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Effect of Rhizobium Inoculation and Supplementation with Phosphorus and Potassium on Growth and Total Leaf Chlorophyll (Chl) Content of Bush Bean *Phaseolus vulgaris*, L.

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Abstract

The study was conducted to investigate the effect of *Rhizobium* inoculation and supplementation of phosphorus and potassium on growth and total leaf chlorophyll content to the three released bush bean varieties in northern Tanzania. To achieve this aim, the glasshouse experiment was conducted at Nelson Mandela African Institution of Science and Technology while field experiment were carried out at Tanzania Coffee Research Institute, in Kilimanjaro, Tanzania between April-July 2014. The experiment was laid out in factorial arrangement. Factor I comprised of three bush bean varieties. Factor II involved two inoculation treatments viz 1) inoculation with *Rhizobium spp.* and 2) without inoculation. Factor III included four fertilizer levels (0 Kg·ha⁻¹ 20 Kg K ha⁻¹, 30 Kg P ha⁻¹ and 20 kg·K + 30 Kg P ha⁻¹). Both screen house and field experiments were replicated four times. Plant growth parameters (plant height (cm), number leaves per plant, stem girth (mm)) were measured at 2, 4 and 6 weeks after planting (WAP). The chlorophyll was extracted by using Dimethylsulphoxide (DMSO) and absorbance was determined at 645 and 663nm using UV/Visible spectrophotometer. Results showed that *Rhizobium* application significantly improved the number of leaves per plant, plant height, plant girth and total leaf chlorophyll content. Furthermore, compared with the zero treatment control, potassium fertilization significantly increased the number of leaves per plant, plant height, plant girth and total leaf chlorophyll content of the three varieties. In general, these parameters were significantly increased with phosphorus supplied at 30 kg/ha. The combination of these supplies at different levels resulted in significant interactions in some parameters and thus indicating need for these inputs in the study area.

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Keywords

Legumes, Total Leaf Chlorophyll Content, Photosynthesis, Plant Height, Rhizobia, Stem Girth

1. Introduction

Nitrogen is the critical limiting element for growth of most plants due to its unavailability [1] [2]. Bush beans need nitrogen more than any other nutrient since it is an important constituent of proteins, enzymes, chlorophyll, and growth regulators [3]. 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 [4] [5]. A commonly known alternative source of nitrogenous fertilizer to legumes is the well documented rhizobial inoculants [6]. Several studies have indicated the potential of rhizobial inoculants in photosynthesis and chlorophyll content of legumes [5] [7]-[12]. However, Ali *et al.* and Malik *et al.* [13] [14] studies on Mungbean (*Vigna radiata* L.) and soybean (*Glycine max* L.) respectively found that *Rhizobium* inoculant significantly affected legume growth.

Phosphorus (P) is frequently one of the most limiting nutrients for plant growth in the tropics, and it is estimated that over 50% of common bean production in tropical soils is limited by phosphate deficiency [15] [16]. P is found in all living plant cell and resumes several key plant functions including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant [17] [18] nucleic acids, phospholipids, ATP, and it makes about 0.2% of plant's dry weight. Successful production systems based on legumes therefore, requires P inputs [19] either from soil reserves or from added fertilizer [20]. Optimum P supply is reported to stimulate the vegetative growth of common bean bush type cultivars by increasing shoot dry weight from 5.34 to 7.10 g·plant⁻¹, root dry weight from 0.41 to 0.5 g·plant⁻¹ [21]. The different behavior exhibited by shoot and root growth indicates that root growth is less sensitive to P deficiency than is shoot growth [22]. Supplementing legumes with P has great potential for increasing legumes growth and yield [23] [24].

Potassium influences the process of photosynthesis at many levels, namely synthesis of ATP, activation of the enzymes involved in photosynthesis, CO₂ uptake, and balance of the electric charges required for photophosphorylation in chloroplasts, and acting as the counter ion to light-induced H⁺ flux across the thylakoid membranes [25]. Studies on cucumber cotyledons, cotton plants and Alfalfa plants have reported that adequate supply of potassium nutrient increase chlorophyll content [26]-[29]. Collins and Duke [30] also confirmed that K deficient in legumes is associated with low chlorophyll content. However, K has other important roles in major plant processes such as photosynthesis, respiration, osmoregulation, growth and yield of plant [31]-[33].

Beside of the studies above, there is little information about the effects of phosphorus, potassium and *Rhizobium* inoculant and their interaction on the chlorophyll formation in new released bush bean *Phaseolus vulgaris* L. in Tanzania. Therefore, it is important to establish and quantify the influence of phosphorus, potassium and *Rhizobium* inoculation on leaf chlorophyll content and growth in such legumes.

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 Nelson Mandela African Institution of science and technology (NM-AIST) which is situated in an area which is 1250 m above the sea level in Arusha, Tanzania of latitude 3°38'33.00"S and longitude 36°38'06.29"E. The field experiment was conducted at the Tanzania Coffee Research Institute which is situated 1390 m 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 1200 mm.

2.2. Experimental Design

The experiment was laid out in factorial arrangement. Factor I comprised of three bush bean varieties. Factor II involved two inoculation treatments viz 1) inoculation with *Rhizobium spp.* and 2) without inoculation. Factor

III included four fertilizer levels ($0 \text{ Kg}\cdot\text{ha}^{-1}$, 20 Kg K ha^{-1} , 30 Kg P ha^{-1} and $20 \text{ kg}\cdot\text{K} + 30 \text{ Kg P ha}^{-1}$). Both screen house and field experiments were replicated four times.

