices like ploughing, harrowing were done first before planting. Before sowing, the soybean seeds were thoroughly mixed with *B. japonicum* inoculants to supply (10^9 cells/g seed), following procedure stipulated by products manufacturer. To avoid contamination, all un-inoculated seeds were sown first, followed by inoculated seeds. Three seeds were sowed and thinned to two plants per hill after full plant establishment.

2.3. Determination of Chlorophyll (Chl) Contents in Plant Leaves

Extraction of chlorophyll concentrations by dimethylsulphoxide (DMSO) was done as described in [18]. A third of the plants leaves from the tip were collected from each pot and/or plot. A hundred (100 mg) of the middle portion of fresh leaf slices was placed in a 15 ml vial containing 7 ml Dimethylsulphoxide (DMSO) and incubated at 4˚C for 72 hours. After the incubation, the extract was diluted to 10 ml with DMSO. The DMSO technique extracts chlorophyll from shoot tissue without grinding or maceration [18]. A 3 ml sample of chlorophyll extract was then transferred into curvets for absorbance determination. A spectrophotometer (2800 UV/VIS Spectrophotometer) was used to determine absorbance values at 645 and 663 (nm), which was then used by [19] to determine total leaf chlorophyll expressed as mgL^{-1} [19].

The equation is expressed as follow; Chlorophyll total ($Chlt = 20.2D_{645} + 8.02D_{663}$).

2.4. Study of Growth Parameters in Soybean Plant

Growth parameters in each experimental site were collected until physiological maturity of the crop. The following growth parameters were collected in each site as follows: Plant height was taken using a meter rule. The height of the plant was measured from the base to the growing tip of the shoot in (cm) in every two weeks in-

terval starting from 2 weeks after planting (WAP) up to 8 WAP. In field experiment, 10 plants were randomly selected in the two middle rows from each field plot for measuring the height of the plant at different stages of the soybean growth. The same procedure was also applied to the glasshouse, but, only the two plants in each pot were measured for height. After recording the data, the average was worked out to get a representative plant height from each plot and pot.

Number of leaves per plant was recorded from 2WAP up to 8WAP. 10 plants were randomly selected from middle rows and the number of leaves counted. This was conducted in the same interval to the height of the plant at different stages of the soybean growth. The same exercise was also conducted for the glasshouse experiment and the average worked out as well.

The number of days to 50% flowering was scored. In glasshouse experiment, the number of days to 50% flowering started at 41 - 44 days after planting, followed by pod formation at 46 - 49 days. In field experiment, the number of days to 50% flowering was scored from 46 - 49 days and 51 - 54 days for pod formation respectively.

Stem girth (mm) was measured at physiological maturity using a veneer caliper in both glasshouse and field experiments and values recorded.

At physiological maturity, a leaf area (LA) was measured using a meter ruler by measuring the length (cm) and width (cm) of the leaf. This was carried out by selecting 5 plants randomly and average worked out. Eventually, the leaf area was used to calculate the leaf area index (LAI) of the soybean plant as follows: (Leaf area index (LAI) = average leaf area (cm^2)/land area covered by the plant (cm^2).

2.5. Statistical Analysis

A 2-way ANOVA was used to analyze data collected. The analysis was done using STATISTICA software program 2013. Fisher's least significant difference was used to compare treatment means at $p = 0.05$ [20].

3. Results

3.1. Effects of *B. japonicum* Inoculation and P Supplementation on Plant Height (cm) and Number of Leaves per Plant

The results in **Tables 1** and **2** indicate that rhizobial inoculation increased plant height (cm) and the number of leaves per plant significantly. The plant height for field experiment increased with rhizobial inoculation for the entire interval of the soybean growth as follows: At 2 weeks after planting (WAP), 4, 6 and 8WAP, the plant height (cm) increased by 16%, 35%, 51% and 29% re-

Table 1. Effects of *B. japonicum* and Phosphorus supplementation on Plant height (cm) measured during the soybean (*G. max* L.) growth.

