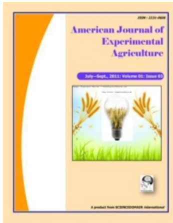

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Effects of Phosphorus and *Bradyrhizobium japonicum* on Growth and Chlorophyll Content of Cowpea (*Vigna unguiculata* (L) Walp)

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Authors' contributions

Authors DN and PAN designed this study, author DN conducted both field and screen house trials and performed statistical analysis with advice from author PAN. Author DN wrote the first draft of this manuscript, author PAN edited the manuscript. All authors read and approved the manuscript.

Original Research Article

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ABSTRACT

The field and screen house experiment was conducted at Tanzania Coffee Research Institute and Seliani Agricultural Research Institute respectively in Tanzania, between March-July 2013, to assess the effects of *Bradyrhizobium japonicum* and phosphorus on growth and total leaf chlorophyll content of cowpea. The experiment was out down in split-plot design whereby the main plots comprised two inoculation treatments (with and without *B. japonicum*), while the sub-plots contained phosphorus (TSP) application at four different levels (0 kg/ha, 20 kg/ha, 40 kg/ha, 80 kg/ha). Both experiments were replicated four times. We measured plant growth parameters such as plant height; number of leaves per plant at different stages of plant growth, the stem girth was measured by vernier caliper at physiological maturity. The chlorophyll content was determined at 3, 5, and 7 weeks after planting (WAP). The chlorophyll was extracted by using dimethylsulphoxide (DMSO) and absorbance was determined at 645 and 663nm using UV/Visible spectrophotometer. *B. japonicum* inoculation significantly increased the plant height, number of leaves per plants, and stem girth above the control. The height was increased by 11.23, 10.43 and 8.99% (screen-house) and by 8.11, 24.05, 9.29% (field) in the measurements taken at 4, 6, and 8 WAP respectively. Number of leaves per plant counted at 6 and 8 WAP increased by 14 and 10.8% (screen-house) and 14 and 11.6% (field) respectively. *B. japonicum* also

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significantly increased leaf chlorophyll content of cowpea by 26% (3 WAP) in screen-house and 52, 37.9 and 13.3% (3, 5 and 7 WAP) respectively on the field. Phosphorus also significantly increased different plant growth parameters and leaf chlorophyll content relative to control. *B. japonicum* inoculation can be as beneficial as inorganic N fertilizers. Therefore, their combined use with phosphorus boosts plant growth and chlorophyll content of the legumes and eventually legume production is increased.

Keywords: Legumes; nitrogen; photosynthesis; plant height; rhizobia; stem girth.

1. INTRODUCTION

Low level of soil P and N are the major constraints to crop growth and production in nutrients depleted sandy soil of Sub-Saharan Africa [1,2]. Phosphorus and nitrogen are essential elements playing major role in plant growth, development and finally determination of yield of the crops. Phosphorus (P) is found in every living plant cell and is involved in several key plant functions including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant [3,4] nucleic acids, phospholipids, ATP, and it makes about 0.2% of plant's dry weight [5]. It stimulates root development and growth of young plants, giving them a good and vigorous start [6,7] and control key enzyme reactions in the regulation of metabolic pathways [8]. Successful production systems based on legumes therefore, requires P inputs [9] either from soil reserves or from added fertilizer [10]. Other workers [11,12,10,4,13] reported that plant height, leaf area, shoot, grain yields, and root dry weights of the legume plants increased with P application. Supplementing legumes with P nutrients enhances symbiotic establishment for increased N_2 fixation [10,14]. Plant cell functions such as photosynthesis, respiration, biosynthesis and the transfer of organic solutes across membranes require inorganic phosphate for its formation from ADP. Phosphorus has been reported to increase chlorophyll content and play an important role in both energy storage and transfer as ADP and ATP (adenosine di- and tri-phosphate) and DPN and TPN (di- and tri- phosphopyridine nucleotide). It has equally been reported that shortage of inorganic phosphate in the chloroplast reduces photosynthesis [15].

