Introduction

Sub-Saharan Africa (SSA) accounts for about 9% of the global population threatened by food and nutritional insecurity. This threat is partly due to poor soil fertility and low crop diversification (Sanchez and Swaminathan, 2005). The nitrogen reserves of arable soils must be replenished periodically to maintain an adequate level for crop production. This replenishment of soil nitrogen is accomplished through the application of mineral fertilizer, or management of biological nitrogen fixation (BNF) (Bekunda *et al*., 2010).

Nitrogen is one of the most abundant elements on earth which constitutes about 78% of the earth's atmosphere, but its availability often limits plant growth and crop production. This situation arises because the N_2 molecule is very stable chemically and is unusable by most biological organisms. It must be "fixed" before it can be assimilated (Fisher and Newton, 2002). Nitrogen can be utilized when it is reduced to ammonia by fixation. It can be reduced by chemical fixation through industrial production and/or biological fixation involving microorganisms. Most plants utilize nitrogen in its ionic forms ammonium (NH_4^+) and nitrate (NO_3^-) from soil (Li *et al*.,2013).

The increasing cost of fertilizers and their impact on the environment have forced people to look for other possible sources of plant nutrients. In this regard, nitrogen fixation which is a process by which elemental atmospheric nitrogen is changed to organic forms by biological nitrogen fixation both by symbiotic and asymbiotic microorganisms in soil has drawn much attention (Kumari and Sinha, 2011). The symbiotic nitrogen fixation is used to maximum advantage in the case of leguminous crops. There is no doubt that specificity exists between rhizobia- strain and the legume, and compatibility between the two is essential for successful nodulation. This necessitates using specific cultures for different legumes. When growing a new legume species on soil, it is necessary practice that the appropriate rhizobia culture is applied (Solomon *et al.*, 2012).

It is therefore important to inoculate seeds with relevant strains of rhizobia before sowing especially if the crop is to be grown for the first time on the land. Inoculation responses are associated primarily with the first planting of a legume in soil having no prior history of the crop (Zerpa *et al*., 2013). The use of commercial rhizobial inoculants in the establishment of soybean has been widely recognized, especially in areas where indigenous nodulation is 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).

Previous studies conducted in Ethiopia showed that the response of soybean to rhizobia inoculation is promising but variable depending on the inherent field variability, difference in environmental and edaphic factors. However, such information is scanty in the study area. Therefore, this study is geared towards the evaluation of different rhizobia strains under different phosphorus supply for improving nodulation and yield of soybean.

Materials and methods

Experimental site

The study was carried out on the farmer's field in 2015 and 2016 at Metema and Tache Aremacheho districts which is located in the North Gondar zone, Amhara Regional State in Ethiopia (Fig.1) The area has a mean elevation of 1080 m.a.s.l, with a maximum of 1608 m a.s.l. and a minimum of 550 m a.s.l. The mean annual rainfall for the area ranges from about 850 to around 1100 mm and the rainy months extend from June to the end of September. However, maximum rainfall is received during July and August respectively. The average annual temperature of the study area ranged between 22 ^{0}C and 28 ^{0}C. The maximum temperature was recorded during March to May. Nearly all of the land in the area is in the lowlands except some mountain tops which fall outside. The major crops grown in the area are sorghum, cotton, sesame and teff. One variety of soybean (Ethio-Yugoslavia) was tested. This variety was obtained from Adet Agricultural Center and its potential grain yield is from 2.5 to 3 t. ha^{-1}. Three strains of soybean rhizobia (Strain MAR-1495, Strain SB- 12, TAL -379) were obtained from Holeta Microbiology Laboratory (Ethiopia).

Figure1. Map of the study area

Experimental design

In each district, the experiments were conducted on farmer's fields where sorghum was grown in the previous season to reduce soil nitrogen effects and soil heterogeneity factor.

The experiment was designed in a randomized complete block design (RCBD) with three replications. The experiment included seven treatments of which three strains of *Bradyrhizobium japonicum* (MAR-1495, SB-12, TAL-379) combined with and without phosphorus fertilizer (10 kg P ha^{-1}) along with one (un inoculated and unfertilized control). Gross plot size was 12 m^2 (4 m x 3 m) and spaced 1 m and 1.5 m between plots and blocks, respectively. The net harvestable area was 6.25 m^2 (2.5 m x 2.5 m). Three seeds were sown per hill, which were later thinned to one after germination, with spacing of 5 cm between plants and 60 cm between rows.

