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Response of soybean to inoculation with *Bradyhrizobia* spp. strains: effect on root nodulation, yield and residual soil nitrogen

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ABSTRACT

Soybean (*Glycine max* (L.) Merrill) is an exotic crop to Ethiopia. Hence, inoculation with effective *Bradyrhizobia* spp. is crucial to improve its productivity. This study was therefore conducted in West Gojam Zone of Amhara Region in 2015 and 2016 aimed at evaluating the effect of bradyrhizobia strains (MAR-1495, SB-12 and TAL-379) with and without phosphorus fertilizer (23 kg P_2O_5 ha^{-1}) on root nodulation and yield of soybean, and on residual soil nitrogen. The treatments were laid in randomized complete block design including a satellite control treatment with three replications. Effect of the use of rhizobia strains and P fertilizer on nodulation, yield and yield components of soybean was found statistically significant ($P \leq 0.05$). The highest grain and dry matter yields of 2.7 and 6.4 t ha^{-1}, respectively, were obtained from MAR-1495+P which was statistically at par with the yields obtained from MAR-1495 alone. A grain yield advantage of 30.8% over the control treatment was found from the use of MAR-1495 alone. Similarly, the maximum number of effective nodules (14.9) per plant was recorded from MAR-1495+P which was statistically at par with the number of effective nodules (11.6) per plant counted from MAR-1495 inoculated treatment. The highest residual soil N exceeding the control treatment by 0.029% (0.29 g total N per kg soil) was obtained from MAR-1495 alone. Therefore, inoculation with the strain MAR-1495 was found the most effective for improved soybean production in the study district and similar agroecologies.

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Keywords**:** fertilizer, inoculation, Jabitehnan, bradyrhizobia, soybean, strain, *Glycine max*, *Bradyrhizobium japonicum*

1. INTRODUCTION

Nitrogen (N) and phosphorus (P) are the two major nutrients that limit plant growth in smallholder farms in Africa. Leguminous plants require large amount of N for grain yield [1], but it is difficult for smallholder farmers with limited resources to supply the needed high N quantities. Most low income farmers tend to plant legumes without any major external input thus obtaining low grain yields. Under such conditions, legumes depend on biological N fixation through symbiosis with rhizobia to partially or fully meet their N requirement.

Rhizobia are bacteria that fix atmospheric N through symbiosis with leguminous plants in a process referred as biological N fixation (BNF). It is estimated that about 50 to 300 kg N ha^{-1} can be fixed by rhizobia bacteria. Thus, their contribution to the N economy of the soil is quite substantial. Moreover, BNF is believed to consume less energy than N fixation through the mineral process. For these reasons, inoculation with strains of rhizobia has become an important agronomic practice to augment N supply to legumes and reduce the amount of inorganic N fertilizers required.

Soybean (*Glycine max* (L.) Merrill) is a grain legume cultivated from tropics to temperate regions in the world on 10^4 million hectares of land with an average seed yield of 1.4-2.0 t ha^{-1}. The seed contains about 18% oil and 38% protein and the extraction residue represents more than 40% of the utilization value of the plant. Soybean fixes up to 200 kg N ha^{-1} $year^{-1}$ when in symbiotic association with *Bradyrhizobium japonicum* reducing the need for potentially environmental damaging N fertilizer [2-18].

Soybean is an extotic crop to Ethiopia and its average yield per hectare is by far below the world's average. Because, as different studies revealed, indigenous rhizobia bacteria are not found effective and competitive as compared to commercial rhizobia strains in producing effective nodulation and increasing yield. This indicates a need for further evaluation and selection of alternative and effective commercial rhizobia strains through research. This study was therefore carried out with the objective of evaluating and selecting the best and effective rhizobia strains for improved nodulation and increased yield of soybean [19-31].

2. MATERIALS AND METHODS

2. 1. Study site description

This study was conducted in Jabi-Tehnan District of West Gojam Zone of Amhara region in Ethiopia in 2015 and 2016 main cropping seasons. The study district is located between the coordinates of 37º5'59" – 37º29'59" E and 10º21'26" – 10º57'20" N with altitudinal ranges of 1500-2500 meters above sea level. The dominant soil type in the study site is Rhodic Nitisols. The district receives a mean annual rainfall of 1250 mm with mean minimum and maximum temperatures of 14 and 32 ºC, respectively. Some physico-chemical characteristics of surface soil (0-20 cm) of the study sites are described below (Table 1).