Rhizobia inoculation in legumes is an alternative to the expensive inorganic nitrogen fertilizers and is known for stimulating plant growth [16]. Rhizobia provides N through symbiotic fixation of atmospheric nitrogen [17,18]. Nitrogen is a building block of proteins and the amino acids [19] which are required for cellular synthesis of enzymes, chlorophyll, DNA and RNA, and therefore, is important in plant growth [18,15]. The element is involved in many processes of plant growth and development which finally lead to yield and the quality of harvested organs (seeds or shoot biomass) [20]. Several research reports have indicated significant achievements in legume growth and yield in many parts of the world due to inoculation with the appropriate rhizobial inoculants [21,22,23,24,25,26,11,27,28,29, 30,12,10,16,17]. Inoculation of legumes with appropriate rhizobia has been reported to increase the leaf chlorophyll content of legume crops [16]. The increased chlorophyll content results in the increased photosynthesis and as a result plant gets enough carbohydrate for its growth and production. However, there is little information on the effects of phosphorus and *B. japonicum* on growth and chlorophyll content of cowpea in northern Tanzania. Therefore, it is important to study and establish the role that could be played by phosphorus and *B. japonicum* on growth and total leaf chlorophyll content of legumes grown under screen house and field conditions.

2. MATERIALS AND METHODS

2.1 Description of Location

Field and pot experiments were conducted at two different locations from mid March to late July 2013. A screen house experiment was conducted at Seliani Agricultural Research Institute (SARI) which is situated in an area which is 1390m above the sea level in Arusha, Tanzania of latitude 3°21'50.08''S and longitude 36°38'06.29''E. The field experiment was conducted at the Tanzania Coffee Research Institute which is situated in an area which is 1390m above the sea level in Kilimanjaro region, Tanzania of latitude (3˚14'44'')S and longitude (37˚14'48)E. The field experiment was conducted in an area with bimodal rainfall pattern and mean annual rainfall of 1200mm.

2.2 Experimental Design

The experiment was laid out in a split plot design. The main plots comprised two inoculation treatments viz. i) with inoculation of *B. japonicum* and ii) without inoculation of *B. japonicum.* Sub plots contained four levels of phosphorus (0, 20, 40, and 80 kg/ha). Both screen house and field experiments were replicated four times.

2.3 Field and Screen House Experiment

The crop plant used for this experiment was Cowpea (*Vigna unguiculata* (L) Walp) supplied by the breeder from Sokoine University of Agriculture, Morogoro, Tanzania. The *B. japonicum* used were Biofix legume inoculants for cowpea, purchased from MEA Fertilizer Company in Nairobi, Kenya. The inoculants packets were supplied with gum Arabic for sticking as many cells as possible into the cowpea seeds. The *B. japonicum* inoculant was applied according to manufacturer's instructions to supply 10^9 cells/g seed. This was done as follows: Three (3) gm of gum Arabic was added to two tablespoonful of distilled water and mixed to form a solution. 1kg of cowpea seeds was weighed and two tablespoonful of gum Arabic solution was added and mixed well. 10gm of legume inoculants was added and mixed well so that all seeds are coated. The inoculated seeds were put under shade to dry and the seeds were then sown immediately in a wet moist soil. The soil for screen house experiment was collected from the site where field experiment was conducted. The soil was packed into 4 kg pots. Four seeds were planted in each pot and later thinned to two after germination and uniform establishment. The field was ploughed and harrowed by using tractor before planting. The plant spacing was 50cm by 20cm and the plot dimension was 4m by 3m. In the field trial, three seeds were planted per hill and then thinned to two plants after germination and establishment. The plots were weeded twice. The first weeding was done two weeks after emergence and the second weeding was done just before flowering. Each plot had six rows. Both screen house and field experiments were conducted at the middle of March 2013, and closely monitored from this point until physiological maturity for field, and pod formation for screen house experiment.

2.4 Growth Data Collection

Data were collected from the four middle rows of each plot. The growth parameters measured from 14 days after planting (DAP) to pod formation in both screen house and field experiment were plant height using a meter rule (from ground surface to the tip), number of leaves per plant, stem girth using a vernier caliper and leaf area using formula

$LA= 11.98 + 0.06LW.$

Where; L= leaf length and W= leaf width according to [31].

Leaf length (L) (cm) and leaf width (W) (cm) were measured by direct leaflet area measurements using a meter rule. Length was measured as the distance between the base and the apex of the leaflet; width was measured at the position on the leaflet yielding the greatest width. Ten randomly selected representative plants from the four central rows in each plot for field experiment were used while one plant from each pot was used for screen house experiment to collect data. The number of days to 50% flowering and number of days to 50% pod formation was also counted as part of the growth data.

2.5 Chlorophyll Content Determination

Extraction of chlorophyll by dimethylsulphoxide (DMSO) was done as described in [32]. The third leaf of each plant counted from the tip was collected from each pot for screen house and five leaf samples were taken from five randomly selected plants for field experiment. A hundred (100) mg of the middle portion of fresh leaf slices was placed in a 15 mL vial containing 7 mL DMSO and incubated at 4ºC for 72 h. After the incubation, the extract was diluted to 10 mL with DMSO. The DMSO technique extracts chlorophyll from shoot tissue without grinding or maceration [32]. A 2 mL sample of chlorophyll extract was then transferred into curvets for absorbance determination. A spectrophotometer (UV/Visible Spectrophotometer) was used to determine absorbance at wavelengths 645 and 663 nm, which was then used in the equation proposed by [33] to determine total leaf chlorophyll content against DMSO blank, as shown below.