	Gl asshouse				Field			
	Plant height (cm)							
Tre atments	2WAP	4WAP	6WAP	8WAP	2WAP	4WAP	6WAP	8WAP
B. japonicum								
−R	32.94 ± 1.02 a	55.80 ± 1.80 a	74.88 ± 2.31 a	97.38 ± 10.33 a	7.74 ± 0.33 b	10.38 ± 0.26 b	21.27 ± 0.38 b	25.37 ± 0.67 b
+R	35.08 ± 0.62 a	57.79 ± 1.29 a	76.25 ± 1.69 a	100.13 ± 12.37 a	9.00 ± 0.12 a	14.03 ± 0.29 a	32.02 ± 0.36 a	32.73 ± 0.47 a
Phosphorus ($kg \cdot ha^{-1}$)								
0	32.61 ± 1.64 a	59.85 ± 2.71 a	74.50 ± 2.76 a	115.13 ± 20.07 a	8.53 ± 0.24 a	12.83 ± 0.73 a	26.82 ± 2.39 a	30.37 ± 0.96 a
20	35.44 ± 1.16 a	56.88 ± 1.13 a	76.88 ± 3.67 a	104.25 ± 16.60 a	8.40 ± 0.29 a	12.35 ± 0.67 a	26.55 ± 2.09 a	29.31 ± 1.78 a
40	34.84 ± 0.58 a	56.50 ± 2.39 a	75.50 ± 2.49 a	102.5 ± 3.09 a	8.66 ± 0.25 a	12.00 ± 0.88 a	26.05 ± 2.07 a	28.59 ± 1.81 a
80	33.14 ± 1.26 a	53.96 ± 2.21 a	75.38 ± 2.79 a	73.13 ± 16.96 a	7.90 ± 0.70 a	11.65 ± 0.83 a	27.17 ± 1.79 a	27.94 ± 1.68 a
2-Way ANOVA (F-Statistics)								
R	3.10 ns	0.77 ns	0.19 ns	0.03 ns	12.10^{**}	90.65^{***}	424.54^{***}	85.58^{***}
P	1.23 ns	1.12 ns	0.09 ns	1.28 ns	0.83 ns	1.72 ns	0.81 ns	1.71 ns
R*P	0.41 ns	0.41 ns	0.08 ns	1.02 ns	0.49 ns	0.61 ns	1.21 ns	0.94 ns

−R: Without *B. japonicum*; +R: With *B. japonicum*. Values presented are means ± SE. **, *** = significant at $p \leq 0.01$, $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter in a given column are not significantly difference from each other at $p = 0.05$. WAP = Weeks after planting.

Table 2. Effects of *B. japonicum* and Phosphorus supplementation on the number of leaves counted during soybean (*G. max* L.) growth.

	Gl asshouse				Field			
	Number of le aves							
Tre atments	2WAP	4WAP	6WAP	8WAP	2WAP	4WAP	6WAP	8WAP
B. japonicum								
−R	2.88 ± 0.09 a	6.44 ± 0.29 a	13.06 ± 0.49 a	17.19 ± 0.52 a	3.25 ± 0.11 b	4.88 ± 0.15 b	10.00 ± 0.26 b	11.88 ± 0.49 b
+R	2.94 ± 0.06 a	6.88 ± 0.33 a	13.25 ± 0.45 a	17.88 ± 0.46 a	3.88 ± 0.09 a	5.75 ± 0.14 a	12.25 ± 0.21 a	18.06 ± 0.68 a
Phosphorus (kg·h a^{-1})								
0	2.75 ± 0.16 a	7.38 ± 0.37 a	14.00 ± 0.33 a	17.75 ± 0.88 a	3.63 ± 0.18 a	5.63 ± 0.18 a	11.38 ± 0.65 a	14.25 ± 0.98 a
20	3.00 ± 0.00 a	6.88 ± 0.39 ab	13.88 ± 0.65 a	17.63 ± 0.71 a	3.63 ± 0.18 a	5.38 ± 0.23 ab	11.00 ± 0.42 a	16.63 ± 1.57 a
40	3.00 ± 0.00 a	6.63 ± 0.49 ab	12.5 ± 0.72 a	17.50 ± 0.73 a	3.63 ± 0.18 a	5.38 ± 0.32 ab	10.63 ± 0.65 a	14.5 ± 1.24 a
80	2.88 ± 0.13 a	5.75 ± 0.33 b	12.25 ± 0.73 a	17.25 ± 0.53 a	3.38 ± 0.18 a	4.88 ± 0.26 b	11.5 ± 0.33 a	14.5 ± 1.74 a
2-Way ANOVA (F-Statistics)								
R	0.33 ns	1.12 ns	0.08 ns	0.85 ns	18.75***	21.00***	46.29***	53.95***
P	1.22 ns	2.71 ns	1.86 ns	0.08 ns	0.75 ns	2.71 ns	1.43 ns	1.7 ns
R*P	0.33 ns	0.45 ns	0.27 ns	0.44 ns	0.75 ns	1.57 ns	0.86 ns	0.28 ns

