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Evaluation of Rhizobia Strain of Soybean (*G. max*) for Symbiotic Performance Grown in Acid-Prone Areas

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Abstract: The development of bio fertilizer for the legume crop is the crucial activity in the enhancement of sustainable agriculture to fulfill the human welfare. This research was focused on such aims through justifying the objective of evaluate the rhizobia isolate naturally associated with soybean obtained from acidic environment during the cropping season of 2019 and 2020. The activities were carried out with the nine treatments (SB AS ARC -010, SB AS ARC-008, SB AS ARC-022, SB Tgp-1, SB Tgp-3, SB SYB-1, TSP+Urea.50kg/ha, TSP and –Ve control) which were arranged in RCBD with three replication. Accordingly, the highest nodule numbers (189.1) were from isolates SB SYB-1 and the highest grain yield (2636.1kg/ha) and biomass weight (6794.7kg/ha) from isolate SB AS ARC-022 in the year 2019. In 2020, the highest nodule number (109.1) was obtained from isolate SB AS ARC-008 and the highest nodule dry weights (3.4) gram weight per plant. Additionally, in the year 2020, there were significant differences among the treatments on above ground biomass of the soybean yield and among different isolates the highest biomass yield (5284 kg/ha) was obtained when the soybean seed is inoculated with SB Tgp-3 rhizobia isolates which have significance difference with the negative control. Similarly, the highest grain yield were obtained when the soybean seed were inoculated with isolate SB Tgp-3 which was almost similar results with seeds inoculated with isolate SB AS ARC-008 and SB AS ARC -010. Overall, SB AS ARC-022 and SB Tgp-3 isolates were given the highest grain yield and can be used for soybean production in acid prone area alternatively.

Keywords: Biofertilizer, Inoculum Legume, Soybean

1. Introduction

Soybean (Glycine max) is an annual legume crop of pea family where its seeds are edible. Its chemical composition, protein quality and nutritional value of soybean meals make special legume crops among all pea family [10]. Soybeans are also important at different field of science. Hence, it has a variety of industrial applications, such as in paper coatings, plastics, textile fibers, and wood adhesives. It's frequently believed that soy oil is too viscous and reactive to air oxygen to be employed as a fuel, lubricant, cosmetic, or chemical ingredient [9]. Additionally, it is used in animal feed; poultry and livestock feed [11]. Increasing soil organic carbon based on adding shoot biomass and fixing atmospheric nitrogen are also another advantage on soybean agricultural activities [6].

Although soybeans are multi-beneficial crops, the yield is not as satisfactory as the crop potential. There are different facts which affect the soybean production. Among those, soil acidity is the common challenges in most cases. Generally speaking, soil pH levels between 6.0 and 7.0 are favorable for soybean growth. The ideal range, however, is between 6.3 and 6.5 because it maximizes food availability and biological nitrogen fixation while minimizing soybean cyst nematode (SCN) population increase [17]. Contrarily, soil pH levels below 5.2 and above 6.5 do not encourage soybean growth; as a result, yields are low under these circumstances Peters et al. [20]. High levels of aluminum and low levels of phosphorus in acidic soils affect the growth of symbiotic nitrogen-fixing bacteria and soybean yield production Bakari, R., et al., [5].

In order to alleviate such challenges, most farmers and soybean producer use lime amelioration. Although liming is essential to ameliorate the acidity of soils but overuse may cause some serious disadvantages such as it may reduce the availability of micronutrients as Zn, Fe, etc, it has some sort of antagonistic effect with phosphorus and potassium availability Rengel, Z., et al., [14]. As an appropriate solution, seeking for acidic tolerant rhizobia strain is the novel solution for such environment than lime application. Although different research findings were reported across the globe, little to no information was exist at study area. Therefore, this research was planned to evaluate the rhizobia isolate naturally associated with soybean obtained from acidic environment.

2. Material and Methods

2.1. The Study Area

The field experiment was carried out in the Benishangul Gumuz Regional state of Metekal Zone at pawe area during rainy season for two consecutive years 2014 and 2015. It has the hot humid agro ecology, maximum rainfall of 1587mm, major soil types:-Nitosols, Vertisols and livesols. The maximum and minimum temperature is 32.6 and 16.3°C respectively. The activity was arranged in complete randomized block design with nine treatment (SB AS ARC - 010, SB AS ARC-008, SB AS ARC-022, SB Tgp-1, SB Tgp-3, SB SYB-1, TSP+Urea.50kg/ha, TSP and –Ve control) in three replication. The inoculum isolates were taken from Holeta Agricultural Research Center in the department of Soil Microbiology.