Chlorophyll total (Chlt = $20.2D_{645} + 8.02D_{663}$

Where “D” is the density at the respective wavelengths which was obtained from Beckman spectrophotometer.

2.6 Statistical Analysis

The data collected were analyzed using analysis of variance (ANOVA), with the computations being performed with the software program STATISTICA. The fisher least significance difference (L.S.D.) was used to compare treatment means at P=0.05 level of significance [34].

3. RESULTS

3.1 Effects of Rhizobia and Phosphorus on the Height of the Cowpea

The results in Table 1 clearly demonstrate that rhizobia inoculation had significant effect on the plant height assessed in this study. Rhizobia inoculation significantly improved the plant height measured at four, six and eight weeks after planting (WAP) in both screen house and field experiments relative to the control treatment (Table 1). For example, rhizobia inoculation increased the plant height by 11.23, 10.43 and 8.99% in the measurements taken at four, six and eight weeks after planting (WAP) respectively in the screen house experiment. For the field experiment, the rhizobia inoculation increased the plant height by

8.11, 24.05 and 9.29% in the measurements taken at four, six and eight weeks after planting respectively compared with the un-inoculated control.

From this study, phosphorus application significantly increased the plant height measured at four, six and eight weeks after planting in both screen house and field experiments as compared with control (Table 1). In this study, phosphorus application in the screen house at the rate of 20, 40 and 80 kg/ha significantly increased the plant height by 19, 24 and 30% (4WAP) respectively over the control. The measurements taken in the screen house at 6 and 8WAP showed that the application of phosphorus at the rate of 20, 40and 80 kg/ha significantly increased the plant height by 15.18, 16.8, 21.7% (6WAP) and 18, 23, 21.9% (8WAP) respectively over control treatment (Table 1). Table 1also indicated that the plant height measured at 4, 6 and 8WAP in the field experiment had positive response to phosphorus application at all levels. For example application of phosphorus at the rate of 20, 40and 80 kg/ha significantly increased plant height by 12.3, 11.8, 15.8% (4WAP); 9.8, 18.4, 27.2% (6WAP); and 14.9, 21.1, 28.8% (8WAP) respectively over the control.

3.2 Interactive Effect of *B. japonicum* and Phosphorus on Plant Height of Cowpea

This study showed significant ($P \leq .01$) interactive effect between *B. japonicum* and phosphorus on plant height of the cowpeas measured at six weeks after planting in the field experiment. Plant height increased with increase in phosphorus. The tallest plants resulted from application of 80 kg/ha while the shortest plants were from the control treatment. *B. japonicum* supplied with P at highest level gave significantly higher values of height compared with other treatments (Fig. 1- A).

3.3 Effects of *B. japonicum* Inoculation and Phosphorus on Number of Cowpea Leaves per Plant

In this study, *B. japonicum* inoculation significantly increased the number of leaves of cowpeas measured at 6 and 8WAP in both the screen house and the field experiments (Table 2) compared with the control treatments. *B. japonicum* did not show significant difference in the number of leaves measured from 2 and 4 WAP. The results in Table 2 shows that in the screen house experiment, supplying P at 40 and 80 kg/ha resulted in significant number of leaves measured at 6 and 8 WAP compared with other treatments. In field experiment, measurements taken at 6 WAP showed that P supplementation at 20, 40 and 80 kg/ha resulted in significant higher number of leaves per plant compared with the control treatment. However, at 8 WAP, supplying P at 40 kg/ha and 80 kg/ha resulted in significant higher number of leaves relative to the other treatments.

3.4 Interactive Effect of *B. japonicum* and Phosphorus on Number of Leaves per Plant

There was significant (P= .05) interactive effect between *B. japonicum* and phosphorus on number of leaves per plant of the cowpeas measured at six weeks after planting in the field experiment (Fig. 1B). *B. japonicum* inoculation supplied with P at any level significantly gave higher number of leaves per plant compared with the control.