2.3. Field and Screen House Experiment

The crop plant used for this experiment were 3 bush bean varieties recently released in mid-altitude zones in northern Tanzania namely: Lyamungo 90, Jesca and Selian 1997. They were supplied by the breeder from Selian Agricultural Research Institute (SARI), Arusha, Tanzania. The *Rhizobium tropici* (CIAT899) used was purchased from United Kingdom (UK). One kg of bean seeds was weighed and 10 gm of *Rhizobium tropici* inoculants was added and mixed according to manufacturer instruction so that all seeds are coated and are supplied with approximately 10^9 cells/g seed. Then inoculated 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 50 cm by 20 cm and the plot dimension was 4 m by 3 m. In the field trial, four 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 2014, 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 were measured from 21 days after planting (DAP) to pod formation in both screen house and field experiment. Plant height was taken using measuring tape (from ground surface to the tip). Stem girth was measured using a vernier caliper. The number of leaves per plant was counted physically. Ten randomly selected representative plants from the four central rows in each plot for field experiment were to collect the growth parameters mentioned above, while two plant from each pot were used for screen house experiment.

2.5. Chlorophyll Content Determination

Extraction of chlorophyll by Dimethylsulphoxide (DMSO) was done as described by Hiscox and Israelstam (1979). 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 4°C 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 (Hiscox and Israelstam, 1979). 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 (Arnon, 1949) to determine total leaf chlorophyll content against DMSO blank, as shown below.

Chlorophyll total ($\text{Chlt} = 20.2\text{D}_{645} + 8.02\text{D}_{663}$), 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) in a factorial arrangement, 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 (Steel and Torrie, 1980).

3. Results

3.1. Effects of Rhizobia, K and P Fertilization on Plant Height of the 3 Bush Bean Varieties

The results in **Table 1** indicate that rhizobial inoculation significantly increased plant height (cm) of all bush

Table 1. Effect of rhizobium inoculation and supplementation of P and K on plant height of 3 bush bean varieties.

Treatments	Field condition			Screen house condition		
	Plant height (recorded) at					
	2 WAP	4 WAP	6 WAP	2 WAP	4 WAP	6 WAP
Rhiz						
-	23.47 ± 0.42b	26.37 ± 0.40b	31.75 ± 0.41b	18.40 ± 0.40b	36.13 ± 0.55b	39.65 ± 0.60b
+	28.85 ± 0.49a	29.13 ± 0.30a	36.65 ± 0.47a	33.38 ± 0.81a	48.18 ± 0.67a	52.10 ± 0.60a
Fert						
Control	24.21 ± 0.84c	26.33 ± 0.45b	31.83 ± 0.66c	24.17 ± 1.72b	39.08 ± 1.43d	42.33 ± 1.44d
20K	25.67 ± 0.85b	26.70 ± 0.52b	33.29 ± 0.68b	25.42 ± 1.78ab	41.25 ± 1.47c	44.92 ± 1.50c
30P	26.54 ± 0.70b	27.80 ± 0.45a	34.67 ± 0.75b	26.13 ± 1.78ab	43.13 ± 1.42b	47.17 ± 1.47b
30P + 20K	28.25 ± 0.80a	30.17 ± 0.51a	37.00 ± 0.75a	27.83 ± 1.85a	45.17 ± 1.51a	49.08 ± 1.46a
Varieties						
L.90	24.31 ± 0.58c	26.18 ± 0.58c	33.97 ± 0.76b	27.84 ± 1.71a	39.97 ± 1.37b	44.47 ± 1.50b
Jesca	26.22 ± 0.71b	27.59 ± 0.28b	33.06 ± 0.48b	25.59 ± 1.46b	42.97 ± 1.32b	46.28 ± 1.38b
S.97	27.97 ± 0.77a	29.47 ± 0.38a	35.56 ± 0.73a	24.22 ± 1.41b	43.53 ± 1.16a	46.88 ± 1.07a
3 Way ANOVA (F stat)						
Rhiz	114.90***	253.7***	173.12***	301.08***	278.21***	355.47***
Fert	11.36***	99.9***	34.87***	3.14*	12.92**	19.41***
Varieties	17.73***	121.2***	15.43***	6.00**	9.35**	4.80***
Rhiz*Fert	0.46ns	1.4ns	0.21ns	0.10ns	0.05ns	0.07ns
Rhiz*Varieties	1.03ns	65.1***	27.43ns	2.95ns	2.07ns	6.76**
Fert*Varieties	0.47ns	1.1ns	0.24ns	0.13ns	0.05ns	0.52ns
Rhiz*Fert*Varieties	1.33ns	1.9ns	0.38ns	0.03ns	0.11ns	0.09ns

+R = With rhizobia; -R = Without rhizobia; P = Phosphorus; K = Potassium; G = Plant girth; WAP = Week after planting; L.90 = Lyamungo 1990; S.97 = Selian 1997. Values presented are means ± SE; ***,**,* Significant at p = 0.05, p ≤ 0.01, p ≤ 0.001 respectively; ns = not significant; SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p = 0.05 according to Fischer least significance difference.

bean varieties under this study in all reading both in the field and glass house experiment. The plant height for field experiment increased with rhizobial inoculation for the entire interval of the bush bean varieties growth. The plant height values recorded at 2, 4, and 6 weeks after planting (WAP), in the field experiment were 23%, 10% and 15% respectively compared with the control. However, in the glass house the relative increase in plant height followed 81.4%, 12.1% and 31.4% for the value recorded at 2, 4, and 6 WAP respectively in the inoculated plots relative to the control.

Supplementing K and P at 20 kg/ha and 30 kg/ha respectively improved plant height both in field and glass house experiment. Result (Table 2) shows K and P application significantly improved plant height in all stages of legume growth for values recorded at 2, 4 and 6 WAP both the field and glass house experiment as compared to control. However, the combined effect of fertilizer gave better result than when K or P applied alone. For instance, for data collected at 2, 4 and 6 WAP, the combined effect of fertilizer increased plant height by 17%, 14% and 16% in the field experiment and 15%, 16% and 17% in the glass house experiment.