2.2. Preparation of the Land, Inoculation and Other Agronomic Procedures

A test crop of the medium-set, high-yielding TGX variety of soybean (Glycine max), which is frequently grown in Pawe district, was employed. The experimental site, which had low nitrogen content and no history of inoculation, was chosen, and the field was prepared according to conventional practice to make it ready for planting in mid-May and early June. The land was then leveled and divided into blocks and individual plots. Blocks were spaced out 1.5 meters apart, with the plots preserved at 1 meter apart. Each plot's beds were created by preparing canals all around it. Each strain's carrier-based inoculants were applied at a rate of 10 g inoculant/kg seed, procured from HARC. The needed amount of inoculant was suspended in a 1:1 mixture with 10% sugar solution to guarantee that all of the applied inoculum adhered to the seed. Dry seeds were carefully combined with the thick slurry of the inoculants so that each seed received a thin coating of the inoculants. To maintain the viability of bacterial cells, all inoculations were completed right before planting in the shade. After a brief period of air drying, seeds were sowed with the necessary rate and spacing. To prevent contamination, plots containing not inoculated seeds were sown first. After being drilled, the infected and un-inoculated seeds were planted in the first week of July at a distance of 5 cm between plants and 60 cm between rows. In the first week of July, the inoculated and un-inoculated seeds were then manually drilled in the prepared rows of each plot at a spacing of 60 cm between rows and 5 cm between plants. To prevent cell death from solar radiation, the soil was immediately covered after sowing. After two weeks, the plants were thinned. After seeding, hand weeding was done three times at intervals of 30 days.

2.3. Techniques for Sampling and Observations

Five randomly chosen second border rows of each plot's flowering plants were used to collect data on nodulation. To determine the precise nodule numbers and nodule dry weight, five plants were carefully removed at random from each plot using a spade, leaving no nodules in the soil. The soil that stuck was gotten rid of by gently running water over a metal sieve over the roots of any intact nodules. The number of nodules per plant was calculated by adding the average number of nodules from each of the five uprooted plants in the plot. When all five plant samples from each plot had been gathered, the nodules were combined, and their dry weight was calculated by drying them at 70°C for 72 hours to a consistent weight. The dry weight was then reported as g/plant.

2.4. Crop Yield and Agronomic Data Collection

At maturity, five plants were randomly selected from each plot, their pods counted, and the average value—the number of pods per plant—was reported. Five randomly selected plants were counted to determine the average quantity of seeds per plant. This average value was then recorded. Each plot's soybean plants were removed after they reached physiological maturity, leaving only the two boundary rows on each plot. Using the formula provided by [18] the moisture content was adjusted to 12.5% to get the desired seed yield and converted to kilogram per hectare.

Adjusted grain yield $= \frac{(100-\mathrm{MC})}{100-12.5} *$ grain yield; Where MC is moisture content of soybean seeds at the time of measurement, and 12.5 is the standard moisture content of soybean seeds at harvest in percent. The weights of one hundred seeds were randomly selected from each plot's seeds while keeping the moisture content of the seeds at 12.5%.

2.5. Methods of Data Analysis

Using statistical analysis systems (SAS) software, the obtained data were subjected to analysis of variance (ANOVA) in a Randomized Complete Block Design (RCBD) that was duplicated three times [19]. Fisher's test with a 0.05 level of significance was used to compare treatment means using the general linear model (GLM).

3. Result and Discussion

3.1. Effects of Different Soybean Rhizobia Isolates On Nodule Parameters

The numbers of nodules per plants were influenced by the different rhizobia isolates and it ranges from (182.2 to 189.1). The highest nodule numbers were obtained when the soybean seed were inoculated with rhizobia isolates SB SYB-1 which was accounted for 189.1 (Table 1). In this study, almost similar results were registered from isolate SB Tgp-1 SB which was 188.7, from isolate SB Tgp-3 and SB-AS ARC-022 which was accounted for 187.9 and 187.8 respectively (Table 1). In contrary to the mentioned results, the lowest nodule numbers (182.2) were obtained when the soybean seeds were inoculated with rhizobia isolate SB AS ARC-008.