Table 1. Effects of *B. japonicum* and phosphorus on plant height of cowpea grown under screen house and field condition

Treatments	Screen House				Field			
	Plant height (cm)				Plant height (cm)			
	2 weeks after planting	4 weeks after planting	6 weeks after planting	8 weeks after planting	2 weeks after planting	4 weeks after planting	6 weeks after planting	8 weeks after planting
Rhizobia								
-R	16.78±0.42a	22.08±0.58b	32.88±0.83b	120.88±3.59b	6.41±0.12a	10.23±0.14b	20.42±0.39b	49.94±1.71b
+R	17.18±0.36a	24.56±0.74a	36.31±0.73a	131.75±2.52a	6.77±0.19a	11.06±0.32a	25.33±0.77a	54.58±2.05a
P Levels ($Kg.ha^{-1}$)								
0	15.13±0.45b	19.69±0.43c	30.50±0.91c	109.13±3.80c	6.33±0.15a	9.67±0.15b	20.09±0.50d	44.98±2.12c
20	17.56±0.38a	23.50±0.82b	35.13±1.26b	128.88±3.22b	6.71±0.25a	10.86±0.36a	22.06±1.09c	51.66±2.35b
40	17.39±0.42a	24.50±0.73ab	35.63±0.91ab	134.25±2.82a	6.59±0.26a	10.81±0.31a	23.78±1.18b	54.48±2.20ab
80	17.84±0.34a	25.60±0.66a	37.13±0.48a	133.0±2.87ab	6.72±0.27a	11.23±0.41a	25.56±1.19a	57.93±2.39a
2 – Way ANOVA (F-Statistics)								
R	1.04ns	23.80***	27.58***	17.90***	2.21 ns	8.76**	181.91***	4.24*
P	10.43***	25.54***	19.07***	20.67***	0.58 ns	5.76**	41.41***	85.96***
R*P	1.53ns	0.6 ns	1.64ns	0.5 ns	0.35 ns	1.42 ns	6.65**	0.09 ns

+R: *With rhizobia;* ***-R:*** *Without rhizobia;* ***R:****Rhizobia;* ***P:*** *Phosphorus; Values presented are means ± SE; *; **; ***; significant at P= .05, P≤ .01, P≤ .001 respectively,* ***ns*** *= not significant,* ***SE*** *= standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at P= .05 according to Fischer least significance difference (LSD).*

Table 2. Effects of *B. japonicum* and phosphorus on number of leaves of cowpea grown under screen house and field condition

Treatments	Screen House				Field			
	Number of leaves (counted at)				Number of leaves (counted at)			
	2 weeks after planting	4 weeks after planting	6 weeks after planting	8 weeks after planting	2 weeks after planting	4 weeks after planting	6 weeks after planting	8 weeks after planting
Rhizobia								
-R	3±0.00a	4.75±0.14a	9.56±0.38b	19.69±0.41b	3.49±0.05a	6.13±0.21a	12.94±0.12b	26.85±0.89b
+R	2.94±0.06a	4.56±0.13a	10.94±0.35a	21.81±0.49a	3.48±0.05a	6.58±1.58a	14.77±0.33a	29.96±1.16a
P Levels ($Kg.ha^{-1}$)								
0	3±0.00a	4.63±0.18a	8.75±0.37c	18.88±0.35b	3.61±0.07a	6.10±0.13a	13.00±0.21b	25.29±0.70c
20	3±0.00a	4.75±0.25a	10.00±0.42b	20.00±0.60b	3.49±0.06ab	6.21±0.31a	14.13±0.52a	27.14±1.36bc
40	3±0.00a	4.50±0.19a	10.75±0.56ab	21.63±0.63a	3.48±0.08ab	6.95±2.21a	14.00±0.43a	29.53±1.58ab
80	2.9±0.13a	4.75±0.16a	11.5±0.42a	22.50±0.63a	3.36±0.08b	6.15±0.33a	14.28±0.60a	31.68±1.54a
2 – Way ANOVA (F-Statistics)								
R	ns	0.82 ns	13.2**	26.68***	0.0 ns	1.28 ns	45.38***	5.89*
P	ns	0.33 ns	9.6***	15.57***	1.8 ns	0.99 ns	4.56*	4.72*
R*P	ns	0.33 ns	0.69 ns	0.52 ns	0.3 ns	0.76 ns	3.85*	0.26 ns

***+R:** With rhizobia; **-R:** Without rhizobia; **R:** Rhizobia; **P:** Phosphorus; Values presented are means ± SE; *; **; ***; significant at P= .05, P≤ .01, P≤ .001 respectively, **ns** = not significant, **SE** = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at P= .05 according to Fischer least significance difference (LSD).*

3.5 Effects of *B. japonicum* Inoculation and Phosphorus Application on the Stem Girth, Leaf Area, Number of Days to 50% Flowering and Number of Days to 50% Pod Formation

