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Response of Faba bean (*Vicia faba* L.) to Rhizobium Inoculation, Phosphorus and Potassium Fertilizers Application at Alicho Wuriro Highland, Ethiopia

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A pot experiment was conducted to evaluate the response of faba bean to rhizobium inoculation, phosphorus and potassium fertilizers application at Worabe Agricultural Research Center, from June to October, 2016. A factorial combination of two levels of rhizobium (inoculated and uninoculated), three levels of phosphorus (0, 30, and 60 kg P ha^{-1}) and three potassium levels (0, 30, and 60 kg K ha^{-1}) was laid in randomized complete block design with six replications. Rhizobium strain significantly affected all parameters studied in this experiment except number of seeds pod^{-1}. Phosphorus fertilizer significantly influenced all parameters, except number of seeds pod^{-1}. Combined application of rhizobium inoculation with phosphorus fertilizer significantly ($p < 0.05$) influenced leaves plant^{-1}, root dry weight, above ground dry matter and days to physiological maturity. Similarly, plant height, leaves plant^{-1}, root dry weight, pods plant^{-1} and above ground dry matter were influenced by rhizobium inoculation and potassium interaction. The combined application of bio-fertilizer and chemical fertilizer improved the performance of faba bean in the study area.

Key words: faba bean, rhizobium strain, growth, yield

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INTRODUCTION

Faba bean is one of the major pulse crops widely produced in the highlands of Ethiopia. It is an annual crop grown by subsistence farmers, during the cool main rainy season (June to September). Faba bean occupies about 28% of the total land area under pulse crops in the country (CSA, 2015). Southern Nations Nationalities and Peoples' Regional State shares 14.08% pulse crop area and holds 12.29% of the national pulse production (CSA, 2015). Of the entire regional area under pulse crop production (219,502.58 hectares), 3.34% is covered by faba bean and accounts for 3.32% of total pulse production (CSA, 2015).

Despite its multifaceted benefits the productivity of faba bean, both national and regional productivity, 18.93 and 16.39 t ha^{-1}, respectively, remained low compared to its attainable yield,>2 t /ha^{-1} (MoA, 2011; CSA, 2015). In low-input agriculture systems of Ethiopia, chemical fertilizers are rarely used in the production of faba bean and other pulse crops; instead, these crops are used as a restorer of soil fertility, subsequently after cereal crops (Mulissa and Fassil, 2012). Hence, this study aimed to evaluate the combined effect of rhizobium inoculation, phosphorus and potassium fertilizers application on growth and yield components of faba bean.

MATERIALS AND METHODS

Experimental Site Description

The pot experiment was conducted from June to October, 2016 under transparent plastic shade at Worabe Agricultural Research Center, Southern Nations Nationalities and Peoples' Regional State of Ethiopia. Worabe Agricultural Research Center is situated at 07^{O}50'49''N and 0 38^{O}10'52''E, and at 2088 m.a.s.l. The soil was collected from Alicho wuriro, the nearby faba bean grown farmers' fields. Alicho wuriro is one of a district in Siltie zone and located at 07^{o}56'96''N and 038^{o}09'39''E, and 2814 m.a.s.l. The area receives a bimodal rainfall with an annual average rainfall of 1008 mm. Rainfall is distributed between the short rainfall season (March to April) and the main rainy season (June to September). The rainfall pattern is, however, extremely variable with high probability. The minimum and maximum temperature of the area is 10 and 24°C, respectively. The soil type of the study area is pellic vertisol. Mixed crop-livestock farming is the dominant economic activity in the rural areas.

Experimental Design and Treatments

The experiment comprised of factorial combinations of three phosphorus levels (0, 30 and 60 kg P ha^{-1}), three potassium levels (0, 30 and 60 ha^{-1}) and rhizobium inoculation with two levels (inoculated and uninoculated). *Degaga* faba bean variety was used as test crop. Since *Degaga* variety is adapted to altitude of 1800-3000 meters above sea level, the agro-ecology of the study area is suitable for its production (EARO, 2004).

Rhizobium strain was applied at the rate of 500 g ha^{-1}. In order to ensure that all the applied inoculum stick to the seed, the required quantity of inoculant was suspended in 10% sugar solution. The sugar slurry was gently mixed with dry seed and then with Carrier-based inoculant so that all the seeds received a thin coating of the inoculants. The inoculated seed was allowed to air dry before sowing. As a precaution of cross contamination, uninoculated treatments were sown first. Four faba bean seeds were planted for every pot and thinned to two plants per pot after 10 days of emergence. The actual rates of fertilizers (phosphorus and potassium) were calculated based on soil weight basis assuming the weight of soil in a hectare at 15 cm depth is 2 $x10^{6}$ kg.

