Available online at www.worldscientificnews.com

World Scientific News

WSN 55 (2016) 15-26 EISSN 2392-2192

Evaluation of Different Commercial *Rhizobial* Strains on Soybean (*Glycine max* L.) Yield at Pawe District, Northwestern Ethiopia

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ABSTRACT

Two experiments were conducted during 2010 and 2011 main cropping seasons under different farmer fields at Pawe District of Northwestern Ethiopia in order to evaluate different commercially produced microbial inoculants, referred to as commercial *rhizobial* strains, on soybean yield. The first experiment was conducted with the aim of evaluating four commercial *rhizobial* strains (Legume fix, TSBF-531, TSBF-442 and MAR-1495) on soybean yield, while the second experiment conducted with the aim of evaluating and selecting the best *rhizobial* strain from the two *rhizobial* strains (Legume fix and MAR-1495). Non-inoculated soybean with and without the recommended rate (RR) of nitrogen (N) fertilizer treatments also included in the experiments. Treatments were arranged in a single replicate per field. Recommended rate of phosphorous (P) fertilizer was applied uniformly to all treatments. The two experiments analysis of variance indicated that there was significant difference ($P < 0.05$) among treatments in number of nodules, nodule weight, biomass yield and grain yield of soybean. Accordingly, the first experiment result indicated that the highest number of nodules, nodule weight, biomass yield and grain yield were recorded by using *rhizobial* strains of legume fix and MAR-1495. The second experiment result revealed that *rhizobial* strain MAR-1495 resulted consistent and highest number of nodules, nodule weight, biomass yield and grain yield of soybean. The increment in grain yield over the un-inoculated control, legume fix and full RR of N fertilized treatments recorded by MAR-1495 was 25.5, 9.7 and 9.9%, respectively. Moreover, inoculation of soybean seeds with *rhizobial* strain MAR-1495 also resulted to higher protein content in soybean plant tissue. Therefore, this study recommends use of the most suitable commercial *rhizobial* strain MAR-1495 for the study area to inoculate seeds before sowing for the production of soybean crop.

Keywords**:** Commercial *Rhizobial* Strain; *Glycine max* L.; Soybean; Inoculation; Nodule

1. INTRODUCTION

Soybean (*Glycine max* L.) is now produced in larger quantities than any other legume crop in the world and is certainly the most important source of vegetable oil, processed in a wide variety of ways to produce soya milk, bean curd, flour and fermented products. As a legume crop, soybean utilizes two sources of nitrogen (N) for its growth via mineral N from the soil and atmospheric N_2 fixed biologically in root nodules formed in symbiosis with different species of *Rhizobium* and *Bradyrhizobium* (Patra *et al., 2012*).

Although N_2 as a macro nutrient is essential for legume crop production and it accounts for about 78% of the Earth's atmosphere, plants and animals do not have an easy time obtaining all the N they need for growth. This situation arises because the N_2 molecule is very stable chemically and so is unusable by most biological organisms. It must be "fixed" in the forms of ammonium (NH_4^+) and nitrate (NO_3^-) before it can be assimilated (Fisher and Newton, 2002).

The most important source of fixed nitrogen derives from the activity of certain soil bacteria that absorb atmospheric N_2 gas and convert it into ammonium through biological nitrogen fixation (BNF) process by which N_2^{14} in the atmosphere is reduced into a biologically useful, combined form of N-ammonia by nitrogen fixing bacteria (Giller, 2001). Moreover, the process of BNF is believed to consume less energy than N fixation through the mineral process (Dubey, 2006). The most important N fixing agents in agricultural systems are the symbiotic associations between legumes and the micro symbiont *rhizobia* via the formation of nodules (Giller, 2001).

Soybean is one of the legume crops that can effectively nodulate by strains of all the recognized species of *rhizobia*. 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 can be quite substantial. In general, symbiotic nitrogen fixation in crop legumes not only reduces fertilizer costs but also improves soil fertility through crop rotation and intercropping. 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 (Gupta, 2004). It is the process of applying *rhizobial* inoculants to the soybean seed before planting in order to increase the nitrogen fixation and nodulation of the soybean roots.

Use of commercial *rhizobial* inoculants in the establishment of soybean has been widely recognized, especially in areas where indigenous nodulation has been found to be inadequate. It has been reported that soybean inoculated with different *rhizobial* strains react differently in the growth, yield and nitrogen fixation (Mmbaga *et al*., 2014).

