

## Full Length Research Paper

# ***Rhizobium* inoculation and sulphur fertilizer improved yield, nutrients uptake and protein quality of soybean (*Glycine max* L.) varieties on Nitisols of Assosa area, Western Ethiopia**

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A greenhouse experiment was conducted to study the effect of inoculation of *Rhizobium* strain and sulphur fertilization on seed and straw yields, nutrients uptake and seed quality protein of two soybean (*Glycine max* L.) varieties. The experiment consisted of four levels of S (0, 20, 30 and 40 kg ha<sup>-1</sup>), three *Rhizobium* strains (MAR-1495, SB-6-1-A<sub>2</sub> and TAL-379) and two soybean varieties (Belessa-95 and Wollo) combined factorially in complete randomized design (CRD) with three replications. Grain and straw yield and nutrients uptake increased by inoculation of *Rhizobium* strain whether used alone or in combination with S. Application of strain and S further increased seed and straw yield and nutrients uptake. Seed yields of Bellessa-95 inoculated with MAR-1495 at 30 and 40 kg S ha<sup>-1</sup> were estimated to be 3864.1 and 3893.8 kg ha<sup>-1</sup>, corresponding to 112 and 114% increase; but Wollo was with seed yields of 3633.3 and 3709.2 kg ha<sup>-1</sup>, corresponding to 160 and 166% increase, respectively over control. Estimated soil N balance was maximum for S application along with inoculation of strains, ranged from -29.25 kg ha<sup>-1</sup> in control to 80.74 kg ha<sup>-1</sup> for Bellessa-95 and from -21.76 kg ha<sup>-1</sup> to 84.66 kg N ha<sup>-1</sup> for Wollo, respectively. Efficient seed nutrients uptake due to combined application further caused significant ( $p < 0.001$ ) increase in protein yield of soybean. With strain MAR-1495 protein yield varied from 26% in the control to 41.5% for Belessa-95 at 30 kg S ha<sup>-1</sup>, while from 28% in the control to 44% for Wollo at 40 kg S ha<sup>-1</sup>, respectively. The results clearly suggested that balanced application of S along with *Rhizobium* strain can affect grain and straw yield and nutrients uptake of soybean varieties, enhance their growth and improve grain quality protein as well as benefit on soil N balance in S-deficient soils.

**Key words:** Sulphur, strain, nutrients use efficiency, quality protein, soil nitrogen balance.

## INTRODUCTION

Soybean (*Glycine max* L.) is one of grain legumes and it is grown for its edible bean, an important source of inexpensive and high quality protein (40%) and oil (20%)

around the world (Laswai et al., 2005). In Ethiopia, soybean is an important food crop widely produced in high rainfall areas, in west and southwestern parts (such

as Assosa) and it is recently integrated into the cropping systems and serves as a cash crop for smallholder farmers of the area (Nigussie et al., 2009). Legumes including soybean are able to fix atmospheric N<sub>2</sub> in association with rhizobia. In this symbiosis they partly supply their own N needs and also provide some nutrients left over to succeeding crops through decomposition of their nodule, roots and biomass (Chianu et al., 2011). The practices appeared to be very useful for smallholder farmers as it is cost effective to improve the N requirement of legumes and succeeding crops (Graham et al., 2004). Furthermore, N<sub>2</sub> fixing soybean crop is of considerable interest for more sustainable agriculture and particularly in organic farming systems (Cazzato et al., 2012). In view of this, biological nitrogen fixation (BNF), a renewable N fertilizer source, holds great promise for smallholder farmers in sub-Saharan legume crops rank second after cereals, with their 12% contribution to national food production and occupy 18% of the total cultivated area in Ethiopia. In recent years, production of haricot bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* L.) has increased as they are exportable and cash earning commodities (Abera et al., 2013).

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 (Zelege et al., 2010). Due to low soil fertility status in the country grain legumes are generally grown in severe soil conditions which are inherently low in fertility including S and low soil pH 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 (Zelege et al., 2010). The Ethiopian Soil Information Service is currently involved in mapping the entire country for all nutrients, and has found extensive areas of S, Zn, and B deficiency (Vanlauwe et al., 2015).

Sulphur is an essential nutrient for plant growth accounting to about 10% of the total N content (Anandham et al., 2007) and legume crops such as soybean generally require it in a similar quantity or more than that of phosphorus for high yield and quality (Jemal et al., 2010). But reductions in S sources from organic matter and less S returned with inadequate use of crop residues and rare addition of manure which often deplete soil organic matter (Habtemichael et al., 2007). In other words, in countries like Ethiopia where subsistence farming is practiced, the turnover of S through SOM is usually insufficient even to meet the small requirement associated with the small yields. Organic S pool which is the large proportion of soil S highly affected by long term

cultivation in the tropics (Solomon et al., 2001) and further aggravated through removing plant residue. In intensive crop rotation S uptake can be very high, especially when the crop residue is removed from field along with the product (Fismes et al., 2002). In addition, less S returned with inadequate use of crop residues and rare addition of manure which often deplete soil organic matter in Ethiopia (Habtemichael et al., 2007).

In addition, using of S-free mineral fertilizers is decreasing soil S levels and threatens the adequate fertilization of most crops (Khalid et al., 2011). Application of N containing fertilizers year after year on S deficient soils can make its deficiency worse because of widen N:S ratio. Mineral fertilizers that are used in Ethiopia such as di-ammonium phosphate (DAP) and urea contain no S. Despite the important roles of S in agriculture, research pertaining to its status in soils and its response in crops are almost nonexistent in Ethiopia (Habtemichael et al., 2007). Sulphur fertilization of soil has significant potential of increasing the amount of N fixed by legumes and their grain yield, thus improving fertility status of soil (Cazzato et al., 2012). Nitrogen fixing capacity of leguminous plants can be increased by the supply of adequate amounts of nutrients such as S. S is a vital part of the ferredoxin, an iron-S protein occurring in the chloroplasts. Ferredoxin has a significant role in NO<sub>2</sub> and sulphate reduction, the assimilation of N by root nodule bacteria and frees living N-fixing soil bacteria (Scherer et al., 2008). A study revealed lower N accumulation and a yield reduction of legumes when S was limiting (Scherer et al., 2006) and also recognized as a limiting factor not only for crop growth and seed yield but also for quality of products (Jemal et al., 2010). Because S is a main component of the amino acids methionine, cysteine, and cystine, coenzymes, thioredoxine and sulfolipids and hence improves protein quality (Jemal et al., 2005). Sharma and Sharma (2014) also reported S-containing amino acids such as methionine and cysteine increased significantly by combined application of N and S and indicated improvement in soybean nutritional value. This is because N and S are both involved in plant protein synthesis, a process that may determines yield of crops (Habtemichael et al., 2013). Therefore, the most important constraints to soybean crop growth may be those caused by the shortage of plant nutrients such as S and N. In addition, there are not many studies available about the effect of S in presence of seed inoculation with effective strains of *Rhizobium* for successful soybean cultivation in Ethiopia. Therefore, a greenhouse trial was conducted to evaluate the effect of S fertilization and inoculation of *Rhizobium* strain on yield and nutrients uptake of grain and straw, grain quality protein content and estimated soil N balance of two soybean varieties.