Table 1. Range of some physico-chemical characteristics of surface soil of the testing sites

Soil parameter	Value
Soil pH (H_2O)	5.4-6.0
Organic carbon (%)	0.55-3.10
Total nitrogen (%)	0.044-0.136
Available phosphorus (mg kg^{-1})	2.60-6.16
Cation exchange capacity CEC (meq 100 gm^{-1})	14.8-22.6
Base saturation (%)	36-55
Texture	Clay

2. 2. Experimental procedure and treatments

The field was ploughed and prepared with an oxen-drawn traditional local tool called *Maresha* and was divided in to experimental plots which had an area of 4 m × 3 m. The space between each plot and block was 1 m. Three rhizobia strains, MAR-1495, SB-12, TAL-379 were factorially combined with 0 and 23 kg P_2O_5 ha^{-1} and there was a control (uninoculated and unfertilized) satellite treatment. The treatments were laid in RCBD design with three replications. The P fertilizer was applied in diammonium phosphate (P_2O_5) form at planting. A starter nitrogen fertilizer at a rate of 9 kg ha^{-1} was applied to all plots where P fertilizer was applied. An improved variety of soybean *Gishama 335* was planted in a row with 40 cm and 10 cm spacings between rows and plants, respectively. Just before planting, soybean seeds were gently rubbed with sugar-dissolved water and inoculated with the strains under a shade without exposure to direct sunlight.

2. 3. Soil sample collection and analysis

Composite surface (0-20 cm) soil samples were collected at planting for the determination of pH, texture, organic carbon, total N and available P. Similarly, surface soil samples at a depth of 0-20 cm were taken plot-wise at harvesting in both experimental years to investigate the residual effect of rhizobia seed inoculation on soil total N in the subsequent cropping season.

Each soil sample collected was thoroughly mixed and air-dried at room temperature. The dried soil samples were ground and passed through a 2 mm sieve. The physicochemical characteristics of the soil samples were analyzed following the standard procedure at Adet Agricultural Research Center soil testing laboratory in Bahir dar, Ethiopia.

2. 4. Data collection

Nodule count data was collected at 50% flowering stage from ten random plants in the boarder rows. The inner rows excluding the boarder were harvested at maturity and yield and yield related parameters were measured. The grain yield measured was adjusted to 14% moisture content. Pod number per plant was measured by counting pod numbers from ten random plant samples at maturity and taking the average. Similarly, seed number per pod was measured by counting seed numbers from ten random sample pods and taking the average. While, 100 seed weight was measured by weighing 100 soybean seeds from the harvested rows on a sensitive balance.

2. 5. Data analysis

The collected data were subjected to analysis of variance (GLM procedure) using SAS software version 9.00. The mixed model procedure was used for the combined analysis over the testing sites in which only the treatments were used as a fixed variable while site and replication were used as random variables. Since experimental sites were not fixed in the two years, year was neither considered as a fixed nor random variable. Mean separation was made by using Least Significance Difference Method (LSD) at 5% level of significance.

3. RESULTS AND DISCUSSION

3. 1. Effect of rhizobia inoculation and P fertilizer on dry matter yield and yield components

There was a significant ($P \leq 0.05$) effect of use of rhizobia strains and P fertilizer on the grain and dry matter yields of soybean at two testing sites in the first experimental year (Table 2). The maximum grain and dry matter yields were obtained from inoculation alone with the strain MAR-1495 followed with insignificant ($P > 0.05$) difference by the yields obtained from inoculation alone with the strain SB-12. However, there was insignificant ($P > 0.05$) difference among plant height due to the effect of the treatments at all the three testing sites.

At the third testing site (at the research station), both use of rhizobia strains and P fertilizer had insignificant ($P > 0.05$) effect on the yield and yield components of soybean (Table 2). This might be due to optimum availability of N and P as a result of long years of N and P fertilization in the surface soil of the research site as compared to the farmers' fields. Different studies also indicated that nodule formation and functioning can be suppressed as the level of soil mineral N in the rhizosphere increases.