Physical and Chemical Soil Characteristics

Soil samples for the pot experiment was taken from 0 to 15 cm depth in May 2016 from randomly selected five faba bean grown farms and composited together. For pre sowing soil properties determination, sub-samples were taken from the collected composite soil sample. The composite soil sample was analyzed for soil pH, cation exchange capacity, texture, available phosphorus, available potassium and total nitrogen at South Nation Nationality People's Region soil laboratory. The pH of the soil was measured in a suspension of a 1: 2.5 soil to water ratio and Cation Exchange Capacity (CEC) was determined by Ammonium Acetate method. The availability of soil phosphorus was analyzed using Olsen method. To determine the available potassium in the soil, the sample was extracted with Morgan solution and K in the extract was measured by flame photometer. The total nitrogen was determined by Kjeldehal method. Particle size was determined by hydrometric method.

Statistical analysis

The data was analyzed using SAS version 9 (SAS, 2004) with three factor analyses of variance (ANOVA) to evaluate the main and interaction effects of the treatments. Least significant difference was used to compare treatment means at 5% probability level.

RESULTS AND DISCUSSION

Pre sowing Soil Chemical and Physical Properties

Pre-sowing soil sample analysis demonstrated that the textural class of experimental soil belongs to clay loam soil texture, with the proportions of 38% sand, 26% silt and 36% clay (Table 1). The soil held 0.145% total N, 13.2 mg kg^{-1} available K and 12.4 mg kg^{-1} available P. The soil exhibited low ratings for N and K, while that of P was medium. Similarly, organic carbon content (1.58%) was in the low range with soil pH being slightly acidic (Hart *et al.,* 2011).

Table 1. Some physical and chemical properties of the 0 to 15 cm soil layer experimental soil before sowing

pH (1: 2.5 soil water suspension)	%OC	Available P (ppm)	Available K (mg/kg)	%Total nitrogen	CEC (meq/100 g)	% Texture			Textural class
						Sand	Clay	Silt	
6.6	1.58	12.4	13.2	0.145	24.6	38	36	26	Clay loam

Table 2. Effect of rhizobium inoculation, phosphorus and potassium rates on days to physiological maturity and growth attributes of faba bean

Treatment	DPM	PH (cm)	LPP	NPP	NDW (mg plant^{-1})	SDW (g plant^{-1})	RDW (g plant^{-1})
Rhizobium							
Un	109.95a	42.67b	17.56b	33.14b	0.31b	5.24b	4.49b
In	108.56b	54.68a	20.96a	55.01a	0.47a	9.08a	6.22a
LSD (5%)	0.72	2.51	1.02	3.96	0.04	0.49	0.45
P (kg ha^{-1})							
0	109.50a	47.09b	18.97a	20.22c	0.24c	5.89c	5.23b
30	108.55b	47.63b	18.88b	43.11b	0.39b	6.97b	4.50c
60	109.50a	53.30a	20.50a	72.55a	0.58a	9.26a	6.60a
LSD (5%)	0.88	3.06	1.24	4.82	0.05	0.60	0.55
K (kg ha^{-1})							
0	110.88a	51.00a	19.91a	42.18b	0.36b	6.82b	4.16b
30	107.72c	49.72ab	19.77ab	43.80b	0.41a	7.73a	6.61a
60	108.94b	47.25b	18.66b	49.91a	0.43a	7.57a	5.59a
LSD (5%)	0.88	3.06	1.24	4.82	0.05	0.60	0.55
CV (%)	1.19	9.17	9.46	15.74	19.33	12.12	14.96

In= inoculated; Un^{-}= uninoculated; PH= plant height; LPP= leaves per plant; NPP= nodule number per plant; NDW= nodule dry weight; SDW= shoot dry weight; RDW= Root dry weight per plant

Effects of P, K and rhizobium inoculation on some faba bean agronomic parameters

Days to physiological maturity

A slightly longer day (110 days) to physiological maturity was recorded in uninoculated plants compared to inoculated plants (108.5 days) (Table 2). Bejandi *et al.* (2012) who reported that inoculation of seeds with rhizobium reduced days to 70% physiological maturity in chickpea as compared to uninoculated treatment.

