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Full Length Research Paper

Growth and nodulation response of soybean (*Glycine max L*) to *Bradyrhizobium* inoculation and phosphorus levels under controlled condition in South Western Ethiopia

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The study was conducted to investigate growth and nodulation response of Clark 63-K soybean variety to *Bradyrhizobium japonicum* inoculation and phosphorus levels under lath house condition at Jima Agricultural Research Center in 2010. The crop was evaluated in terms of nodule number, nodule volume, nodule dry weight, shoot nitrogen content, plant height, number of pod bearing branches, shoot dry matter and root dry matter. The result revealed that an interaction effect of the main factors did not significantly ($P < 0.05$) influence number of pod bearing branches, shoot dry matter and root dry matter yield of the crop. In terms of shoot nitrogen content and plant height, Clark 63-K soybean had no response to inoculation when it was grown without phosphorus. However, inoculation significantly ($p < 0.05$) increased these parameters over the uninoculated control when 60, 120 and 180 mg kg^{-1} phosphorus were applied. For uninoculated and inoculated treatments, phosphorus significantly ($p < 0.05$) influenced all nodulation and growth traits. The three phosphorus treatments (60, 120 and 180 mg kg^{-1}) significantly ($p < 0.05$) improved pod bearing branches, shoot and root dry matter yield over the untreated control. Inoculation coupled with 120 mg kg^{-1} phosphorus level being considered an optimal combination for Clark 63-K soybean production in the area.

Key words: Growth, nodulation, phosphorus, soybean.

INTRODUCTION

In Ethiopia, declining soil fertility requires approaches that include, but go beyond application of chemical fertilizers (Beyene, 1988; IFPRI, 2010). Bio-fertilizer as an alternative to commercial fertilizer N for pulses is gaining priority due to its economical and ecological benefits (Beyene, 1988; Hailemariam and Tsige, 2006; Wijnands et al., 2011; Jensen et al., 2012). Biological nitrogen fixation (BNF) is a renewable source of nitrogen to replace inorganic nitrogen fertilizer (Beyene, 1988; Bejiga, 2004).

Nitrogen is the most important nutrient element which limits yield in crop production. Feasibility of biological nitrogen fixation in Ethiopia has been well reviewed and documented (Bejiga, 2004; Hailemariam and Tsige, 2006). Although, industrial fertilizer has helped to improve yields of pulse crops, its escalating price caused by a nexus to fossil fuel prices has seriously limited its use. Accordingly, production potential of grain legumes is still low and as a result producers are now looking for other alternatives to these fertilizers.

Legumes have been an essential component of crop production since ancient times because of their role in improving soil fertility via N_2 fixation (Paau, 1998; Ellafi et al., 2011). Leguminous crops can be self-sufficient for all or part of their nitrogen requirements, when their roots are nodulated with effective nitrogen fixing strains of rhizobia. Specifically, 70-80% of soybean nitrogen demand is met by biological N_2 fixation on average.

Study on growth and yield response of soybean to inoculation and varying phosphorus levels was reported

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(Malik et al., 2006; Fatima et al., 2007; Zewdu, 2009). Phosphorus plays key roles in many plant processes such as energy metabolism, nitrogen fixation, synthesis of nucleic acids and membranes, photosynthesis, respiration and enzyme regulation. It influences nodule development through its basic functions as an energy source. However, the element is generally deficient and limits biological nitrogen fixation in highly weathered tropical soils (Nyemba, 1986; Tsvetkova and Georgiev, 2003; Kumaga and Ofori, 2004). Additionally, the use of biofertilizer as a nitrogen source and the amount of phosphorus needed during inoculation is still open to question. Therefore, the study was initiated with the following objectives:

1. To investigate nodulation, shoot nitrogen content and growth response of soybean to *Bradyrhizobium japonicum* strain, TAL379, inoculation.
2. To determine the optimal amount of phosphorus fertilizer needed for soybean (cv. Clark) inoculated with TAL 379 strain.

MATERIALS AND METHODS

A pot experiment with two levels of inoculation and four levels of phosphorus fertilizer was conducted at Jima Research Center during August to November of 2010. Jima Agricultural Research Center is situated 363 km away from Addis Ababa, in South Western Ethiopia, Oromiya Regional State. The Center is found at 7°40'47"N latitude and 36°49'47"E longitude. The me an maximum and minimum temperature of the Center are 26.2 and 11.3°C respectively. The elevation of the Center is 1,753 m above sea level and it receives 1,529.5 mm average annual rainfall. The two factors (inoculation and phosphorus) were combined in factorial arrangement as follows: 1) No inoculation + 0 mg P kg^{-1}; 2) No inoculation + 60 mg P kg^{-1}; 3) No inoculation + 120 mg P kg^{-1}; 4) No inoculation + 180 mg P kg^{-1}; 5) Inoculation + 0 mg P kg^{-1}; 6) Inoculation + 60 mg P kg^{-1}; 7) Inoculation + 120 mg P kg^{-1}; 8) Inoculation + 180 mg P kga^{-1}.