Table 2. Effect of use of rhizobia strains and P fertilizer on the mean yield and yield components of soybean at the three testing sites in 2015

Treatment*	Site I			Site II			Site III		
	PH	GY	DM	PH	GY	DM	PH	GY	DM
Cntrl.	64.9	1547.1	4357.7	83.0	2238.8	6423.6	84.7	3024.4	7320.2
MAR-1495	75.9	2642.2	6628.5	90.0	3034.6	7472.4	80.5	3100.3	6937.5
SB-12	73.1	2375.7	6083.3	82.7	3141.1	7484.4	81.9	3012.8	6843.8
TAL-379	71.6	1837.8	5138.9	81.9	1984.8	5618.4	78.7	2938.8	6812.9
MAR-1495+P	76.5	2610.0	6524.3	95.9	3374.9	8430.6	87.3	3124.4	7621.5

SB-12+P	75.9	2474.9	6371.5	84.1	2574.8	6894.2	86.9	3105.2	7350.7
TAL-379+P	72.3	1682.9	5343.7	92.7	2554.9	7326.4	87.2	3229.8	7201.4
Mean	72.9	2167.2	5778.3	87.2	2678.5	7073.3	83.9	3076.5	7155.4
CV (%)	6.9	15.3	10.3	9.2	14.9	7.1	5.3	7.6	6.6
LSD (5%)	ns	588.7	1062.6	ns	740.6	935.6	ns	ns	ns

PH = Plant height (cm), GY = Grain yield (kg ha^{-1}), DM = dry matter yield (kg ha^{-1}). *Means within a column followed by the same letter are not significantly different at $\leq$0.05 significance level; ns = non-significant at P = 0.05.

The pooled analysis over the three testing sites revealed a statistically significant ($P \leq 0.05$) difference among plant height, hundred seed weight (HSW), grain and dry matter yields due to the effect of treatments (Table 3). The maximum plant height of 86.6 cm, HSW of 17.4 g, grain yield of 3.0 t ha^{-1} and dry matter yield of 7.5 t ha^{-1} were obtained from the use of MAR-1495+P followed with insignificant difference ($P>0.05$) by the yield and yield components obtained from the use of MAR-1495 alone. Phosphorus treated plots exhibited the maximum HSW as compared to inoculated alone treatments. Though the treatment by site interaction effect on the grain and dry matter yields was significant ($P \leq 0.05$), the effects of MAR-1495+P, MAR-1495 and SB-12 alone were found uniformly dominant across all testing sites.

Table 3. Effect of P fertilizer and inoculation with rhizobia strains on the yield and yield components of soybean pooled over the three testing sites in 2015

Treatment*	Plant height (cm)	Pod no. per plant	Seed no. per pod	100 Seed weight (g)	Grain yield (kg ha^{-1})	Dry matter (kg ha^{-1})
1. Control	77.6	31.4	2.8	15.6	2270.1	6033.8
2. MAR-1495	82.1	28.6	2.7	16.8	2925.7	7012.8
3. SB-12	79.2	32.9	3.6	16.5	2806.0	6718.8
4. TAL-379	77.4	27.8	3.3	15.9	2253.8	5856.7
5. MAR-1495 + P	86.6	29.3	2.8	17.4	3036.4	7525.5
6. SB-12 + P	82.3	27.2	2.6	17.3	2718.3	6872.1
7. TAL-379 + P	84.1	29.6	3.0	16.4	2489.2	6623.8
Mean	81.3	29.5	2.9	16.6	2640.1	6662.5

CV (%)	7.4	18.6	39.6	6.6	13.8	8.6
LSD (5%)	5.8	ns	ns	1.05	350.1	554.9
Loc*Trt	ns	ns	ns	ns	*	**

*Means within a column followed by the same letter are not significantly different at $\leq$ 0.05 significance level; ns = non-significant at P = 0.05; * and ** = significant at 5 and 1% probability level, respectively.

The second year data analysis results also indicate that grain and dry matter yields were significantly ($P \leq 0.05$) affected by the effect of rhizobial inoculation and addition of P fertilizer. (Table 4). However, other yield component parameters such as plant height and pod number per plant were not significantly ($P > 0.05$) affected by the effect of treatments. At the fourth testing site, the highest grain yield of 2.6 t ha^{-1} was measured from SB-12+P and MAR-1495+P being statistically at par with the yields obtained from MAR-1495 alone. However, in the fifth testing site, except the control treatment, there was no significant ($P > 0.05$) yield difference among treatments. The lowest yield at both testing sites was recorded from the control treatment. The combined analysis over the two testing sites showed that rhizobial inoculation with P fertilizer had significant ($P \leq 0.05$) effect on the yield of soybean (Table 5). Inoculation alone with the strains MAR-1495 and SB-12 gave statistically similar grain and dry matter yields with their combined use along with P (Table 5).

