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EVALUATING RHIZOBIUM STRAINS FOR SOYBEAN (*GLYCINE MAX L.*) PRODUCTION IN HAWASSA ZURIA DISTRICT, SIDAMA REGION

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ABSTRACT

Urea is the main source of N applied to legume crops grown in Ethiopia, but it is very expensive. In order to substitute the use of chemical fertilizers in legume production, there is a need to produce rhizobial inoculants, which are capable of fixing N and, thus, can be used as biofertilizers. To achieve this, an experiment was conducted on farmer's field in Hawassa Zuria district for two consecutive years using three types of strains combined with TSP fertilizer and soybean variety Hawassa 95. Five treatments, including control (no fertilizer and inoculant), recommended N (46 kg ha^{-1}), SB 12 + 50 kg TSP, SB MAR 1495 + 50 kg ha^{-1} TSP and SB 14 + 50kg/ha TSP, were laid out in randomized complete block design with three replications. It was observed that higher yield was obtained from the rhizobium strains combined with TSP, compared to the control and recommended N. Strain SB 12 + 50 kg TSP gave the highest yield (2303.3 kg ha^{-1}), while the lowest yield was recorded for the untreated plot. Hence application of rhizobial inoculants can be an option for farmers producing soybean in the area. The highest net benefits with acceptable marginal rate of returns were obtained from the plots treated with rhizobium strains. Therefore, these strains could be recommended as alternative options for soybean production around Hawassa zuria district. However, as the experiment was carried out only in one region, similar undertakings should be carried out over years at different region to determine the long-term effect of rhizobium strains on the soil and the crop.

Key words: Rhizobium strains, Soybean, Nodule, Economic benefit, Yield

1. INTRODUCTION

It is estimated that Rhizobia can fix about 50 to 300 kg N ha^{-1} (Bokhtiar and Sakurai, 2005). Thus, their contribution to the N economy of the soil is quite substantial. In general, symbiotic nitrogen fixation in legume crops through crop rotation and intercropping not only reduces fertilizer costs, but also improves soil fertility. For these reasons, inoculation with strains of rhizobia has become an important agronomic practice to ensure adequate N supply to legumes such as soybean and reduce the amount of inorganic N fertilizers required by the crop as well as by non-leguminous subsequent crops (Vargas and Hungria, 1997; Chen *et al*., 2002; Gupta, 2004). Soybean (*Glycine max* (L.) Merr.) is one of the most important and extensively grown crops that accounts for 30% of the world's processed vegetable oil and also has been used as a source for bio-diesel fuels (Graham and Vance, 2003). The use of inorganic fertilizers is constrained by their high cost to most small scale farmers. The use of farm yard manure is also limited by little or no availability of cattle in most soybean growing areas. Soybean has a high capacity to improve soil fertility by adding nitrogen from the atmosphere through symbiotic relationship with Rhizobia. On average, 50–60% of soybean N demand is met by biological N_2 fixation for a wide range of yield levels and environments and the proportion of plant N derived from fixation decreases with increasing inputs of N fertilizer (Salvagiotti *et al*., 2008). The estimated quantities of nitrogen which can be biologically fixed from the atmosphere range from 15 to 450 kg per hectare (Salvagiotti *et al*., 2008). Under optimum conditions of nitrogen fixation, atmospheric nitrogen fixed meets both the needs of the crop and provides residual benefits (Woomer, 2010). To bridge the wide gap between production and consumption of edible oil in Ethiopia, it is imperative to improve the yield of oil seed plants (Jalaluddin, 2005). The soil of Hawassa zuria district is low in N and needs application of the nutrients for optimum crop production. Therefore this study was designed to test the possibility of enhancing soybean production under the small holder farmers condition in Hawassa Zuria district through the use of *Rhizobium* strain inoculation.