As an additional information, the highest nodule number (195.6) were obtained when the application of the inorganic fertilizer tri super phosphate (100kg/ha) is applied and whereas the lowest nodule number (163.6) registered when the application of full recommendation of TSP with 50kg/ha of urea. In general, although the different figures of nodule number were obtained in this study, statistically, there were no significance differences among the isolates. In the current results based on the nodule dry weight, almost all similar results were observed among the treatments and statistically there were no significance difference among them (Table 1). Similar findings were reported by Abera Y., et al., [3] who revealed that application of rhizobia isolate could significantly influence the nodule number and nodule dry weight when compared with untreated or control treatment. In line with this findings effective nodules, were highly ($P < 0.01$) affected by the interaction of inoculum and inorganic fertilizers and lime at different locations and years Abeje, A., et al., [1]. Solomon T., et al., [16] also reported that all the nodulation parameters, namely, nodulation rating, nodule number per plant, nodule volume per plant, and nodule dry weight were significantly influenced by the main effect of *Bradyrhizobium japonicum* strains alone. In the same manner, inoculation of rhizobium strain significantly increased the percentage of effective nodules per plant in contrast to no inoculated seeds and the highest percentage of effective nodules was obtained at the application of seeds with inoculum and a lower percentage of effective nodules was recorded without inoculation were applied as stated by [7].

3.2. Soybean Biomass and Grain Yield as Influenced by Different Rhizobia Isolates

The above ground biomass and grain yield were influenced by different rhizobia isolates. The results indicated that the highest grain yield (2636.1kg/ha) and biomass weight (6794.7kg/ha) was obtained when the seeds were inoculated with rhizobia isolate SB AS ARC-022. However, the lowest grain yield (1904.9kg/ha) was obtained when the rhizobia isolate (SB AS ARC-008) inoculated with the soybean seed (Table 1).

Table 1. *Nodule parameters and grain yield of soybean as influenced by rhizobium inoculation in pawe district (2019).*

	Treatments	NN	NDW	BMS (kg/ha)	GY(kg/ha)
1.	–Ve control	175.3	0.8	4480.9b	1759.7b
2.	TSP	195.6	0.9	5317.2ab	1898.3ab
3.	TSP+Urea.50kg/ha	163.6	0.9	7271.2a	2773.9a
4.	SB SYB-1	189.1	0.8	5778.2ab	2149.4ab
5.	SB Tgp-3	187.9	0.9	5628ab	2395.4ab
6.	SB Tgp-1	188.7	0.8	6169.9ab	2306.8ab
7.	SB AS ARC-022	187.8	1.1	6794.7ab	2636.1ab
8.	SB AS ARC-008	182.2	0.8	5059.9ab	1904.9ab
9.	SB AS ARC -010	186.8	0.8	5429ab	2065.5ab
	LSD (p=0.05)	30.6	0.5	2682.2	961.5
	CV (%)	Ns	ns	39.9	37.4

Although there were none significant effects among the all treatments, the lowest average grain yield (1759.7b kg/ha) of soybean were recorded from the negative control where there was no any application of fertilizer (Table 1). Inline to this finding, different research findings were also reported the same scenario. The low result from the negative control might be due to the low nutrient content of the soil and absence of native rhizobia around the study area. The largest figures were obtained from the application of recommended inorganic fertilizer on both grain yield (2773.9akg/ha) and above ground biomass (7271.2). This might be the plant can easily obtained the nutrients needed for its growth to complete its life cycle (Table 1). According to the report of, Abeje, A., et al., [1], the interaction of SB12 + MAR1495, 10 t·ha−1 fresh cattle manure, without lime, and NPSB at 19-46-7-0.1 increased the grain yield of soybean in two location Assosa and Bambassi districts of Benishangul Gumuz.