Result also indicates that the overall Varietal performance, Selian 1997 performed better in plant height in all stages of its growth followed by Jesca and Lyamungo. The performance of Selian 1997 for data recorded at 4 WAP in the field experiment and 6 WAP in the 6 WAP were significantly influenced by rhizobial inoculation.

In this study there was significant (p = 0.001) interactive effect between *Rhizobium* inoculation and varieties on the plant height of bush bean varieties measured at fourth weeks after planting in the field experiment and during the sixth week for measurements taken from the glass house experiment (Figure 4(a) & Figure 4(b)) where Selian 1997 reached the maximum height of 29 cm tall relative to 26 and 27 cm tall exhibited by Lyamungo and Jesca respectively in the field experiment and also in screen house experiment Selian 1997 reached 47 cm tall relative to Lyamungo 44 cm and Jesca 46 cm relative to control.

Table 2. Effect of rhizobium inoculation and of P and K fertilization on number of leaves, stem girth of 3 bush bean varieties.

Treatments	Field condition				Screen house			
	Number of leaves plant girth (recorded) at				Number of leaves plant girth (recorded) at			
	No. of Leaves 4 WAP	No. of Leaves 6 WAP	Plant G 3 WAP	Plant G 6 WAP	No. of Leaves 4 WAP	No. of Leaves 6 WAP	Plan G 3 WAP	Plant G 6 WAP
Rhiz								
-	19.93 ± 0.51b	29.40 ± 0.47b	3.90 ± 0.05b	4.75 ± 0.03b	7.50 ± 0.22b	11.00 ± 0.00b	4.25 ± 0.05b	4.54 ± 0.05b
+	21.04 ± 0.33a	34.33 ± 0.42a	3.98 ± 0.04a	4.81 ± 0.05a	8.00 ± 0.00a	11.67 ± 0.14a	4.50 ± 0.06a	4.78 ± 0.06a
Fert								
Control	19.88 ± 0.54b	30.42 ± 0.66b	3.72 ± 0.05c	4.61 ± 0.04c	7.00 ± 0.29c	11.08 ± 0.06b	4.05 ± 0.06d	4.35 ± 0.07d
20K	20.42 ± 0.55b	31.25 ± 0.80b	3.81 ± 0.05c	4.67 ± 0.05c	7.00 ± 0.29c	11.33 ± 0.13b	4.25 ± 0.05c	4.52 ± 0.06c
30P	21.50 ± 0.70b	34.88 ± 0.82b	4.01 ± 0.05b	4.80 ± 0.05b	7.38 ± 0.25b	11.17 ± 0.10b	4.47 ± 0.06b	4.72 ± 0.06b
30P + 20K	18.88 ± 0.47a	33.41 ± 0.81a	4.21 ± 0.05a	5.03 ± 0.06a	7.63 ± 0.21a	11.75 ± 0.24a	4.73 ± 0.06a	5.04 ± 0.06a
Varieties								
L.90	18.88 ± 0.47b	31.25 ± 0.47b	4.00 ± 0.03a	4.56 ± 0.04b	6.88 ± 0.26b	11.38 ± 0.14b	4.60 ± 0.05a	4.92 ± 0.06a
Jesca	23.41 ± 0.41a	31.18 ± 0.73a	3.97 ± 0.07ab	4.87 ± 0.03a	8.00 ± 0.00a	11.41 ± 0.16a	4.22 ± 0.06c	4.47 ± 0.06c
S.97	18.71 ± 0.20b	32.47 ± 0.76a	3.85 ± 0.05b	4.89 ± 0.05a	6.88 ± 0.26b	11.22 ± 0.10c	4.31 ± 0.07b	4.58 ± 0.07b
3 Way ANOVA (F stat)								
Rhiz	16.21***	116.94***	2.51ns	120.33***	123.43***	27.93***	37.79***	29.28***
Fert	5.93***	19.88***	20.16***	91.49***	5.14**	5.53**	49.98***	44.25***
Varieties	76.31***	11.56***	3.75*	1.85ns	30.86***	0.85ns	32.08***	36.68***
Rhiz*Fert	0.44ns	3.11*	0.41ns	0.16ns	5.14**	5.53**	0.42ns	0.03ns
Rhiz*Varieties	14.74***	1.09ns	0.49ns	9.28***	30.86***	0.85ns	2.09ns	4.55**
Fert*Varieties	0.76ns	1.94ns	1.07ns	1.28ns	1.71ns	0.70ns	0.93ns	0.53ns
Rhiz*Fert*Varieties	0.42ns	0.33ns	0.17ns	0.69ns	1.71ns	0.70ns	0.20ns	0.47ns

+R = with rhizobia; -R = Without rhizobia; P = Phosphorus; K = Potassium; G = Plant girth; WAP = Week after planting; L.90 = Lyamungo 1990; S.97 = Selian 1997. Values presented are means ± SE; ***,**,* Significant at $p = 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively; ns = not significant; SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at $p = 0.05$ according to Fischer least significance difference.

3.2. Effects of Rhizobia, K and P Fertilization on the Number of Leaves of the 3 Bush Bean Varieties

Rhizobia inoculation, fertilization (P and K) and the varieties tested had significant effect on the plant leaves assessed in this study (Table 2). For instance, *Rhizobium* inoculation significantly increased the number of leaves of the 3 bean varieties at 4 weeks after planting (WAP) by 6% and 7% both in the field experiments and screen house (Table 1) compared with the control treatments. However, for data collected 6WAP *Rhizobium* inoculation increased the number of leaves by 17% and 6% respectively relative to control.