2. MATERIALS AND METHODS

This study was carried out for two consecutive years in 2016 and 2017 main growing season in Hawassa zuria district of Sidama Region. Soybean seeds of variety Hawassa 95 obtained from Hawassa Agricultural Research Centre (HARC) were surface sterilized by Mercuric chloride (0.1%) for 2 minutes, and thoroughly rinsed with distilled water. Thereafter, seeds were soaked in distilled water for a minute. Seeds were moistened in sugar solution (48%) before application of peat based inoculums (*Rhizobium* strain SB12, SB14 and SB MAR 1495) to get a thin uniform coating of inoculums on seeds immediate before sowing. The field experiment was laid out in a Randomized Complete Block Design (RCBD) having five treatments; control (no inoculants and fertilizer), recommended N (46 kg ha^{-1}), SB 12+50 kg ha^{-1} TSP, SB 14+50 kg ha^{-1} TSP and SB MAR1495+TSP. Each treatment was replicated three times. Soybean was planted in 10 cm and 40 cm spacing between plants and rows, respectively. The plot size was 4.00 m by 3.00 m (12.00 m^2) and plots were separated by 1.00 m to avoid treatment mixing up. Fertilizer was applied on the sides of soybean seed on ridges and covered with soil to avoid direct contact. Urea and TSP were used as sources of nutrients and all dose of Urea and TSP fertilizers were applied at planting. Earthing-up, weeding and other cultural practices were carried out based on the recommendation for the crop.

Soil sampling and analysis

Representative composite sample (0-20 cm depth) from experimental site was taken before planting from the surface of 8 spots following zigzag sampling pattern. Soil samples were also taken from each plot for analysis of selected chemical properties after harvesting the crop. The soil samples collected were air dried, crushed and passed through a 2 mm sieve for the analysis of selected soil chemical properties following standard procedure. The soil analysis was carried out at the Soil Laboratory of Hawassa Agricultural Research Center.

All soil samples were analyzed for soil pH, OC, TN and available P. Potentiometric method using a glass-calomel combination electrode was used to measure pH of the soils in water suspension in a 1:2.5 soil to water ratio (Barauah and Barthakulh, 1997). The Walkley and Black (1934) wet digestion method was used to determine soil OC content. Total N of the soil was analyzed using the Kjeldahl method as described by Bremner and Mulvaney (1982). Available P was determined using the standard Bray-II extraction method (Bray and Kurtz, 1947).

Table1.Geographic position and initial soil physical and chemical characteristics of experimental sites

Characteristics	**Values**
Location	Hawassa zuria
Latitude	7° 02' 47" N
Longitude	38° 23' 18" E
Texture	Loamy sand
pH	7.68
Organic carbon	3.16%
Available phosphorus	32.02 mg kg^{-1}
Total N	0.23%
C: N ratio	13.73

Important agronomic parameters including plant height, nodule numbers/plant, pod number/plan, total above ground biomass and grain yield were recorded for five randomly sampled plants per plot. Plant height and nodule number were determined at 50% flowering. Plant height measurement was done from the base of the plants to the apex and counting of nodules was carried out by digging the soil around and taking out the root system of soybean plant. Pod number, biomass and grain yield were determined at harvest. Data were subjected to analysis of variance (ANOVA) using the statistical analysis system (SAS) version 9.2 software (SAS, 2009). The least significant difference (LSD) test was used to separate the treatment means at 5% level of significance.

3. RESULTS AND DISCUSSION

3.1. Selected chemical properties of the experimental soil before planting

The pH of the experimental soil before planting was 7.68 which is slightly alkaline classes according to Foth and Ellis (1997) and loamy sand in texture (Table 1). The OC and TN of the soil before planting were 3.16% and 0.23% (Table 1), which were rated as low and moderate respectively (Landon, 2014). This might be due to low input of organic sources such as animal manure, compost and household wastes. To improve soil properties and productivity, organic amendment of soil of the study area is vital. Available P contents of soils was 32.02 mg kg^{-1} indicating that the soil is optimum in available P according to the rating of EthioSIS (2016). The C: N ratio was 13.73, which is far below 30, indicating that immobilization of inorganic N might not be a concern (Muhammad *et al.,* 2011).

3. 2. Effect of Rhizobial Strains on the selected chemical properties

The soil analysis revealed that its pH were decreases compared to control treatment. This may be due to application of bio-fertilizer (OM). Improvement in soil organic matter was observed for the plots which received *rhizobium* strain compared to chemical fertilizer. This was in agreement with the finding of Cao *et al*., (2002), who reported that combination of low levels of nitrogen (25 and 50 kg N ha^{-1}) with rhizobial inoculation significantly increased organic matter content of the top soil. Also in this study , the TN content (0.36%) was recorded by inoculation of rhizobial strain SB12 + 50 kg ha^{-1} TSP, whereas the lowest TN (0.23%) was recorded by un-inoculated control. The result indicated that inoculation of soybean seeds with rhizobial strain MAR-1495 could have contributed to the higher content of TN in plant tissue (Fitsum *et al*., 2016). Singh and Rai (2005) also found out that inoculated soybean with BNF rhizobial strains resulted to higher TN content than un-inoculated treatment plots. The available P of the soil varied from 32.02 to 37.84 mg kg^{-1} after incubation (Table 2). The highest available P was obtained when SB Mar 1495 and 50 kg/ha of TSP were applied in combination. This might be due to the significant increase in soil OM due to the effect of bio-fertilizer, which in turn reduced soil P sorption making both the soil native P and the applied P fertilizer available for plant uptake. Similar results were reported by Kisinyo *et al.* (2012).

