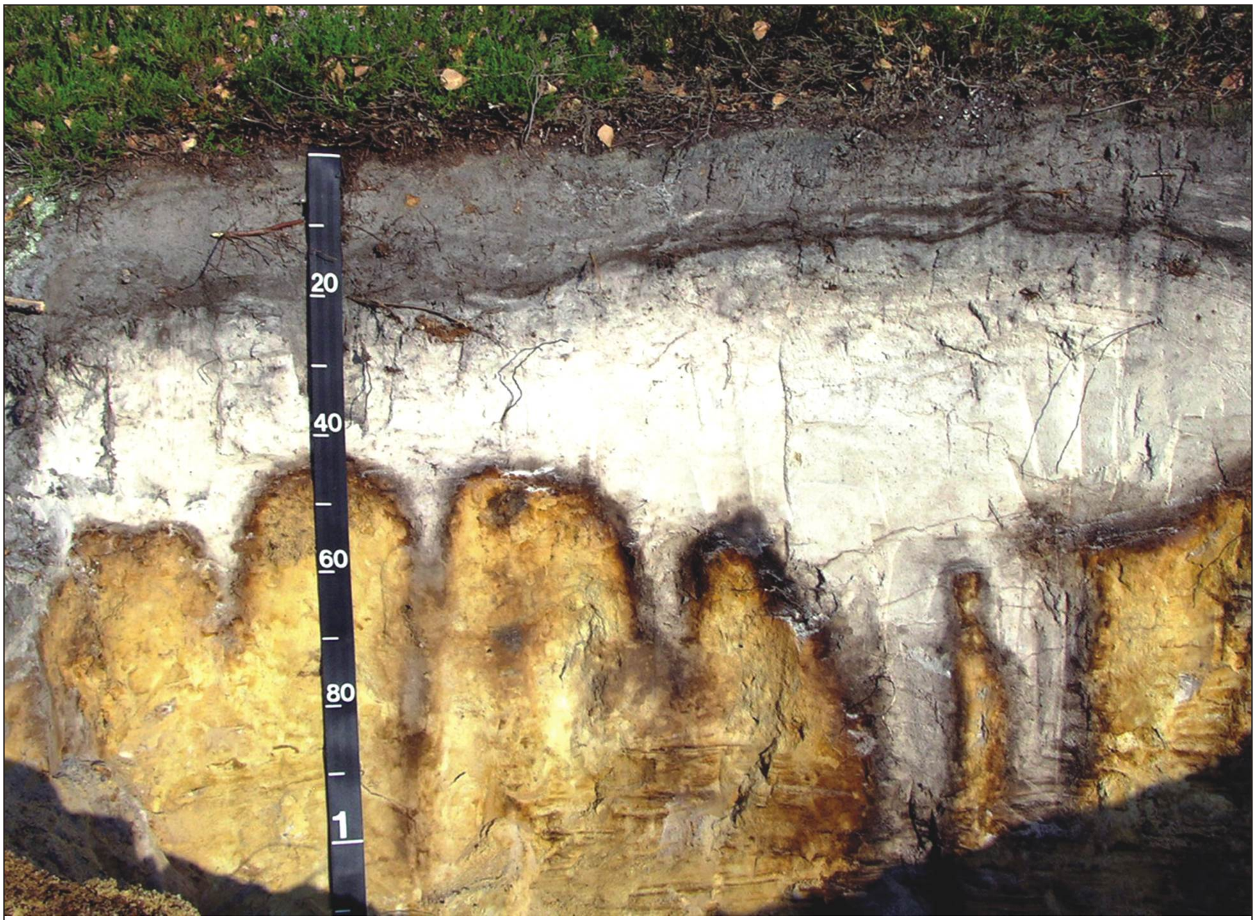

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Growth and Nodulation Response of Soybean (*Glycine max* L.) to Lime, *Bradyrhizobium japonicum* and Nitrogen Fertilizer in Acid Soil at Melko, South Western Ethiopia

