ORIGINAL ARTICLES

Sulfur fertilizer and *rhizobia* inoculation improved soybean (*Glycine max L.* Merill) growth performance and N_2 fixation on acidic Nitisols of western Ethiopia

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ABSTRACT

Effects of sulfur fertilization and rhizobia inoculation on nodulation, N_2 fixation and growth performance of soybean on acidic Nitisols of Assosa district, western Ethiopia were assessed under greenhouse condition. The experiment was consisted of four levels of S (0, 20, 30 and 40 kgha^{-1}), three rhizobia strains (MAR-1495, SB-6-1-A_2 and TAL-379) and two soybean varieties (Belessa-95 and Wollo) that was factorial combined in a completely randomized design with three replications. Nodulation, N_2 fixation and growth performance of soybean were significantly ($P < 0.001$) affected by the main effects, two-way and three-way interactions of S fertilizer, rhizobia strains and variety. Nodule volume, nodule dry weight, shoot dry weight and amount of N_2 fixed were increased with inoculation of rhizobia strains and S fertilizer application on acid soil. Across S applications, inoculation of MAR-1495 increased the amount of fixed N by 759% over non-inoculated, showing that it is highly competitive and efficient strain as compared to others. At 30 kg S ha^{-1} application, inoculation of MAR-1495 with Belessa-95 and Wollo varieties of soybean have fixed 89.6 and 92.5% more N_2, respectively over control. Inoculation of MAR-1495 rhizobia strain with application of 30 kg S ha^{-1} to Belessa-95 and Wollo varieties of soybean produced 2.5-3 fold increase of soybean shoot dry weight, over the control. The results demonstrate that application of 30 kg S ha^{-1} and inoculation of MAR-1495 strain were improved N_2 fixation, growth performance and shoot nutrients uptake of soybean varieties on acidic Nitisols of Assosa area.

Keywords: Rhizobia strains, legume, nitrogen fixation, soybean, sulfur

INTRODUCTION

Nitrogen is a scarce resource in the tropics that widely limits crop productivity after water availability (Burke et al., 1997). It is an expensive mineral fertilizer beyond the reach of the poor smallholder farmers. In view of this, biological nitrogen fixation (BNF), a renewable N fertilizer source, holds great promise for smallholder farmers in Sub-Saharan Africa. In Ethiopia, legume crops rank second after cereals, with their 12% contribution to national food production and occupy 18% of the total cultivated area (Girma et al., 2013). Currently, production of soybean (*Glycine max* L.) and haricot bean (*Phaseolus vulgaris* L.) has increased as they are exportable and cash earning commodities (Girma et al., 2013). There is also a wide diversity of legumes that grow from the lowlands to the highlands of Ethiopia. A large number of rhizobial strains that can nodulate and fix atmospheric N_2 have been isolated and identified in Ethiopia (Woldemeskel et al., 2005; Argaw, 2014). However, their effectiveness and efficiency were not well examined and hence rigorous study is required to enhance their contribution to soil fertility improvement and crop productivity.

Soybean (*Glycine max* L.) is one of the grain legumes and native to East Asia, perhaps in North and Central China (Laswai et al., 2005) and it is grown for its edible bean, an important source of inexpensive and high quality protein (40%) and oil (20%) around the world. In Ethiopia, soybean is an important food crop widely produced in the west and southwestern parts (Nigussie et al., 2009). Its production is highly concentrated in high rainfall areas of Ethiopia like Assosa, Wollega and Jimma areas. It was recently integrated into the cropping systems of smallholder farmers in Assosa areas and serves as a cash crop for farmers (Nigussie et al., 2009). The soybean symbiosis with rhizobia can fix about 300 kg N ha $^{-1}$ under good conditions (Harold and Fudi, 1992).

Declining soil fertility, particularly low soil N availability is often the major factor resulting in decreased crop plant yields and recognized as a major problem to continue cereal cropping in soils of Ethiopia, specifically in soils of Assosa area (Zeleke et al., 2010). Of the total land mass, about 40% of arable lands of Ethiopia, like Assosa, Jimma and Wollega areas are plagued by soil acidity problems (Mesfine, 2007). Under such acidic conditions, nutrient availability may limit crop growth and productivity. Due to low soil fertility status in the country and priority given to cereal production, grain legumes are generally grown in sever soil conditions which are inherently low in fertility including sulfur especially in western Ethiopia (ATA, 2013). Fertility situation is further deteriorated by nutrient depletion by crops and other related processes, such as leaching and removal of crop residues in the area (Zeleke et al., 2010). The Ethiopian Soil Information Service is currently engaged in mapping the entire country for all nutrients, and has found extensive areas of S, Zn, and B deficiencies (Vanlauwe et al., 2015).