In this study, supplying K and P alone could not significantly improve the number of leaves per plant for measurements taken 4 and 6 WAP. However, combined effect between 30 Kg·ha⁻¹ and 20 Kg·ha⁻¹ showed significant increase of leaves number as compared with all other treatments. Result indicate that variety Jesca performed better in the leaf number count followed by Selian 1997 and then Lyamungo 1990 both in field and glass house experiment.

This study showed significant ($p \leq 0.001$) interactive effect between *Rhizobium* inoculation and varieties on number of leaves both in the field and screen house experiment (Figure 5(a) & Figure 5(b)). The significant effect was recorded at 4 WAP both in the screen house and field experiment. However, the *Rhizobium* inoculation also showed significant interactive effect with fertilizer both in the field and glass house experiment. In field experiment the interaction was significant ($p \leq 0.05$) on leaf number counted at 6 WAP, whereas in the glass house the interaction was significant ($p \leq 0.01$) on leaf number counted at 4 WAP and 6 WAP (Figures 1(a)-(c)).

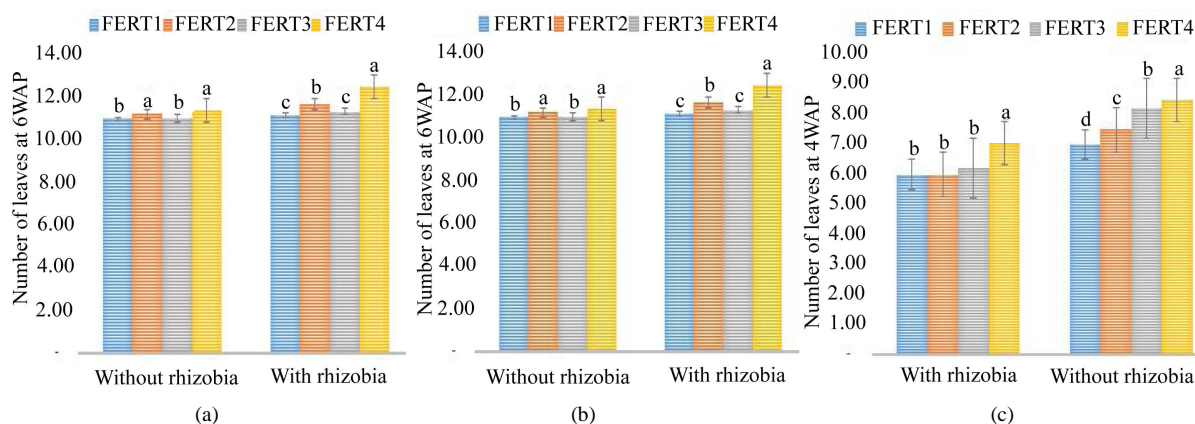


Figure 1. Interactive effects of rhizobia and fertilizer on number leaves in field and screen house experiment. L.90 = Lyamungo 1990; S.97 = Selian 1997. FERT1 = Zero fertilizer; FERT2 = 20 kg/ha K; FERT3 = 30 kg/ha P; FERT4 = Combination of 20 kg/ha K and 30 kg/ha P. Bars followed by dissimilar letters are significantly different at $p = 0.05$ according to Fischer least significance difference (LSD). (a) Field experiment; (b) Screen house experiment; (c) Glass house experiment 4 WAP.

3.3. Effects of Rhizobia, K and P Fertilization on Plant Girth of the 3 Bush Bean Varieties

Result in **Table 1** indicate that *Rhizobium* inoculation had significant effect to the plant girth recorded at 6 WAP in the field experiment and 3 WAP and 6 WAP in green house compared with control. *Rhizobium* inoculation increased plant girth by 1.3% 6 WAP in field experiment and 5.1% and 11.67% in green house for 3 WAP and 6 WAP respectively. In the field experiment, *Rhizobium* inoculation numerically, but not significantly increased plant girth at 3 WAP.

Application of phosphorus 30 kg·ha⁻¹ generally increased plant girth both in field experiment and glasshouse experiment for all stages of legume growth. Plant girth, increased numerically but statistically insignificant by applying potassium at 20 kg/ha. Applying a combination of 30 kg·ha⁻¹ P and 20 kg·ha⁻¹ K improved plant girth both in field and glasshouse experiment. In the field experiment, the combination increased plant girth by 13% and 9% in 3 WAP and 6 WAP, whereas, in glasshouse plant girth increased by 17% and 16% in 3 WAP and 6 WAP respectively compared with control. Generally, Lyamungo 1990 outperformed better than the other varieties both in the field and glass house experiment in all stages of their growth circle when girth of three varieties was to be compared. This was followed by Selian 1997 and the least was Jesca as shown in **Table 2**.

The interactive effect between *Rhizobium* inoculation and fertilizer was significant in both field and glass house experiment. However, the significant ($p \leq 0.001$) at the field level on plant girth was found on the 6 WAP. Result from the glass house also proved the interactive effect was significant ($p \leq 0.01$) on plant girth in glass house experiment at 6 WAP (**Figure 2(a)** & **Figure 2(b)**).

3.4. Effects of Rhizobia, Phosphorus and Potassium on the Total Leaf Chlorophyll of 3 Bush Bean Varieties

The results in **Table 3** indicated that the *Rhizobium* inoculation had positive effects on the total leaf chlorophyll content of bush bean varieties measured from three and six weeks after planting. *Rhizobium* inoculation significantly increased leaf chlorophyll content by 44% and 19% in the field experiment for 3 WAP and 6 WAP respectively compared with un-inoculated plots. However, in the screen house the *Rhizobium* inoculation increased leaf chlorophyll content by 98% and 40% for data collected 3 and 6 week after planting compared with the control.