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ABSTRACT

The study was conducted to investigate the effect of liming, Bradyrhizobium inoculation and nitrogen fertilization on nodulation and growth response of Clark 63-K soybean variety at Melko, Jimma in 2012 growing season. The crop was evaluated for nodule number, nodule volume, nodule dry weight, pod bearing branch number, shoot dry weight and plant height. The combined effect of liming and Bradyrhizobium inoculation significantly ($p<0.05$) increased nodule number, nodule volume and nodule weight dry per plant compared to un-limed and non-inoculated treatments. On the other hand, nitrogen fertilization did not improve nodulation parameters of the crop nor its interaction with lime and Bradyrhizobium was significant. Application of lime and nitrogen gave more branch number, shoot dry weight and taller soybeans than non-limed and unfertilized treatment when the crop was grown without inoculation. However, regardless of nitrogen fertilization, only lime significantly ($p<0.05$) improved those investigated growth parameters when the soybean was grown symbiotically with Bradyrhizobium. In acid soils, co-treatment of Bradyrhizobium japonicum and lime could complement for chemical fertilizer N in soybean production.

Key words: *Bradyrhizobium*, growth, nitrogen fertilizer, nodulation, lime

INTRODUCTION

Soil acidity has long been known to induce N deficiency in legumes if they depend solely on symbiotic N_2 fixation. Aluminum and manganese toxicity as well as calcium and phosphorus deficiency in this soil inhibit *Rhizobium* growth and root infection resulting in symbiotic failure (Bambara and Ndakidemi, 2010; Negi *et al.*, 2006; Bakker *et al.*, 1999; Zahran, 1999; Keyser and Munns, 1979). So, for economically feasible and sustainable agricultural production in the acid soils, liming is required. Application of lime to acidic soil supply Ca^{+2} or Mg^{+2} which is essential to plant growth and neutralize toxicity effect of H^{+1}, Al^{+3} and Mn^{+2} in the soil. On the other hand, it raises pH of the soil at which *Bradyrhizobium* acts best and an important plant macronutrient P is made available to them (Guo *et al.*, 2009; Negi *et al.*, 2006).

Impairment of nodulation and N_2 fixation by legume *Rhizobium* symbiosis is noticed when legumes are grown on acid soil (Mohammadi *et al.*, 2012; Anetor and Akinrinde, 2006; Munns *et al.*, 1981). Therefore, legume crops face N deficiency resulting in growth and yield reduction. In this regard, Rice *et al.* (2000) reported that soil pH had significant effect on plant biomass, pink nodule number and pink nodule dry weight of field pea. Indeed, the quantity of nitrogen fixed by legumes vary depending on a level of soil nitrogen, effectiveness of strains, management practices, estimation method and length of growing season (Mabrouk and Belhadj, 2012; Jensen *et al.*, 2012; George *et al.*, 1987).

Both symbiotic N_2 fixation and mineral fertilizer N contributes to the N requirement of legumes. In addition to fulfilling their own nitrogen requirement, they are known to leave residual nitrogen in soil. It is reported that application of large quantities of fertilizer N inhibits N_2 fixation but lower levels stimulate early growth of legumes and increase N_2 fixation (Bekere and Hailemariam, 2012; Keyser and Li, 1992; Munns *et al.*, 1981). It is now increasingly being realized that integrated soil fertility management involving combinations of microbial inoculants, inorganic and organic fertilizers are essential to sustain productivity of acid soil and maintain soil health and biodiversity for the long run (Ellafi *et al.*, 2011; Bejiga, 2004). This is especially important for developing countries like Ethiopia where farming will continue to be in the hands of small scale farmers.

The negative effect of acid soil on legume production can be explained in many folds. In addition to its cost and cause of environmental contamination, urea which is a commonly used chemical fertilizer N, release H^+ to the soil and increase soil acidity. On the other hand, the use of *Rhizobium* as bio fertilizer is limited because they are sensitive to acidic soil reaction (Anetor and Akinrinde, 2006). In Ethiopia, soil acidity covers about 41% of arable land though its severity extent varies. It is also true that many small scale farmers of the country depend on this soil for their livelihoods so that it has been given due attention (Abebe, 2007). Some leguminous crops like soybean can successfully be grown on this type of soil. So, the experiment was conducted to investigate the combined effect of lime, *Bradyrhizobium* and nitrogen fertilizer on nodulation and growth of Clark 63-K soybean at Melko.

MATERIAL AND METHODS

Field experiment was conducted at Jimma Agricultural Research Center, Melko, during June to October in 2012. Jimma Agricultural Research Center is found in South western Ethiopia; in Oromiya National Regional State. It is located at 7°40'47"N latitude and 36°49'47"E longitude. The mean 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.