Physico-chemical analysis of Soil

Experimental soils were sampled for their Physico-chemical properties analysis from one representative composite sample which were taken 0 to 20 cm depth from the entire field before planting. Similarly, soil samples were collected at each plot after harvest. Sampleswere analyzed for the following parameters viz., pH, organic carbon, cation exchange capacity, and available P from the representative bulk soil sample before planting and at harvest. soil pH was estimated by potentiometric method at soil: water ratio of 1:2.5 (Van Reeuwijk, 1992). Cation exchange capacity was determined by 1M ammonium acetate method at pH 7 (Chapman, 1965) whereas organic carbon was determined by the dichromate oxidation method (Walkley and Black, 1934) and available P was analyzed by Olsen method (Olsen *et al*., 1954) and determined colorimetrically by the ascorbic acid- molybdate blue method (Watanabe and Olsen, 1965). Ca^{++} and Mg^{++} values were found out from Atomic Absorption. Spectrophotometer reading while Na^{+} and K^{+} was determined using flame photometer.

Seed Inoculation

The lignite -based inoculants were added to the soybean seeds in a container after moistening the seeds. The inoculants and the seeds were mixed thoroughly until the seeds were adequately coated with the inoculants and allowed to air-dry in the shade for 30 minutes after which they were planted on the ridges.

Data collection

Nodule Data Collection

Four plants were sampled randomly from the second border rows of each plot at mid-flowering. The whole plant was carefully uprooted using a spade to obtain intact roots and nodules. Uprooting was done by exposing the whole-root system to avoid loss of nodules. The adhering soil was removed by washing the roots with intact nodules gently with water. The number of nodules per plant was determined by counting the number of nodules from all the four uprooted plants per plot and then averaged as per plant.

Crop Yield and Agronomic Data Collection

Five plants were sampled randomly at maturity from each plot, pods were counted for all the five plants, and the average value was reported as the number of pods per plant. The number of seeds per pod was determined from five pods randomly sampled, and the average was reported as a number of seeds per pod. The number of plants per plot was recorded at harvesting from the central four rows, and the mean was computed and used for the analysis of the final plant stand. Soybean plants were harvested from each plot at physiological maturity leaving the border rows and 0.5m row length on every end of each row. Seed yield was obtained by adjusting the moisture level to 10% according to the formula indicated by Abebe (1979) as follows and converted into kg ha $^{-1}$:

$\text{Adj. grain yield (kg ha}-^{1}) = \frac{100-\text{M.C}}{100-\text{SMC}} \text{X Plot yield (g)x} \frac{10}{\text{Net plot size (m2)}}$, where Adj. grain yield (kg/ha) = adjusted grain yield in kg ha $^{-1}$, M.C = the % moisture content of the seed sample, Plot yield (g) = unadjusted grain yield in gram, SMC= % standard moisture content (which is 10 for soybean), Net plot size = Area harvested (plot size) in m^{2}.

or

$\text{Adj. grain yield (per plot)} = \frac{100-\text{M.C}}{100-10} \text{X Plot yield (g)}$, where 10 is the SMC; then convert this into ha basis.

where MC is moisture content of soybean seeds at the time of measurement, and 10 is the standard moisture content of soybean seeds at harvest in percent. The weights of hundred seeds randomly counted from the seeds of each plot maintaining the seed moisture content at 10% were reported as hundred seeds weight.

Statistical analysis

All statistical analysis was carried out using SAS software version 10. One-way analysis of variance (ANOVA) was performed to determine the statistical differences. When significant differences (p < 0.05) were noticed, all treatment means were compared using the Least Significant Difference (LSD) at a 5% level of significance.

Results and discussion

Selected Soil Physical and Chemical Properties before planting

The soil analysis results of the pre-sowing selected physical and chemical properties of the experimental sites are presented in Table 1. Soil analysis of the experimental field has shown that the soil had a pH of 6.84 to 7.93, which was rated as moderately alkaline (Tekalign, 1991). Soil organic matter of the experimental site was high (4.8%) (Tekalign, 1991). The analysis further indicated that the soil had low available phosphorus (4.5 to 7.01 ppm) (Table 1) according to the ratings of Marx *et al*. (1996). Besides, the cation exchange capacity (CEC) of the soil was rated in the range of very high as reported by Landon (1991).

Table 1. Intial soil c

Table 3. Soil chemical properties after harvesting at Tach-Armachiho (2015).