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## MATERIALS AND METHODS

### Description of the study area

A greenhouse experiment was conducted using Dystric Nitiosols (AsARC, 2007) at Assosa Agricultural Research Center (EIAR) to investigate the effects of integrated application of S and *Rhizobium* strains on grain and straw nutrients yield and grain quality protein content of two soybean varieties (Belessa-95 and Wollo). Assosa is located about 670 km west of Addis Ababa, the capital city of Ethiopia. It is a capital city of 'Benishangul Gumuz' Region State of Ethiopia and lies on altitude of 1,480 m above sea level, and located at 09°58'41.7" N, 034°38'09.5" E coordinates. The study soil was silty clay loam in texture with clay 33%; silt 25%; sand 42%, acidic in pH (5.30), medium in soil organic carbon (OC, 1.90%), medium in soil nitrogen content (TN, 0.12%), very low in available P (14.55 ppm) and low in CEC (14.7, milliequivalents per 100 g soil). Available S was low (2.97 ppm) can be categorized under S-deficient soil.

### Experimental set up

Prior to the experiment, 32 surface (0-20 cm) random sub 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 *Rhizobium* inoculation. Surface soil samples (0-20 cm) from Megele-32 was 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. The experiment consisted of four levels of S (0, 20, 30, and 40 kg ha<sup>-1</sup> S) and three strains of *Rhizobium* (MAR-1495, SB-6-1-A<sub>2</sub>, and TAL-379) along with uninoculated control and two soybean varieties (Belessa-95 and Wollo) that was arranged in a Completely Randomized Design (CRD) with three replications in greenhouse conditions. For the purpose of assessing BNF, a non-N fixing reference crop (wheat, variety called Digalu) was grown in similar environmental condition with soybean.

### Soil fertilization and sowing

Sulphur was applied as potassium sulfate (K<sub>2</sub>SO<sub>4</sub>) in solution form. In addition to sulphur, other nutrients, such as starter dose of N fertilizer at 18 kg N ha<sup>-1</sup> as Urea (Solomon et al., 2012) and phosphorus fertilizer as TSP at recommended dose of 23 kg P ha<sup>-1</sup> were applied at sowing for each treatment pots (5 kg of soil). Since sulphur fertilizer was applied as K<sub>2</sub>SO<sub>4</sub>, 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<sup>-1</sup> Mg as MgCl<sub>2</sub>, 10 kg ha<sup>-1</sup> Zn as ZnCl<sub>2</sub>, 1 kg ha<sup>-1</sup> Mo as Na<sub>2</sub>MoO<sub>24</sub>·2H<sub>2</sub>O. S 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 pF 2.5.

### Seed treatment with *Bradyrhizobium* strains

Soybean seeds were selected based on size and healthiness (able to shoot). Then the seeds were weighed and surface sterilized by soaking them first with 70% (v/v) ethanol for 10 s 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 100 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 *Bradyrhizobium* inoculum was mixed thoroughly 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 (that is, 15 days after germination) into three plants per pot and maintain plant distance of 5 cm. Soil N balance (Nba) considering the aboveground biomass (straw) was calculated by subtracting N output from N input using Equation 1 (Habtemichial et al., 2007). Roots were not removed from the soil, and hence the calculated potential N benefits are conservative estimates, as they do not include root N.

$$Nba = (N_f + N_2 \text{ fixed}) - N_g \quad 1$$

Where: N<sub>f</sub> = Applied N fertilizer (kg ha<sup>-1</sup>), N<sub>g</sub> = N removed by soybean grain.

### Chemical analysis

At physiological maturity, plants were harvested and partitioned into grain and straw and samples from each treatment were collected for analysis of N, P, K and S. Each plant part was dried in an oven at 70°C for 48 h, ground and sieved with 0.5 mm mesh for analysis of nitrogen, sulphur, phosphorus and potassium. N was determined by Micro Kjeldahl's method (Nelson and Sommers, 1973). After samples were digested with di-acid mixture (HNO<sub>3</sub> and HClO<sub>4</sub>), P was determined using spectrophotometric vanadium phosphomolybdate method, K using digested solution on a flame photometer and S by turbidimetric, Barium sulfate precipitation by turbidimetric, Barium sulfate precipitation method of estimating available S adapted from Motsara and Roy (2008). N, P, K and S uptakes in the grain and straw was determined quantitatively by multiplying N, P, K and S content of the seed and straw with that of seed and straw yield, respectively.

### Statistical analysis

The analysis of variance was carried out using SAS statistical software version 9.00 (SAS, 2004) after parameters taken were converted into kg ha<sup>-1</sup>. Three-factor analysis of variance also were performed to evaluate the effects of treatments (*Rhizobium* strain, S rate and variety) and their interactions on grain and straw yield and N, P, K and S uptake. Means were separated using Tukey's procedure (P < 0.05). A correlation analysis between grain and straw yield and nutrients uptake were also carried out.

## RESULTS

### Seed and straw yields and nutrients uptake of soybean

Seed and straw yields, and nutrient uptake (N, P, K and S) were significantly (P<0.05) varied between the two

**Table 1.** Seed and straw yield, estimated soil N balance and protein yield of soybean varieties as affected by S fertilizer rates and *Rhizobium* strains.

Variations	Seed yield (kg ha <sup>-1</sup> )	Straw yield (kg ha <sup>-1</sup> )	Nba (kg ha <sup>-1</sup> )	Protein yield (%)
<b>Variety</b>				
Belessa-95	2493.21 <sup>a</sup>	4195.26 <sup>b</sup>	21.30 <sup>b</sup>	31.98
Wollo	2158.58 <sup>b</sup>	4403.22 <sup>a</sup>	37.26 <sup>a</sup>	32.37
LSD	52.19	110.36	3.30	NS
<b>S (kg ha<sup>-1</sup>)</b>				
0	1972.42 <sup>c</sup>	4487.79 <sup>a</sup>	18.27 <sup>b</sup>	31.31 <sup>b</sup>
20	2323.04 <sup>b</sup>	4507.17 <sup>a</sup>	13.27 <sup>b</sup>	31.82 <sup>b</sup>
30	2540.12 <sup>a</sup>	4032.97 <sup>b</sup>	41.25 <sup>a</sup>	32.04 <sup>b</sup>
40	2468.01 <sup>a</sup>	4169.05 <sup>b</sup>	44.33 <sup>a</sup>	33.52 <sup>a</sup>
LSD	97.46	206.09	6.17	1.02
<b>Rhizobium strain</b>				
Uninoculated	1493.63 <sup>d</sup>	4181.67 <sup>b</sup>	-14.43 <sup>c</sup>	24.55 <sup>d</sup>
TAL-379	2020.77 <sup>c</sup>	3935.12 <sup>c</sup>	44.06 <sup>a</sup>	31.14 <sup>c</sup>
MAR-1495	3342.15 <sup>a</sup>	4855.62 <sup>a</sup>	37.55 <sup>a</sup>	39.10 <sup>a</sup>
SB-6-1-A <sub>2</sub>	2447.05 <sup>b</sup>	4224.57 <sup>b</sup>	49.95 <sup>a</sup>	33.89 <sup>b</sup>
LSD	97.46	206.09	6.17	1.02

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

soybean varieties. The straw and seed yields and their respective N, P, K and S uptakes of soybean were significantly ( $P < 0.001$ ) improved by S fertilization and *Rhizobium* inoculation (Tables 1 and 3). Seed yield of soybean was increased by 28.8 and 25.2%, when 30 and 40 kg ha<sup>-1</sup> S was applied as compared to control (no S application, S<sub>0</sub>) (Table 1).