Supplementation of K at 20 kg/ha and P at 30 kg/ha all growth stages showed significant effect on the leaf chlorophyll content in both field and glass house experiment at 3, and 6 WAP. However, the combined effect of K and P indicated high total leaf chlorophyll content than when K or P were applied alone (**Table 3**). The combined effect of 20 kg·ha⁻¹ and 30 kg/ha increased the leaf chlorophyll content by 4% and 46% at 3 WAP and 6 WAP relative to control in the field experiment and by 89% and 43% for data collected at 3 WAP and 6 WAP in the glass house experiment.

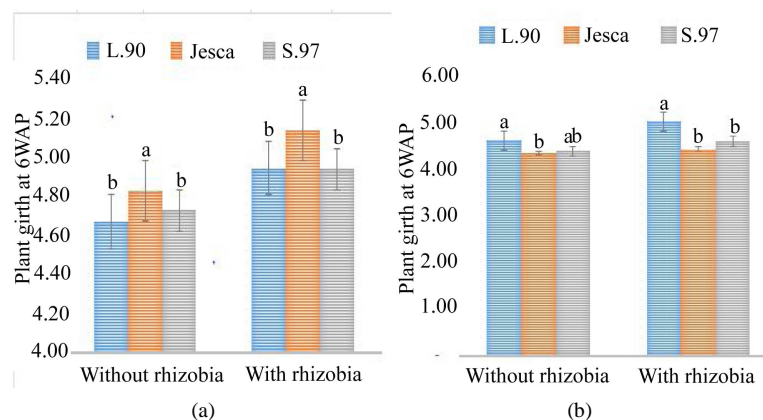


Figure 2. Interactive effects of rhizobia and varieties on plant girth in field and glasshouse experiment. L.90 = Lyamungo 1990; S.97 = Selian 1997. Bars followed by dissimilar letters are significantly different at $p = 0.05$ according to Fischer least significance difference (LSD). (a) Field experiment; (b) Glass house experiment.

Table 3. Effect of Rhizobium inoculation and supplementation of P and K on total chlorophyll content 3 bush bean varieties.

Treatment	Field condition		Screen house	
	Total chlorophyll (recorded) at		Total chlorophyll content (recorded) at	
	3 WAP	6 WAP	3 WAP	6 WAP
Rhiz				
-	4.07 ± 0.09b	2.28 ± 0.07b	33.11 ± 1.21b	11.94 ± 0.59b
+	5.84 ± 0.21a	2.72 ± 0.08a	44.34 ± 1.29a	23.61 ± 1.03a
Fert				
Control	4.28 ± 0.25c	2.00 ± 0.11c	31.79 ± 2.20c	12.26 ± 1.07d
20K	4.69 ± 0.23ab	2.34 ± 0.09ab	36.34 ± 1.84b	15.78 ± 1.20c
30P	4.99 ± 0.21b	2.73 ± 0.08b	41.28 ± 1.66b	19.87 ± 1.51b
30P + 20K	5.85 ± 0.37a	2.91 ± 0.08a	45.57 ± 1.63a	23.20 ± 1.95a
Varieties				
S.90	5.18 ± 0.18a	2.64 ± 0.07a	38.30 ± 1.31b	19.24 ± 1.24a
Jesca	5.13 ± 0.27b	2.46 ± 0.13b	34.44 ± 2.23c	19.12 ± 1.63b
S.97	4.55 ± 0.29c	2.40 ± 0.10b	43.49 ± 1.46a	17.97 ± 1.47c
3 Way ANOVA (F stat)				
Rhiz	98.365***	57.900***	87.713***	280.687***
Fert	13.874***	48.232***	24.647***	46.794***
Varieties	5.062**	6.742**	18.971***	5.792***
Rhiz*Fert	1.415ns	2.112ns	0.136ns	6.040***
Rhiz*Varieties	3.865*	20.867**	0.665ns	8.592***
Fert*Varieties	0.572 ns	2.298*	1.542ns	1.223ns
Rhiz*Fert*Varieties	2.591*	1.431ns	1.234ns	0.747ns

+R = With rhizobia; -R = Without rhizobia; P = Phosphorus; K = Potassium; G = Plant girth; WAP = Week after planting; L.90; Lyamungo 1990; S.97; Selian 1997. Values presented are means ± SE; *, **, *** Significant at $p = 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively; ns = Not significant; SE = Standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at $p = 0.05$ according to Fischer least significance difference (LSD).

This study indicated that the varietal response to total leaf chlorophyll content was significant both in the field and screen house experiment. However, when the 3 bean varieties were compared, Lyamungo 1990 scored higher amount of chlorophyll content followed by Jesca in the field experiment and Selian 1997 in the glass house experiment (Table 3).

The results (Figure 6(a) & Figure 6(b) and Figure 3(b)) show that there were significant interactive effect between *Rhizobium* and varieties both in the field and glasshouse experiment in total leaf chlorophyll content. For the field experiment, the interactions was significant for the total leaf chlorophyll content in bean varieties measured both in 3 WAP ($p \leq 0.05$) and 6 WAP ($p \leq 0.01$). However, in the glass house experiment, there was significant ($p \leq 0.001$) interactive between *Rhizobium* and varieties at 6 WAP. Generally *Rhizobium* inoculation supplied with bean varieties significantly gave higher amount of leaf chlorophyll content compared with the control.

The interaction between rhizobium \times fertilizers \times varieties was significant on the total leaf chlorophyll content only the field experiment. In the field experiment results showed the significant ($p \leq 0.05$) interactive effects on total leaf chlorophyll content between rhizobium \times fertilizers \times varieties measured at 3 WAP (Figure 8). The result generally indicated that *Rhizobium* combined with the application of fertilizer influenced growth parameters such as number of leaves and plant girth and eventually total leaf chlorophyll content in all bean varieties under the study.

