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Response of Haricot Bean (*Phaseolus Vulgaris* L.) to Lime Application and *Rhizobium* Inoculation on Nitosols at Bench-Maji Zone, Southwestern Ethiopia

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Abstract

Haricot bean (*Phaseolus Vulgaris* L.) is one of the most economically important legume crops cultivated in Ethiopia. However, its productivity has never reached its full potential of production that it has in supporting income of resource poor farmers in the nation. This is partly due to infertility caused by acidic soils which have low nutrient contents including calcium. The present study was conducted to evaluate response of haricot bean to liming and *Rhizobium* inoculation in the vicinity of Mizan-Teferi, Bench-Maji Zone in Southwestern Ethiopia. The experiment comprised a factorial combination of four lime levels: 0, 1025, 2050 and 3075 kg ha-1; and *Rhizobium leguminosarum* biover *phaseoli* inoculation - with and without inoculation. The experiment was laid out in a randomized complete block design with three replications. Data on nodulation yield and yield component parameters were collected and analyzed. The result revealed that the interaction effect of lime and *Rhizobium* inoculation had a significant influence on the majorities of parameters tested except pod number per plant and thousand seed weight. Higher values of nodule number per plant, nodule weight per plant, pod length, number of seed per pod and yield of haricot bean were obtained at *Rhizobium* inoculated treatments receiving lime rate of 2050 and 3075 kg ha-1. However, further increases in rate of lime beyond 2050 kg ha-1 even got worsen with respect to seed yield. Therefore, lime at a rate of 2050 kg ha-1 in combination with *Rhizobium* inoculation might be tentatively recommended to be used as a bio-fertilizer in the studied area. Further research must be needed to confirm the result presented from this finding.

Introduction

Haricot bean is the second most important grain legume cultivated as cash crop in Ethiopia. The national production of haricot bean in 2015 cropping season is estimated at over 540.24 thousand tones, with a production area of 357.29 thousand hectares. The average yield per hectare is 1.48 tones [1]. It is one of the main cash crop and protein sources of farmers in many low lands and mid altitude zones of Ethiopia [2]. The crop is grown by subsistence farmers either as a sole crop and/or intercropped with either cereal crops. It is grown for its dry seed and edible immature pods and to a lesser extent for green-shelled beans. Haricot bean is the most important food and export crop in Ethiopia and cash for poor farmers [3]. Apart from being food and a source of income, it is also replenishes of soil fertility through nitrogen fixation. Meanwhile, the productivity has never reached its full potential of production that it has in supporting income generation of resource poor farmers.

This is attributed to lack of proper management of the soil, adapted varieties and different diseases and insect incidence [4]. The poor yields are also partly due to infertility caused by acidic soils which have low nutrient contents [5]. Soil acidity may affect all stages of haricot bean growth and specifically the legume, - *Rhizobium* symbiosis, from strain survival in soil and on the seed, to root-hair infection, nodule initiation and nitrogen fixation [6]. Higher concentrations and contents of hydrogen ion, aluminum and manganese in acidic soils are known to be the major causes of poor growth to plants due to their toxicity effects to plants and microorganisms such as N fixing bacteria [7]. The most common management practice to ameliorate acid soils is through the surface application of lime [8]. The major influence of lime when applied in the soil is on its ability to supply Ca^{2+} which is essential for plant growth and neutralizing the toxicity effects of H^+, Al^{3+} and Mn^{2+} in the soil [9,10]. Lime may also increase soil pH resulting suitable environment for survival of *Rhizobium*.

As soils become more acid, particularly when the pH drops below 4.5, it becomes increasingly difficult to produce food crops. Also the status and magnitude of acidity in Ethiopia are not well known, some 40% of arable lands were reported as acidic and large areas of acidic soils occur in South and Southwestern regions of Ethiopia [11,12]. Moreover, most of the soils in Southwestern Ethiopia are acidic with pH of < 5.5 [13]. Furthermore, more than 80% farmers live in Bench-Maji Zone identify “ Kub-Kub” which literally means that acid soils in Benchnon, as a major crop production constraints [14]. Haricot bean however, thrives well in the pH range of 6 - 7.5 [5]. It is therefore justifiable to introduce lime in acidic soils of Bench-Maji Zone, Southwestern Ethiopia. In Mizan-Teferi where this experiment was conducted, very little information is available on how to improve acid soils. Keeping in view all these points, the current study was conducted with the objectives to investigate the effect of liming and *Rhizobium* inoculation on yield and yield components of haricot bean with the view to determine appropriate treatment combination that gave higher haricot bean yield to the studied area.