In Ethiopia and in other countries, evaluation and selection of different *rhizobial* strains have given promising results in different studies. However, for the study area of Pawe District, no information is available on either isolation of recognized *rhizobial* strains from root nodules of soybean (indigenous soil microbial isolates) or selection of suitable *rhizobial* strain/s from different commercially available *rhizobial* strains for soybean production. Therefore, this study is geared towards evaluation of different commercial *rhizobial* strains for better nodulation and improving soybean yield.

2. MATERIALS AND METHODS

2. 1. Description of the Study Area

Pawe District is located in Metekel Zone of the Benishangul-Gumuz Regional State, Ethiopia (Figure 1). It is located at about 570 kilometers distance from Addis Ababa in Northwestern direction. The altitude of the study area ranges between 1000 – 1200 meters above sea level. The dominant soil types in Pawe District are broadly categorized as Vertisols (40 – 45% of the area), Nitisols, (25 – 30%), and intermediate soils of a blackish brown color (25 – 30%) (Viezzoli, 1992). According to the meteorological data gathered by the Pawe Meteorological Station and Pawe Agricultural Research Center from 1987 to 2012, the mean annual minimum and maximum temperatures of the District are 16 and 32 ^{0}C, respectively, and the mean annual rainfall is 1596.3 mm. The area has a uni-modal rainfall pattern with high rainfall that extends from May to October. The mean annual potential evapotranspiration is about 1300 mm. The climate of the area characterized as hot humid.

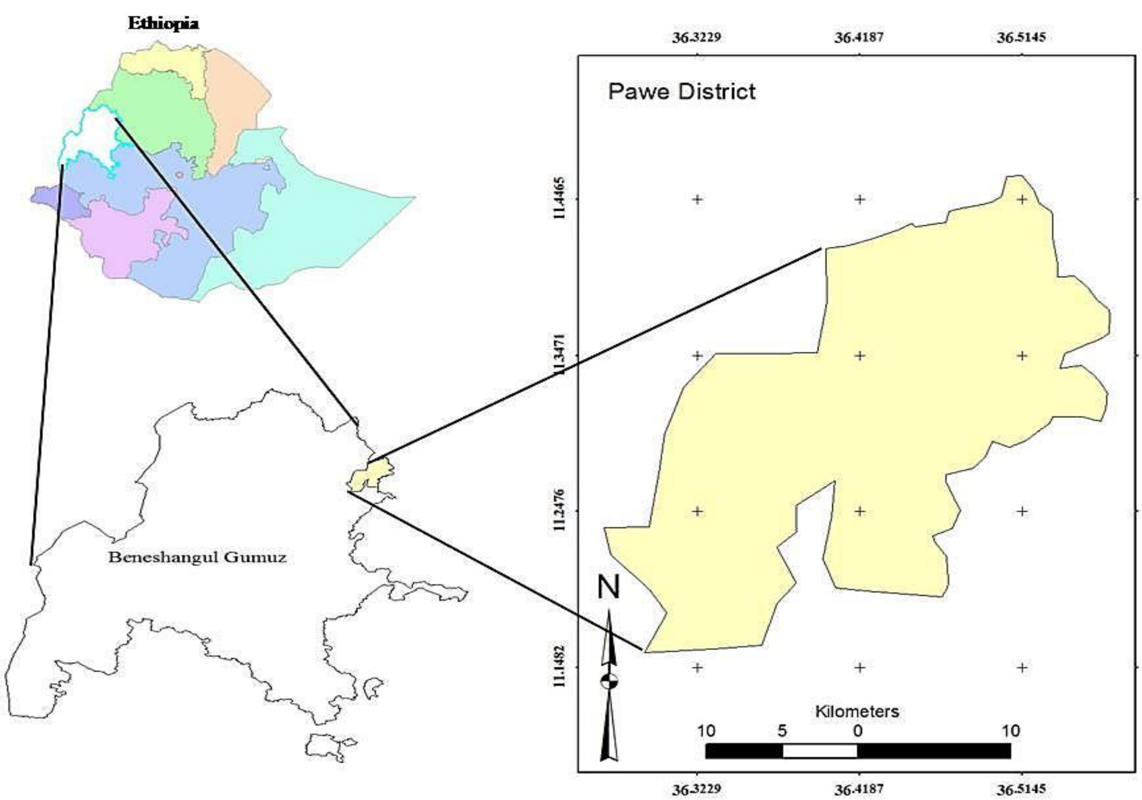

Figure 1. Location map of the study area, Pawe District, Northwestern Ethiopia

The major annual and perennial crops of the area include sorghum (*Sorghum bicolour*), finger millet (*Eleusine coracana*), maize (Zea mays), haricot bean (*Phaseolus vulgaris*) sweet potato (*Ipomoea batatas*) and mango (*Mangifera indica*). While the major cash crops of the area includes soybean (*Glycine max*), sesame *(Sesamum indicum*) and groundnut (*Arachis hypogaea*), where a number of improved and new varieties of these crops were released for the farmers from Pawe Agricultural Research Center.