A field of unknown history of soybean cultivation and *Bradyrhizobium* inoculation was selected and an area of 546 m^2 was prepared. The field was then divided in to three replications and each replication was divided in to six experimental units making a total of eighteen plots with an area of 16 m^2. Before planting, a composite soil sample was taken from the upper 0-0.3 m of the experimental field and analyzed for selected physical and chemical properties (Bray and Kurtz, 1945; Walkley and Black, 1934). In this line, exchangeable acidity which is sum total of exchangeable aluminum and hydrogen ion in the soil solution, pH, organic carbon, available phosphorus and nitrogen content of the experimental soil were 2.31 cmol kg^{-1}, 4.43, 1.87%, 3.85 mg kg^{-1} and 0.17%, respectively.

Treatments and their application: Legumefix, Urea and $CaCO_3$ were used as a source of inoculum, nitrogen and lime in the investigation, respectively. The experiment consisted of two factors of inoculation, with and without inoculation; two factors of lime; with and without lime; two factors of nitrogen; with and without nitrogen. Treatments were control (without inoculation+without lime+without N), inoculation only, N only, lime only, inoculation+lime, inoculation+N, lime+N and inoculation+lime+N. Clark 63-K, a well performing soybean variety was used as a test crop. The experiment was conducted in factorial RCB design with three replications. The amount of lime applied was determined based on exchangeable acidity, mass per 0.15 m furrow slice and bulk density of the soil (Van Lierop, 1983; Tran and van Lierop, 1982; Shoemaker *et al.*, 1961). In this line, 2598.75 kg ha^{-1} of lime in the form of $CaCO_3$ was uniformly applied and incorporated in to the soil a month ago before sowing. Splitting in to two, recommended rate of nitrogen (46 kg N ha^{-1}) was applied for nitrogen treatment.

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. The seeds were then sown on 10th June. Agronomic practices were uniformly applied for all treatments throughout the experimental period.

Data collection and analysis: Nodulation parameters such as nodule number per plant, nodule volume per plant, nodule dry weight per plant were recorded at mid flowering. Shoot dry matter yield and number of pod bearing branches and plant height were evaluated at maximum growth after flowering. Count data such as nodule number and pod bearing branches per plant were transformed by Square Root Transformation method before analysis (Gomez and Gomez, 1984). The data were subjected to analysis of variance using SAS packages and treatment means were separated by Least Significant Difference ($LSD_{0.05}$) method.

RESULT AND DISCUSSION

Nodulation response of soybean to lime, *Bradyrhizobium* and N fertilizer: Nodule number, nodule volume and nodule dry weight per plant of the crop were significantly influenced by the interaction effect of lime and *Bradyrhizobium* (Table 1). Liming the soil significantly ($p<0.05$) improved nodule number, nodule volume and nodule dry weight per plant over non-limed soil when

Table 1: Interaction effect of lime and *Bradyrhizobium* on nodules of soybean at Melko, Jimma

		Bradyrhizobium	
Parameter	Lime (kg ha^{-1})	No inoculation	Inoculation
Nodule No. per plant	0	10.4^{d}	52.6^{b}
	2598.75	22.5^{c}	79.8^{a}
$LSD_{0.05}$ = 10			
Nodule volume (mL $plant^{-1}$)	0	1.0^{c}	4.1^{b}
	2598.75	1.5^{c}	5.9^{a}
$LSD_{0.05}$ = 0.6			
Nodule dry weight (g $plant^{-1}$)	0	0.1^{c}	1.5^{b}
	2598.75	0.2^{c}	3.0^{a}
$LSD_{0.05}$ = 0.4			

Values followed with the same letter/s for a parameter are not significantly different at $p<0.05$

the crop was grown symbiotically with *Bradyrhizobium* (Table 1). When the crop was grown without inoculation, lime had no effect on nodule volume and nodule dry weight per plant. However, soybeans grown in lime treated soil had significantly ($p<0.05$) more nodules than those grown in non-limed soil even when the bradyrhizobia were not added (Table 1). This is because of lime created better soil environment for the activity of indigenous soybean nodulating bacteria existing in that soil. On the other hand, inoculation of the crop with *Bradyrhizobium* produced significantly ($p<0.05$) more nodule number, nodule volume and nodule dry weight per plant than uninoculated soybeans under both limed and non-limed soil (Table 1). Lime and *Bradyrhizobium* application at the same time produced the highest nodule number, nodule volume and nodule dry weight per plant of all the combinations. This finding is in agreement with reports of Munns *et al*. (1981), Guo *et al*. (2009) and Chalk *et al*. (2010). The result also revealed that few nodules were observed on uninoculated soybeans. This is an indicator of the presence of indigenous soybean nodulating bacteria in the experimental soil (Bekere and Hailemariam, 2012). Generally, inoculation significantly ($p<0.05$) improved nodulation parameters over uninoculated treatment in both limed and non-limed condition.