Sulfur is an essential nutrient for plant growth, and legume crops such as soybean generally require it in a similar quantity or more than that of phosphorus for high yield and quality (Jamal et al., 2010). But reductions in sulfur sources from organic matter and less S returned with inadequate use of crop residues and rare addition of manure often deplete soil organic matter, a major source of S in the soil (Habtemichial et al., 2007). In Ethiopia where subsistence farming is practiced, the turnover of S through soil organic matter (SOM) is usually insufficient even to meet the small requirement associated with the small yields. In addition, the use of sulfur-free mineral fertilizers such as di-ammonium phosphate (DAP) and urea are decreasing soil sulfur levels and threaten the adequate fertilization of most crops (Solomon et al., 2001). Sulfur like N is involved in plant protein synthesis, a vital process determining yield. The shortage of sulfur supply to crops decreases their nutrient use efficiency (NUE) (Ceccotti, 1996). Generally, sulfur uptake accounts for 9-15% of N uptake (Inal et al., 2003). Despite the important roles of sulfur in agriculture, research pertaining to its status in soils and crop response studies related to this element is almost non-exist or very much limited in Ethiopia (Itana, 2005).

In most parts of Sub-Saharan Africa use of rhizobia inoculants for improvement in N-fixation and productivity of grain legumes is still in the developing stage (Abdullahi et al., 2013). Even if several field demonstrations of leguminous crops show remarkable growth and yield response to rhizobia inoculation in different agro-ecologies of Ethiopia, the potential of these rhizobia inoculants in combination with other deficient nutrients is scarce and need extensive work. Nitrogen fixing capacity of leguminous plants can be increased by the supply of adequate amounts of nutrients such as sulfur. Sulfur is a vital part of the ferredoxin, an iron-sulfur protein occurring in the chloroplasts. Ferredoxin has a significant role in NO_2 and sulphate reduction, the assimilation of N by root nodule bacteria and free living N-fixing soil bacteria (Scherer et al., 2008). Several findings have reported that nodule formation, N_2 fixation and shoot N concentration improved with sulfur application (Zhao et al., 1999; Scherer, 2001; Scherer et al., 2006). Lower N accumulation and yield reduction of legumes when sulfur was limiting (Scherer et al., 2006) and also recognized S as a limiting factor not only for crop growth and seed yield but also for quality of products (Jemal et al., 2010). Biological nitrogen fixation is very useful for smallholder farmers as it is cost effective and environmentally friendly that improves the N requirement of legumes and other succeeding crops.

Inoculation with compatible and effective rhizobia may be necessary to optimize nitrogen fixation and legumes grain yields, where a low population of native rhizobial strains predominates

(Chianu et al., 2011). Therefore, evaluation and identification of appropriate and effective rhizobial strains are crucial to enhance nitrogen fixation and yield of soybean.

Sulfur is not included in fertilization practices in Ethiopia and very little is known about sulfur status of soils in the country for decades. However, recently, assessment of soil fertility status indicated that some soils of western Ethiopia were predicted to be deficeint in sulfur contents (ATA, 2013). In general, information is scant with regard to the combined effects of sulfur fertilization and seed inoculation with effective strains of rhizobia for successful and profitable soybean production in Ethiopia. Therefore, the objective of this study was to investigate the effects of sulfur fertilizer and inoculation of rhizobia strains on growth performance and N_2 fixation of two soybean varieties.

MATERIALS AND METHODS

Description of the study area

A greenhouse experiment was conducted using Dystric Nitiosols (AsARC, 2007) at Assosa Agricultural Research Center in Benishangul Gumuz' National Regional State. Assosa is located at about 670 km west of Addis Ababa. It lies on altitude of 1,480 m above sea level and located at 09°58′41.7″ N, 034°38′09.5″ E latitude and longitude, respectively. The soil type is slity clay loam in texture, acidic in pH (5.30), medium in soil organic carbon (1.90%) and total nitrogen content (0.12%), very low in available P (14.55, mg kg^{-1}) and low in CEC (14.7, meq $100g^{-1}$ soil).

Soil analysis

Prior to the experiment, 32 surface (0-20 cm) random soil samples were collected in a zigzag walk from different villages of Assosa district. The measurement of soil pH was performed to identify and select the experimental soil with acidic soils pH range of 5.1 to 5.5. Thirty-two farmers' fields were considered from sampling villages with known soil acidity problems based on past and present management and production of soybean and with no previous history of rhizobia inoculation. Surface soil samples (0-20 cm) collected were air dried, passed through a 0.5 cm sieve and filled in 5 kg soil pots containing holes at the bottom to ensure free drainage with saucers placed under the pots to prevent losses of nutrients.

Experimental set up

The experiment consisted of four levels of S (0, 20, 30, and 40 kg ha^{-1} S) as factor A and three strains of rhizobia (MAR-1495, SB-6-1-A_2, and TAL-379) along with uninoculated control as factor B and two soybean varieties (Belessa-95 and Wollo) as factor C that were arranged factorial with a completely randomized design with three replications in greenhouse condition. The two rhizobia strains, MAR-1495, and TAL-379 are the commercial ones obtained from Holetta Agricultural Research Center, while SB-6-1-A_2, is a local strain identified from Assosa area. For the purpose of assessing BNF, a non-N fixing reference crop (wheat, variety called Digalu) was grown in similar environmental condition with soybean.