Materials and Methods

Description of the study area

The study was conducted from January 2015 to June 2015 at Plant Science research field, Mizan-Tepi University. It is found in Mizan

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Teferi, Bench-Maji Zone of the Southern Nation Nationality and People Regional State (SNNPRS). It is situated at 6°58'34" N latitude and 35°34'6.34" E longitudes at an elevation of 1350 meters above sea level. It is found at about 561 km South-West of Addis Ababa and 842 km from regional capital of Hawassa. The annual rain fall in the study area ranges from 1250 to 2000 mm and annual temperature range from 20 to 40 °C. The main rain occurs in April and from June to September. The soil in the study area is acidic in reaction and red in color.

Treatments and experimental design

The experiment comprised a factorial combination of four levels of lime (0, 1025, 2050 and 3075 kg ha^{-1}) and *Rhizobium leguminosarum* biover *phaseoli* inoculation (with and without inoculation). It was laid out in a randomized complete block design with three replications. A 2 m X 2 m (4 m^2) gross plot size was used as one experimental unit. Each of these experimental units was accommodated five rows at spacing of 40 cm X 10 cm between rows and plants, respectively. A 1 m wide open strips was used to separate the block where as plots within each blocks was separated by 0.5 m.

Experimental procedure

N-fixing *Rhizobium* bacteria (*Rhizobium leguminosarum* biover *phaseoli*) were obtained from National Soil Testing Center, Addis Ababa, Ethiopia. While, liming material ($CaCO_3$) was obtained from Plant Science Department, College of Agriculture and Natural Resource, Mizan-Tepi University. Local haricot bean variety was used as a test variety for this experiment. The experimental field was ploughed and harrowed before planting. The lime ($CaCO_3$) was broad casted and thoroughly mixed with the soil manually by hand 15 days before planting [15]. *Rhizobium* inoculation was done manually on the seed. Un-inoculated haricot bean seeds were sown first to avoid contamination. All the other recommended agronomic managements were performed uniformly to all plots.

Data collected

From each plot, five plants were randomly uprooted with the bulk of soil and root mass at flowering for recording nodulation parameters. After separating effective nodules from the root, numbers and weights of nodules were determined. Accordingly, five plants from each border plots were randomly taken at harvest for recording yield components such as; numbers of pod per plant, pod length and seed number per pod. For thousand seed weight and seed yield, the three central rows were harvested at final harvest. Finally, yield per hectare was determined by converting the yield per three central rows (kg plot^{-1}) into kg ha^{-1}.

Data analysis

The means of the above parameters were subjected to analysis of variance (ANOVA) using SAS version 9.1.3 computer software. Mean comparison was done by using least significant difference test at 5% probability level.

Result and Discussion

Nodule number per plant

Nodule number per plant of haricot bean was very highly significantly ($p \leq 0.001$) influenced by the interaction effects of *Rhizobium leguminosarum* biover *phaseoli* and lime application (Table 1). The highest nodule number per plant (43) was scored at treatment combination of inoculated and 3075 kg lime ha^{-1}, which however was statistically similar with that obtained from the *Rhizobium* inoculation and 2050 kg lime ha^{-1}. The lowest nodule number (10.8) was scored when haricot bean was sown without inoculation and lime application (Figure 1).

Liming the soil significantly improved nodule number over non-limed soil when the crop was grown symbiotically with *Rhizobium*. The finding is in agreement with the report of Bekere W et al. who reported that lime and *Bradyrhizobium* inoculation at the same time produce the highest nodule number and nodule volume of soybean in Southwestern Ethiopia [16]. The increase in number of nodules production in plants under optimum *Rhizobium* inoculated and liming combination could be due to the fact that liming improved supply of calcium to plants through enhancing symbiotic association with *Rhizobium*. Studies have indicated that calcium deficiency in legumes depressed the calcium content of nodules, impairing nitrogen fixation due to inadequate calcium for nodule structure and/or metabolism [17].

Nodule weight

Nodule weight was significantly influenced by the interaction effect of inoculation and lime application (Table 1). The maximum weight of nodule (0.533 g plant $^{-1}$) was recorded from the inoculation of the *Rhizobium* after the application of 3075 kg lime per hectare followed by the application of 2050 kg lime per hectare. In contrast, the lowest value of this parameter (0.13 g plant $^{-1}$) was obtained from the interaction effect of zero lime application and without inoculation (Table 2).