2. 2. Treatment Structure of the Two Experiments

The field experiments were conducted during 2010 and 2011 main cropping seasons for experiment one and two, respectively. The 1st experiment was conducted on five representative farmer and one on-station fields with the aim of evaluating four commercial *rhizobial* strains (Legume fix, TSBF-531, TSBF-442 and MAR-1495) on soybean yield. While, the 2nd experiment was conducted on fourteen representative farmer and one on-station fields with the aim of evaluating and selection of the best *rhizobial* strain from the two *rhizobial* strains (Legume fix and MAR-1495) that were selected according to their performance during the 1st experiment, on soybean yield. Treatment structure for the two experiments was as shown below Table 1.

Table 1. Treatment structure for the two experiments in 2010 and 2011 cropping seasons.

Experiment one	
Treatment number	Treatment description
1	Control /no strain and no N fertilizer/
2	No strain + half RR N fertilizer
3	No strain + full RR N fertilizer
4	Legume fix + no N fertilizer
5	TSBF-442 + no N fertilizer
6	TSBF-531 + no N fertilizer
7	MAR-1495 + no N fertilizer
Experiment two	
1	Control /no strain and no N fertilizer/
2	MAR-1495 + no N fertilizer
3	Legume fix + no N fertilizer
4	No strain + full RR N fertilizer

RR = Recommended Rate where, full RR of N = 18 kg N ha^{-1}; ha = hectare

2. 3. Site Selection, Experimental Design and Procedure

From the study area, Pawe District, representative fields were selected using systematic random sampling method. During selection of fields, poor or degraded and extremely fertile lands were avoided. The experiment was arranged in a single replicate in each site, however, fields across sites were considered as replicates (multi-location design). Soybean variety Belessa-95, which is already recommended for the area was used as a test crop for this study. Planting was done during late June to early July at a seed rate of 60 kg ha^{-1}. For the 1^{st} experiment, the size of each treatment plots was 4.8 x 5 m^2 consisting of eight rows per plot (6 harvestable and 2 boarder rows). While for the 2^{nd} experiment, the size of each treatment plots was 10 x 10 m^2. The plant spacing was 60 cm and 5 cm between rows and plants, respectively. In all treatments, to avoid phosphorous (P) limitations, basal dose of P at 46 kg P_2O_5 ha^{-1} in the form of triple super phosphate (0, 46, 0) was applied by banding 5 cm below the seed at a time of planting. Nitrogen in the form of urea (46, 0, 0) was applied in split. The first split was applied at the time of planting, whereas the remaining N was applied 4 weeks after planting by banding along the row at a distance of about 10 cm away from the plant and at about 5 cm below the surface.

Inoculated seeds were used for the treatments that require inoculation with respective commercial *rhizobial* strains. For inoculation, seeds were taken in small polythene bags equal in weight for each treatment plots and mixed with a small portion of sticking agent (10% sugar solution) until seeds were uniformly moistened and able to stick the inoculant. Care was taken for not damaging/ removal of seed coats because of over moistening with the sugar solution. Then, the inoculant (commercial *rhizobial* strain with carrier) was gently mixed with the moist seeds at the rate of 40 g inoculant kg^{-1} seeds so that all the seeds received a thin coating of the inoculant. Inoculated seeds were dried under shade for half an hour and then immediately planted and covered with soil. For each inoculation, separate plastic bag was used to avoid contamination of the inoculated with un-inoculated seeds.

Weeds were controlled manually and relevant data were collected during the crop growing period. Agronomic data such as number of soybean nodules $plant^{-1}$, nodule weight $plant^{-1}$, total biomass yield and grain yield were collected.

2. 4. Soil and Plant Sampling, Preparation and Laboratory Analysis

Representative composite sample (0-30 cm depth) from each site was taken before planting from the surface of 8 spots following "W" design. 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 Pawe Agricultural Research Center.