Sulfur was applied as potassium sulfate (K_2SO_4) in solution form. Nutrients, such as starter dose of N fertilizer at 18 kg N ha^{-1} as Urea (Solomon et al., 2012) and phosphorus fertilizer as TSP at recommended dose of 20 kg P ha^{-1} were applied at sowing for each treatment pots (5 kg of soil). Since sulfur fertilizer was applied as K_2SO_4, the disproportionate addition of K in different treatments was counter balanced by the addition of proportionate amount of potassium chloride. Basal nutrients were also added to each pot to prevent deficiency of other nutrients. These include 5 kg ha^{-1} Mg as $MgCl_2$, 10 kg ha^{-1} Zn as $ZnCl_2$, 1 kg ha^{-1} Mo as $Na_2MoO_{24}\cdot 2H_2O$. Sulfur and other basal nutrients were dissolved in deionized water and applied to each pot in required amounts. Soils were then mixed thoroughly, and deionized water was added to raise the soil moisture to pF2.5. Moisture was retained at around pF2.5 by compensating water loss measured using time domain refelectrometery (TDR).

Improved clean soybean seeds were used for the experiment. Then the seeds were weighed and surface sterilized by soaking them first with 70% (v/v) ethanol for 10 seconds and 4% (v/v) sodium hypochlorite (NaOCl) solution for five minutes and later washed five times with sterilized water as indicated in Somasegaran and Hoben (1994). Each strain was applied at the rate of 10 g peat-based powder inocula per 1000 g of seed. In order to ensure that all the applied inoculum stick to the seed, the required quantity of inoculants was suspended in 1:1 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 inoculant. Then rhizobia inoculum was mixed thoroughly soaked with these seeds. For each inoculation, separate plastic bag was used and care was taken to avoid contamination of the inoculated and uninoculated seeds. Seeds were allowed to air dry for a few minutes and were then sown at the required rate and spacing. Pots with uninoculated seeds were planted first to avoid contamination. Seeds were sown at 3-4 cm depth of soil. Five seeds per pot were sown, and plant populations was maintained by thinning at four to six leaf stages (i.e. 15 days after germination) into three plants per pot and to maintain plant distance of 5 cm.

At 50 % flowering nodule (nodule number, nodule volume and nodule dry weight) and shoot dry weight data were recorded from three plants. The plants were carefully uprooted using a scoop and exposing the whole-root system to avoid loss of nodules. The adhering soil was removed by washing the roots with intact nodules gently with running tap water over a metal sieve. Nodules were dissected and checked for presence of pink coloration, which was used to distinguish between those actively fixing nitrogen from the inactive ones. The number of nodules per plant was counted from a representative three uprooted plants per pot and then averaged as per plant. To determine nodule volume, the nodules collected were carefully immersed in 25 and 50 ml capacity plastic cylinders which were filled up to 10 and 25 ml with water, respectively. The volume of water displaced by the nodules obtained from three plants was recorded. Finally, the nodules were oven dried at 70 ^{O}C for 48 h (Argaw, 2014). The dry nodules were then weighed and nodule dry weights were recorded.

Tissue Analysis

Plant tissue was cut above the soil surface and washed with deionised water. Then dried in an oven at 70 ^{O}C for 48 h, ground and sieved with 0.5 mm mesh, for analysis of nitrogen, sulfur, phosphorus and potassium concentrations. N was determined by Micro Kjeldahl's method (Nelson and Sommers, 1973). After samples were digested with di-acid mixture (HNO_3 and $HClO_4$), P was determined using spectrophotometric vanadium phosphomolybdate method, K using digested solution on a flame photometer and S by turbidimetric, barium sulfate precipitation method adapted from Motsara and Roy (2008). The N, P, K and S uptake in the shoot of soybean was determined quantitatively by multiplying shoot dry weight with that of N, P, K and S content of the shoot.

For assessing biological nitrogen fixation, non-fixing reference crop (wheat, variety called *Digalu*) was used. The amount of N fixed was calculated by subtracting total nitrogen of soybean from the reference crop (wheat). The difference value is assumed as N derived by BNF (N_2 fixed) (Belachew and Hailemariam, 2010). Therefore, the quantity (Q) of N derived from N_2 fixation symbiotically as:

$$Q\ (N_2 \text{ fixed}) = [N \text{ yield (legume)} - N \text{ yield (ref)}] + [N \text{ soil (legume)} - N \text{ soil (ref)}] \quad (1)$$

$$\text{Where N yield or N uptake (g plant}^{-1}) = \frac{(\text{Shoot dry weight (g plant}-1) \times \%\text{N in plant})}{100} \quad (2)$$

$$\%\ \text{Ndfa} = \frac{[\text{N uptake in legume} - \text{N uptake in reference crop}] \times 100}{\text{N uptake in legume}} \quad (3)$$

Where % Ndfa is the percentage of N_2 derived from the atmosphere determined as the ratio of N_2 fixed to plant nitrogen yield (Shoot N uptake).