Table 1: Mean squares of haricot bean as affected by *Rhizobium* inoculation and lime levels.

Source of variation	DF	NNPP	NWPP	NPPP	TSW	PL	NSPP	SY
Replication	2	242.89***	12.89***					21815.22ns
Rhizobium	1	824.85***	0.35***	15.68***	7238.43***	28.64***	18.37***	3455537.31***
Lime	3	660.61***	0.05***	20.36***	998.14***	4.60***	3.53***	404080.92***
Rhizobium * Lime	3	47.82***	0.012***	1.38ns	116.05ns	2.68***	0.44**	68415.9*
Error	14	5.71	0.00097	1.12	80.65	0.12	0.105	18724.16

DF: Degree of Freedom; NNPP: Number of Nodule Per Plant; NWPP: Nodule Weight Per Plant; NPPP: Number Of Pod Per Plant, TSW: Thousand Seed Weight; PL: Pod Length; NSPP: Numbers Of Seed Per Pod; SY: Seed Yield; NS: Non Significant at 5% Probability Level; *: Significant at 5% Probability Level; **: Significant at 1% Probability Level; ***: Significant at 0.1% Probability Level

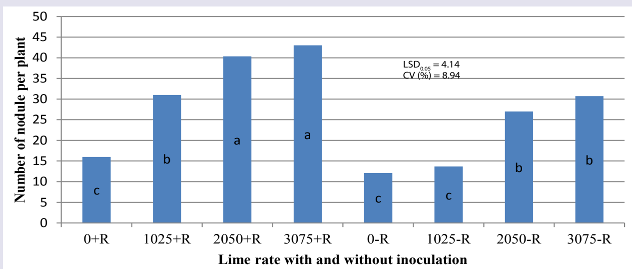

Figure 1: Interaction effect of lime rate with *Rhizobium* inoculation on mean number of nodule per plant of haricot bean.

From the data presented above, mean nodule weight of haricot bean is determined by the interaction effect of liming and *Rhizobium* inoculation. The result was in agreement with the finding of Bekere W et al. who observed 50% increases in nodule weight when soybean inoculated with *Bradyrhizobium* and receiving at a rate 2598.75 kg per hectare [16]. The highest nodule weight under greater liming and inoculation combination could be due to the fact that liming neutralizes the toxicity effects of H^+, Al^{3+} and Mn^{2+} in the soil resulting suitable environment for survival of *Rhizobium* [9]. *Rhizobium* inoculation and lime level had a significant effect on mean nodule number of haricot bean (Figure 1). The increase in nodule weight at higher rate of liming and nodulation combination thus might be due to the fact that higher lime and *Rhizobium* inoculation increased nodule number per plant, and consequently increased mean nodule weight per plant.

Number of pod per plant

Numbers of pod per plant were very highly significantly ($p < 0.001$) influenced by the main effect of liming and inoculation treatments (Table 1). Inoculation of haricot bean plant with *Rhizobium* bacteria significantly increased numbers of pod per plant by 22% (Table 3). The same parameters were also affected by liming treatments. The lowest numbers of pod per plant (6.63) were recorded at non-limed treatments (Table 3). Mean number of pod per plant increase with increasing lime level, reaching maximum at 2050 kg ha^{-1}; thereafter lime application even got worsen. Interaction effects between these factors were not significant with respect to numbers of pod per plant (Table 1).

Higher values of pod per plant with *Rhizobium* inoculation can be attributed to the inoculated *Rhizobium leguminosarum* biover *phaseoui* could be adapted to the harsh soil conditions such as acidity condition of the studied area. The *Rhizobium* strain used in the current study therefore enhanced soil fertility and increased mean number of pods in haricot bean plant. This result is in agreement with the report of Beyene S et al. who reported that *Rhizobium* isolated from chickpea and lentils at Meskan and Halaba area are uniquely adapted to the harsh environmental conditions encountered in these regions [18]. Similarly, the use of liming also increased yield components in haricot bean plant. This can be explained by the fact that liming provide sufficient amount of calcium to the soil. The availability of Ca in the soil enhances the symbiotic N fixation, thus promote productivity in haricot bean through increasing yield components. In support of the current study, Lucrecia M et al. demonstrated that supply of Ca^{2+} through lime significantly increased plant productivity in *Pirulgaris* [19].