−R: Without *B. japonicum*; +R: With *B. japonicum*. Values presented are means ± SE. *** = significant at $p \leq 0.001$ respectively, ns = not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$. WAP = Weeks after planting.

spectively relative to the control treatment. This increase was also observed on the number of leaves by 19%, 18%, 23% and 52% at 2, 4, 6 and 8 WAP respectively. However, P supplementation did not show any significant effects on plant height in both glasshouse and field experiment.

3.2. Effects of *B. japonicum* Inoculation and P Supplementation on Stem Girth (mm)

Results in **Table 3** shows that ***B. japonicum*** inoculation significantly increased the stem girth in the glasshouse and field experiment. Stem girth increased by 6% and 25% due to inoculation for the glasshouse and field experiment respectively. In glasshouse, phosphorus supply showed significant increase in stem girth by 9%, 15%, and 12% by supplementing with 20, 40 and 80 kgP·ha^{-1} respectively relative to the control treatment. However, in the field experiment, phosphorus significantly increased the stem girth by 18%, 22% and 32% by supplementing with 20, 40 and 80 kgP·ha^{-1} respectively relative to the control treatment.

3.3. Effects of *B. japonicum* Inoculation and P Supplementation on the Number of Days to 50% Flowering and Number of Days to 50% Pod Formation

Table 3 results indicate that rhizobial inoculation significantly induced late flowering and pod formation in soybean in both glasshouse and field experiment. Rhizobia inoculation significantly delayed flowering by 6% and 3% in both glasshouse and field experiment respectively relative to the control treatment. Furthermore, rhizobial inoculation in field study significant increased the number of days to 50% pod formation in field experiment by 3% relative to the control. Phosphorus supplementation in field experiment at rate of 20, 40 and 80 kg·Pha^{-1} significantly delayed pod formation compared with the control (0 kgP·ha^{-1}) treatment.

3.4. Effects of *B. japonicum* Inoculation and P Supplementation on Leaf Area (LA) and Leaf Area Index (LAI)

In this study, rhizobial inoculation had significant effects on Leaf area (LA) in both glasshouse and field experiment relative to the control treatment. Inoculated soybean significantly increases LA by 31% and 157% in the glasshouse and field experiment respectively relative to the control. Phosphorus supplementation in field experiment showed significant increase in LA by 11%, 29% and 44% through supplying 20, 40 and 80 kgP·ha^{-1} respectively relative to the control (**Table 3**).

The LAI was not significantly influenced by rhizobial inoculation and phosphorus supplementation in glass-

Table 3. Effects of *B. japonicum* and Phosphorus supplementation on stem girth, number of days to 50% flowering, number of days to 50% pod formation and Leaf area (LA) and Leafarea index (LAI) in soybean (*G. max* L.) grown during 2013 cropping season.