Treatment	PH (H2O)	EC(ms/cm)	AVAILABLE P (ppm)	OC (%)	CEC (cmol(+)/kg)
1	8	0.08	4.55	0.7	53.23
2	7.9	0.07	4.17	0.92	53.46
3	7.7	0.08	3.27	25.6	53.09
4	7.8	0.07	3.71	0.85	52.29
5	7.7	0.07	4.49	0.97	56.25
6	7.8	0.08	5.75	1.1	51.83
7	7.8	0.09	3.29	0.84	53.91

Where: OC: Organic content; P: Phosphorus; CEC: Cation Exchange Capacity.

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

There was a significant ($P<0.05$) effect 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 4).The maximum grain and dry matter yields were obtained from inoculation alone with the strain SB-12 followed with insignificant ($P>0.05$) difference by the yields obtained from inoculation alone with the strain MAR-1495. However, there was insignificant ($P>0.05$) difference among plant height due to the effect of the treatments at all testing sites.

Table 4. Mean yield and yield components of soybean influenced by rhizobia strains and P at Metema and Tach armachiho in 2015

Treatment*	Metema PH (cm)	Metema GY (kg ha^{-1})	Metema DM (kg ha^{-1})	Tach armachiho PH (cm)	Tach armachiho GY (kg ha^{-1})	Tach armachiho DM (kg ha^{-1})
Control.	61.6	1543.8	4354.4	79.7	2235.5	6420.3
SB-12	72.6	2638.9	6625.2	86.7	3031.3	7469.1
MAR-1495	69.8	2372.4	6080	79.4	3137.8	7481.1
TAL-379	68.3	1834.5	5135.6	78.6	1981.5	5615.1
SB-12+P	73.2	2606.7	6521	92.6	3371.6	8427.3
MAR-1495+P	72.6	2471.6	6368.2	80.8	2571.5	6890.9
TAL-379+P	69	1679.6	5340.4	89.4	2551.6	7323.1
CV (%)	6.9	15.3	10.3	9.2	14.9	7.1
LSD (5%)	ns	588.7	1062.6	ns	740.6	935.6

*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 < 0.05 significance level; ns = non-significant at P = 0.05.*

The pooled analysis over the testing sites revealed a statistically significant ($P<0.05$) difference among plant height, hundred seed weight (HSW), grain and dry matter yields due to the effect of treatments (Table 5). The maximum plant height of 85.1 cm, HSW of 15.9 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 SB-12+P followed with insignificant difference (P>0.05) by the yield and yield components obtained from the use of SB-12 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<0.05), the effects of SB-12+P, SB-12 and MAR-1495 alone were found uniformly dominant across all testing sites.

Table 5. Effect of P and rhizobia on the yield and yield components of soybean pooled over the two testing sites in 2015

Treatment*	PH (cm)	PN per plant	SN per pod	100SW (g)	GY (kg ha^{-1})	DM (kg ha^{-1})
1. Control	76.1	31.4	2.8	14.1	2268.6	6032.3
2. SB-12	80.6	28.6	2.7	15.3	2924.2	7011.3
3. MAR-1495	77.7	32.9	3.6	15	2804.5	6717.3
4. TAL-379	75.9	27.8	3.3	14.4	2252.3	5855.2
5. SB-12 + P	85.1	29.3	2.8	15.9	3034.9	7524
6. MAR-1495 + P	80.8	27.2	2.6	15.8	2716.8	6870.6
7. TAL-379 + P	82.6	29.6	3.0	14.9	2487.7	6622.3
Mean	79.8	29.5	2.9	15.1	2638.6	6661
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 < 0.05 significance level; ns = non-significant at P = 0.05; * and ** = significant at 5 and 1% probability level, respectively. PH; plant height, PN ; pod number, SN ; seed number, 100SW; 100 seed weight, GY; grain yield, DM; dry matter.*

The second year data analysis results also indicated that grain and dry matter yields were significantly (P<0.05) affected by the effect of rhizobial inoculation and addition of P fertilizer. (Table 6). 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 Metema, the highest grain yield of 2.6 t ha^{-1} was measured from MAR-1495+P and SB-12+P being statistically at par with the yields obtained from SB-12 alone. However, at Tach Armachiho, 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<0.05) effect on the yield of soybean (Table 6). 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 6).