Table 2. Some soil properties as affected by N fertilizer and *rhizobial* inoculant after soybean harvest

No	Treatment	pH	OC (%)	Total N (%)	OM (%)	Available P (mg kg^{-1})
1	Recommended N (46 kg/ha)	7.78	2.68	0.27	4.62	33.00
2	SB 12+50 kg/ha TSP	7.46	3.67	0.36	6.30	33.22
3	SB 14+50kg/ha TSP	7.63	3.46	0.32	5.97	34.03
4	SB Mar1495+ 50 kg/ha TSP	7.60	4.14	0.29	7.14	37.84
5	Control (No inoculation and no fertilizer)	7.68	3.16	0.23	5.50	32.02

3.3. Effect of Rhizobium strains on the nodule number, pod number and plant height of soybean

Rhizobium strains, SB 12+50 kg ha^{-1} TSP and SB Mar1495+ 50 kg ha^{-1} TSP, significantly (P <0. 05) increased nodule number per plant compared to the control treatment (Table 3). Among the strains, the highest number of nodules (22.8) was recorded by rhizobial strain SB 12+50 kg ha^{-1} TSP followed by MAR-1495. The reason might be due to inoculation with Rhizobia, which increased the number of bacteria and hence more nodules plant-1 were produced. Again result could probably be due to the ability of legumes to nodulate and fix nitrogen, which is a genetic factor affected by environmental conditions and that nodulation and nitrogen fixation require large amounts of plants dry matter that nodulation will be at the expense of dry matter production (Giller *et al*., 2001). This was in agreement with the findings of Joshi (2011), who reported that highest number of nodules was associated with plants raised from seeds inoculated with soybean *Rhizobium* isolate Indore-II followed by isolate Delhi.

Significantly higher pod number per plant was recorded for inoculated plots, whereas the lower pod number was recorded at control plot treatment (Table 3). Among the strains, the highest number of pod number per plant (27.2) was recorded by rhizobial strain SB 14+50 kg ha^{-1} TSP followed by MAR-1495. This result were in line with the findings of Lamptey *et al*. (2014) who reported that number of pods harvested from the inoculated and un-inoculated plants were 55 and 37 per plant, respectively.

All inoculants significantly (P <0. 05) improved plant height, whereas the recommended N did not show difference from the control (Table 3). But among the strains, the largest plant height (49.8cm) was recorded by rhizobial strain SB 12+50 kg ha^{-1} TSP. The present result is in agreement with the findings of Lamptey *et al.* (2014) who reported that inoculation with Rhizobium produced significantly ($P < 0.05$) taller soybean plants throughout the sampling period with the greatest effect (53.4 cm) being experienced at 9 week after planting compared to the heights (48.9 cm) of un-inoculated plants at the same stage.

3.3. Effect of Rhizobium strains on the biomass and grain yield of soybean

Significant ($P < 0.05$) improvements in both biomass and grain yields of soybean were obtained due to inoculation of seeds with *Rhizobium* strains which out yielded both recommended N (46 kg ha^{-1}) and the control plots (Table 3). The inoculation of strain SB 12 + 50kg ha^{-1} produced significantly more dry biomass yield (5555.6 kg/ha) and grain yield (2303.3kg/ha) as compared to biomass yield (2552.1 kg/ha) and grain yield (572.9 kg/ha) of uninoculated /control treatment/ respectively. The result indicated that using rhizobial strain SB 12 + 50 kg/ha for soybean production in the study area resulted to higher grain yield and replace the need for inorganic N fertilization. An increase in the above-ground dry biomass was observed because rhizobial inoculation is known to increase the yields of several legumes by way of increasing the nodulation and the biomass of root and shoot (Tamiru *et al.,* 2012). Similarly, Fisum *et al.* (2016) reported highest biomass (2804.7 kg ha^{-1}) and grain yields (1245.9 kg ha^{-1}) for a rhizobia strain legume fix, followed by MAR-1495 which gave 2628.7 kg biomass and 1182.5 kg grain yield ha^{-1} and legume fix strain resulted more than 100 and 300% advantage for biomass and grain yield, respectively, over the control.