Even though most parameters were significantly ( $P < 0.05$ ) affected by main effect and two-way interaction of the factors, but three-way interactions significantly ( $P < 0.001$ ) affected all seed and straw yields, and nutrients (N, P, K and S) uptake more than their respective two way interaction and/or main effects (Tables 2 and 4). The combined application of S (at 30 and 40 kg S ha<sup>-1</sup>) along with inoculation of MAR-1495 further showed highest seed yield for Bellessa-95 and Wollo, respectively (Table 2). For Bellessa-95, seed yields of 3864.1 and 3893.8 kg ha<sup>-1</sup> were obtained, corresponding to 112% and 114% increases at 30 and 40 kg S ha<sup>-1</sup>, respectively with strain MAR-1495. For Wollo, seed yields of 3633.3 and 3709.2 kg ha<sup>-1</sup> were obtained, corresponding to 160 and 166% increases for S at 30 and 40 kg ha<sup>-1</sup>, respectively with strain MAR-1495. While, S application at the rate of 30 kg S ha<sup>-1</sup> was significantly at par with 40 kg S ha<sup>-1</sup> for both varieties.

In addition, application of different doses of S increased straw yield of soybean significantly ( $p < 0.001$ ) (Table 1). The highest straw yield of soybean (4507 kg ha<sup>-1</sup>) was recorded with 20 kg S ha<sup>-1</sup> which was statistically at similar content with S<sub>0</sub> treatment (4487 kg ha<sup>-1</sup>), whereas, the lowest straw yield were recorded in the highest S

rates (30 and 40 kg S ha<sup>-1</sup>). Consequently, straw yield was higher in the inoculated treatments than uninoculated, with and/or without S application (Table 1).

N, P, K and S uptakes in soybean seed and straw were increased significantly with application of S application and inoculation of *Rhizobium* strains individually as well as in combination (Tables 3 and 4). The maximum increase in N uptake (3-folds) was obtained for Wollo at 40 kg S ha<sup>-1</sup> along with inoculation of MAR-1495, while at 30 kg S ha<sup>-1</sup> for Bellessa-95 inoculated with MAR-1495 was obtained an N uptake (256.6 kg ha<sup>-1</sup>) increased by two and half folds over control. The same is true for P and K seed uptakes which were increased significantly by addition of combined treatments of S fertilizer and inoculation of *Rhizobium* strain. Like N uptake, significantly the highest increase in S uptake (seventeen folds) was obtained for Wollo at 40 kg S ha<sup>-1</sup> along with inoculation of MAR-1495, while at 30 kg S ha<sup>-1</sup> for Bellessa-95 inoculated with MAR-1495 induced an S uptake of 39.02 kg ha<sup>-1</sup>, increased by about fifteen folds over control (Table 4).

### Seed protein yield

Significant variation ( $p < 0.001$ ) was observed in the seed protein yield of soybean with different doses of S application, resulted increased with increasing S rates (Table 2). Application of S at 40 kg ha<sup>-1</sup> resulted with significantly the highest protein content, ranged from 31.3% for unfertilized treatment to 33.5%, corresponding

**Table 2.** Interaction effects of S rates, *Rhizobium* strains and soybean varieties on seed and straw yield, estimated soil N balance and grain protein yield.