Table 3 clearly indicates that *B. japonicum* inoculation significantly affected almost all growth parameters measured in this study compared with the control. All the growth parameters presented in Table 3 were significantly improved by *B. japonicum* inoculation except that leaf area with or without inoculation in the screen house experiment (Table 3). Inoculation of cowpeas with *B. japonicum* lowered the number of days to 50% flowering and pod formation in the screen house by 4.5 and 4% respectively, while it was vice versa in the field experiment where inoculation of cowpea seeds with *B. japonicum* increased the number of days to 50% flowering and pod formation by 2.32 and 2.4% respectively. Table 3 also shows that phosphorus supplementation at any level significantly affected the stem girth and leaf area of cowpea in both screen house and field experiments relative to control treatment. In addition, phosphorus supplementation at 20 kg/ha showed significant effects on number of days to 50% flowering and 50% pod formation in the screen house experiment compared with all other treatments. Supplying P at 20 kg/ha resulted in early flowering and pod formation in screen house experiment. However, in the field study, the results showed that supplying P at any level significantly affected the number of days to 50% flowering and pod formation compared with control.

3.6 Effects of *B. japonicum* Inoculation and Phosphorus on Total Leaf Chlorophyll Content of Cowpea

The results in Table 4 indicated that the *B. japonicum* inoculation had positive effects on the total leaf chlorophyll content of cowpeas measured from three weeks after planting at two weeks interval. The total leaf chlorophyll content measured at 3 WAP significantly increased with *B. japonicum* inoculation in both screen house and field experiment compared with un-inoculated pots and plots (Table 4). The total leaf chlorophyll content of cowpea measured at five and seven weeks after planting were also significantly increased with inoculation of *B. japonicum* in the field experiment. However, the total leaf chlorophyll content measured at the same time from the screen house experiment did not show significant difference from rhizobia inoculated and un-inoculated treatments (Table 4). In the screen house, P supplementation at all levels did not showed significant effect on the chlorophyll content of the cowpeas measured at 3, 5 and 7 WAP. Table 4 also shows that supplementation of phosphorus at all levels significantly increased the chlorophyll content of cowpea measured at 5 and 7 WAP compared with the control. Supplementation of P at 80 kg/ha (5 WAP) and 40 kg/ha (7 WAP) resulted in greater values of chlorophyll content compared with other treatments.

3.7 Interactive Effect of *B. japonicum* and Phosphorus on the Total Leaf Chlorophyll Content of Cowpea

There was significant (P= .05) interactive effect between *B. japonicum* and phosphorus on the total leaf chlorophyll content of cowpeas measured at three weeks after planting in the screen house and seven weeks after planting in the field experiment. (Fig. 2 A and B).

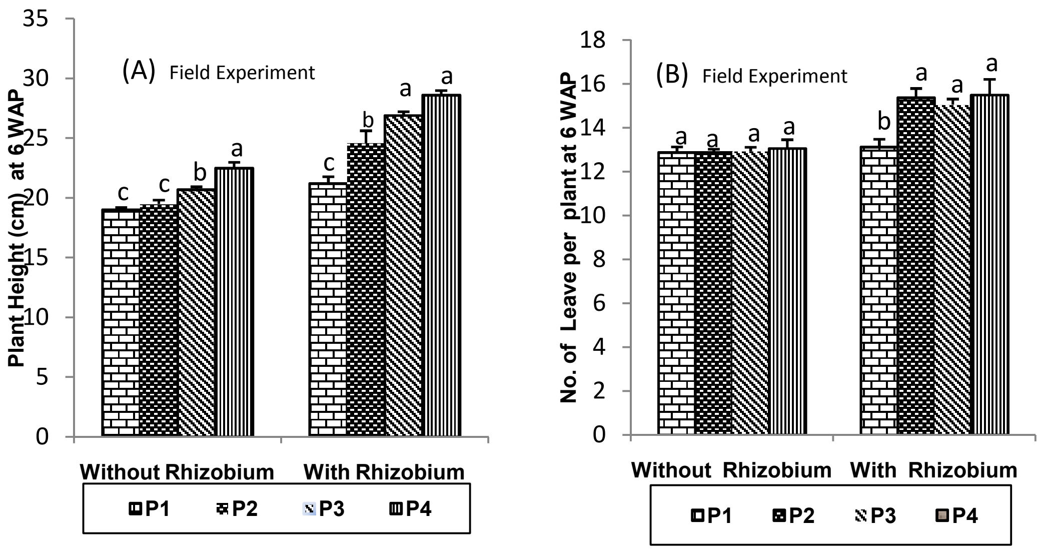

Fig. 1. Interactive effects of *B. japonicum* and phosphorus (P) on: (A) Plant height (cm) measured at 6 WAP, (B) Number of leaves per plant counted at 6 WAP