The result also indicated that nitrogen fertilization did not improve nodule number, nodule volume and nodule dry weight as well as its interaction with lime and *Bradyrhizobium* was not significant (Table 2). This result is in agreement with Cassman *et al*. (1980) and Seneviratne *et al*. (2000) findings who reported the presence or absence of N in soybean nutrient do not significantly affect nodulation parameter of soybean. This indicates that bio fertilizer inoculants have a potential to substitute chemical fertilizer N for legumes.

Number of pod bearing branch, shoot dry weight and plant height response of soybean to lime, *Bradyrhizobium* and nitrogen fertilizer: Number of pod bearing branches, shoot dry weight and plant height of the soybean were significantly ($p<0.05$) affected by an interaction effect of lime, *Bradyrhizobium* and nitrogen fertilizer (Table 3). When the crop was grown without inoculation, application of lime and nitrogen fertilizer separately as well as in combination gave significantly higher number of pod bearing branches, shoot dry weight and taller soybeans than those crops grown without lime and nitrogen. This result is similar with finding of Chalk *et al*. (2010) who reported that dry matter and N_2 fixation of red clover were increased significantly with lime addition. On the other hand, application of nitrogen had no significant effect on plant height and number of pod bearing branches in both limed and non-limed soil when the crop was grown symbiotically with *Bradyrhizobium*. However, lime significantly ($p<0.05$) increased pod bearing branches of the crop to which nitrogen was applied and with held. Beneficial effect of lime is also reported by Kisinyo *et al*. (2005, 2012), Negi *et al*. (2006) and Zaroug and Munns (1979) for

Table 2: The effect of nitrogen fertilizer on nodules of Clark 63-K soybean at Melko, Jimma

N (kg ha^{-1})	Nodule number per plant	Nodule volume (mL plant^{-1})	Nodule dry weight (g plant^{-1})
0	51.3	2.5	1.3
46	49.2	2.3	1.1
$LSD_{0.05}$	ns	ns	ns
CV (%)	35.34	32.9	30.02

ns: Not significantly different at $p<0.05$

Table 3: Effect of lime, *Bradyrhizobium* and nitrogen fertilizer on branch number, shoot dry weight and plant height of Clark 63-K soybean at Melko, Jimma

			Bradyrhizobium	
Parameter	Lime (kg ha^{-1})	N (kg ha^{-1})	No inoculation	Inoculation
Pod bearing branches per plant	0.00	0.0	3.1^{c}	4.0^{bc}
	46.00	5.2^{a}	4.0^{bc}	
	2598.75	0.0	5.2^{a}	5.0^{ab}
	46.00	4.9^{ab}	5.1^{a}	
$LSD_{0.05}$ = 1.1				
Shoot dry weight (g plant $^{-1}$)	0.00	0.0	42.2^{d}	53.0^{cd}
	46.00	57.5^{b}	74.2^{ab}	
	2598.75	0.0	64.1^{b}	76.3^{a}
	46.00	59.6^{b}	76.7^{a}	
$LSD_{0.05}$ = 12				
Plant height (cm)	0.00	0.0	46.9^{b}	60.0^{a}
	46.00	56.6^{a}	54.0^{ab}	
	2598.75	0.0	56.6^{a}	60.2^{a}
	46.00	61.0^{a}	61.3^{a}	
$LSD_{0.05}$ = 5.5				

Values followed with the same letter/s for a parameter are not significantly different at $p<0.05$

symbiotically grown legumes. The tallest and highest shoot dry weight were recorded in a treatment which received lime, *Bradyrhizobium* and nitrogen fertilizer whereas the lowest values were observed from soybeans which were grown without these inputs.

CONCLUSION

In acid soils, it is important to advice farmers to practice the use of economically viable and environmentally friendly *Bradyrhizobium* together with lime rather than fuel based chemical fertilizer N for effective soybean production. However, comparison of net economic return of the inputs could be considered for clear cut recommendation in the investigation area.

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