4. Discussion

The study assessed the effect of *Rhizobium* inoculation with K and P fertilization on growth and total leaf chlorophyll content leaf to bush bean varieties (*Phaseolus vulgaris*, L.). The study showed that *Rhizobium* inoculation had positive effects on plant growth parameters (plant girth, height and number of leaves) for glass house and field experiment compared with un-inoculated treatment. Commonly, the un-inoculated controls showed relatively poor growth in all growth parameters measured. The increased plant height, stem girth, number of leaves per plant observed in the inoculated pots and plots might be due to symbiotic relationship between *Rhizobium* inoculation and root nodules of legumes which fix atmospheric nitrogen into a usable form used for the plant growth reflected in the measured parameters. It is well established that leguminous plants in partnership with *Rhizobium* have the ability to convert the atmospheric nitrogen into usable form [20] [34]-[36], which can be used by the plants. Similar to our results Tairo and Ndakidemi, Bambara and Ndakidemi, Nyoki and Ndakidemi, Ndakidemi and Semoka, Ravikumar [5] [11] [12] [20] [37] have reported significant improvement in various legumes growth in Tanzania when inoculated with specified legume inoculant.

Rhizobial inoculant however improved the total leaf chlorophyll content of all three bush bean varieties under the study. The study proved that rhizobial inoculation had significant effect to total leaf chlorophyll content to bush bean varieties both in the field and glass house experiment (Table 3). Inoculated treatments showed more dark green and found to be rich in leaf chlorophyll content as compared with un-inoculated treatments. In a study involving common bean by Bambara and Ndakidemi [38] in glasshouse and field experiment the leaf

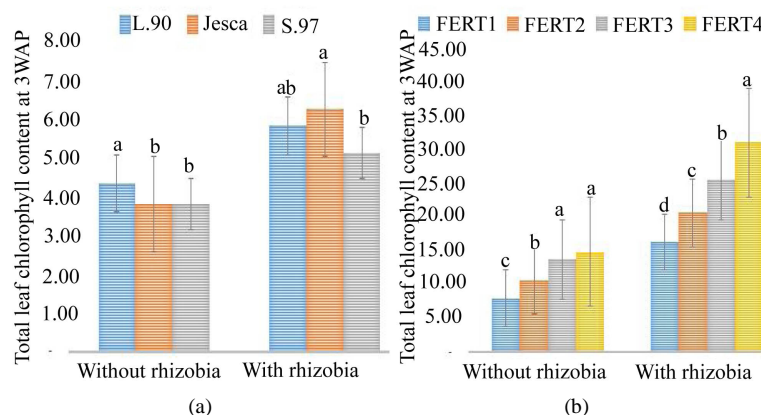


Figure 3. Interactive effects of (a) Rhizobia and varieties (b) Rhizobia and fertilizer on total leaf chlorophyll content recorded at 3 WAP and 6 WAP respectively. L.90 = Lyamungo 1990; S.97 = Selian 1997; FERT1 = Zero fertilizer; FERT2 = 20 kg/ha K; FERT3 = 30 kg/ha P; FERT4 = Combination of 20 kg/ha K and 30 kg/ha P. Bars followed by dissimilar letters are significantly different at $p = 0.05$ according to Fischer least significance difference (LSD). (a) Field experiment; (b) Glass house experiment.

chlorophyll (Chl) content increased significantly with rhizobial inoculation. These improvements in inoculated treatments could be accredited to improved biological nitrogen fixation by rhizobial inoculants which increased nitrogen supply to the plants and subsequently increased total leaf chlorophyll contents of the legumes. The results in the current study showed that *Rhizobium* inoculation increased leaf chlorophyll content by 19% and 44% in the field experiment and 40% and 98% in glasshouse experiment which is within the range of those reported earlier by Jairo and Ndakidemi, Nyoki and Ndakidemi, Bambara and Ndakidemi [5] [12] [38] in *Phaseolus vulgaris* bush type, *Vigna unguiculata* and *Glycine max*. The promising results attained from this study conclude that rhizobia inoculation may substitute the expensive inorganic N fertilizers in improving plant growth and chlorophyll synthesis.

In this study, phosphorus and potassium significantly improved all growth parameters assessed in the study both in the field experiment and glass house experiment. For instance, supplying 30 kg/ha of P increased the height of bush bean varieties by 10% and 8% at the field and glass house experiment. Other parameters including plant girth and number of leaf were significantly improved by application of phosphorus 30 kg/ha (Table 2). P-fertilizer application resulted in more vigorous plants with more intense green colour indicating higher chlorophyll content and greater photosynthetic ability [39]. This improvement could be due to functional importance of phosphorus in legume growth such as sugar and starch utilization, cell division and organization, photosynthesis use efficiency and formation of green pigment in plant which finally improve plant growth and chlorophyll synthesis. Such significant result in legumes has also been reported by Jairo and Ndakidemi, Nyoki and Ndakidemi, Berg and Lynd, Pacovsky, Kasturikrishna and Ahlawat [5] [12] [40]-[42].

Supplementing beans with 20 kg/ha of K relatively improved plant height both in field and glass house experiment. The increase in plant height might be influenced by the role of potassium in photosynthesis, respiration, osmoregulation, growth and yield of plant [33]. The combination of 30 Kg/ha and 20 Kg/ha of P and K had significant effects in all parameters which were under observation. This may be accounted by the role played by phosphorus and potassium in legume nutrition, particularly in energy acquisition, storage and utilization [43]. Similar to our work, other related studies have reported on the effect of P and K on legume growth [20] [24] [44]-[50].

This study also showed significant interactive effects ($p = 0.001$) between *Rhizobium* inoculation and varieties on plant height on the field and green house experiment (Figure 4(a) & Figure 4(b)) and number of leaves (Figures 5(a) & Figure 5(b)). For instance, whether inoculated with *Rhizobium* inoculation or un-inoculated, Selian 1997 significantly gave greater height compared with other variety in the field experiment. Generally, all 3 varieties recorded greater height when inoculated with *Rhizobium* inoculation both in field and glass house experiment (Figure 4(a) & Figure 4(b)). However, the maximum number of leaves per plant was also observed where *Rhizobium* inoculation was supplied with varieties (Figure 5(b)).