All soil samples were analyzed for soil pH, organic carbon (OC), total N 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: liquid ratio) (Van Reeuwijk, 1992). The Walkley and Black (1934) wet digestion method was used to determine soil OC content. Then, percent soil organic matter (OM) was obtained by multiplying percent soil OC by a factor of 1.724, following the assumptions that OM is composed of 58% OC. Total N was analyzed using the Kjeldahl digestion, distillation and titration method as described by Blake (1965). Available P was determined using the standard Bray-II extraction method (Bray and Kurtz, 1945).

During experiment two, above ground plant sample was taken at 50% of soybean maturity from each treatment across sites. The sampled plants were dried immediately in an oven at 70 ^{0}C until constant weight then grinded and passed through 1 mm sieve size. The plant samples were analyzed for total N using modified kjeldhal method. Then, percent plant crude protein was computed by multiplying percent total N by a conventional factor of 6.25 (Jackson, 1973).

2. 5. Statistical Analysis

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. Initial soil Chemical Properties of Experimental Sites

Some selected soil chemical properties of all experimental sites and their summary are presented below Table 2. Soil pH of the study sites ranged from 5.00 to 6.41 with mean soil pH of 5.55, that qualify for moderately acidic reaction classes (5.3- 5.9) according to the classification ranges suggested by Tekalign (1991). The low pH values could be due to the study area receives high mean annual rainfall (> 1500 mm) that results in loss of base forming cations through leaching.

Table 2. Initial soil chemical properties of the experimental sites (Experiment 1 and 2).

Experiment No.	Farmers name and location	pH	Available P (mg kg^{-1})	Total N (%)	OM (%)	CN ratio
1	Fentaw Temesgen, V-23/45	5.50	1.01	0.148	3.96	15.5
	Deginet Sodono, *V-9	5.63	0.62	0.162	4.69	16.8
	Yigrem Gessese, V-10	5.59	1.14	0.162	3.96	14.2
	On-station, V-7	5.31	4.64	0.148	4.25	16.7
	Alebachew Ahmed, V-30	5.52	2.97	0.162	3.96	14.2
	Getiso Sodono, V-5	5.70	2.89	0.134	3.08	13.3
2	Girma Rango,V-4	5.60	3.38	0.134	3.66	15.8
	Admasu Bora,V-5	5.32	2.31	0.134	4.18	18.1
	Matyos Areko,V-9	5.25	4.70	0.190	6.14	18.7
	Bergena Bekelo,V-10	5.72	4.53	0.197	6.32	18.6
	Awol Hassen,V-28	5.32	1.07	0.169	4.93	16.9
	Alemu Assefa, Almu town	5.35	8.58	0.112	3.97	20.6

	Zemen Damte,V-17	5.66	2.67	0.190	4.52	13.8
	On Station, V-7	5.00	3.16	0.155	4.52	16.9
	Yeshaneh Demeke,V-10	5.14	1.86	0.176	4.52	14.9
	Awol Endris,V-130	6.24	2.43	0.155	4.59	17.2
	Umer Adem,V-1	6.44	4.45	0.119	3.38	16.5
	Sheh Musa Ali, Almu town	5.81	4.94	0.105	3.42	18.9
	Yilma Bekele , V-16	5.81	1.70	0.112	3.35	17.3
	Ashuro Bedare,V-9	5.27	1.13	0.155	3.66	13.7
	Letebo Lendebo,V-10	5.29	0.73	0.148	3.52	13.8
	Mean	5.55	2.90	0.151	4.22	16.3

*V = village in Pawe District; CN ratio = Carbon to Nitrogen ratio

Available P contents of soils were far below 15 mg kg^{-1} indicating that the soils are low in available P according to the ratings for some tropical soils (Landon, 1991) extracted by the Bray II method. The very low available P status in the soils of the study area is illustrative of P deficiency and may be due to the low level of soil pH that can have the acidic cations such as exchangeable Al, H, and oxides of Al and Fe that could fix the soluble P in the soil solution (Abreha *et al*., 2012). According to Tekalign (1991) ratings of soil OM and total N contents, the mean values of OM (4.22%) and total N (0.151%) recorded by soils of the experimental fields qualify for medium (2.59 - 5.17 %) and high (0.12 - 0.25 %) status, respectively. The C: N ratio ranged from 13.3 to 20.6, which was 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 Nodule Number and Weight

The two experiments analysis of variance indicated that there was significant difference ($P < 0.05$) among treatments in number of nodules and nodule weight plant^{-1} (Table 3). Accordingly, the highest number of nodules and nodule weight plant^{-1} recorded by using all four *rhizobial* strains than un-inoculated treatment plots. Among the strains during the 1st experiment, the highest number of nodules (63.17) and nodule weight plant^{-1} (0.20) were recorded by *rhizobial* strain TSBF-531 followed by MAR-1495.