Table 6. Effect of rhizobial and P on the yield and yield components of soybean at Metema and Tach Armachiho in 2016

Treatment*	Metema				Tach Armachiho			
	PH (cm)	PNPP	GY (kg ha^{-1})	DM (kg ha^{-1})	PH (cm)	PNPP	GY (kg ha^{-1})	DM (kg ha^{-1})
1. Control	69.6	27.7	1996.3	3869	56	20	899.2	2224.6
2. SB-12	63	28.2	2383.5	4514.9	61.2	23.8	1539.4	3346.5
3. MAR-1495	69.5	26.6	2085.6	5538.7	60.2	23.6	1469.3	3178.1
4. TAL-379	65	29.8	1926.4	4126	57.4	20.1	1615.5	3518.3
5. SB-12 + P	74.1	27.3	2578.3	5021.8	66.9	25.3	1573.6	3310
6. MAR-1495 + P	76.8	33.8	2625.6	5136.4	59.6	25.2	1307.6	3018.3
7. TAL-379 + P	67	33.4	2183.2	4271.8	63	27.3	1324.5	3063.5
Mean	69.3	29.5	2254.1	4639.8	60.6	23.6	1371.7	3062.8
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 7. Mean yield and yield components of soybean affected by rhizobia and P fertilizer pooled over the two testing sites in 2016.

Treatment*	PH (cm)	PNPP	GY (kg ha^{-1})	DM (kg ha^{-1})

1. Control	65.3	26.3	1450.3	3049.3
2. SB-12	64.6	28.5	1964.0	3933.2
3. MAR-1495	67.4	27.6	1779.9	4360.9
4. TAL-379	63.7	27.5	1804.5	3885.4
5. SB-12 + P	73.0	28.8	2179.0	4339.6
6. MAR-1495 + 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

PH = Plant height (cm), GY = Grain yield (kg ha^{-1}), DM = dry matter yield (kg ha^{-1}).

**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 all testing sites indicated that there was a highly significant effect of treatments on the plant height, grain and dry matter yields of soybean (Table 8). However, pod number per plant and seed number per pod were not significantly affected by the effects of treatments. The combined use of SB-12 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 SB-12 alone. Inoculation with the strain SB-12 alone had a 30.8% yield advantage over the yield obtained from the control treatment. Inoculation of soybean with the strain MAR-1495 had also statistically comparable yield advantage with the strain SB-12 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 SB-12 was the best strain among the strains evaluated in their study in increasing the yield of soybean.

Table 8. Mean yield and yield components of soybean affected by rhizobia strains and P combined over locations and years

Treatment*	PH (cm)	PNPP	SNPPD	GY (kg ha^{-1})	DM (kg ha^{-1})
1. Control	69.7	26.3	2.9	1939.2	4837

2. SB-12	72.1	25.5	2.8	2538	5777.9
3. MAR-1495	71.5	27.8	3.4	2363.2	5705.2
4. TAL-379	68.9	24.6	3.2	2090.3	5149.7
5. SB-12+P	78.1	26.1	2.9	2727.2	6384.6
6. MAR-1495+P	74.7	26.1	2.8	2494.8	5947.5
7. TAL-379+P	74.4	27.9	3.0	2193.1	5439.4
Mean	72.7	26.3	2.9	2331.8	5598.4
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. PH = plant height, PNPP = pod no per plant, SNPPD=seed number per pod, GY = Grain yield (kg ha^{-1}), DM = dry matter*

Effect of rhizobial inoculation and P fertilizer on root nodule formation

The maximum number of effective nodules per plant (NENP) at Mtema was recorded from TAL-379+P statistically at par with the NENP recorded from MAR-1495 and SB-12. While at Tach Armachiho, the maximum NENP was recorded from SB-12+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 (15.1) was recorded from the use of SB-12+P which was statistically at par with the average NENP (11.8) recorded from the use of the strain SB-12 alone (Table 9).

Although the effect of treatment by site interaction 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.

Table 9. Effect of inoculation with rhizobia strains and P fertilizer on root nodulation of soybean in 2016

Treatment*	Number of effective nodules per plant

	Metema	Tach Armachiho	Combined
1. Control	0.6	1.8	1.2
2. SB-12	5.2	18.3	11.8
3. MAR-1495	4.4	13.1	9.6
4. TAL-379	1.5	8.5	6.1
5. SB-12 + P	4.8	20.4	15.1
6. MAR-1495 + P	6.4	16.1	10.3
7. TAL-379 + P	6.8	11.5	9.1
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.*

Conclusion

The result in this study indicated that the combined use of the rhizobia strains SB-12 and MAR-1495 were found to increase yield of soybean significantly as compared to the control treatment. However, the sole use of the rhizobia strain SB-12 had statistically similar yield advantage with its combined use with P fertilizer.Thus, inoculation of soybean seeds with SB-12 primarily and MAR-1495 alternatively prior to planting can be recommended for improved soybean production in Metema and Tach Armachiho districts and similar agro-ecologies.

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