Table 4. Effect of use of rhizobial inoculation and P fertilizer on the yield and yield components of soybean at testing site IV and V in 2016

Treatment*	Site IV				Site V			
	PH	**PNPP**	**GY**	**DM**	**PH**	**PNPP**	**GY**	**DM**
1. Control	72.1	30.2	1998.8	3871.5	58.5	22.5	901.7	2227.1
2. MAR-1495	65.5	30.7	2386.0	4517.4	63.7	26.3	1541.9	3349.0
3. SB-12	72.0	29.1	2088.1	5541.2	62.7	26.1	1471.8	3180.6
4. TAL-379	67.5	32.3	1928.9	4128.5	59.9	22.6	1618.0	3520.8
5. MAR-1495 + P	76.6	29.8	2580.8	5024.3	69.4	27.8	1576.1	3312.5
6. SB-12 + P	79.3	36.3	2628.1	5138.9	62.1	27.7	1310.1	3020.8
7. TAL-379 + P	69.5	35.9	2185.7	4274.3	65.5	29.8	1327.0	3066.0
Mean	71.8	32.0	2256.6	4642.3	63.1	26.1	1374.2	3065.3
CV (%)	8.4	12.9	9.6	10.0	11.5	10.3	14.9	13.9
LSD (5%)	ns	ns	385.6	826.5	ns	ns	416.3	873.3

PH = plant height (cm), PNPP = pod no per plant, GY = Grain yield (kg ha^{-1}), DM = dry matter yield (kg ha^{-1}). *Means followed by the same letters are not significantly different at 5% level of probability: ns = Non-significant at 5% significance level.

Table 5. Mean yield and yield components of soybean as affected by the use of rhizobia inoculation and P fertilizer pooled over the two testing sites in 2016

Treatment*	Plant height (cm)	Pod no. per plant	Grain yield (kg ha^{-1})	Dry matter (kg ha^{-1})
1. Control	65.3	26.3	1450.3	3049.3
2. MAR-1495	64.6	28.5	1964.0	3933.2
3. SB-12	67.4	27.6	1779.9	4360.9
4. TAL-379	63.7	27.5	1804.5	3885.4
5. MAR-1495 + P	73.0	28.8	2179.0	4339.6
6. SB-12 + P	70.7	31.9	2100.9	4291.7
7. TAL-379 + P	67.5	32.9	1756.3	3670.1
Mean	67.4	29.1	1849.3	3914.5
CV (%)	10.1	12.7	12.4	11.6
LSD (5%)	ns	4.4	286.1	567.4
Loc*trt	ns	ns	*	Ns

*Means followed by the same letter/s are not significantly different at 5% level of probability: ns = Non-significant at P = 0.05.

The combined analysis result over the two experimental years and over the five testing sites indicated that there was a highly significant effect of treatments on the plant height, grain and dry matter yields of soybean (Table 6). However, pod number per plant and seed number per pod were not significantly affected by the effects of treatments. The combined use of MAR-1495 with P gave the highest grain and dry matter yields of 2.7 and 6.4 t ha^{-1}, respectively which are statistically at par with the yields recorded from MAR-1495 alone. Inoculation with the strain MAR-1495 alone had a 30.8% yield advantage over the yield obtained from the control treatment. Inoculation of soybean with the strain SB-12 had also statistically comparable yield advantage with the strain MAR-1495 and had 21.8% yield advantage over the yield obtained from the control treatment. The finding in this paper is supported, who concluded that the strain MAR-1495 was the best strain among the strains evaluated in their study in increasing the yield of soybean.

Table 6. Mean yield and yield components of soybean as affected by the use of rhizobia strains and P fertilizer pooled over all testing sites in the two experimental years

Treatment*	Plant height (cm)	Pod no. per plant	Seed no. per pod	Grain yield (kg ha^{-1})	Dry matter (kg ha^{-1})
1. Control	72.7	29.3	2.9	1942.2	4840.0
2. MAR-1495	75.1	28.5	2.8	2541.0	5780.9
3. SB-12	74.5	30.8	3.4	2366.2	5708.2
4. TAL-379	71.9	27.6	3.2	2093.3	5152.7
5. MAR-1495+P	81.1	29.1	2.9	2730.2	6387.6
6. SB-12+P	77.7	29.1	2.8	2497.8	5950.5
7. TAL-379+P	77.4	30.9	3.0	2196.1	5442.4
Mean	75.7	29.3	2.9	2334.8	5601.4
CV (%)	8.6	16.7	31.1	13.6	9.6
LSD (5%)	4.8	ns	ns	236.2	401.4
Loc*Trt	ns	ns	ns	ns	ns

*Means followed by the same letter/s are not significantly different at 5% level of probability; ns = Non-significant at P = 0.05.