A significant interactive effect was observed between *Rhizobium* inoculation and fertilizers on the number of leaves both in the field and screen house experiment (Figures 1(a)-c). Good results were recorded in *Rhizo*

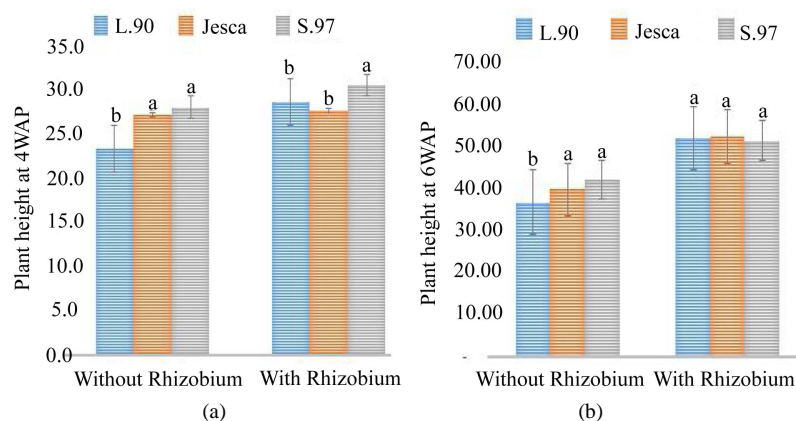


Figure 4. Interactive effect of rhizobia and variety on plant height in field and glass house experiment. L.90 = Lyamungo 1990; S.97 = Selian 1997. Bars followed by dissimilar letters are significantly different at $p = 0.05$ according to Fischer least significance difference (LSD).

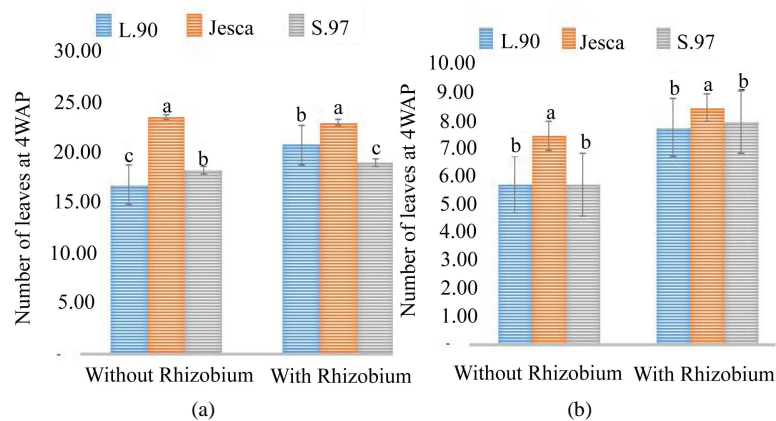


Figure 5. Interactive of rhizobia inoculation and varieties on number of leaf in the glasshouse and field Experiment 2014. L.90 = Lyamungo1990; S.97 = Selian 1997. Bars followed by dissimilar letters are significantly different at $p = 0.05$ according to Fischer least significance difference (LSD). (a) Field experiment; (b) Screen house experiment.

bium inoculated treatments in combination with the highest rate of fertilizer (Figures 1(a)-(c)), suggesting significant additive results by mixing these inputs. Similar to our findings, the role of rhizobia inoculation when supplement with inorganic fertilizer in legumes on leaf count have been reported by other researchers [5] [12] [14] [51] [52].

The interactive effect between *Rhizobium* inoculation and varieties on the plant girth was also observed (Figure 2(a) & Figure 2(b)). However, the significant interaction ($p \leq 0.001$) *Rhizobium* inoculation and varieties on plant girth at the field level were noticeable on the 6 WAP. 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 plant girth and other growth parameters [37] [53].

The results (Figure 6(a) & Figure 6(b)) show that there were significant interactive ($p \leq 0.01$) effect between *Rhizobium* and varieties both in the field and glasshouse experiment. For the field experiment, the interaction was significant for the value of leaf chlorophyll content of three bush bean varieties measured in 6 WAP ($p \leq 0.01$). All three bush bean varieties positively responded to increased total leaf chlorophyll content with *Rhizobium* inoculation suggesting the incumbent role of rhizobia in legume production. Interactive effects of fertilizer and varieties indicated significant interactive effects ($p \leq 0.05$) in leaf chlorophyll content (Figure 7). The three varieties yielded high amount of total leaf chlorophyll content when the combination of 20 kg/ha of K and 30 kg/ha of P were added to the soil indicating the importance of P and K in legume nutrition. Our result corresponds to the previous researchers who reported that adequate supply of potassium and phosphorus nutrient increase chlorophyll content in plants [27]-[29].

In the presence of all three treatments (*Rhizobium*, + P & K fertilization and varieties), a significant interaction was observed on the total leaf chlorophyll content of *Phaseolus vulgaris* L. The greater leaf amount of chlorophyll content was observed in the highest rates of P and K in combination with *Rhizobium* inoculation in all 3 varieties under study (Figure 8). However, increased total leaf chlorophyll was recorded on the inoculated plots relative to un-inoculated plots. The result generally indicated that *Rhizobium* combined with the application of fertilizer influenced growth parameters such as number of leaves, plant height, plant girth and eventually total leaf chlorophyll content in all bean varieties in the field experiment. Other Studies by Shahid, Saleem, Khan, & Anjum [18] [54]-[56] concluded that improved growth in these parameters suggest compatibility of rhizobia, fertilizer and varieties which in turn enhanced root formation, cell division and elongation, nodulation, N-fixation and eventually plant growth with dark green color for plant food production.