A commercial effective *B. japonicum* strain, TAL 379, was obtained from National Soil Testing Center. Clark 63-K, a well adapted soybean cultivar in the area with 94% viability, was obtained from Jima Agricultural Research Center and used as a test crop. Triple super phosphate was obtained from the Institute of Agricultural Research, Jima Research Center and used as phosphorus source.

Before commencement of the experiment, composite soil sample was analyzed for selected physical and chemical properties. Texture was determined by the Hydrometer principle whereas soil pH was measured from the suspension of 1: 2.5 soil: H_2O by pH meter. Soil organic carbon was determined by the Wakley and Black (1934) and FAO (2008) method. Available phosphorus was determined based on Bray II procedure (Bray and Kurtz, 1945; FAO, 2008). In this regard, the soil was sandy clay loam (sand 61%, clay 28% and silt 11%), acidic (pH H_2O = 5.4) with higher organic matter (4.3%) and lower phosphorus (0.3 mg P kg^{-1}).

Soil was taken randomly from the upper 0.30 m of ten spots from a field of Haru Agricultural Research Sub Center. Soils from these spots were thoroughly homogenized and taken to Jima Agricultural Research Center. It was then dried and crushed into a diameter less than 2 mm and sterilized by fumigating with 10% formaldehyde solution for eliminating indigenous *Bradyrhizobium*, if any. The sterilized soil was watered with distilled water for a week to leach the persisting formaldehyde from the soil. Homogenized, dried and sterilized 3 kg soil was added to carefully sterilized pots and the pots were arranged in Factorial Completely Randomized Design (CRD) with four replication.

Soybean seeds were washed with distilled water and surface sterilized with 70% ethanol. Seeds were then rinsed 3 to 4 times with tap water; moistened with a 0.2 M dilute sucrose solution and inoculated by covering them with paste of inoculum which was made from a rate of 10 g of peat-based powder inocula per 100 g (Somasegaran and Hoben, 1985; Deaker et al., 2004) of seed just before planting. Triple Super Phosphate (TSP) was applied to the pots as per the treatment and mixed with the soil evenly before seeding operation. Finally, 5 seeds were sown per pot. Seedlings were thinned when they attained two pairs of true leaves and three uniformly growing ones were left. Uniform agronomic managements such as weed control, pest and diseases inspection and control as well as, watering were applied to all treatments as per the schedule.

Data collection and analysis

Plant height, number of pod bearing branches per plant, nodule number per plant, nodule volume per plant, nodule dry weight per plant, root dry weight and shoot dry weight were recorded at mid flowering during the maximum growth. Tissue nitrogen content was analyzed following Kjeldahl procedure (FAO, 2008). Analysis of variance was conducted using the General Linear Model procedure of Statistical Analysis System (SAS) and Least Significant Difference (LSD) method at 0.05 probability level was used for mean separation (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Nodulation response of soybean to inoculation and phosphorus level

Nodule number per plant

Nodules were not generally observed in uninoculated treatment. Phosphorus level significantly ($P < 0.05$) affected nodule number per plant (Table 1). In this regard, 60, 120 and 180 mg P kg^{-1} resulted in significantly ($P < 0.05$) more nodule numbers per plant than the unfertilized controls. Nodule number per plant of the 120 and 180 kg P kg^{-1} supplied soybeans was significantly ($P < 0.05$) higher than those recorded from 60 mg P kg^{-1} fed ones. Nevertheless, nodule number per plant of 120 and 180 mg P kg^{-1} supplied soybeans was not significantly different when inoculation was employed. *Bradyrhizobium* inoculation coupled with 120 and 180 mg P kg^{-1} of soil resulted in significantly higher nodule number per plant than other combinations, though they were statistically at par. This report is in agreement with the previous findings of Tsvetkova and Georgiev (2003), Malik et al. (2006).

Nodule volume per plant

The three phosphorus levels (60, 120 and 180 mg P kg^{-1}) resulted in significantly ($P < 0.05$) higher nodule volume per plant than the unfertilized control. Higher phosphorus

Table 1. Interaction effect of inoculation and phosphorus level on nodule number, nodule volume, nodule dry weight per plant and height of soybean at mid-flowering.