	Glasshouse					Field				
Treatments	Stem girth (mm)	No. days to 50% flowering	No. days to 50% pod form ation	Leaf area (cm)	Leaf area index (cm)	Stem Girth (mm)	No. days to 50% flowering	No. days to 50% pod formation	Leaf area (cm)	Leaf area index (cm)
B. japonicum										
-R	3.46 ± 0.10 b	40.69 ± 0.18 b	47.88 ± 0.20 a	63.12 ± 1.89 b	0.46.00 ± 0.01 a	4.17 ± 0.01 b	46.06 ± 0.27 b	51.81 ± 0.23 b	107.97 ± 8.62 b	0.38 ± 0.02 b
+R	3.73 ± 0.09 a	42.94 ± 0.28 a	48.00 ± 0.18 a	82.94 ± 4.01 a	0.47.00 ± 0.01 a	5.20 ± 0.01 a	47.31 ± 0.35 a	53.13 ± 0.29 a	277.32 ± 10.38 a	0.67 ± 0.08 a
Phosphorus ($kg \cdot h\ a^{-1}$)										
0	3.33 ± 0.10 b	42.00 ± 0.20 a	48.00 ± 0.27 a	70.48 ± 5.67 a	0.47.00 ± 0.01 a	3.97 ± 0.04 b	46.50 ± 0.50 a	51.75 ± 0.41 b	158.59 ± 29.78 c	0.36 ± 0.04 c
20	3.55 ± 0.18 ab	41.75 ± 0.18 a	48.00 ± 0.19 a	75.94 ± 6.52 a	0.46.00 ± 0.01 a	4.70 ± 0.03 a	46.75 ± 0.37 a	53.25 ± 0.25 a	178.03 ± 33.08 bc	0.43 ± 0.04 bc
40	3.79 ± 0.15 a	41.88 ± 0.17 a	47.63 ± 0.32 a	73.82 ± 7.04 a	0.44.00 ± 0.01 a	4.84 ± 0.04 a	46.63 ± 0.56 a	52.38 ± 0.42 ab	204.88 ± 35.28 ab	0.62 ± 0.12 ab
80	3.71 ± 0.07 a	41.63 ± 0.22 a	48.13 ± 0.30 a	71.89 ± 3.72 a	0.46.00 ± 0.01 a	5.23 ± 0.05 a	46.88 ± 0.58 a	52.50 ± 0.55 ab	229.08 ± 35.26 a	0.68 ± 0.12 a
2-Way ANOVA (F-Statistics)										
R	5.76^{*}	55.54^{***}	0.21 ns	17.24^{***}	0.01 ns	23.85^{***}	8.69^{**}	15.56^{***}	273.57^{***}	16.61^{***}
P	3.19^{*}	0.29 ns	0.62 ns	0.25 ns	0.94 ns	6.26^{**}	0.14 ns	3.42^{*}	9.06^{***}	4.56^{*}
R*P	2.44 ns	1.94 ns	1.17 ns	0.41 ns	0.51 ns	0.60 ns	2.61 ns	0.69 ns	0.30 ns	1.52 ns

-R: Without ***B. japonicum***; +R: With ***B. japonicum***. Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

house experiment. However, in field experiment, inoculation improved LAI by 76% and phosphorus supply improved LAI by 19%, 72% and 89% by supplementing 20, 40 and 80 kgP·ha^{-1} relative to the control.

3.5. Effects of *B. japonicum* Inoculation and P Supplementation on Chlorophyll (Chl) Content in Soybean Leaves

Results in **Table 4** shows that rhizobial inoculation significantly increased the total leaf chlorophyll content in both glasshouse and field experiments. Inoculated treatment showed significant increase in leaf chlorophyll content by 29% for measurements taken 4WAP for the glasshouse relative to the control. In the field experiment, rhizobial inoculation significantly increased the chlorophyll content by 70%, 62% and 90% for measurements taken at 2, 4, and 8WAP respectively. With regard to P supply, chlorophyll content was uniform in all treatments and was numerically but not significantly decreasing with increasing phosphorus (**Table 4**). However, in the field study, the leaf chlorophyll content was significantly influenced by P at 6 WAP. The highest chlorophyll content was recorded by supplying 40 kgP·ha^{-1}.