P1 = Control, P2 = 20 kg/ha, P3 = 40 kg/ha, P4 = 80 kg/ha, WAP = Weeks After Planting. Bars followed by similar letter(s) are not significantly different from each other

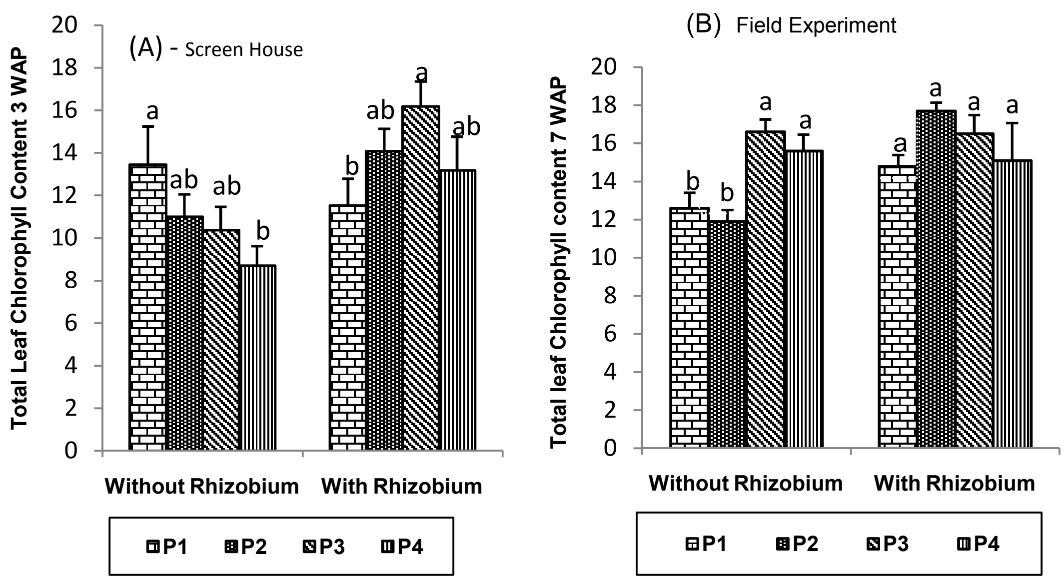

Fig. 2. Interactive effects of *B. japonicum* and phosphorus (P) on: (A) Total leaf chlorophyll content at 3 WAP, (B) Total leaf chlorophyll content at 7 WAP

P1 = Control, P2 = 20 kg/ha, P3 = 40 kg/ha, P4 = 80 kg/ha, WAP = Weeks after planting. Bars followed by similar letter(s) are not significantly different from each other

Table 3. Effects of *B. japonicum* and phosphorus on growth parameters of cowpea grown under screen house and field condition

Treatments	Screen House				Field			
	Stem girth (mm)	Leaf area (cm^2)	Days to 50% flowering	Days to 50% pod formation	Stem girth (mm)	Leaf area (cm^2)	Days to 50% flowering	Days to 50% pod formation
Rhizobia								
-R	0.47±0.01b	28.46±071a	65.31±0.28a	68.81±0.29a	7.64±0.12b	29.17±0.39b	70.25±0.64a	75.63±0.51b
+R	0.53±0.02a	29.90±0.81a	62.50±0.70b	66.19±0.66b	8.31±0.14a	30.49±0.55a	71.88±0.49b	77.44±0.48a
P Levels $K.gha^{-1}$								
0	0.45±0.02b	26.83±0.66b	64.88±0.77a	68.63±0.63a	7.48±0.18b	28.21±0.42b	69.63±0.73b	75.25±0.62b
20	0.52±0.02a	30.31±1.00a	62.13±1.14b	65.75±1.01b	7.95±0.26a	29.88±0.64ab	71.75±0.56a	76.63±0.42ab
40	0.50±0.02a	29.79±1.18a	64.38±0.56a	67.75±0.56a	8.22±0.20a	30.36±0,53a	72.00±1.10a	77.5±1.00a
80	0.54±0.02a	29.79±1.18a	64.25±0.84a	67.88±0.91a	8.25±0.10a	30.88±0.87a	70.88±0.77ab	76.75±0.80ab
2 – Way ANOVA (F-Statistics)								
R	9.97**	1.84 ns	20.18***	18.77***	20.44***	4.58*	4.47*	7.40*
P	4.66*	2.22 ns	3.78*	4.11*	5.67**	3.52*	1.95 ns	1.98 ns
R*P	0.08 ns	0.07 ns	2.61 ns	2.06 ns	1.46 ns	0.42 ns	0.96 ns	1.06 ns