S (kg ha <sup>-1</sup> )	Strain	Variety	SY (kg ha <sup>-1</sup> )	StY (kg ha <sup>-1</sup> )	Nba (kg ha <sup>-1</sup> )	Protein content (%)
0	Uninoc.	Belessa-95	1819.5 <sup>ijk</sup>	4120.0 <sup>efghi</sup>	-29.25 <sup>p</sup>	25.99 <sup>klm</sup>
	MAR-1495		2782.5 <sup>de</sup>	4259.4 <sup>defghi</sup>	24.32 <sup>hijkl</sup>	37.89 <sup>bcdef</sup>
	SB-6-1-A <sub>2</sub>		2185.5 <sup>fg hij</sup>	4249.3 <sup>defghi</sup>	23.03 <sup>hijklm</sup>	27.91 <sup>ijk</sup>
	TAL-379		1926.2 <sup>ghijk</sup>	3981.2 <sup>efghi</sup>	32.97 <sup>ghijk</sup>	34.63 <sup>defg</sup>
	Uninoc.	Wollo	1390.5 <sup>lmn</sup>	4672.0 <sup>bcdefg</sup>	-21.76 <sup>nop</sup>	27.96 <sup>ijk</sup>
	MAR-1495		2535.7 <sup>ef</sup>	5374.1 <sup>ab</sup>	55.20 <sup>bcdefg</sup>	37.23 <sup>bcdef</sup>
	SB-6-1-A <sub>2</sub>		1896.7 <sup>ghijk</sup>	3705.3 <sup>ijk</sup>	21.43 <sup>hijklm</sup>	29.12 <sup>ijk</sup>
	TAL-379		1242.8 <sup>n</sup>	5801.1 <sup>a</sup>	40.25 <sup>efghij</sup>	33.89 <sup>fgh</sup>
20	Uninoc.	Belessa-95	1840.5 <sup>hijk</sup>	4374.7 <sup>cdefgh</sup>	-37.60 <sup>p</sup>	27.03 <sup>ijk</sup>
	MAR-1495		3184.7 <sup>cd</sup>	5218.7 <sup>abc</sup>	7.49 <sup>klm</sup>	38.35 <sup>bcde</sup>
	SB-6-1-A <sub>2</sub>		2788.5 <sup>de</sup>	4400.0 <sup>cdefgh</sup>	18.41 <sup>ijklm</sup>	34.86 <sup>defg</sup>
	TAL-379		2178.2 <sup>fg hij</sup>	3840.0 <sup>ghij</sup>	24.75 <sup>hijkl</sup>	28.33 <sup>ijk</sup>
	Uninoc.	Wollo	1794.0 <sup>kl</sup>	4228.0 <sup>defghi</sup>	-28.81 <sup>op</sup>	23.40 <sup>lm</sup>
	MAR-1495		3134.0 <sup>cd</sup>	4716.0 <sup>bcdef</sup>	14.59 <sup>klm</sup>	39.23 <sup>bc</sup>
	SB-6-1-A <sub>2</sub>		2007.0 <sup>ghijk</sup>	4640.0 <sup>bcdefg</sup>	41.42 <sup>defghi</sup>	30.92 <sup>ghi</sup>
	TAL-379		1657.5 <sup>klm</sup>	4643.0 <sup>bcdefg</sup>	65.92 <sup>abcde</sup>	28.41 <sup>ijk</sup>
30	Uninoc.	Belessa-95	1336.5 <sup>mn</sup>	3953.3 <sup>efghi</sup>	4.85 <sup>lm</sup>	25.28 <sup>klm</sup>
	MAR-1495		3864.1 <sup>a</sup>	4594.7 <sup>bcdefg</sup>	34.60 <sup>efghij</sup>	41.52 <sup>ab</sup>
	SB-6-1-A <sub>2</sub>		3356.4 <sup>bc</sup>	4067.2 <sup>efghi</sup>	57.70 <sup>bcdefg</sup>	34.29 <sup>efgh</sup>
	TAL-379		2473.5 <sup>ef</sup>	3445.3 <sup>ijk</sup>	15.36 <sup>ijklm</sup>	26.54 <sup>ijk</sup>
	Uninoc.	Wollo	1386.0 <sup>lmn</sup>	3705.3 <sup>hij</sup>	0.903 <sup>lmn</sup>	22.72 <sup>lm</sup>
	MAR-1495		3633.3 <sup>ab</sup>	4827.1 <sup>bcde</sup>	84.13 <sup>a</sup>	40.21 <sup>ab</sup>
	SB-6-1-A <sub>2</sub>		2247.7 <sup>fgh</sup>	4654.7 <sup>bcdefg</sup>	72.21 <sup>abc</sup>	35.61 <sup>cdef</sup>
	TAL-379		2023.5 <sup>ghijk</sup>	3016.0 <sup>jk</sup>	60.29 <sup>abcdef</sup>	30.16 <sup>hij</sup>
40	Uninoc.	Belessa-95	1233.0 <sup>n</sup>	4162.7 <sup>efghi</sup>	-2.62 <sup>mno</sup>	22.05 <sup>m</sup>
	MAR-1495		3893.8 <sup>a</sup>	4761.7 <sup>bcde</sup>	39.74 <sup>efghij</sup>	34.50 <sup>defgh</sup>
	SB-6-1-A <sub>2</sub>		2808.7 <sup>de</sup>	3869.3 <sup>fg hij</sup>	80.74 <sup>ab</sup>	44.00 <sup>a</sup>
	TAL-379		2219.9 <sup>fghi</sup>	3826.7 <sup>ghij</sup>	46.40 <sup>cdefgh</sup>	28.56 <sup>ijk</sup>
	Uninoc.	Wollo	1149.0 <sup>n</sup>	4237.3 <sup>defghi</sup>	-1.153 <sup>lmn</sup>	22.05 <sup>m</sup>
	MAR-1495		3709.2 <sup>ab</sup>	5093.3 <sup>abcd</sup>	40.34 <sup>efghij</sup>	43.90 <sup>a</sup>
	SB-6-1-A <sub>2</sub>		2286.0 <sup>fg</sup>	4470.7 <sup>cdefgh</sup>	84.66 <sup>a</sup>	34.50 <sup>defgh</sup>
	TAL-379		2444.5 <sup>ef</sup>	2930.7 <sup>k</sup>	66.57 <sup>abcd</sup>	38.64 <sup>bcd</sup>
LSD			413.82	875.02	26.21	4.34

Non-inoculated (0)=Uninoc.; SY-Seed yield; StY-Straw yield; Nba- Estimated soil N balance.

to an average increase of 7.0%. In addition, inoculation of MAR-1495 significantly increased protein content, ranged from 24.5% for uninoculated treatment to 39.1%, corresponding to an average increase by 59.2% over control. But, results showed that combined application of S with *Rhizobium* strain further increased protein yield for the two soybean varieties, resulted with highest protein content (Table 2). Consequently, Wollo inoculated with MAR-1495 along with S application at 40 kg ha<sup>-1</sup> showed the highest seed protein content (43.9%) which was statistically at similar content with Wollo inoculated with

SB-6-1-A<sub>2</sub> at similar S rate (40 kg ha<sup>-1</sup>). While Belessa-95 produced higher percentage of protein when inoculated with MAR-1495 along with S at 30 kg ha<sup>-1</sup>. Whereas, the lowest protein content of soybean (27.9 and 25.9%) were recorded in the uninoculated unfertilized (R<sub>0</sub>S<sub>0</sub>) treatment combination for Wollo and Belessa-95, respectively.

#### Estimated soil N balance (Nba)

Nitrogen fixed by legumes represents a key contribution

**Table 3.** Seed and straw nutrients (N, P, K and S) uptake of soybean varieties as affected by S fertilizer rates and *Rhizobium* strains.

Variations	Seed nutrient uptake (kg ha <sup>-1</sup> )				Straw nutrient uptake (kg ha <sup>-1</sup> )			
	N	P	K	S	N	P	K	S
<b>Variety</b>								
Belessa-95	133.46 <sup>a</sup>	17.88 <sup>b</sup>	64.66 <sup>a</sup>	14.50 <sup>a</sup>	30.82 <sup>b</sup>	5.93	51.20 <sup>b</sup>	11.68 <sup>b</sup>
Wollo	118.42 <sup>b</sup>	19.62 <sup>a</sup>	49.82 <sup>b</sup>	12.13 <sup>b</sup>	42.89 <sup>a</sup>	6.11	58.05 <sup>a</sup>	12.44 <sup>a</sup>
LSD	3.34	0.963	1.56	0.614	2.13	NS	2.37	0.625
<b>S (kg ha<sup>-1</sup>)</b>								
0	102.16 <sup>c</sup>	15.17 <sup>c</sup>	45.23 <sup>d</sup>	6.45 <sup>d</sup>	43.06 <sup>a</sup>	6.25 <sup>ab</sup>	47.68 <sup>c</sup>	4.45 <sup>d</sup>
20	120.89 <sup>b</sup>	18.61 <sup>b</sup>	55.74 <sup>c</sup>	11.02 <sup>c</sup>	35.57 <sup>b</sup>	6.48 <sup>a</sup>	54.53 <sup>b</sup>	14.88 <sup>b</sup>
30	138.71 <sup>a</sup>	19.09 <sup>b</sup>	67.67 <sup>a</sup>	18.53 <sup>a</sup>	34.84 <sup>b</sup>	5.79 <sup>ab</sup>	59.40 <sup>a</sup>	11.42 <sup>c</sup>
40	141.98 <sup>a</sup>	22.13 <sup>a</sup>	60.32 <sup>b</sup>	17.25 <sup>b</sup>	33.95 <sup>b</sup>	5.55 <sup>b</sup>	56.89 <sup>ab</sup>	17.48 <sup>a</sup>
LSD	6.24	1.79	2.92	1.14	3.73	0.743	4.42	1.16
<b>Rhizobium strain</b>								
Uninocu.	59.13 <sup>d</sup>	8.87 <sup>c</sup>	38.89 <sup>d</sup>	7.52 <sup>c</sup>	32.17 <sup>bc</sup>	4.11 <sup>c</sup>	51.95 <sup>b</sup>	12.85 <sup>b</sup>
TAL-379	100.38 <sup>c</sup>	16.55 <sup>b</sup>	54.07 <sup>c</sup>	11.27 <sup>b</sup>	35.87 <sup>b</sup>	6.10 <sup>b</sup>	55.01 <sup>ab</sup>	8.37 <sup>c</sup>
MAR-1495	209.69 <sup>a</sup>	24.65 <sup>a</sup>	76.70 <sup>a</sup>	22.44 <sup>a</sup>	48.78 <sup>a</sup>	6.86 <sup>a</sup>	59.21 <sup>a</sup>	12.31 <sup>b</sup>
SB-6-1-A <sub>2</sub>	134.54 <sup>b</sup>	24.93 <sup>a</sup>	59.30 <sup>b</sup>	12.03 <sup>b</sup>	30.60 <sup>c</sup>	7.00 <sup>a</sup>	52.28 <sup>b</sup>	14.70 <sup>a</sup>
LSD	6.24	1.79	2.92	1.14	3.99	0.743	4.42	1.16