Statistical analysis

The analysis of variance was carried out using SAS statistical software version 9.0 (SAS, 2004) after shoot dry weight and N_2 fixation parameters were converted into kg ha^{-1}. Means were separated using Tukey's procedure at $P \leq 0.05$.

RESULTS

Effects of sulfur and rhizobia on nodulation and shoot dry weight of soybean

Nodule number, volume and dry weight and shoot dry weight of soybean were significantly ($P < 0.05$) affected by the main effects of sulfur rate, rhizobia strain and soybean variety and their two and three way interactions (Tables 1 and 2). Sulfur application at 30 and 40 kg S ha^{-1} increased nodule volume by 63% and 121% and nodule dry weight by 33% and 54%, respectively, as compared to the control (without sulfur). Similarly, application of sulfur at 30 and 40 kg ha^{-1} increased shoot dry weight of soybean by 29% and 26%, respectively over the control (Table 1).

Nodulation parameters were significantly higher in inoculated plants as compared to uninoculated. Inoculation of strain MAR-1495 significantly increased nodule number, nodule volume and dry weight and shoot dry weight relative to the other strains (SB-6-1-A_2 and TAL-379) and uninoculated control. The number of nodule plant^{-1} produced with inoculation of the other two strains showed interaction with varieties and sulfur fertilizer application. Soybean variety Wollo responded in a better way to inoculation resulting in higher nodulation compared to Belessa-95, but Belessa-95 gave higher shoot dry weight (Table 1). Mean nodule volume and nodule dry weight showed superior and linear increasing trends in response to S fertilizer rates when inoculated with MAR-1495, whereas the least nodule number, volume, dry weight and shoot dry weight were recorded in non-inoculated ones. Although most parameters were significantly ($P <$ 0.05) affected by main effect and two-way interaction of the factors, three-way interactions significantly (P < 0.001) affected all nodulation parameters and shoot dry weight more than their respective two-way interaction and/or main effects (Tables 1, 2, 3 and 4).

Table 1 : Nodulation and shoot dry weight of soybean varieties as affected by sulfur fertilizer rates and rhizobia strains

Source of Variations	NN (No. plant^{-1})	NV (ml plant^{-1})	NDW (g plant^{-1})	SDW (kg ha^{-1})
Variety				
Belessa-95	11.40^{b}	1.36^{b}	0.208^{b}	4481.14^{a}
Wollo	13.59^{a}	1.72^{a}	0.252^{a}	4419.00^{b}
LSD	0.422	0.051	0.004	21.10
Sulfur (kg ha^{-1})				
0	12.04^{b}	1.02^{d}	0.1908^{c}	3811.67^{d}
20	12.21^{ab}	1.23^{c}	0.1823^{c}	4294.58^{c}
30	12.93^{a}	1.66^{b}	0.2545^{b}	4900.00^{a}
40	12.81^{ab}	2.25^{a}	0.2942^{a}	4793.75^{b}
LSD	0.789	0.095	0.014	39.41
Rhizobium strain				
Uninoculated	0.53^{d}	0.005^{c}	0.0037^{c}	2471.25^{d}
TAL-379	9.24^{c}	1.47^{b}	0.2300^{b}	4507.92^{c}
MAR-1495	29.16^{a}	3.14^{a}	0.4695^{a}	5975.42^{a}
SB-6-1-A_2	11.05^{b}	1.55^{b}	0.2186^{b}	4845.42^{b}
LSD	1.403	0.170	0.014	39.41

Nodule number = NN; Nodule volume (ml plant^{-1}) = NV; Nodule dry weight (g plant^{-1}) = NDW; Shoot dry weight at late flowing and early pod setting stage (kg ha^{-1}) = SDW. Note: Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

No nodulation was observed with both Belessa-95 and Wollo, when both sulfur fertilizer application and rhizobia strain inoculation were not performed (Table 2). Significantly higher nodule number was produced when Wollo was inoculated with rhizobia strain MAR-1495 in all rates of sulfur fertilizer applied except the unfertilized control (Table 2). Furthermore, higher nodule volume and nodule dry weight were recorded for Wollo inoculated with rhizobia strain MAR-1495 at sulfur rate of 40 kg ha^{-1}. For Belessa-95 variety, inoculation of MAR-1495 at 30 kg S ha^{-1} gave higher nodule volume and nodule dry weight, while no nodulation was obtained from the control treatment for the two varieties (Table 2). Shoot dry weight was significantly affected by the interaction of S application with inoculation of rhizobia strain. The shoot dry weight at 30 kg S ha^{-1} with inoculation of MAR-1495 for Belessa-95 and Wollo were 6723 kg ha^{-1} and 7140 kg ha^{-1}, corresponding to about 2.5 fold and 3 fold increases, respectively, as compared to the control.