***+R**: With rhizobia; **-R:** Without rhizobia; **R:** Rhizobia; **P:** Phosphorus; Values presented are means ± SE; *; **; ***; significant at P= .05, P≤ .01, P≤ .001 respectively, **ns** = not significant, **SE** = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at P= .05 according to Fischer least significance difference (LSD).*

Table 4. Effects of *B. japonicum* and phosphorus on total leaf chlorophyll content of cowpea grown under screen house and field condition

	Screen House			Field		
	Total leaf chlorophyll content (mg/l)			Total leaf chlorophyll content (mg/l)		
Treatments	**3 weeks after planting**	**5 weeks after planting**	**7 weeks after planting**	**3 weeks after planting**	**5 weeks after planting**	**7 weeks after planting**
Rhizobia						
-R	10.88±0.72b	13.30±0.71a	13.54±0.51a	7.65±0.79b	11.61±0.76b	14.17±0.61b
+R	13.74±0.72a	12.49±0.81a	13.76±0.44a	11.17±0.42a	16.01±0.65a	16.05±0.60a
P Levels (Kg.ha^{-1})						
0	12.48±1.08a	12.73±1.30a	14.59±0.71a	8.90±1.34a	12.39±1.58b	13.70±0.63b
20	12.54±0.90a	13.63±1.24a	13.93±0.48a	10.34±1.03a	13.92±1.22ab	14.81±1.16ab
40	13.27±1.33a	13.00±1.01a	13.01±0.66a	8.41±0.99a	13.55±0.82ab	16.55±0.55a
80	10.93±1.20a	12.23±0.83a	13.07±0.72a	10.00±0.98a	15.38±1.33a	15.37±0.99ab
2 – Way ANOVA (F-Statistics)						
R	10.03**	0.62 ns	0.10 ns	14.33***	21.20***	7.50*
P	1.19 ns	0.32 ns	1.23 ns	0.96 ns	1.67*	2.97*
R*P	3.50*	2.79 ns	0.56 ns	0.30 ns	1.38 ns	4.45*

***+R**: With rhizobia; **-R**: Without rhizobia; **R:** Rhizobia; **P:** Phosphorus; Values presented are means ± SE; *; **; ***; significant at P= .05, P≤ .01, P≤ .001 respectively, **ns** = not significant, **SE** = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at P= .05 according to Fischer least significance difference (LSD).*

4. DISCUSSION

The field and screen house experiments was carried out in 2013 at northern Tanzania to assess the effects of *Bradyrhizobium japonium* on growth and total leaf chlorophyll content of cowpea (*Vigna unguiculata* (L) Walp). The results of this study revealed that *B. japonium* inoculation had significant effect on different plant growth parameters. For example, *B. japonium* inoculation significantly increased the plant height, number of leaves per plant, stem girth and leaf area of cowpea (Tables 1, 2 and 3 respectively) in both screen house and field experiments over the un-inoculated control. The fresh and dry weight of shoots and roots of the plants and the number of nodules formed were also significantly increased by inoculation and P supplementation (data not shown). The positive fresh and dry weight of shoots and roots of the plants, the number of nodules formed, plant height, stem girth, leaf area and maximum number of leaves per plant observed in the inoculated pots and plots might be due to symbiotic relationship between *B. japonium* and root nodules of legumes which fix atmospheric nitrogen into a usable form by plants [10,35,36] and ultimately results in better plant growth than the control. The fixed nitrogen is an important input in plant growth [37,38,39]. Therefore, if there is reduced nitrogen fixation due to different factors in nutrient depleted soils, there will be reduced plant growth [17]. Similar to our study, other workers [36,40,4] have reported that rhizobial inoculation significantly increased the plant height, number of leaves per plant and biomass of groundnuts (*Arachis hypogaea*) and soybean (*Glycine max*).

This study also showed significant improvement on the total leaf chlorophyll content of cowpea as a result of *B. japonium* inoculation in both screen house and field experiment relative to un-inoculated treatments. These findings concur with the results found by [16] in which *B. japonium* inoculation improved chlorophyll content in *Phaseolus vulgaris*. Furthermore, inoculation with *B. japonium* significantly increased the number of days to 50% flowering and pod formation in the field experiment. The late maturity of legumes in the field experiment may be attributed to biological nitrogen fixation by *B. japonium* inoculation on cowpea seeds which produce adequate nitrogen and make the plant to grow luxuriantly vegetative. These results differ from the study by [41] on the effect of rhizobial inoculant on nodulation, yield and yield traits of chickpea (*Cicer arietinum* L) who found that *B. japonium* inoculation resulted in the early maturity of chickpea. The improved plant growth parameters in the inoculated treatments over the control is an indication that *B. japonium* were efficient in fixing nitrogen which is a building block of plant proteins many of which determine the size and structure of plant tissues and consequently plant growth is enhanced.