3.6. Interactive Effects of *B. japonicum* and P Supplementation on Leaf Chlorophyll Content (mgL^{-1}) in Soybean Plant

Results presented in **Figure 1** shows significant interaction between Rhizobia inoculation and phosphorus supplementation on the leaf chlorophyll content for glasshouse experiment. In the inoculated treatments, the highest chlorophyll value was recorded at 20 kgP·ha^{-1} compared with other levels. However, for un-inoculated treatment, the highest value was recorded at 0 kgP·ha^{-1} (**Figure 1**).

4. Discussion

In the present study, we assessed the effects of *B. japonicum* inoculation and phosphorus (P) supplementation on growth and chlorophyll accumulation in soybean (*G. max* L). This study clearly shows that, *B. japonicum* inoculation was supportive in improving growth parameters of soybean. The treatments supplied with *B. japonicum* inoculation had great positive response in leaf chlorophyll content and growth parameters measured such as plant height, number of leaves per plant, stem girth (mm), LA (cm^2) and LAI (cm^2), number of days to 50% flowering and number of days to 50% pod formation as compared with the control (**Tables 1-4**). These improvements in inoculated treatments could be attributed to improved biological nitrogen fixation by rhizobial inoculants which increased nitrogen supply to the plants and consequently improved the growth parameters of plant. Generally, the un-inoculated controls showed reduced growth in all growth parameters measured. Our results are similar to those reported by [15,21-29].

Table 4. Effects of *B. japonicum* inoculation and Phosphorus supplementation on leaf chlorophyll content (mg·L^{-1}) of the soybean (*G. maxi* L.).

	Glasshouse		Field		
	Leaf Chl content (mgL^{-1})				
Treatments	2WAP	4WAP	2WAP	4WAP	6WAP
B. japonicum					
−R	15.42 ± 0.82 a	19.81 ± 1.75 b	13.78 ± 0.48 b	15.49 ± 0.93 b	12.65 ± 0.38 b
+R	17.35 ± 0.72 a	25.47 ± 1.14 a	23.49 ± 0.71 a	25.08 ± 1.31 a	24.02 ± 1.14 a
Phosphorus (kg·ha^{-1})					
0	17.69 ± 1.25 a	25.77 ± 1.71 a	19.74 ± 1.55 a	18.08 ± 1.97 a	17.45 ± 2.18 ab
20	15.82 ± 1.09 a	22.98 ± 3.77 a	18.06 ± 1.79 a	19.38 ± 2.19 a	18.47 ± 2.41 ab
40	15.93 ± 0.79 a	21.61 ± 1.76 a	19.41 ± 2.31 a	23.01 ± 2.78 a	21.17 ± 2.84 a
80	16.09 ± 1.40 a	20.21 ± 0.85 a	17.34 ± 2.25 a	20.68 ± 2.42 a	16.25 ± 2.03 b
2-Way ANOVA (F-Statistics)					
R	3.11 ns	15.14***	138.32***	37.20***	113.42***
P	0.65 ns	2.66 ns	1.87 ns	1.79 ns	3.85*
R*P	1.39 ns	10.08***	0.83 ns	0.67 ns	0.74 ns

−R: Without *B. japonicum*; +R: With *B. japonicum*. Values presented are means ± SE. *, *** = significant at $p \leq 0.05$, $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$. WAP = Weeks after planting.