Thousand seed weight

Thousand seed weight was very highly significantly ($p \leq 0.001$) influenced by *Rhizobium* inoculation (Table 1). The maximum thousand seed weight (162.95 g) was obtained at *Rhizobium* inoculation treatments (Table 3). Application of lime had also a highly significant ($p \leq 0.001$) effect on thousand seed weight (Table 1). The lowest thousand weed weight (130.14 g) was obtained at 0 kg lime ha^{-1} while the highest (158.87 g) was recorded at 3075 kg lime ha^{-1} which however, was at par with that recorded at 2050 kg lime ha^{-1}; all the other liming treatments performed in between the two (Table 3). The interaction effect of *Rhizobium* inoculation and lime application on thousand seed weight was not significant (Table 1).

Inoculation of haricot bean with *Rhizobium* bacteria increases thousand seed weight of haricot bean plant by 27% compared with un-inoculated treatment. This may be due to the presence of indigenous haricot bean nodulating bacteria in the experimental soil. In agreement with this Bekere W and Hailemariam A who reported such arguments on soybean [20]. Thousand seed weight also increased with increase in liming level up to 3075 kg ha^{-1}. The increase in thousand seed weight of haricot bean with increase in lime levels may be attributed to the fact that lime application improves productivity through enhancement of soil fertility by reducing the toxicity effects of Al, Fe and Mn. Similar results were obtained by Dejene T et al. who reported that hundred seed weights of common bean increases with increasing lime level up to 2.7 t ha^{-1} [21].

Pod length (cm)

Pod length of haricot bean plants were very highly significantly ($p < 0.001$) influenced by interaction effect of inoculation and liming levels (Table 1). The lowest pod length (4.43 cm) was obtained at zero levels of liming and non-inoculated treatment combinations. The highest pod length (9.11 cm) was obtained at lime rate of 3075 kg ha^{-1} and *Rhizobium* inoculated combinations. However, all liming levels inoculated with *Rhizobium leguminosarum biover phaseoli* varied none significantly with respect to pod length (Table 4).

The use of lime and *Rhizobium* bacteria in combination in the current study resulted improvement in pod length in haricot bean plant. This can be explained by the fact that lime application can promote the survival of *Rhizobium* through neutralizing the toxicity effects of H^+, Al^{3+} and Mn^{2+} in the soil resulting suitable environment for activity of N fixing bacteria. This result is similar with the finding of Bekere W et al. who reported the N_2-fixation of soybean were increased significantly with the addition of lime and *Rhizobium* inoculation [23].

Table 2: Interaction effects of *Rhizobium* inoculation and liming on nodule weight (g plant $^{-1}$).

Treatments *Rhizobum* inoculation	Lime rate (kg ha^{-1})			
	0	1025	2050	3075
- R	0.130^d	0.153^d	0.213^c	0.223^c
+ R	0.253^c	0.373^b	0.520^a	0.533^a
LSD (5%) = 0.054 **CV %** = 10.41				

Means across all rows and columns followed by the same letter are not statistically different at $p \leq 0.05$. -R: without inoculation; +R: with inoculation

Table 3: Yield components of haricot bean as influenced by *Rhizobium* inoculation and liming.

Treatments		Yield components	
		Number of pod per plant	Thousand seed weight
Rhizobium inoculation	*-R*	*7.47^b*	*128.22^b*
	+R	*9.10^a*	*162.95^a*
	$LSD_{0.05}$	*0.92*	*7.77*
Lime rate (kg ha^{-1})	*0*	*6.63^c*	*130.14^b*
	1025	*6.98^c*	*140.27^b*
	2050	*10.60^a*	*153.11^a*
	3075	*8.92^b*	*158.87^a*
$LSD_{0.05}$		*1.29*	*10.99*
CV (%)		*12.78*	*6.17*

Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$ probability level. -R: without inoculation; +R: with inoculation

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Table 4: Interaction effects of *Rhizobium* inoculation and liming on yield and yield component of haricot bean plant.