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

**Table 4.** Interaction effects of S rates, *Rhizobium* strains and soybean varieties on seed and straw nutrients (N, P, K and S) uptake.

S (kg ha <sup>-1</sup> )	Strain	V	Seed nutrient uptake (kg ha <sup>-1</sup> )				Straw nutrient uptake (kg ha <sup>-1</sup> )			
			N	P	K	S	N	P	K	S
0	Uninoc.	B	75.5 <sup>mnp</sup>	12.4 <sup>klmn</sup>	51.64 <sup>ijkl</sup>	2.46 <sup>qr</sup>	27.43 <sup>ij</sup>	4.03 <sup>fgh</sup>	50.93 <sup>defg</sup>	4.12 <sup>k</sup>
	MAR-1495		168.7 <sup>fg</sup>	18.80 <sup>ghijk</sup>	59.24 <sup>ghij</sup>	12.2 <sup>fghijk</sup>	44.4 <sup>cdefgh</sup>	6.47 <sup>bcdefgh</sup>	25.28 <sup>i</sup>	3.34 <sup>k</sup>
	SB-6-1-A <sub>2</sub>		97.53 <sup>klm</sup>	12.4 <sup>klmn</sup>	49 <sup>ijklmn</sup>	7.49 <sup>klmnop</sup>	20.56 <sup>j</sup>	6.55 <sup>bcdefgh</sup>	31.55 <sup>hi</sup>	4.75 <sup>jk</sup>
	TAL-379		106.6 <sup>ijk</sup>	16.06 <sup>hijkl</sup>	47 <sup>ijklmno</sup>	6.08 <sup>opqr</sup>	35 <sup>cdefghij</sup>	5.80 <sup>bcdefgh</sup>	42.17 <sup>ghi</sup>	3.05 <sup>k</sup>
	Uninoc.	W	62 <sup>nopqr</sup>	11.29 <sup>klmn</sup>	29.71 <sup>p</sup>	1.90 <sup>r</sup>	28.58 <sup>hij</sup>	3.60 <sup>h</sup>	61.49 <sup>bdef</sup>	5.03 <sup>jk</sup>
	MAR-1495		151.1 <sup>gh</sup>	15.32 <sup>ijklm</sup>	51.44 <sup>ijkl</sup>	9.5 <sup>ijklmno</sup>	91.01 <sup>a</sup>	6.87 <sup>bcdefg</sup>	53.45 <sup>defg</sup>	5.55 <sup>jk</sup>
	SB-6-1-A <sub>2</sub>		88.2 <sup>klmn</sup>	25.31 <sup>cdefg</sup>	43 <sup>lmno</sup>	7.35 <sup>lmnop</sup>	25.99 <sup>ij</sup>	7.57 <sup>abcde</sup>	50.01 <sup>defgh</sup>	2.97 <sup>k</sup>
	TAL-379		67.3 <sup>nopq</sup>	9.62 <sup>lmn</sup>	29.35 <sup>p</sup>	4.56 <sup>pqr</sup>	70.56 <sup>b</sup>	9.15 <sup>a</sup>	66.54 <sup>bcd</sup>	6.84 <sup>ijk</sup>
20	Uninoc.	B	79.6 <sup>lmno</sup>	9.41 <sup>lmn</sup>	50 <sup>ijklm</sup>	11 <sup>fghijklm</sup>	36 <sup>cdefghij</sup>	3.74 <sup>gh</sup>	50.21 <sup>defgh</sup>	16.9 <sup>cdef</sup>
	MAR-1495		195.4 <sup>de</sup>	23.28 <sup>defgh</sup>	71.95 <sup>def</sup>	12 <sup>fghijkl</sup>	45.64 <sup>cdefg</sup>	8.41 <sup>abc</sup>	63.25 <sup>bode</sup>	15.8 <sup>cdefg</sup>
	SB-6-1-A <sub>2</sub>		155.57 <sup>g</sup>	28.88 <sup>bcde</sup>	66.46 <sup>fgh</sup>	10 <sup>ghijklmn</sup>	24.47 <sup>j</sup>	6.85 <sup>bcdefg</sup>	49.93 <sup>defgh</sup>	20.51 <sup>bc</sup>
	TAL-379		98.37 <sup>klm</sup>	13.3 <sup>ijklmn</sup>	61.58 <sup>fghi</sup>	12.44 <sup>fghij</sup>	24.87 <sup>j</sup>	8.16 <sup>abc</sup>	51.55 <sup>defg</sup>	5.84 <sup>jk</sup>
	Uninoc.	W	67.1 <sup>nopq</sup>	9.99 <sup>lmn</sup>	45.5 <sup>klmno</sup>	12.98 <sup>fghij</sup>	35 <sup>cdefghij</sup>	4.86 <sup>defgh</sup>	53.87 <sup>defg</sup>	16.9 <sup>cdef</sup>
	MAR-1495		196.6 <sup>de</sup>	26.67 <sup>bcdef</sup>	64.87 <sup>fgh</sup>	15.71 <sup>defg</sup>	49.89 <sup>cd</sup>	7.00 <sup>abcdef</sup>	58.17 <sup>bdefg</sup>	11.28 <sup>ghi</sup>
	SB-6-1-A <sub>2</sub>		98.97 <sup>klm</sup>	27.97 <sup>bcde</sup>	46.1 <sup>klmno</sup>	6.62 <sup>nopqr</sup>	30.9 <sup>efghij</sup>	7.56 <sup>abcde</sup>	50.69 <sup>defg</sup>	22.36 <sup>b</sup>
	TAL-379		75.4 <sup>mnpq</sup>	9.33 <sup>lmn</sup>	38.7 <sup>mnpq</sup>	5.61 <sup>opqr</sup>	30.9 <sup>efghij</sup>	5.32 <sup>cdefgh</sup>	58.55 <sup>bdefg</sup>	9.33 <sup>hij</sup>
30	Uninoc.	B	54.0 <sup>opqr</sup>	7.42 <sup>n</sup>	37.73 <sup>nop</sup>	8.2 <sup>ijklmnop</sup>	30.60 <sup>ghij</sup>	3.95 <sup>fgh</sup>	48.87 <sup>defgh</sup>	14.8 <sup>defg</sup>
	MAR-1495		256.6 <sup>ab</sup>	33.23 <sup>ab</sup>	102.46 <sup>a</sup>	39.02 <sup>a</sup>	29.55 <sup>ghij</sup>	8.68 <sup>ab</sup>	51.27 <sup>defg</sup>	6.98 <sup>ijk</sup>
	SB-6-1-A <sub>2</sub>		184.16 <sup>ef</sup>	19.11 <sup>fghij</sup>	92.12 <sup>ab</sup>	19.92 <sup>cd</sup>	27.29 <sup>ij</sup>	6.47 <sup>bcdefgh</sup>	62.46 <sup>bdef</sup>	13.28 <sup>fgh</sup>
	TAL-379		105.0 <sup>ijkl</sup>	21.32 <sup>efghi</sup>	79.02 <sup>cde</sup>	18.68 <sup>cde</sup>	22.87 <sup>j</sup>	4.95 <sup>defgh</sup>	57.47 <sup>bdefg</sup>	12.73 <sup>fgh</sup>
	Uninoc.	W	50.40 <sup>pqr</sup>	8.25 <sup>mn</sup>	36.53 <sup>op</sup>	8.8 <sup>ijklmnop</sup>	30.68 <sup>fghij</sup>	3.55 <sup>h</sup>	46.43 <sup>efgh</sup>	14.3 <sup>efgh</sup>