In general, as level of phosphorus fertilizer increased the days to physiological maturity shortened as compared to control (Table 2). This result is in agreement with that of Gifole *et al.* (2011) who found that phosphorus application significantly shortened days to physiological maturity as compared to the control in haricot bean. Phosphorus application without rhizobium inoculation elongated days to physiological maturity. Conversely, as phosphorus was applied with rhizobium inoculation, days to physiological maturity shortened and attained its lowest record at application of phosphorus at 30 kg ha^{-1}. Another investigation by Bejandi *et al.* (2012) reported that inoculation of seeds with rhizobium reduced days to 70% physiological maturity in chickpea as compared to uninoculated treatment.

Faba bean supplied with both phosphorus and potassium nutrients matured earlier compared to control and separate applications (Table 3). This implies that balanced nutrition facilitate health growth, good

Table 3. Interaction effect of phosphorus and potassium rates on days to physiological maturity, number of nodules per plant and shoot dry weight per plant

Treatments		DPM	NPP	SDW (g plant^{-1})
P (kg ha^{-1})	K (kg ha^{-1})			
0	0	107.33c	15.16d	3.15e
	30	109.00c	16.33d	3.48de
	60	113.00ab	15.66d	3.37e
30	0	115.33a	28.33c	3.33e
	30	107.00c	30.00c	4.40cd
	60	108.66c	44.16b	4.02cde
60	0	112.33b	56.33a	6.18a
	30	107.00c	59.16a	7.97a
	60	108.0c	59.50a	4.99c
LSD (5%)		2.38	6.24	0.99

DPM= days to physiological maturity; NPP=number of nodules per plant; SDW=shoot dry weight per plant at mid flowering

Table 4. Interaction effect of rhizobium inoculation and potassium rate

Treatments		PH (cm)	LPP	RDW (g plant^{-1})	PPP
R	K(kg ha^{-1})				
Un	0	39.83b	17.00c	3.15c	5.00c
	30	40.75b	17.33bc	3.48c	6.50c
	60	37.16b	16.00c	3.37c	6.66c
In	0	58.00a	20.50ab	4.59b	10.66b
	30	51.66a	21.83a	8.33a	11.16ab
	60	55.16a	21.16a	8.46a	13.16a
LSD (5%)		8.95	3.36	0.75	2.27

photosynthesis and enhanced flowering, pod setting and physiological maturity (Nadia, 2012).

Plant height

Rhizobium inoculation resulted significantly ($p< 0.001$) taller plants (55 cm) compared to uninoculated plants (43 cm) (Table 2). The present work is in agreement with the findings of Endalkachew *et al*. (2016) who reported that an increase in plant height of lentil in response to inoculation with rhizobium strain.

Phosphorus fertilization enhanced the height of faba bean (Table 2). Correspondingly, Shakori and Sharifi (2016) showed that an increase in plant height of faba bean as level of phosphorus application increased.

The combined effect of inoculation and potassium fertilization resulted in taller plants. The current result is in concurrence with the result that potassium supply had positive effect on nitrogen fixation and on shoot growth (Abebe and Tolera, 2014).

Number of Leaves per Plant

Inoculated plants produced more leaves as compared to uninoculated plants (Table 2). Similarly, the study by Mmbaga *et al.* (2015) with climbing bean varieties inoculated with rhizobium and fertilized with phosphorus and potassium showed increased number of leaves per plant after six weeks of planting.

Application of potassium along rhizobium inoculation resulted in relatively higher number of leaves per plant as compared to applying without rhizobium inoculation (Table 4). Similarly, study by Mmbaga *et al.* (2015) revealed that number of leaves per plant increased by 20% four weeks after planting relative to control in the green house experiment.

Similarly, higher number of leaves per plant was recorded due to phosphorus application along rhizobium

Table 5. Interaction effect of rhizobium inoculation and phosphorus rate

Treatments		LPP	RDW (g)
R	P (kg ha^{-1})		
Un	0	17.00b	3.15d
	30	20.16ab	3.33cd
	60	21.15a	6.18a
In	0	20.50ab	4.59b
	30	20.50ab	3.66c
	60	20.16ab	4.05bc
LSD (5%)		4.00	0.75

Un=uninoculated with rhizobium strain In= inoculated with rhizobium strain; LPP=number of leaves per plant; RDW= root dry weight

inoculation compared to plants supplied phosphorus alone (Table 5).

Nodule number per plant

Rhizobium inoculation showed increase of nodules per plant (Table 2). Correspondingly, Bejandi *et al*. (2012) reported that seed inoculation with *Rhizobium cicerea* produced significantly highest nodule number of active nodule per plant than control.