Table 2: Interaction effects of sulfur rates, rhizobia strains and soybean varieties on nodulation and shoot dry weight

Sulfur (kg ha^{-1})	Strain	Variety	Nodulation NN	NV	NDW	SDW (kg ha^{-1})
0	Uninoculated	Belessa-95	0.00^{f}	0.00^{f}	0.000^{j}	1823.3^{s}
	MAR-1495		22.17^{c}	1.98^{d}	0.413^{d}	5380.0^{f}
	SB-6-1-A_2		7.67^{e}	0.40^{e}	0.141^{h}	3606.6^{m}
	TAL-379		9.33^{de}	1.03^{e}	0.188^{g}	4736.6^{h}
	Uninoculated	Wollo	0.00^{f}	0.00^{f}	0.000^{j}	1670.0^{s}
	MAR-1495		35.16^{a}	2.58^{c}	0.352^{e}	5636.6^{e}
	SB-6-1-A_2		9.33^{de}	0.43^{f}	0.103^{i}	3346.6^{n}
	TAL-379		12.67^{d}	1.80^{d}	0.331^{ef}	4293.3^{j}
20	Uninoculated	Belessa-95	1.00^{f}	0.01^{g}	0.005^{j}	3383.3^{n}
	MAR-1495		31.00^{ab}	2.75^{d}	0.375^{e}	5496.6^{ef}
	SB-6-1-A_2		10.67^{de}	0.92^{e}	0.174^{g}	4756.6^{h}
	TAL-379		3.17^{f}	0.50^{f}	0.077^{i}	4070.0^{kl}
	Uninoculated	Wollo	0.58^{f}	0.01^{g}	0.005^{j}	2563^{pq}
	MAR-1495		29.83^{b}	3.66^{b}	0.509^{b}	5373.3^{f}
	SB-6-1-A_2		13.33^{d}	1.42^{e}	0.209^{g}	4233.3^{jk}
	TAL-379		8.17^{e}	0.65^{f}	0.107^{h}	4480.0^{i}
30	Uninoculated	Belessa-95	0.75^{f}	0.01^{g}	0.005^{j}	2700^{op}
	MAR-1495		33.50^{ab}	3.33^{b}	0.539^{b}	6723.3^{b}
	SB-6-1-A_2		12.67^{d}	1.63^{de}	0.206^{g}	5996.6^{d}

	TAL-379		2.33^{f}	0.83^{f}	0.133^{h}	4006.6^{l}
	Uninoculated	Wollo	0.58^{f}	0.01^{g}	0.005^{j}	2796.6^{o}
	MAR-1495		22.67^{c}	3.17^{bc}	0.459^{c}	7140.0^{a}
	SB-6-1-A$_2$		14.83^{d}	2.17^{d}	0.311^{f}	5026.6^{g}
	TAL-379		16.17^{d}	2.17^{d}	0.379^{d}	4810.0^{h}
40	Uninoculated	Belessa-95	0.83^{f}	0.01^{f}	0.005^{j}	2340.0^{r}
	MAR-1495		25.50^{c}	3.50^{b}	0.457^{c}	5603.3^{e}
	SB-6-1-A$_2$		11.50^{de}	2.83^{c}	0.312^{f}	6393.3^{c}
	TAL-379		10.50^{de}	2.17^{d}	0.304^{f}	4680.0^{h}
	Uninoculated	Wollo	0.50^{f}	0.01^{f}	0.005^{j}	2493.3^{qr}
	MAR-1495		33.50^{ab}	4.17^{a}	0.654^{a}	6450.0^{c}
	SB-6-1-A$_2$		8.50^{e}	2.67^{c}	0.294^{f}	5403.3^{f}
	TAL-379		11.67^{de}	2.66^{c}	0.323^{ef}	4986.6^{g}
	LSD		5.60	0.435	0.0358	167.36

Note: Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

Effects of sulfur and rhizobia inoculation on growth parameters of soybean

The main effects of sulfur rates, rhizobia strains and varieties and their interaction significantly ($P < 0.05$) affected all the growth parameters of soybean (plant height, number of leaf number and number of branches except the main effect of variety and the interaction effect of S and variety on number of branches (Table 3). Three-way interaction of S rates, *rhizobia* strains and varieties resulted in significant variation on growth parameters of soybean varieties (Table 3).

Table 3: Growth parameters of soybean varieties as affected by sulfur fertilizer rates and rhizobia strains

Source of Variations	Plant height (cm)	Leaf number (No. plant^{-1})	Branch number (No. plant^{-1})
Variety			
Belessa-95	53.89^{b}	32.12^{b}	10.28
Wollo	65.99^{a}	33.64^{a}	10.35
LSD	1.35	0.663	NS
Sulfur (kg ha^{-1})			
0	57.99^{c}	31.37^{c}	10.16^{b}
20	53.46^{d}	29.59^{d}	9.85^{b}
30	67.27^{a}	36.26^{a}	11.23^{a}
40	61.02^{b}	34.30^{b}	10.02^{b}
LSD	2.53	1.23	0.445
Bradyrhizobium strain			
Uninoculated	49.23^{d}	25.85^{d}	7.99^{d}
TAL-379	64.90^{b}	33.24^{b}	11.08^{b}
MAR-1495	70.27^{a}	40.55^{a}	11.84^{a}
SB-6-1-A$_2$	55.35^{c}	31.87^{c}	10.35^{c}
LSD	2.53	1.239	0.445