Table 4. Contd.

	MAR-1495	233.7 <sup>bc</sup>	26.3 <sup>bcdefg</sup>	84.71 <sup>bc</sup>	26.73 <sup>b</sup>	51.22 <sup>c</sup>	6.53 <sup>abcdefgh</sup>	89.80 <sup>a</sup>	6.87 <sup>ijk</sup>
	SB-6-1-A <sub>2</sub>	127.84 <sup>hi</sup>	21.56 <sup>efghi</sup>	56.88 <sup>hijk</sup>	14.16 <sup>efgh</sup>	47.78 <sup>cde</sup>	7.75 <sup>abcd</sup>	74.32 <sup>abc</sup>	17.6 <sup>bcdef</sup>
	TAL-379	97.75 <sup>klm</sup>	15.48 <sup>ijklm</sup>	51.94 <sup>ijkl</sup>	12.59 <sup>ghij</sup>	44.5 <sup>cdefgh</sup>	4.44 <sup>efgh</sup>	44.62 <sup>efgh</sup>	4.75 <sup>jk</sup>
	Uninoc.	43.50 <sup>qr</sup>	5.94 <sup>n</sup>	30.47 <sup>p</sup>	7.00 <sup>mnpq</sup>	32.9 <sup>defghij</sup>	4.33 <sup>fgh</sup>	54.27 <sup>defg</sup>	15.0 <sup>defg</sup>
40	MAR-1495	214.9 <sup>cd</sup>	23.40 <sup>defgh</sup>	97.73 <sup>a</sup>	29.13 <sup>b</sup>	30.9 <sup>efghij</sup>	5.95 <sup>bcdefgh</sup>	75.40 <sup>ab</sup>	19.67 <sup>bcd</sup>
	SB-6-1-A <sub>2</sub>	198.0 <sup>de</sup>	25.6 <sup>bcdefg</sup>	69.53 <sup>defg</sup>	20.72 <sup>c</sup>	28.39 <sup>hij</sup>	5.65 <sup>bcdefgh</sup>	43.67 <sup>fghi</sup>	18.6 <sup>bcde</sup>
	TAL-379	101 <sup>ijklm</sup>	15.3 <sup>ijklmn</sup>	66.86 <sup>efgh</sup>	13.69 <sup>fghi</sup>	30.9 <sup>efghij</sup>	4.86 <sup>defgh</sup>	60.94 <sup>bcdefg</sup>	11.33 <sup>ghi</sup>
	Uninoc.	40.54 <sup>r</sup>	6.15 <sup>n</sup>	28.84 <sup>p</sup>	6.93 <sup>mnpq</sup>	35 <sup>cdefghij</sup>	4.85 <sup>defgh</sup>	49.53 <sup>defgh</sup>	15.5 <sup>defg</sup>
	MAR-1495	260.35 <sup>a</sup>	30.20 <sup>bcd</sup>	81.21 <sup>bcd</sup>	35.01 <sup>a</sup>	47.59 <sup>cdef</sup>	4.96 <sup>defgh</sup>	57.10 <sup>bcdefg</sup>	29.01 <sup>a</sup>
	SB-6-1-A <sub>2</sub>	125.9 <sup>hij</sup>	38.51 <sup>a</sup>	50.2 <sup>ijklm</sup>	9.0 <sup>ijklmnop</sup>	39.4 <sup>cdefghi</sup>	7.63 <sup>abcd</sup>	55.66 <sup>cdefg</sup>	17.5 <sup>bcdef</sup>
	TAL-379	151.1 <sup>gh</sup>	31.92 <sup>abc</sup>	57.7 <sup>ghijk</sup>	16.51 <sup>cdef</sup>	26.35 <sup>ij</sup>	6.17 <sup>abcdefgh</sup>	58.59 <sup>bcdefg</sup>	13.07 <sup>fgh</sup>
	LSD	26.51	7.63	12.42	4.87	16.94	3.15	18.8	4.95

B- Belessa-95; W- Wollo. Note: Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

to nutrient cycling in legume-based farming systems by increasing soil organic N status. Soil N balance (Nba) was negative in the control treatments. Application of S had significantly increased soil N balance over the control (unfertilized) except S at 20 kg ha<sup>-1</sup> (Table 1). On the other hand, response of inoculation of *Rhizobium* strain was accompanied by significant increase in estimated soil N balance compared to uninoculated control.

Inoculation of SB-6-1-A<sub>2</sub> had showed maximum increase in N balance with corresponding variation from -14.43 kg N ha<sup>-1</sup> (uninoculated) to 50.0 kg N ha<sup>-1</sup>. Further benefit of estimated N in soil increased with both S fertilization and inoculation *Rhizobium* strain for the two soybean varieties (Table 2). Deficit N balance (Nba) of soybean was lowest (-29.25 kg ha<sup>-1</sup>) in control and maximum (80.74 kg ha<sup>-1</sup>) for Bellessa-95 treated with S application at 40 kg ha<sup>-1</sup> along with inoculation of SB-6-1-A<sub>2</sub>. For Wollo, lowest (-21.76 kg ha<sup>-1</sup>) in control and maximum (84.66 kg N ha<sup>-1</sup>) along with S application at 40 kg ha<sup>-1</sup> and inoculation of SB-6-1-A<sub>2</sub> (Table 2).