Parameter	Inoculation	Phosphorus level (mg P kg^{-1})			
		0	60	120	180
Nodule number per plant	No inoculation	0.00	0.00	0.00	0.00
	Inoculation	22.38^{c}	30.38^{b}	36.50^{a}	37.50^{a}
	LSD $_{0.05 =}$ 1.41				
Nodule volume (ml/plant)	No inoculation	0.0	0.0	0.0	0.0
	Inoculation	1.3^{c}	1.8^{b}	2.0^{a}	2.1^{a}
	LSD $_{0.05}$ = 0.18				
Nodule dry weight (g plant^{-1})	No inoculation	0.00	0.00	0.00	0.00
	Inoculation	0.14^{d}	0.24^{c}	0.33^{b}	0.36^{a}
	LSD $_{0.05}$= 0.02				
Shoot nitrogen content (%)	No inoculation	0.84^{e}	2.17^{d}	2.88c	3.32^{bc}
	Inoculation	1.02^{e}	3.88^{ab}	4.14a	4.34^{a}
	LSD $_{0.05}$= 0.65				
Plant height (cm)	No inoculation	33.88^{c}	46.00^{c}	54.75^{b}	56.38^{b}
	Inoculation	45.38^{c}	56.25^{b}	59.1^{3a}	59.05^{a}
	LSD $_{0.05}$= 2.36				

Means followed by the same letter for a parameter are not significantly different at P **<** 0.05.

levels (120 and 180 mg P kg^{-1} of soil) produced significantly (P < 0.05) higher nodule volume per plant than those soybeans fertilized with 60 mg P kg^{-1}. There was no significant difference between nodule volume per plant of the 120 and 180 mg P kg^{-1} fertilized soybeans, suggesting that the requirement was satisfied.

Nodule dry weight per plant

Mean nodule dry weight was significantly increased with phosphorus levels (Table 1). Though nodule dry weights of 120 and 180 mg P kg^{-1} supplied soybean was statistically indistinguishable, they were significantly higher than of 60 mg P kg^{-1}. The result also showed that the three levels of phosphorus (60, 120 and 180 mg P kg^{-1}) significantly improved nodule dry weight of the crop over the unfertilized treatment (Table 1). This confirmed the findings of Tsvetkova and Georgiev (2003), Ali et al. (2010), who concluded that phosphorus limits nodulation of soybean and mung bean.

Shoot nitrogen content

An interaction effect of the main factors significantly (P < 0.05) influenced shoot nitrogen content of the soybean, Clark 63-K. Inoculation did not influence shoot nitrogen content when the crop was grown without phosphorus. On the other hand, inoculation significantly increased shoot nitrogen content over the control when the soybean was supplied with 60, 120 and 180 mg P kg^{-1}. Supplementations of 60, 120 and 180 mg P kg^{-1} significantly (P < 0.05) increased shoot nitrogen content over the unfertilized control. However, they did not give significantly (P > 0.05) different shoot nitrogen content when the crop was grown symbiotically.

Soybean growth and dry matter yield response to inoculation and phosphorus level

Plant height

Inoculation did not significantly influence height of soybean when phosphorus was not applied (Table 1). However, inoculation resulted in significantly ($P \leq 0.05$) taller soybeans than the uninoculated check when 60,120 and 180 mg P kg^{-1} were applied. Phosphorus level significantly influenced soybean height. The result revealed that 120 and 180 mg P kg^{-1} resulted in significantly taller soybeans than unfertilized check and 60 mg P kg^{-1} whereas height of 120 and 180 mg P kg^{-1} supplied soybeans were not significantly (P > 0.05) different when the crop was grown without inoculation. Similarly, the height of soybean supplied with the 60 mg

Table 2. Number of pod bearing branches and shoot dry matter per plant of Clark 63-K soybean as affected by inoculation and phosphorus level.

Treatment	Parameters		
	NPBBPP	Shoot dry matter (g plant^{-1})	Root dry matter (g plant^{-1})
Inoculation			
No inoculation	3^b	8.99^b	3.5^b
Inoculation	4^a	10.91^a	6.2^a
LSD $_{0.05}$	0.5	0.9	0.5
Phosphorus level			
0 mg P kg^{-1}	1^c	3.29^c	1.8^c
60 mg P kg^{-1}	4^b	11.80^b	4.4^b
120 mg P kg^{-1}	5^a	12.67^b	5.2^{ab}
180 mg P kg^{-1}	5^a	13.89^a	6.3^a
LSD $_{0.05}$	0.70	1.19	1.20
CV (%)	15.67	16.60	18.00

Means within a column with the same letter are not significantly different at p < 0.5. PBBPP = pod bearing branch per plant.