Note: Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

Higher plant height was recorded when Wollo was inoculated with MAR-1495 at 40 kg ha^{-1} S supply, followed by Wollo inoculated with TAL-379 and Wollo inoculated with MAR-1495 at 30 kg ha^{-1} S fertilizer application. Inoculated plants showed significantly higher plant height as compared to uninoculated plants across sulfur rates. Higher leaf number was produced when Wollo variety inoculated with MAR-1495 at 30 and 40 kg ha^{-1} sulfur application followed by Belessa-95 inoculated with MAR-1495 at 40 kg ha^{-1} sulfur application. At application of 30 kg S ha^{-1} inoculation with all strains resulted in significantly higher number of branches for both varieties as compared to the control (Table 3).

Effects of sulfur rate and rhizobia on N_2 fixation, shoot N concentration and nutrient uptake

Main effects of sulfur rates, rhizobia strains and soybean varieties and their interactions significantly ($P < 0.01$) affected amount of N_2 fixed, %Ndfa, shoot N concentration and shoot nutrients (N, P, K and S) uptake. Shoot N concentration, %Ndfa, K and S uptake were varied between the two soybean varieties (Table 4).

Application of sulfur significantly increased shoot N concentration, amount of N_2 fixed and percentage of N_2 derived from the atmosphere (%Ndfa). Likewise, inoculation of MAR-1495 significantly increased shoot N concentration, N_2 fixed and %Ndfa over other tested strains and the uninoculated control. Tissue analysis showed that plants grown at the highest level of S application (40 kg S ha^{-1}) and those inoculated with MAR-1495 accumulated more N in shoot of soybean than the other treatments (Table 4).

Table 4: Interaction effects of sulfur rates, rhizobia strains and varieties on shoot N concentration, amount of N_2 fixed, % Ndfa and shoot nutrients uptake

Source of Variations	Shoot N (%)	N_2 fixed (kg ha^{-1})	% Ndfa	Shoot nutrient uptake (kg ha^{-1})			
				N	P	K	S
Variety							
Belessa-95	3.23^b	136.76	57.09^b	155.76	10.03	101.11^b	21.60^b
Wollo	3.26^a	137.68	58.31^a	156.68	9.86	108.42^a	22.80^a
LSD	0.025	NS	0.426	NS	NS	3.30	0.481
Sulfur (kg ha^{-1})							
0	3.06^c	102.43^d	51.04^d	121.43^d	7.94^c	81.86^c	18.44^c
20	2.96^d	116.17^c	56.84^c	135.17^c	10.32^b	97.53^b	20.79^b
30	3.41^b	161.97^b	59.58^b	180.97^b	11.21^a	121.34^a	24.80^a
40	3.56^a	168.32^a	63.35^a	187.32^a	10.31^b	118.34^a	24.78^a
LSD	0.047	2.72	0.796	2.72	0.758	6.17	0.899
Rhizobium strain							
Uninoculated	1.92^d	26.70^d	15.39^d	45.70^d	6.13^c	32.48^c	5.35^d
TAL-379	3.20^c	126.45^c	64.93^c	145.45^c	10.31^b	119.45^b	24.00^c
MAR-1495	4.12^a	229.24^a	79.55^a	248.24^a	13.46^a	151.65^a	33.47^a
SB-6-1-A_2	3.75^b	166.49^b	70.93^b	185.49^b	9.88^b	115.48^b	25.99^b
LSD	0.047	2.72	0.758	2.72	0.758	6.17	0.899

Note: Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

MAR-1495 rhizobia strain had fixed significantly higher N at S 30 kg ha^{-1} application for *Wollo* soybean variety, followed by the same strain and S level for Bellessa-95 soybean variety. At 30 kg S ha^{-1}, Wollo variety inoculated with MAR-1495 fixed 92.51% more N, while Bellessa-95 variety fixed 89.63 % more N over the non-inoculated. This corresponds to N_2 fixation of 161.2 kg N ha^{-1} for Wollo and 150.8 kg N ha^{-1} for Bellessa-95. Thus, S fertilizer application and rhizobia inoculation enhanced the percentage of N derived from the atmosphere in the whole plant of soybean from 18.31 to 84.63 %, and from 9.53 to 83.23 % for Wollo and Bellessa-95, respectively (Table 4).

Application of significantly ($P < 0.01$) increased shoot N, P, K and S uptake and shoot N concentration of soybean varieties (Table 4). Application of S at 40 kg ha^{-1} resulted in the highest N uptake, followed by 30 and 20 kg S ha^{-1}, while the least N uptake was obtained from the unfertilized control. In addition, S application at 30 kg S ha^{-1} had showed the highest shoot P, K and S uptake, which were increased by 29.8, 48.2 and 34.5 % over the unfertilized control. Shoot uptake of N, P, K and S in soybean varieties were significantly increased with S rates, when applied with inoculation of rhizobia strain, showing strong positive interactions ($P < 0.01$). There were increases of 87, 94, 89 and 96% in N, P, K and S uptake, respectively, relative to the control with inoculation of MAR-1495 combined with application of 30 kg S ha^{-1} for Wollo variety. For Belessa-95 variety, inoculation with MAR-1495 strain and application of 30 kg S ha^{-1} rate increased the uptakes of N, P, K and S by 84, 89, 87 and 93%, respectively, over control (Table 4).