Treatments combinations			Yield and yield components		
Lime rate (kg ha^{-1})	+	*Rhizobium* inoculation	Pod length (cm)	Number of seed per pod	Yield (kg ha^{-1})
0	+	$^{-}$R	4.43^{d}	2.93^{f}	581.7^{f}
1025	+	$^{-}$R	6.46^{c}	4.03^{e}	832.1^{e}
2050	+	$^{-}$R	7.52^{b}	4.73^{d}	982.7^{de}
3075	+	$^{-}$R	7.85^{b}	4.83^{d}	939.8^{e}
0	+	$^{+}$R	8.53^{a}	5.23^{cd}	1190.6^{d}
1025	+	$^{+}$R	8.59^{a}	5.50^{bc}	1434.5^{c}
2050	+	$^{+}$R	8.81^{a}	5.87^{b}	1998.4^{a}
3075	+	$^{+}$R	9.11^{a}	6.93^{a}	1748.3^{b}
LSD 0.05			0.60	0.56	239.28
CV (%)			4.55	6.48	11.39

Means within a column followed by the same letters are not significantly different at 5% probability level. $^{-}$R: without inoculation; $^{+}$R: with inoculation

Number of seed per pod

The interaction effect of *Rhizobium* inoculation and application of lime had a significant ($p < 0.01$) effect on numbers of seed per pod (Table 1). The maximum numbers of seed per pod (6.93) was recorded from haricot bean plant inoculated with *Rhizobium* bacteria after the application of 3075 kg lime per hectare. In contrast, the minimum numbers of seed per pod (2.93) was recorded from un-limed and non-inoculated treatment combinations (Table 4). The more numbers of seed per pod at inoculated and limed treatment combination can be explained by the fact that, liming created better soil environment through supply of calcium in the soil. This condition results in a better activity of *Rhizobium leguminosarum* to exist in the soil [22]. Similar result was also reported by Bambara S and Ndakidemi PA who conducted a research on effects of *Rhizobium* inoculation, lime and molybdenum on nitrogen fixation of inoculated *Phaseolus vulgaris* [15].

Seed yield (kg ha^{-1})

Interaction effects between *Rhizobium* inoculation and lime levels were significant ($p < 0.05$) with respect to seed yield of haricot bean plant (Table 1). The highest (1998.4 kg ha^{-1}) and the lowest (581.7 kg ha^{-1}) yield of haricot beans were obtained at lime-dose of 2050 kg ha^{-1} with *Rhizobium* inoculation and at un-limed plus un-inoculated treatment combinations, respectively; all the other treatments combinations performed in between the two (Table 4). The increase in yield of haricot bean plants at higher liming and *Rhizobium* inoculation could be due to the fact that the use of *Rhizobium* bacteria and liming in combination provide sufficient amount of available N and Ca^{+} to the soil. These ultimately increased yield of haricot bean plant through better utilization of such nutrients. Furthermore, interaction effects of inoculation and liming had a significant effect on yield components such as pod length and number of seed per pod. The influence of inoculation and liming on yield may thus be due to their effects on these parameters.

This result is in conformity with many of the earlier authors such as; Beyene S et al., Dejene T et al., and Kassa M et al. working with *Rhizobium* inoculation and liming on chickpea, lentil and common bean [18,21,23]. Each of these studies clearly reported that *Rhizobium* inoculation and lime application neutralizes soil acidity, reduces toxicity levels of Al, Fe and Mn and improves productivity by improving the physiological, chemical and biological properties of soil.

Conclusion

Responses of haricot bean to liming and *Rhizobium* inoculation were assessed on acid soil of Mizan-Teferi, Bench-Maji Zone; Southwestern Ethiopia. The result of the current study revealed that interaction effects between liming and *Rhizobium* inoculation had a significant effect on the majorities of yield and yield component parameters except; number of pod per plant and thousand seed weight. Higher level of liming (2050 and 3075 kg ha^{-1}) with *Rhizobium* inoculation resulted in higher values of nodule number per plant, nodule weight, pod length, number of seed per pod and seed yield of haricot bean. However, in most cases further increase in lime application beyond 2050 kg N ha^{-1} did not bring significant effect. Therefore, lime level of 2050 kg ha^{-1} in combination with *Rhizobium* inoculation sounds economical and can be tentatively recommended for haricot bean production to the studied area. Furthermore, the use of appropriate rate of liming in combination with *Rhizobium* inoculation provides sufficient amount of available N and other essential nutrients to the soil. These low cost practices will therefore maintain the product and productivity of haricot bean and other crop in a legume-cereal rotation system. Since this finding is one season research at one location; further research across more locations and years is of paramount importance.

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