In the year 2020, there were variations of results among the treatments on all parameters (Table 2). Among the all rhizobia isolates, the highest nodule number were obtained from the co-inoculation of the seed with SB AS ARC-008 followed by isolate SB Tgp-1 (104.6) and SB Tgp-3 account for (103.5). On the findings of the nodule dry weight, similar results were obtained as usual and the highest nodule dry weight were obtained from isolate SB AS ARC-008 which was recorded as 3.4gram weight whereas the lowest nodule dry weight 2.6 were obtained when the soybean seed inoculated with SB AS ARC-022 and the other treatment which have only tri super phosphate fertilizer (Table 2). According to the finding of Ishaq, A. S., et al., [8], rhizobia strain(s) form the basis increased soybean yields and BNF while all inoculants enhance nodulation. Similarly, [4] reported that application of rhizobium *leguminosarum* bv significantly ($p<0.05$) affect common bean nodulation at different site of study in Eastern Hararghe. Nodule numbers increased with increasing rates of applied rhizobia from log 4.59 up to the highest rate of log

9.59 viable cells/cm as reported by Smith, R. S., et al., [15]. On average, inoculation + fertilization treatments had higher nodule fresh weight than uninoculated soils (no inoculum or fertilizer) as suggested by Mathenge, C., et al., [12].

Table 2. *Nodule parameters and grain yield of soybean as influenced by rhizobium inoculation in pawe district (2020).*

	Treatments	NN	NDW	BMS (kg/ha)	GY(kg/ha)
1.	–Ve control	87.0^{ab}	2.8	4370^{c}	1557^{c}
2.	TSP	95.4^{ab}	2.6	4642^{c}	1686^{bc}
3.	TSP + Urea (50kg/ha)	85.5^{b}	3.0	6226^{a}	2280^{a}
4.	SB SYB-1	100.5^{ab}	3.2	4989^{bc}	1772^{bc}
5.	SB Tgp-3	103.5^{ab}	3.0	5284^{b}	1911^{b}
6.	SB Tgp-1	104.6^{ab}	2.8	4481^{c}	1596^{bc}
7.	SB AS ARC-022	92.3^{ab}	2.6	4772^{bc}	1688^{bc}
8.	SB AS ARC-008	109.1^{a}	3.4	4654^{c}	1732^{bc}
9.	SB AS ARC -010	98.5^{ab}	3.2	4824^{bc}	1782^{bc}
10.	Murdock	97.7^{ab}	3.2	4698^{bc}	1657^{bc}
	cv (p=0.05)	28.35	40	15.86	24.26
	lsd (%)	22.36	ns	628.44	346.85

In the year 2020, there were significant differences among the treatments on above ground biomass of the soybean yield (Table 2). From the all treatments, the highest biomass yield was obtained when the soil is treated with TSP + Urea (50kg/ha). However, among different isolates the highest biomass yield (5284 kg/ha) was obtained when the soybean seed is inoculated with SB Tgp-3 rhizobia isolates which have statistically non significance difference with isolate SB AS ARC-022, SB SYB-1, SB AS ARC -010, Murdock, but have only significance difference with the negative control, TSP alone and SB AS ARC-008. Overall, the lowest biomass yield (4370kg/ha) were obtained from the negative control which have no any fertilizer input (Table 2).

In the year 2020, although there were no significance difference among the rhizobia isolates, the highest grain yield were obtained when the soybean seed were inoculated with isolate SB Tgp-3 which was almost similar results with seeds inoculated with isolate SB AS ARC-008 and SB AS ARC - 010 (Table 2). Among all treatments, the highest grain yield (2280kg/ha) were obtained when the soil is treated with the application of TSP + Urea (50kg/ha) whereas, the lowest grain yield (1557) with no application of any fertilizer which non significance difference with almost isolates (Table 2). Abeje, A., et al., [2] reported that the maximum grain yield (2621.67 kg) was obtained from (SB12+MAR1495) + NPSB at Assosa and the maximum grain yield (2460.20 kg) was obtained from SB12+NPS at Bambassi. In line with this finings Muleta, D., et al., [13] inoculation of soybean seeds with commercial and /or locally available rhizobia isolate improved soybean grain yield.

4. Conclusion

To sum up, the numbers of nodules per plants, nodule dry weight per plant, above ground biomass and grain yield per hectare were influenced by the different rhizobia isolates. Accordingly, SB AS ARC-022 and SB Tgp-3 isolates were given the highest grain yield and can be used for soybean production in acid prone area alternatively. However, the overall investigation demonstrated that the application of rhizobia isolate to soybean seeds have influenced the soybean nodule parameters nodule number and nodule dry weight in partial amount which brought insignificant effects and similar results were achieved for yield parameters above ground biomass and grain yield per hectare in both year. This result gives forward direction for the researcher to investigate the isolation of new native rhizobia strain from the research areas to bring significant effect.

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Conflicts of Interest

The authors declare no conflict of interest.

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