DISCUSSION

Effects of sulfur and rhizobia strain on nodulation and shoot dry weight of soybean

The number of nodule plant^{-1} and nodule dry weight of soybean varieties were higher with inoculation as compared to non-inoculated. Most of the nodules showed pink coloration indicating that the tested strains are effective in inducing N_2 fixation. The highest nodulation was displayed with MAR-1495 than the other two strains, implying the existence of specificity and compatibility amongst rhizobial strains and the soybean varieties. The higher nodule and shoot dry weight were registered with MAR-1495 among strains and when 30 kg ha^{-1} S was applied.

Increasing nodule weight by inoculation was generally a prerequisite for increasing N_2 fixation (Singleton and Tavares, 1986). MAR-1495 was more effective than the other rhizobia strains in influencing nodulation and shoot dry weight (Table 1). This could be due to the fact that the available resident rhizobia strains maybe insufficient and/or ineffective to nodulate the host adequately in conformity with many earlier findings (Chianu et al., 2011; Argaw, 2012; Argaw, 2014). Shoot dry weight was significantly higher in inoculated than non-inoculated revealing the benefits of rhizobial inoculation in affecting growth performance of soybean. Specifically, inoculation increased soybean shoot dry weight by 108% over non-inoculated controls, while nodule dry weight was increased by 135% when MAR-1495 was inoculated as compared to the other two strains (Table 2). These results are assertive of the statement that continuous and coordinated selection of the most effective combinations of host and microbial symbionts is a prerequisite for profitable and sustainable agricultural systems (Meghvansi et al., 2010). The nodulation tests showed that the rhizobia strains were effective in nitrogen fixation on the host soybean plant. But the most striking differences were noted among rhizobia strains in affecting nodulation and shoot dry weight soybean.

Inoculation of MAR-1495 combined with application of sulfur gave significantly higher nodulation and shoot dry weight followed by SB-6-1-A_2 and TAL-379 in decreasing order. This shows that the three strains were different in competitiveness in the rhizosphere. There was also variation between the two soybean varieties. Consequently, the amount of N_2 fixed or the proportion of plant N derived from biological N_2 fixation was different depending the tested strains and the variety. Similarly, Patra et al. (2012) and Solomon et al. (2012) reported variation in nodulation and N_2-fixation capacity of rhizobia strains. Therefore, the results have particular significance in strain selection for superior competitiveness. Application of sulfur produced higher nodulation parameters as compared to unfertilized soybean or S-deficient soil. This result confirms that nodule development on the roots of soybeans is promoted by availability of S (Scherer and Lange, 1996).

Enhanced nodule development which stimulates the growth of soybeans was attributed to the action of sulfates on the soybean bacteria. Legumes fertilized with sulfates showed higher amount of fixed N and higher N content in the plants than unfertilized controls. Nodulation and subsequent N_2 fixation were higher in the inoculated soybean plants than in the uninoculated ones with increased sulfur application, showing that for enhanced symbiotic N_2 fixation, inoculation of rhizobia and application of sulfur are required in S deficient soil. Nodule parameters were increased with increasing sulfur rates and inoculation with rhizobia strain. Significantly increased soybean volume of nodule and nodule dry weight by the combined application of sulfur fertilizers with inoculation of rhizobia strains showing sulfur application was effective in nitrogen fixation through increasing nodule volume and dry biomass.

There was a highly positive interaction between sulfur application and rhizobia strains on nodulation and shoot dry weight. Similarly, Singleton et al. (1985) and Grham et al. (2004) reported in soybeans where nodules are effective, there will be fewer, but larger nodules (higher volume and biomass) resulting in increased shoot dry weight of soybean. Likewise effect of sulfur on nodulation has been previously observed (Scherer and Lange, 1996) and attributed to the greater partitioning of root dry matter into nodule tissue when sulfur supply is adequate. The absence of nodules in the control treatment in this study may be due to the poor or no existing native rhizobial population in the soil of the study area confirming the previous results of (Argaw, 2012) in Assosa area.

Effects of sulfur and rhizobia strain on growth parameters of soybean

The increase in plant height, number of leaf and biological nitrogen fixation may be due to the favorable effects of rhizobia strains and sulfur on N metabolism and consequently on the vegetative growth of soybean plant. This is because growth of plant organs depends on proper supply of mineral nutrients such as nitrogen and sulfur. Sulfur is required in higher amounts specifically by legumes for their proper growth and development (Falk et al., 2007). The highest plant height might have resulted from the synergistic effect of nitrogen and sulfur on the growth and yield of soybean plant.