## DISCUSSION

The results revealed that the interactive positive effect of S fertilization and inoculation of *Rhizobium* strain on grain and straw yield and nutrients uptake and quality protein of soybean varieties, grown as a major crop on Nitisols of Assosa area, Ethiopia. Application of S with *Rhizobium* strains plays an important role in physiological and developmental processes in plant life and the favorable effect of these important nutrients combination (N and S) might accelerate the growth processes, which ultimately resulted in increased seed yield and quality of the crop. Increased in root nodulation due to *Rhizobium* inoculation

and S fertilization resulted in absorption of higher concentration of mineral nutrients from soil and hence increased shoot dry weight by soybean. The highest nutrient in shoot was due to total N accumulation enhanced biomass yield and showed the synergistic effect of nutrients in shoot. In addition, the positive effect of S fertilization with *Rhizobium* strain on nitrogen fixing potential and shoot dry weight attributed to seed yield production. *Rhizobium* strain treatments to soybean significantly increased seed weight per plant either alone or in combination with S. Each *Rhizobium* strain has own synergetic effect on production of seed weight as seed weight also increased when *Rhizobium* strains (significantly greater for MAR-1495) were applied individually but greater in combination with S. That shows S application is important nutritional element to get better soybean yield. Similarly, results for beneficial effect of S and *Rhizobium* strain application on yield and yield attributing characteristics have also been recorded by other workers (Habtemichial et al., 2007; Scherer et al., 2008).

This study revealed that *Rhizobium* inoculation with S fertilizer increased sufficient yield of quality soybean seed. The synergistic effect of N and S may be due to utilization of high quantities of nutrients through their well-developed root system and nodules which might have resulted in better growth and yield. These results confirm the earlier findings reported by Hussain et al. (2011). The application of S might have increased the availability of nutrient to soybean plant due to improved nutritional environment, which in turn, favorably influenced the energy transformation activation of enzymes, chlorophyll synthesis as well as increased carbohydrate metabolism (Dhage et al., 2014). It constitutes the main element of

**Table 5.** Correlation analysis between grain and straw yield and nutrients uptake and protein yield of soybean.

Correlation	SY	Nba	Protein	Seeds Nup	Seeds Pup	Seeds Kup	Seeds Sup	Straw Nup	Straw Pup	Straw Kup	Straw Sup
SY	0.19 <sup>NS</sup>	0.38 <sup>***</sup>	0.76 <sup>***</sup>	0.96 <sup>***</sup>	0.70 <sup>***</sup>	0.93 <sup>***</sup>	0.84 <sup>***</sup>	0.08 <sup>NS</sup>	0.34 <sup>***</sup>	0.27 <sup>**</sup>	0.20 <sup>*</sup>
SY	1	0.02 <sup>NS</sup>	0.32 <sup>**</sup>	0.26 <sup>**</sup>	0.004 <sup>NS</sup>	0.03 <sup>NS</sup>	0.07 <sup>NS</sup>	0.61 <sup>***</sup>	0.40 <sup>***</sup>	0.41 <sup>***</sup>	0.18 <sup>NS</sup>
Nba		1	0.57 <sup>***</sup>	0.44 <sup>***</sup>	0.51 <sup>***</sup>	0.32 <sup>**</sup>	0.32 <sup>**</sup>	0.22 <sup>*</sup>	0.40 <sup>***</sup>	0.26 <sup>*</sup>	0.05 <sup>NS</sup>
Protein			1	0.88 <sup>***</sup>	0.71 <sup>***</sup>	0.60 <sup>***</sup>	0.60 <sup>***</sup>	0.34 <sup>***</sup>	0.46 <sup>***</sup>	0.18 <sup>NS</sup>	0.12 <sup>NS</sup>
SeedsNup				1	0.73 <sup>***</sup>	0.85 <sup>***</sup>	0.83 <sup>***</sup>	0.18 <sup>NS</sup>	0.37 <sup>***</sup>	0.25 <sup>*</sup>	0.21 <sup>*</sup>
SeedsPup					1	0.61 <sup>***</sup>	0.55 <sup>***</sup>	-0.03 <sup>NS</sup>	0.46 <sup>***</sup>	0.16 <sup>NS</sup>	0.27 <sup>***</sup>
SeedsKup						1	0.85 <sup>***</sup>	-0.09 <sup>NS</sup>	0.24 <sup>*</sup>	0.28 <sup>**</sup>	0.18 <sup>NS</sup>
SeedsSup							1	0.003 <sup>NS</sup>	0.18 <sup>NS</sup>	0.30 <sup>**</sup>	0.32 <sup>**</sup>
Straw Nup								1	0.31 <sup>**</sup>	0.27 <sup>**</sup>	-0.08 <sup>NS</sup>
Straw Pup									1	0.21 <sup>*</sup>	-0.07 <sup>NS</sup>
Straw Kup										1	0.22 <sup>*</sup>

NS- non significant; \*, \*\*, \*\*\* significant at 0.05, 0.01 and 0.001, respectively.

amino acids such as cysteine and methionine, which are of essential nutrient value and can increase seed yield. Therefore, soybean protein quality could be significantly improved by increasing the concentration of the sulfur-containing amino acids through S fertilization. The yield and quality of legume seeds are limited by the amount of S (S) partitioned to the seeds (Tan et al., 2010). The role of S in the seed production of soybean has also been reported by (Jamal et al., 2005). Dhage et al. (2014) found that soybean seed yield and straw yield increased significantly due to application of 60 kg ha<sup>-1</sup> S followed by 40 kg ha<sup>-1</sup> S over control.

Each *Rhizobium* strain has its own synergetic effect on seed yield and nutrients uptake increased substantially when applied in combination with S. Generally, the two soybean varieties, Belessa-95 and Wollo showed highest seeds N, P, K and S uptakes when inoculated with MAR-1495 at S rate of 30 and 40 kg ha<sup>-1</sup>, respectively, indicated that balanced S application is important nutritional aspect for increasing soybean yield and nutrients uptake efficiency along with *Rhizobium* strain. Islam et al. (2012) also reported that S fertilization enhanced crop yield and uptake of macronutrients such as nitrogen, phosphorus and potassium especially nitrogen. According to Cazzato et al. (2012), S fertilization rates were increased nutrient levels in lupin seeds when compared with the control unfertilized treatment. Seed yield and N, P, K and S uptakes of seeds were significantly improved by *Rhizobium* inoculation and S fertilization. Results indicated a significant positive correlation (Table 5) between seed yield and N, P, K and S uptakes ( $r^2=0.96$ ,  $r^2=0.70$ ,  $r^2=0.93$ , and  $r^2=0.84$ , respectively) showing the importance of nutrients uptake on improvement in growth and seed yield of soybean. The magnitude of response on straw yield of soybean was more in case of inoculated with or without S fertilization. This may be due to the enhanced shoot nutrient uptake and N<sub>2</sub> fixation due to combined application of *Rhizobium* strain with S during

the vegetative growth; therefore most of the absorbed forms are re-translocated in seed than to straw during the reproductive stages. Therefore, S input modifies N allocation more to storage organs (seeds) than to straw.