P kg^{-1} was not significantly (P > 0.05) different from height of the crop grown in unfertilized plots. Soybean grown in 60, 120 and 180 mg P kg^{-1} had significantly taller soybeans than the unfertilized check when the crop was inoculated. On the other hand, fertilization of 120 and 180 mg P kg^{-1} resulted in significantly (P $\leq$ 0.05) taller soybeans than 60 mg P kg^{-1} though, there was no significant (P > 0.05) variation between them. Similar findings were also reported by Kumaga and Ofori (2004), Malik et al. (2006) and Zewdu (2009).

Pod bearing branches per plant

Inoculation significantly influenced the number of pod bearing branches per plant of soybean although the interaction effect did not (Table 2). Inoculation resulted in significantly higher pod bearing branches per plant than the control treatment. Pod bearing branches per plant of 120 mg P kg^{-1} supplied soybeans (4.5 branches plant^{-1}) was not significantly (P > 0.05) different from those of 180 mg P kg^{-1} fertilized soybeans (5.06 branches plant^{-1}). However, both 120 and 180 P mg kg^{-1} gave a significantly (P < 0.05) higher number of pod bearing branches per plant than 60 mg P kg^{-1} and unfertilized control (Table 2). The result also showed that even 60 mg P kg^{-1} significantly increased the number of pod bearing branches per plant over the unfertilized control. This may be attributed to the lower (0.21 mg kg^{-1}) availability of phosphorus in the experimental soil.

Shoot dry matter yield

Inoculation and phosphorus level operated independently on shoot dry matter per plant of the soybean. Inoculated soybeans gave significantly higher shoot dry matter per plant than uninoculated ones (Table 2). Phosphorus level significantly influenced shoot dry matter per plant of soybean. The highest mean shoot dry matter per plant (13.89 g plant^{-1}) was recorded from 180 mg P kg^{-1} fertilized soybeans and closely followed by 120 mg P kg^{-1} (12.67 g plant^{-1}) supplied ones. Fertilization of 180 mg P kg^{-1} significantly improved shoot dry matter per plant of soybean over the preceding levels (60 and 120 mg P kg^{-1}). The shoot dry matter yields of the crop obtained under applications of 60 and 120 kg P ha^{-1} were not significantly different (P > 0.05). However, mean shoot dry matter per plant of 60, 120 and 180 mg P kg^{-1} supplied soybeans was significantly greater than mean shoot dry matter of the unfertilized crop. Previous research findings also indicate that omission of P from optimum nutrition dramatically reduced shoot dry matter yield of cereals (Mengel and Kirkiby, 1987; Tena and Beyene, 2011).

Root dry matter

Both factors significantly (p < 0.05) influenced root dry matter of soybean. In this regard, inoculated soybeans gave significantly higher root dry matter than uninoculated ones. The three phosphorus levels (60, 120 and 180 mg kg^{-1}) significantly increased root dry matter over the control (Table 2).

Correlation between the studied parameters

Analysis of correlation showed that all nodulation and growth parameters of the crop were significantly related to each other (Table 3). Nodule number, nodule volume and nodule dry weight were highly significantly (P< 0.01)

Table 3. Correlation coefficients among the studied parameters.

Parameter	NN	NV	NDW	SNC	PH	NPBB	SDM	RDM
NN	1.00	0.99***	0.99***	0.97**	0.89*	0.77*	0.94*	0.82*
NV		1.00	0.98**	0.95**	0.78*	0.76*	0.68*	0.89*
NDW			1.00	0.96**	0.89*	0.93*	0.92*	0.87*
SNC				1.00	0.96*	0.99**	0.99**	0.98**
PH					1.00	0.91*	0.83*	0.87*
NPBB						1.00	0.98**	0.76*
SDM							1.00	0.77*
RDM								1.00

NN= Nodule number, NV= Nodule volume, NDW= Nodule dry weight, SNC= Shoot nitrogen content, PH= Plant height, NPBB= Number of pod bearing branches, SDM= Shoot dry matter, RDM= Root dry matter.

related to shoot nitrogen content whereas they were significantly ($P< 0.05$) correlated to plant height, number of pod bearing branches, shoot dry matter and root dry matter of the crop (Table 3).

Conclusion

The soybean crop did not respond to inoculation when phosphorus was not applied. Therefore, when Clark 63-K is to be grown by inoculating with *B. japonicum* at the location under investigation, it is mandatory to apply up to 120 mg P kg^{-1} of soil. However, net economic return of the inocula and phosphorus levels might be considered from yield of the crop to choose optimum combination.

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