Response to rhizobia inoculation and sulfur fertilizer application were visible by the more vigorous growth habit and dark green colour of plants. Plants inoculated with rhizobia strains produced greener leaves and more branches than non-inoculated plants suggesting that inoculation significantly influenced growth parameters of soybean. Similarly, Shahid et al. (2009) and Argaw (2012) found significant increase in growth performance (plant height and number of leaf)and biological nitrogen fixation of soybean plants in response to rhizobia inoculation in different tropical soil indicating positive interaction between sulfur application and rhizobia inoculation on soybean growth. Plant height was significantly increased by inoculation of rhizobia strain, especially MAR-1495 and SB-6-1-A_2, at 30 kg S ha^{-1}. Similar finding was reported by Hussain et al. (2008) that the maximum plant height was observed at 30 kg S ha^{-1}on inoculated plants. These growth parameters are expected to be a good attributes for higher nitrogen accumulation in the plant tissue that could be used as organic fertilizer and/or livestock feed via legume crops residue.

Effects of sulfur and rhizobia strain on shoot N concentration, N_2 fixation and nutrients uptake of soybean varieties

Shoot N accumulation and amount of N_2 fixed were showed more pronounced disparities among strains performance (Table 4). Likewise Kadiata et al. (2012) reported that total N yield is a better parameter in assessing the ability of different rhizobial strains to enhance N status of soybean. It is important to note that balanced fertilizer application (all macro and micro nutrients) along with the treatment effect of S play significant roles in N_2 fixation. Perhaps the applied starter dose of N (18 kg ha^{-1}) had synergetic effect with other nutrients resulting in high nodulation, shoot dry weight and N_2 fixation.

There was significant difference between rhizobia strains combined with applied sulfur fertilizer in N_2 fixation ability indicating the necessity of high care in selecting rhizobia strain to improve N yield of soybean. Increased sulfur application resulted in increased amount of N_2 fixed and %Ndfa. These results are in line with Scherer (2001) on pea, Habtemicheal et al. (2007) on faba bean and Cazzato et al. (2012) on lupin. Similarly Scherer and Lange (1996) and Scherer (2008) have also reported increased N_2 fixed and the %Ndfa with increased sulfur application. The amount of N_2 fixed and the %Ndfa were significantly affected by combined application of sulfur and inoculation of rhizobia strain.

The amount of N_2 fixed was in a range of 28 to 273 kg ha^{-1} for Belessa-95 and 22 to 300 kg ha^{-1} for Wollo, which were much higher as compared to earlier report of (Habtemichial et al., 2007; Hussain et al., 2011). The higher results may be attributed to the balanced fertilizer supply including Sulfur. Similarly Zahran (1999) reported the amounts of N fixed by symbiotic systems may differ according to the method used to study N_2 fixation; i.e., use of non-nitrogen fixing reference crop method and nitrogen isotope method. Zhao et al. (1999) in a greenhouse had recorded more than double amount of N_2 fixed in pea by the addition of Sulfur indicating that sulfur fertilization has a direct effect on N_2 fixation.

The plant tissue analysis showed rhizobia inoculation and sulfur application alone or in combination influenced N_2 fixation, N, P, K and S uptake and concentration in shoot of both varieties. Applications of sulfur at 30 and 40 kg ha^{-1} along with inoculation of MAR-1495 resulted in significantly higher nutrient uptake and N_2 fixation. Increase in root nodulation due to rhizobia inoculation and sulfur application were gave higher absorption of mineral nutrients from soil and hence increased shoot dry biomass of soybean. Any effect of sulfur and rhizobial strains on nodulation was due mainly to their effects on plant vigor, N accumulation, nitrogen fixation and shoot dry weight. Increase of nodulation brought about by S fertilization was associated with an increase in percentage of N in shoot and N_2 fixed as well as shoot dry weight. Therefore, better nodulation, higher nitrogen fixation and improved growth parameters observed were due to increased sulfur application along with inoculation of rhizobia strain.

CONCLUSION

Sulfur fertilizer application and rhizobia inoculation improved nodulation, N_2-fixation and soybean growth attributes on acid soil of Assosa, western Ethiopia. Notably, the commercial inoculant MAR-1495 produced high amount of nodule, greater shoot dry weight and higher N concentration in soybean plant tissue across all S fertilizer rates than others, displaying its higher N_2 fixating capacity. Amount of nitrogen fixed was varied in response to the rhizobia strains and sulfur fertilizer rates in terms of dry matter production and N content in the shoots of soybean. The inoculation of MAR-1495 with application of 30 and 40 kg S ha^{-1} was the most superior combination that improved nodulation, N_2-fixation and growth attributes. The most beneficial effect of S with rhizobia inoculation can be realized when the soil is deficient in available sulfur as well as low in effective resident rhizobial population. Therefore, proper fertilization program including sulfur supply in combination with bio-fertilization should be implemented to improve the productivity of soybean in Assosa area, western Ethiopia. Further studies to fully exploit the potential of rhizobial strains in the area for repeated seasons under different agroclimatic conditions with sulfur fertilizer application are suggested for improvement of soybean productivity on acid soils of Ethiopia.

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