Results also revealed that *Rhizobium* strain alone or combined with S application increased protein content, while inoculation of strain with S increased the protein content up to 44.0%. The increased in protein content with increasing level of S may be because in the absence of S, amino acids cannot be transformed into proteins, which results in reduced N acquisition (Zuber et al., 2013). Because of central role of S and N in the synthesis of proteins, the supplies of these nutrients in plants are highly inter-related (Jamal et al., 2005), which suggested that an insufficient S supply can affect yield and quality of crops. In fact, there is close link between S supply and N requirement of plant in addition. There was accumulation of one nutrient in plant when other nutrient was lacking and accumulated nutrient was used in protein synthesis when treatments were reversed (Jamal et al., 2010). The least protein content was produced in the uninoculated plants (Tables 1 and 2); resulted with decreasing protein content with increasing S application. This may be due to nutrient imbalance between the two nutrients. Fismes et al. (2000) also found that their interactions, reflected by plant uptake, are synergistic at optimum rates and antagonistic at excessive levels of one of the both. But when applied in combination known to create a more synergism, which was helpful in improved plant growth, including nutrient uptake in the plants and the improved protein content. In legumes, previous studies showed that S deficiency decreases N assimilation and fixation (Scherer et al., 2006). In addition, modifies seed protein composition by decreasing the abundance of storage proteins with the highest content of S-containing amino acid, while increasing the level of S-poor globulins (Zuber et al., 2013). Similarly, Sharma and Sharma (2014) also reported that a significant increase in S-containing amino acids such as methionine and cysteine



obtained by combined application of N and S for soybean. Therefore, the results of this study indicated that S fertilization is required to improve N-use efficiency and thereby maintaining protein quality (Fismes et al., 2000). The increase in seed nutrients uptake was correlated with the enhancement of N<sub>2</sub> fixation due to S increased the nitrogenase activity when applied with *Rhizobium* inoculation. A significant positive correlation between seed protein content and N, P, K and S uptakes of seeds ( $r^2=0.88$ ,  $r^2=0.71$ ,  $r^2=0.60$ , and  $r^2=0.60$ , respectively) may be due to fertilization of S facilitated the growth of plants by improving the uptake of nutrients in shoot and seeds and stimulating seeds and straw production. Accordingly, the present observations strongly support the view that seed yield, nutrients uptake and quality protein of soybean varieties are improved with combined application of *Rhizobium* strain and S-fertilization.

Amount of N taken by plant from soil and fertilizer was significantly higher due to inoculation and S application as compared to control. Even though the estimation only considers the chemical nature of the plant residues, it should be further estimated considering the magnitude and timing of N and S release from residues, and any subsequent immobilization. The subsequent decomposition of N rich residues replenish N removed by harvesting without the addition of fertilizer N and contribute fixed N to subsequent crops (Jensen and Hauggaard, 2003). Nitrogen inputs from grain legumes are highly dependent on the crop N harvest index, that is, the proportion of total above-ground N production removed as grain (Ravuri and Hume, 1993). Some studies show a positive effect of grain legumes on the soil N balance due to S fertilization and *Rhizobium* inoculation (Habtemichial et al., 2007), whereas other study show a negative effect (Hussain et al., 2011). However, most of these N balances have probably underestimated the below-ground input of fixed N by legumes due to problems of root sampling and quantifying root exudates or rhizodeposition (Khan et al., 2003). Negative N balance in the uninoculated and unfertilized control may be due to the fact that nitrogen input (starter N at 18 kg ha<sup>-1</sup> fertilizer) was not enough to meet crop demand. Therefore, large amounts of N moved away from the soil for uninoculated as well as unfertilized control. (Amanuel et al., 2000) also reported that N balance after legume harvest is positive when crop residues are returned to soil and only seed or grain is removed, which was not included in this study. Habtemichial et al. (2007) reported positive soil N balance in range of 12 to 52 kg ha<sup>-1</sup> after harvest of faba bean crop in Northern Ethiopia, but major difference was that crop residues were returned to soil. Higher positive soil N balance estimated with S application and inoculation might be due to the fact that amount of N fixed increased from 22.4 to 300 kg ha<sup>-1</sup> and 28.3 to 273.2 kg ha<sup>-1</sup> for Wollo and Belessa-95, respectively. Therefore, the estimated soil nitrogen balance became

more positive, because the estimation only considers the chemical nature of the plant residues (straw nutrients content) that soybean straw can be a benefit for soil nutrients replenishment. Therefore, soybean inoculation with S fertilizer can be beneficial to farmers whose aim is to increase N balance in the soil in addition to maximize soybean yields since most of the N is removed in seeds as well as in straw harvested. Because, the amount of straw N in soybean varieties depended on how completely N was translocated to seed, total amount of N remaining in the field after soybean has been harvested for seed (Ravuri and Hume, 1993).

## Conclusion

In this experiment the two soybean varieties performed better when inoculation of *Rhizobium* strain was applied with S fertilization than when both were applied alone. The combined application increased the availability and uptake of N, P, K and S by seeds and straw, and the growth and yield of the two varieties of soybean. The most beneficial effect of S with inoculation on N<sub>2</sub> fixation, plant growth and yield can be understood given the fact that the experimental soil was severely deficient in available S as well as *Rhizobium* strain, which can be considered major limiting factors for quality soybean production on acidic soils of Assosa area. Hence, the integrated application of *Rhizobium* strain with S could be a viable strategy to improve the yield and quality of soybean, in soils containing suboptimal S and N.

The two varieties responded to the combined application of *Rhizobium* strain MAR-1495 with S fertilizer at 30 and 40 kg ha<sup>-1</sup>. Even though with the highest yield achieved at the highest S rate (40 kg ha<sup>-1</sup>) and indicated that the yield of soybean can still be improved at further higher S rate, but were significantly at par for most parameters. Therefore, S at 30 and 40 kg ha<sup>-1</sup> with MAR-1495 was the most profitable interaction between inorganic S levels and *Rhizobium* strain treatments for N<sub>2</sub> fixation, yield and quality attributes of the two soybean varieties. In addition, strain SB-6-1-A<sub>2</sub> with S fertilization also suggesting a promising way for enhancing the growth and yield of soybean. Therefore, proper fertilization programs including S integrated with inoculation of *Rhizobium* strain should be implemented to improve the productivity of food legumes and thereby increase total food production, enhance the supply of good quality proteins in Ethiopia.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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