5. Conclusion

Rhizobium inoculation and supplementation with phosphorus and potassium improved plant growth parameters (plant girth, height and number of leaves) and total leaf chlorophyll content of all three bush bean varieties tested in this study. *Rhizobium* inoculation alone had significant effect on all parameters under the study fol-

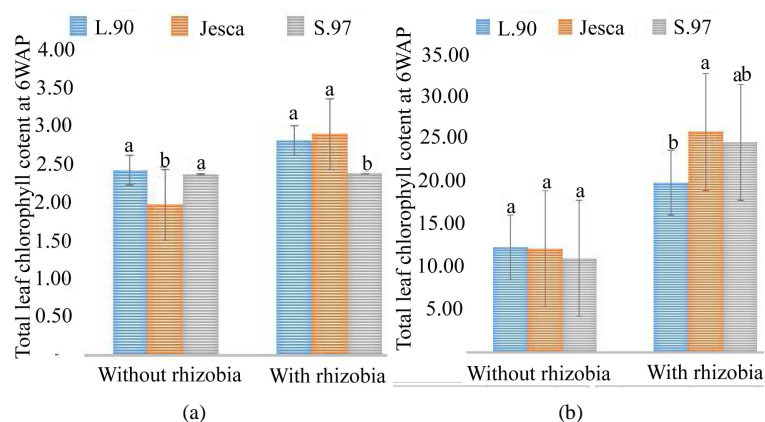


Figure 6. Interactive effect of rhizobia and varieties on total leaf chlorophyll content of three bush bean varieties recorded at 6 WAP in field and glass house experiment 2014. L.90 = Lyamungo 1990; S.97 = Selian 1997. Bars followed by dissimilar letters are significantly different at $p = 0.05$ according to Fischer least significance difference. (a) Field experiment; (b) Glass house experiment.

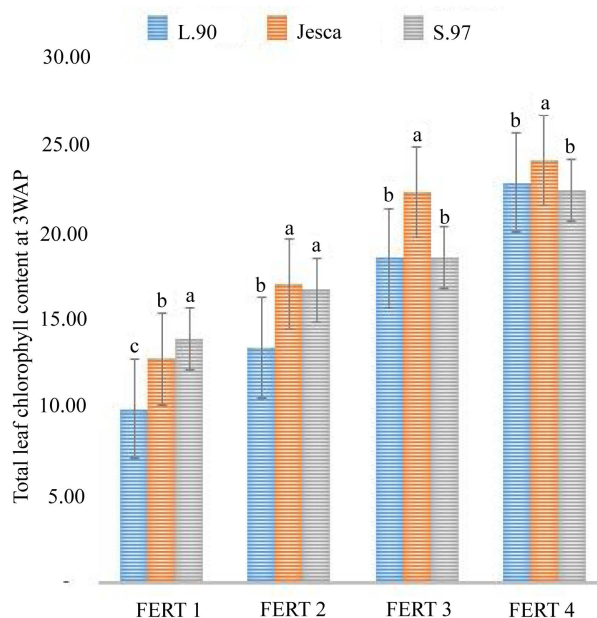


Figure 7. Interactive effect of varieties and fertilizer on total leaf chlorophyll content recorded at 3 WAP in the screen house experiment. L.90 = Lyamungo 1990; S.97 = Selian 1997. FERT1= Zero fertilizer; FERT2 = 20 kg/ha K; FERT3 = 30 kg/ha P; FERT4 = Combination of 20 kg/ha K and 30 kg/ha P. Bars followed by dissimilar letters are significantly different at $p = 0.05$ according to Fischer least significance difference (LSD).

lowed by phosphorus and potassium. Better results were recorded in plots supplied with the both rhizobial inoculant, and combination of phosphorus and potassium at 30 kg/ha and 20 kg/ha respectively. Significant interactive effects exhibited by inoculating the soil with *Rhizobium*, and supplementing it with phosphorus and potassium suggest the need of this technology in the study area.

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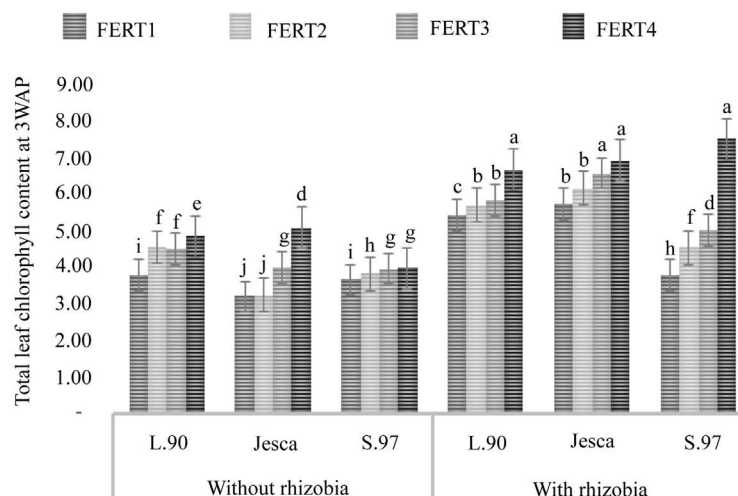


Figure 8. Interactive effect of rhizobia inoculation, fertilizer and varieties on the total leaf Chlorophyll content recorded in the field experiment at 3 WAP 2014. L.90 = Lyamungo 90; S.97 = Selian 97 FERT1 = Zero fertilizer; FERT2 = 20 kg/ha K; FERT3 = 30 kg/ha P; FERT4 = Combination of 20 kg/ha K and 30 kg/ha P. Bars followed by dissimilar letters are significantly different at $p = 0.05$ according to Fischer least significance difference (LSD).

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