Table 3. Effect of *Rhizobium* strains on the yield and yield components of soybean in Hawassa zuria district

Treatments	Plant height (cm)	Nodule number $Plant^{-1}$	Pod number $Plant^{-1}$	Biomass kg ha^{-1}	Grain kg ha^{-1}
1. Control (No inoculation and no fertilizer)	35.7c	5b	12.0c	2552.1b	572.9b
2. Recommended N (46 kg/ha)	39.4bc	13ab	17.3bc	3391.2b	740.8b
3. SB 12+50kg/ha TSP	49.8a	22.8a	25.4ab	5555.6a	2303.3a
4. SB 14+50kg/ha TSP	45.8ab	4.8b	27.2a	5324.1a	1967.6a
5. SB Mar1495+ 50kg/ha TSP	45.7ab	17.3a	23.3ab	5364.6a	2158.6a
Mean	43.3	12.6	21.0	4437.5	1549.0
CV (%)	15.3	30.5	33.4	27.4	26.4
LSD at 0.05	8.0	10.9	8.5	1476.7	495.0

Mean values followed by the same letter with in a column for a given variable are no significantly different at P<0.05

Table 4. Economic analysis (dominance and marginal rate of return) of treatments

Variables	**Treatments**				
	No inoculation and fertilizer	SB 14+50 kg ha^{-1} TSP	SB Mar1495+ 50 kg ha^{-1} TSP	SB 12+50 kg ha^{-1} TSP	Recommended N (46 kg ha^{-1})
Av. Yield	572.9	1967.6	2158.6	2303.3	740.8
Adj. yield	515.6	1770.8	1942.7	2073.0	666.7
TVC (Birr ha^{-1})	0.0	905.0	905.0	905.0	1327.0
Gross Field Benefits (Birr ha^{-1})	10054.4	34531.4	37883.4	40422.9	13001.0
Net Benefit (Birr ha^{-1})	10054.4	33626.4	36978.4	39517.9	11674.0
MRR					D

Av. Yield=average yield, yield adjustment=10%, TCV= total variable cost, MRR= marginal rate of return

Table 5. Economic (partial budget and marginal rate of return) analysis of treatments

Variables	**No inoculation and fertilizer**	**SB 14+50 kg ha^{-1} TSP**	**SB Mar1495+ 50 kg ha^{-1} TSP**	**SB 12+50kg ha^{-1} TSP**
TVC (Birr ha^{-1})	0.0	905.0	905.0	905.0
Net Benefit (Birr ha^{-1})	10054.4	33626.4	36978.4	39517.9
MRR (%)	-	2604.6	2975.0	3255.6

Yield adjustment=10%, TCV= total variable cost, MRR= marginal rate of return

3.4. Economic analysis

Based on the dominance analysis it was observed that, except for the recommended N (46 kg ha^{-1}) all *rhizobium* strains combined with TSP were not dominated by the treatments with low TVC. Therefore, recommended N was eliminated from further analysis. The partial budget analysis revealed that the highest net benefit (3255.6 ETB ha^{-1}) was obtained from SB 12 + 50 kg TSP ha^{-1}, followed by SB Mar1495+ 50 kg ha^{-1} TSP and SB 14+50 kg ha^{-1} TSP with acceptable MRR of 3255.0, 2975.0 and 2604.6, respectively. The marginal rate of return (MRR) above 100% is high and acceptable to farmers (CIMMYT, 1988). The non-dominated strains were produced 34.5 to 93.0% of net benefit advantage over the control (Table 5).

4. CONCLUSIONS AND RECOMMENDATION

In conclusion, the experiment revealed that treating the soybean seeds with *rhizobium* strains has an advantage in improving the grain yield. The economic analysis also clearly showed the benefits of *rhizobium* strains compared to inorganic fertilizer alone and the untreated plot. Therefore, SB12 followed by SB MAR1495 and SB14 combined with 10 kg P ha^{-1} (50 kg TSP ha^{-1}) could be recommended for soybean (particularly variety Hawassa 95) producers Hawassa Zuria district, where farmers can choose based on availability of the strains. However, as the experiment was carried out only in one region, similar undertakings should be carried out over years and at different region to determine the long-term effect of *rhizobium* strains on the soil and the crop.

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