3. 2. Effect of rhizobial inoculation and P fertilizer on root nodule formation

The maximum number of effective nodules per plant (NENP) at site I was recorded from TAL-379+P statistically at par with the NENP recorded from SB-12 and MAR-1495. While at site II, the maximum NENP was recorded from MAR-1495+P statistically at par with the NENP recorded from MAR-1495 and SB-12 alone. The combined analysis over the two testing sites indicated that the maximum average NENP (14.9) was recorded from the use of MAR-1495+P which was statistically at par with the average NENP (11.6) recorded from the use of the strain MAR-1495 alone (Table 7).

Although the treatment by site interaction effect on nodulation was significant, use of the strains MAR-1495 and SB-12 alone was found to perform uniformly better than the other treatments in producing effective nodules. According to Pedersen, a successful nodulation by the V3 to V4 growth stages of soybean should produce 8 to 10 healthy nodules per plant. A similar finding was reported who found higher nodule dry weight and nodule number from soybean inoculated with the strain MAR-1495 as compared to soybean inoculated with the strain SB-12 and non inoculated treatments. Also revealed that application of *rhizobial* inoculant alone significantly increased nodulation of soybean.

Table 7. Effect of inoculation with rhizobia strains and P fertilizer on root nodulation of soybean in 2015

Treatment*	Number of effective nodules per plant		
	Site I	Site II	Combined
1. Control	0.4	1.6	1.0
2. MAR-1495	5.0	18.1	11.6
3. SB-12	4.2	12.9	9.4
4. TAL-379	1.3	8.3	5.9
5. MAR-1495 + P	4.6	20.2	14.9
6. SB-12 + P	6.2	15.9	10.1
7. TAL-379 + P	6.6	11.3	8.9b
Mean	4.1	12.6	8.8
CV (%)	40.7	34.2	48.9
LSD (5%)	3.4	7.7	5.12
Trt*Loc	-	-	**

*Means followed by the same letter/s are not significantly different at 5% level of probability; ** = significant at 1% significance level.

3. 3. Effect of rhizobial inoculation and P fertilizer on residual soil nitrogen

The statistical analysis result of the average residual symbiotically derived total nitrogen on the surface soil pooled over two testing sites in the first year indicated that although there was no statistically significant (P>0.05) difference among treatments, the maximum residual total N difference of 0.029% (0.29 g N per 1 kg soil) from the control treatment was measured from the plots where the strain MAR-1495 had been inoculated (Table 8).

Similarly, sizable residual soil N was also measured from the plots where TAL-379 and SB-12 bacteria inoculated seeds were planted. A number of crop sequence studies from Midwest US indicated that soybean harvested for grain leaves an average residual N of 45-67 kg ha^{-1}. In contrast, soybean N budget studies indicated that N removal in grain may substantially exceed biological N fixation.

Table 8. Effect of inoculation with rhizobia and P fertilizer on residual total N (%) of the surface soil in 2015

Treatment	Site I	Site II	Combined
1. Control	0.1877	0.1731	0.1804
2. MAR-1495	0.2392	0.1796	0.2094
3. SB-12	0.1758	0.2145	0.1913
4. TAL-379	0.1849	0.2244	0.2007
5. MAR-1495+P	0.1878	0.1944	0.1911
6. SB-12+P	0.2007	0.1743	0.1901
7. TAL-379+P	0.1872	0.1661	0.1787
Mean	0.194	0.188	0.1918
CV (%)	17.5	17.6	17.9
LSD (0.05)	ns	ns	ns

ns = Non-significant.

4. CONCLUSION

The result in this study indicated that the combined use of the rhizobia strains MAR-1495 and SB-12 were found to increase yield of soybean significantly as compared to the control treatment. However, the sole use of the rhizobia strain MAR-1495 had statistically similar yield advantage with its combined use with P fertilizer. Moreover, the maximum residual nitrogen on the surface soil was measured from plots where MAR-1495, TAL-379 and SB-12 strains were inoculated which is beneficial for subsequent crop production. Thus, inoculation of soybean seeds with MAR-1495 primarily and SB-12 alternatively prior to planting can be recommended for improved soybean production in Jabitehnan district and similar agro-ecologies.

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