In the second experiment, the highest number of nodules (38.54) and nodule weight plant^{-1} (1.56) were recorded by *rhizobial* strain MAR-1495. Whereas, the lowest number of nodules (9.62) and nodule weight plant^{-1} (0.71) were recorded by un-inoculated control treatment. Even though *rhizobial* strain legume fix in the 1st experiment resulted to higher number of nodules and nodule weight plant^{-1}, it was lower in the 2nd experiment as compared to full RR of N fertilized treatment indicating that *rhizobial* strain legume fix did not show consistent result with regard to nodulation. Moreover, *rhizobial* strain MAR-1495 resulted consistent and better soybean nodulation than un-inoculated control and full RR N fertilized treatments.

In line with this, the results of Mahmood *et al*. (2009) revealed that application of *rhizobial* inoculant alone significantly increased nodulation of soybean. The reason might be due to inoculation with *Rhizobia*, which increased the number of bacteria and hence more nodules plant^{-1} were produced. Ravikumar (2012) also reported that the seed inoculation with *rhizobial* strain caused an increase in the number of root nodules in inoculated plants. The study of Solomon *et al*. (2012) also indicated the strain TAL 379 produced significantly higher number of nodules than TAL 378 and the un-inoculated control.

3. 3. Effect of *Rhizobial* Strains on Biomass and Grain yield of Soybean

The two season experiments analysis of variance indicated that there was significant difference ($P < 0.05$) among treatments in biomass and grain yields (Table 3).

During the 1st experiment, the highest biomass and grain yields respectively were recorded by *rhizobial* strain legume fix (2804.7 and 1245.9 kg ha^{-1}) followed by MAR-1495 (2628.7 and 1182.5 kg ha^{-1}). Whereas, the other two strains (TSBF-531 and TSBF-442) showed lower biomass and grain yields than un-inoculated control treatments. Furthermore, considering the four strains, TSBF-531 and TSBF-442 *rhizobial* strains recorded higher number of root nodules, but didn't result to higher soybean grain yield as compared to un-inoculated treatment plots. This indicated that higher number of nodules did not guarantee the higher grain yield unless they are effective. Weaver (1974) indicated that the presence of nodules on roots does not mean that the sufficient N is being fixed for good growth of the host plant.

Table 3. Effect of *rhizobial* strains on nodulation and yield of soybean.

Experiment No.	Treatments	Nodule number (No. plant^{-1})	Nodule weight (gm plant^{-1})	Biomass yield (kg ha^{-1})	Grain Yield (kg ha^{-1})
1	Control	34.71^{bcd}	0.10^{b}	2419.7^{ab}	1085.2^{ab}
	Half RR N fertilizer	23.70^{d}	0.08^{b}	2309.5^{ab}	1024.2^{ab}
	Full RR N fertilizer	30.13^{cd}	0.11^{b}	2408.3^{ab}	1103.8^{ab}
	Legume fix	49.73^{abc}	0.10^{b}	2804.7^{a}	1245.9^{a}
	TSBF-442	53.17^{ab}	0.13^{b}	2189.6^{ab}	1041.5^{ab}
	TSBF-531	63.17^{a}	0.20^{a}	2050.7^{b}	804.6^{b}
	MAR-1495	55.13^{ab}	0.14^{ab}	2628.7^{ab}	1182.5^{a}
	CV%	40.8	60.7	23.4	26.5
	LSD(0.05)	21.28	0.07	663.8	334.6
	ANOVA	*	*	*	*

2	Control	9.62^{c}	0.71^{b}	3696.0^{b}	1731.5^{b}
	MAR-1495	38.54^{a}	1.56^{a}	4263.8^{a}	2172.3^{a}
	Legume fix	21.58^{b}	0.65^{b}	3787.1^{ab}	1981.1^{ab}
	Full RR N fertilizer	37.35^{a}	1.36^{a}	4109.8^{ab}	1976.8^{ab}
	CV%	54	51.3	17.9	24.5
	LSD(0.05)	10.67	0.40	544.5	355.16
	ANOVA	*	*	*	*

Means within a column followed by same letters in superscripts are not significantly different from each other at $P > 0.05$; * = Significant

For these reasons, *rhizobial* strains of legume fix and MAR-1495 were selected for further evaluation on soybean in the 2nd experiment and the result revealed that the highest biomass yield (4263.8 kg ha^{-1}) and grain yield (2172.3 kg ha^{-1}) were recorded by *rhizobial* strain MAR-1495 (Table 3). The increment in grain yield over the un-inoculated control, legume fix and full RR of N fertilized treatments recorded by MAR-1495 was 25.5, 9.7 and 9.9%, respectively. The result indicated that using *rhizobial* strain MAR-1495 for soybean production in the study area resulted to higher grain yield and replace the need for inorganic N fertilization.