As the level of applied phosphorus increase, number of nodules per plant also increased (Table 2). Accordingly, application of phosphorus at 60 kg ha^{-1} P resulted in the highest number of nodules (72.55 nodules plant^{-1}) while the lowest (20.22 nodules plant^{-1}) was recorded from control (0 kg ha^{-1}P).

Similarly, application of potassium significantly affected number of nodules per plant. Even though plants supplied with potassium at 30 kg ha^{-1} K, possessed higher number of nodules, it was not significantly different from that of plants not supplied with potassium (Table 2).

Comparably, study by Mmbaga *et al.* (2015) disclosed that nodule number per plant increased with increasing potassium supply on of faba bean and other legume crops.

In this study, nodule number per plant was positively affected by the interactive effect of potassium and phosphorus. As the rate of applied phosphorus and potassium increased, number of nodules per plant increased. Accordingly, the higher number of nodules was recorded due to application potassium at 60 kg ha^{-1} K compared to the respective rates of phosphorus with 0 and 30 kg ha^{-1}K. The increase of nodule number per plants due to application of phosphorus and potassium together could be due to the fact that phosphorus role in increasing nutrient uptake in legumes (Tairo and Ndakidemi, 2014).

Nodule dry weight

Rhizobium inoculation significantly increased nodule dry weight by 34.04% compared with un-inoculated plants (Table 2). Similarly, Bejandi *et al.* (2012) reported that seed inoculation with *Rhizobium cicerea* produced significantly highest nodule dry weight and active nodule per plant than control.

Similarly, significant ($p< 0.001$) variation was observed due to different levels of phosphorus application on nodule dry weight. Application of phosphorus at rate of 60 kg P ha^{-1} resulted in the highest nodule dry weight (0.58 mg plant^{-1}), while the lowest (0.24 mg plant^{-1}) was recorded due to control (Table 2). Also, recent study reported that application of phosphorus influenced nodulation and nitrogen fixation of faba bean (Kiros *et al*., 2015).

Nodule dry weight increased due to potassium application. For instance, from application of potassium at rate of 60 kg ha^{-1} K, 0.43 mg plant^{-1} nodule dry weight was recorded while the control produced 0.36 mg plant^{-1} (Table 2).

Shoot dry weight per plant

Shoot dry weight per plant was significantly ($p< 0.01$) affected by the main effect of rhizobium inoculation. The mean shoot dry weight 5.24 and 9.08 g plant^{-1} were recorded for uninoculated and inoculated plants, respectively (Table 2). This result is in agreement with the study by Huang and Erickson (2007) who reported that inoculation with *Rhizobium leguminosarum*, increased shoot biomass of lentil.

As level of phosphorus fertilizer increased, shoot dry weight also increased. Accordingly, the largest shoot dry weight (9.26 g plant^{-1}) was obtained due to 60 kg P ha^{-1}

while the smallest was recorded from control (Table 2). There was increasing trend on shoot dry weight as level of applied potassium increased (Table 2). Accordingly, application of the highest level of potassium (60 kg K ha^{-1}) resulted in the highest shoot dry weight (7.57g $plant^{-1}$) while the lowest value (6.82 g $plant^{-1}$) was recorded from the control.

Root Dry Weight per plant

Rhizobium inoculation increased root dry weight by 27.81% compared with un-inoculated plants. Similarly, Application of the highest rate of phosphorus (60 kg ha^{-1} P) produced the highest root dry weight per plant. Conversely, the control produced higher root dry weight compared to application of 30 kg ha^{-1} P (Table 2).

Also, root dry weight per plant was significantly ($p < 0.05$) influenced by potassium fertilization. Accordingly, application of potassium increased shoot dry weight. As, the maximum level (60 kg ha^{-1} K) of potassium produced 5.59 g $plant^{-1}$ while control treatment resulted in significantly lower shoot dry weight (4.16 g $plant^{-1}$) (Table 2). Similarly, Kurdali *et al*. (2002) reported that the higher level of potassium fertilizer increased dry matter production of faba bean.

Interaction effect of rhizobium inoculation and phosphorus application significantly ($p < 0.05$) affected root dry weight. Higher root dry weight was recorded from rhizobium inoculation with lower rate of phosphorus. Conversely, higher root dry weight was recorded from uninoculated plants when the phosphorus dose increases beyond 30 kg ha^{-1} (Table 5).

Similarly, significant variation at $p < 0.001$ was observed on root dry weight due to interaction effect of potassium and rhizobium inoculation. Root dry weight increased as the level of potassium with rhizobium inoculation and attains its highest value at the level of potassium is 30 kg ha^{-1} along rhizobium inoculation. There was no change in root dry weight with application of potassium fertilizer when this was not combined with inoculation showing clearly the synergistic effects of the two factors.