In this study, phosphorus supplementation significantly increased the plant height, number of leaves per plant and stem girth (Tables 1, 2 and 3 respectively) of cowpea in both screen house and field experiment. These findings are supported by other workers [42] who concluded that supplying P irrespective of the rate significantly increased the plant height, the number of leaves per plant, stem girth and leaf area. These results suggest that P is an important element for plant growth and development. In relation to our findings, other workers [43,44] have reported that supplying P to legume and cereal crops significantly improved the plant height. The improvement in growth parameters and chlorophyll content observed in this experiment might have been caused by the activity of phosphorus which plays major role in structural and metabolic function in the plant leading to growth and development [45]. Therefore, an adequate supply of phosphorus is needed for the promotion of early root formation and plant growth because phosphorus is a component of numerous cell constituents and performs major roles in several key processes such as photosynthesis, respiration, energy storage and transfer, cell division and cell enlargement [46,47]. However, other studies [48] have reported that in symbiosis *Rhizobium. leguminosarum* bv. *trifolii* with clover, excessive phosphorus reduced number of nodules, nodule occupancy and shoot dry weight. Other researchers [49] also have reported that high concentration of phosphorus (90 kg P_2O_5 per hector) decreased the plant height, number of branches, shoot dry weight, number of pods, seed yield and biomass yield indicating that application of phosphorus above threshold may cause decrease in growth and yield parameters of plants.

This study also showed significant interactive effects between *B. japonium* and phosphorus on plant height, number of leaves per plant (Fig. 1A & B) and chlorophyll content of cowpeas (Figs. 2A & B). For instance, whether supplied alone or with *B. japonium*, P significantly gave greater plant height at its highest level 80 kg/ha (Fig. 1A). The maximum number of leaves per plant was also observed where *B. japonium* was supplied with phosphorus relative to control (Fig. 1B).There was also significant interactive effect between *B. japonium* and phosphorus on the chlorophyll content of cowpea where the greatest value of chlorophyll content was recorded in *B. japonium* supplied with 40 kg/ha relative to the control. P supplementation alone without *B. japonium* decreased chlorophyll content in the screen house compared with control (Fig. 2A). The decreasing values of chlorophyll content with increasing P without *B. japonium* inoculation suggests that increasing P alone does not increase chlorophyll concentration in the leaves, but rather nitrogen is a necessary building block of chlorophyll molecule in the plant and therefore, it influences the formation of green pigment in the plant leaves. These results also suggest that there was limited amount of nitrogen in the small volume of soil in the pots compared with the field experiment where the plant roots can move far searching nitrogen and consequently chlorophyll content of plant leaves was improved in both inoculated and un-inoculated treatments in the field trial. Fig. 2B showed interactive effects between *B. japonium* and phosphorus on the total leaf chlorophyll content of cowpea in which P supplementation alone produced greater values of chlorophyll content at 40 and 80 kg/ha relative to other treatments while both *B. japonium* inoculation and P supplementation had no significant difference over the control. However, treatments which received *B. japonium* inoculation and supplemented with P produced greater values of chlorophyll content compared with the treatments received P alone (Fig. 2B). The increased plant height, number of leaves and leaf chlorophyll content of cowpea might have been contributed by combination of *B. japonium* and phosphorus which enhance root formation, nodulation, N-fixation and finally plant growth with dark green color for food production [28,30,11,12].

5. CONCLUSION

In conclusion, our results showed the importance of rhizobia inoculation and phosphorus on the growth and total leaf chlorophyll content of legume crops in the nutrient depleted soils. The growth parameters such as plant height, number of leaves per plant, leaf area and stem girth were significantly improved with *B. japonium* inoculation and application of P over the control treatments. Inoculation with *B. japonium* also significantly improved the total leaf chlorophyll content of the cowpea. There was also significant interaction between *B. japonium* and phosphorus on the plant height, number of leaves per plant and total leaf chlorophyll content of cowpeas. Therefore, for improved plant growth, number of leaves and chlorophyll content in the legume production system. It is important to supplement the depleted soils of sub-Saharan Africa with phosphorus and rhizobia inoculation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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