The current result also similar with Wondwosen *et al*. (2016) report that evaluations of *rhizobial* strains on lentil revealed the highest grain yield was recorded by *rhizobial* strain Lt29 than others tested *rhizobial* strains, un-inoculated control and N fertilized treatments. They also suggested that inoculation of lentil with *rhizobial* strain Lt29 replace the need for inorganic nitrogen fertilization to optimize lentil yields in soils of southern Ethiopia. Similarly, Appunu *et al*. (2008) reported that inoculation of soybean with *rhizobial* strain ASR011 recorded the highest nodulation and grain yield as compared to inoculation with others tested *rhizobial* strains and un-inoculated treatments. Bhuiyan *et al*. (1998) also reported that *rhizobial* inoculation increased nodulation and yield up to 35%.

3. 4. Effect of *Rhizobial* Strains on Soybean Protein Content

Soybean plant sample analysis showed that there was significant difference ($P < 0.05$) in total nitrogen or crude protein among treatments (Table 4). Accordingly, the highest protein content (19.5 %) was recorded by inoculation of *rhizobial* strain MAR-1495, whereas the lowest protein (15.56 %) was recorded by un-inoculated control. The increment in crude protein over the un-inoculated control, legume fix and full RR of N fertilized treatments recorded by MAR-1495 was 25.3, 21.3 and 5.1%, respectively. The result indicated that inoculation of soybean seeds with *rhizobial* strain MAR-1495 could have contributed to the higher content of protein in plant tissue. This result is in agreement with the findings of Wondwosen *et al*. (2016). Singh and Rai (2005) also found out that inoculated soybean with BNF *rhizobial* strains resulted to higher protein content than un-inoculated and N fertilized treatment plots.

Table 4. Effect of *rhizobial* strains on above ground soybean plant nitrogen and protein content.

Treatments	Total N (%)	Crude Protein (%)
Control	2.489[b]	15.56[b]
MAR-1495	3.120[a]	19.50[a]
Legume fix	2.571[b]	16.07[b]
full RR N fertilizer	2.967[a]	18.55[a]
LSD (0.05)	0.353	2.21
C.V. (%)	12.2	12.2
ANOVA	*	*

Means within a column followed by same letters in superscripts are not significantly different from each other at $P > 0.05$; * = Significant at $P < 0.05$

4. CONCLUSION

The two experiments analysis of variance indicated that there was significant difference ($P < 0.05$) among treatments in number of nodules, nodule weight, biomass yield and grain yield of soybean. Accordingly, the 1st experiment result indicated that the highest number of nodules and nodule weight plant^{-1} recorded by using all four *rhizobial* strains than un-inoculated treatment plots. However, the highest biomass and grain yields were recorded by only two *rhizobial* strains (legume fix and MAR-1495).

The other two strains (TSBF-531 and TSBF-442) showed lower grain yield than un-inoculated control treatments. As a result, legume fix and MAR-1495 strains were evaluated in the 2nd experiment and the result revealed that *rhizobial* strain MAR-1495 resulted consistent and highest number of nodules, nodule weight, biomass yield and grain yield of soybean. The increment in grain yield over the un-inoculated control, legume fix and full RR of N fertilized treatments recorded by MAR-1495 was 25.5, 9.7 and 9.9%, respectively. Moreover, inoculation of soybean seeds with *rhizobial* strain MAR-1495 also resulted to higher protein content of soybean plant tissue. Therefore, *rhizobial* strain MAR-1495 was found to be the most suitable commercial *rhizobial* inoculant for soybean production in the study area.

ACKNOWLEDGMENTS

This research was funded by the Bill and Melinda Gates Foundation through the Tropical Soil Biology and Fertility of Centro International de Agricultura Tropical (TSBF-CIAT) with the COMPRO project on commercial products. We acknowledge with gratitude the contributions of this project in disseminating the products for agricultural sector to increase the productivity of legume crops.

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(Received 22 August 2016; accepted 07 September 2016)