Number of pods per plant

Number of pods per plant showed significant ($p < 0.001$) response to phosphorus fertilization and rhizobium inoculation but not for potassium fertilization. Rhizobium inoculation increased number of pods per plant (Table 6).

As the level of phosphorus application increased, also number of pods per plant increased accordingly. For instance, the highest number of pods (11.94 pods $plant^{-1}$) was recorded from application of phosphorus at 60 kg P ha^{-1} increasing the number of pods per plant by 25.79% and 13.48% compared to control and due to the rate of 30 kg ha^{-1} P respectively (Table 6). The current study is in agreement with Abebe and Tolera (2014) and Kiros *et al*. (2015) reporting that phosphorus fertilization significantly increased number of pods per plant on faba bean.

Number of Seeds per Pod

The number of seeds per pod did not vary significantly ($p > 0.05$) among the treatments. Even though, it was not significant, inoculation and supplementation with phosphorus and potassium tend to improve number of seeds per plant (Table 6). Similarly, Abebe and Tolera (2014) showed that number of seed per pod of faba bean was not significantly affect due to fertilizer rate, rhizobium inoculation and lime rate. This is due to the fact that the number of seeds per plant is mainly under genetic control can be little affected by the environmental factors (Gemechu *et al*., 2006).

Hundred Seed Weight

Inoculation increased hundred seed weight by 11.44%, compared to uninoculated plants (Table 6). The recent study on lentil also indicated that 100-grain weight was significantly higher in inoculated treatments in the pot experiment (Endalkachew *et al*., 2016).

Application of phosphorus fertilizer significantly increased hundred seed weight compared to the control (Table 6). But further increase the level of applied phosphorus form 30 kg ha^{-1} to 60 kg ha^{-1} had no significant difference on hundred seed weight (Table 2). Similarly, Getachew and Rezene (2006) and Shakori and Sharifi (2016) indicated that application of phosphorus fertilizer increased hundred seed weight compared to unfertilized plants on faba bean on Ethiopian and elsewhere soils. This may be due to the fact that phosphorus enhances root system which provides greater root-soil contact and eventually higher uptake of phosphorus and other important and low mobility nutrients and absorption of higher concentration of mineral nutrients (Zafar *et al*., 2013).

Seed Yield per Plant

Rhizobium inoculation independently increased the seed yield as compared to uninoculated seed (Table 6). This may be due to the fact that inoculation of seeds with rhizobium increase nitrogen uptake (Bejandi *et al*., 2012) and thereby plant growth and performance were enhanced. Rhizobium inoculation can increased the potential of plants to produce more plant height,

Table 6. Effect of rhizobium inoculation, phosphorus and potassium rates on yield attributes of faba bean

Treatments	PPP	SPP	HSW (g)	SYPP (g plant^{-1})
Rhizobium				
Un	7.00b	2.95	44.86b	9.30b
In	13.08a	3.17	50.66a	19.58a
LSD (5%)	0.68	NS	3.22	1.59
P (kg ha^{-1})				
0	8.86c	2.94	43.84b	11.99c
30	10.33b	3.00	48.73a	14.07b
60	11.94a	3.27	51.68a	18.98a
LSD	0.82	NS	3.93	1.94
K (kg ha^{-1})				
0	10.05	2.97	46.92	14.01
30	10.58	3.19	49.75	15.90
60	10.50	3.05	47.58	15.13
LSD (5%)	NS	NS	NS	NS
CV (%)	11.80	19.17	19.13	12.07

branches, pods and seeds that ultimately resulted in high grain yield. Hence, increase in seed yield might be associated with high number of pods plant^{-1} and 100-seed weight (Mansouri-far, *et al.,* 2010).

The result also indicated that there was a linear increase in grain yield of faba bean as a function of increasing phosphorus application rates. Several research reports under Ethiopia condition and elsewhere in Africa also disclosed significant crop yield increment in response to the application of phosphorus fertilizers (Fisseha and Yayis, 2015).

CONCLUSION

Rhizobium inoculation significantly improved grain yield of faba bean. Also, application of phosphorous improved the grain yield of faba bean. Accordingly, the highest level of phosphorous application resulted in the highest grain yield compared to 30 kg/ha P and the control. Due to potassium levels, leaves plant^{-1}, pods plant^{-1}, seeds pod^{-1}, total seed weight plant^{-1} and hundred seed weight were not significantly affected while the other traits did. The combined application of bio-fertilizer and chemical fertilizer improved the performance of faba bean in the study area.

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