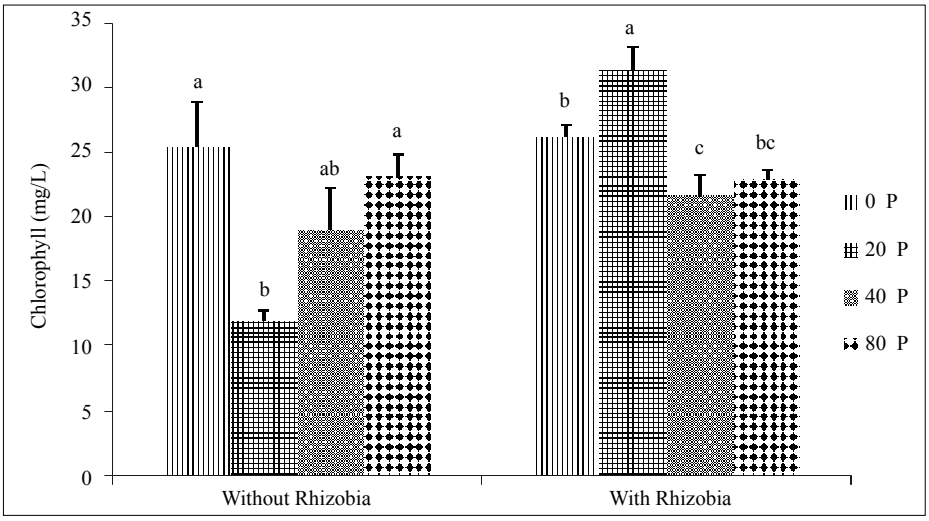

Figure 1. Interactive effects of *B. japonicum* and Phosphorus (kg·ha^{-1}) on leaf Chlorophyll content (mg·L^{-1}). 0 P = 0 kgP·ha^{-1}, 20 P = 20 kgP·ha^{-1}, 40 P = 40 kgP·ha^{-1}, 80 P = 80 kgP·ha^{-1}. Bars followed by similar letter(s) are not significantly different at *p* = 0.05.

Flowering and pod formation are the important stages in legume plant growth and development. The present study reveals that inoculated soybean delayed period for flowering and pod formation respectively. This could be due to sufficient nutrients present in the soil which influence the plant to complete its life cycle successful by growing vegetatively.

Inoculated treatments were as well more rich in leaf chlorophyll content as compared with un-inoculated treatments (**Table 4**). Anjum, Ahmed and Rauf, 2006 [30], report that beneficial rhizobia bacteria may influence the physiological growth conditions of leguminous plants by increasing chlorophyll contents in leaves. Similar to our work, other studies have also shown that *B. japonicum* increased the chlorophyll content in legume plant and finally ending up with improved plant growth [31-33]. The encouraging results obtained from this study demonstrates that rhizobia inoculation may substitute the expensive inorganic N fertilizers in improving plant growth and chlorophyll synthesis.

Results from this study revealed that phosphorus supplementation significantly improved some attributes of growth of the *G. max* L. (stem girth, LA, LAI and number of days to 50% pod formation) in the glasshouse and/or field studies compared with the control treatment. This improvement could be due to functional importance of phosphorus in legume growth which serves many functions such as sugar and starch utilization, cell division and organization, photosynthesis use efficiency and formation of green pigment in plant which finally improving plant growth and chlorophyll synthesis. Similar to our work, studies by [13,23,34-39] also revealed the significance of phosphorus in the growth and chlorophyll synthesis in legumes. Other workers have also reported that phosphorus is not only necessary for plant growth, but its availability might be very prominent in influencing the performance of the biological nitrogen fixation [40] an important process reputable in enhancing legume growth and productivity. However, phosphorus shortage has been revealed to be an important fertility problem limiting legume production mainly in the tropics. Therefore its exogenous supply to support plant growth in such system is of paramount importance as reported in this study.

5. Conclusion

In conclusion, *B. japonicum* inoculation was effective in improving most of growth parameters as well as leaf chlorophyll content. Furthermore, supplementing phosphorus significantly increased some growth parameters compared with the control. Therefore, it is strongly recommended to use appropriate rhizobial strain in the cultivation of legumes such as soybean. Such inoculants are good and cheaper alternative to expensive commercial nitrogen fertilizers to most small scale farmers in Africa.

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