

Evaluation of Co-inoculation of *Bradyrhizobium japonicum* and Phosphate Solubilizing *Pseudomonas spp.* Effect on Soybean (*Glycine max* L. (Merr.)) in Assossa Area

A. Argaw¹

ABSTRACT

An experiment was conducted at Assossa Agricultural Research Center (AARC) station during 2008 cropping season in order to study the effects of co-inoculation of *Bradyrhizobium japonicum* (TAL-378 and TAL-379) and phosphate-solubilizing bacteria (PSB) (*Pseudomonas spp.*), and conventional farmers' fertilizer level (combined and individual application of 46N kg ha⁻¹ and 46 P₂O₅ kg ha⁻¹) on nodulation, seed yield and yield components of soybean (*Glycine max* L. (Merr.)). Analyses of variance indicated that most of the parameters measured were significantly ($P \geq 0.05$) affected by the treatments. Accordingly, dual inoculation with TAL-378 and PSB significantly increased plant height at harvest, number of nodules per plant, nodule volume per plant, nodule fresh weight per plant, and shoot height at late flowering and early pod setting compared to the other treatments. Similarly, co-inoculation with TAL-378 and PSB, and dual fertilization of nitrogen (N) and phosphorus (P) fertilizers significantly increased number of pods per plant, number of seeds per pod per plant and seed yield ha⁻¹ compared to the other treatments. Analysis of plant tissue also indicated that dual inoculation of TAL-379 and PSB yielded the highest total N whereas the lowest was recorded for the negative control. Likewise, dual inoculation with TAL-378 and PSB and the positive control gave the highest plant P compared to all other treatments. The data revealed that, there was no significant difference ($P \geq 0.05$) in days required for emergence, flower bearing and maturity, number of seeds per pod, 300 seeds weight and root length among the investigated treatments. Thus, the dual inoculation of TAL-378 and PSB is recommended to achieve maximum seed yield of soybean in Assossa area.

Keywords: Assossa, *Bradyrhizobium japonicum*, Phosphate solubilizing bacteria (PSB), Soybean (*Glycine max* L. (Merr.)).

INTRODUCTION

Soybean (*Glycine max* L. (Merr.)) is a subtropics member of the Leguminosae family, an erect bushy annual crop with considerable morphological diversity. Grain legumes are a good source of protein. The protein content in the pulses seed is high, ranging from 17%-42%. Legume seeds contain significant concentrations of minerals (calcium, zinc, iron) and vitamins (folic acid and vitamin B including riboflavin, thiamin and niacin). Soybean is

one of the most important oil seed crops in the world. It contains 18 to 22% oil, highly desirable in diet and has 40 to 42 % protein. Soybean protein provides all eight amino acids in the amount needed for human health; hence it is the best source of protein and oil and truly claims the title of the meat/oil on plants (Ali, 2010). Generally, soybean is used in the food industry for flour, oil, cookies, candy, milk, vegetable cheese, lecithin and many other products. At present, the USA has the largest area under soybean cultivation. Soybean is also grown in other parts of the world including Brazil,

¹ School of Natural Resources Management and Environmental Sciences, Haramaya University, Ethiopia, Dire Dawa, e-mail: antarga@yahoo.com



China, Argentina, Indonesia, Korea, and Japan. In Ethiopia soybean occupies around 95 thousand hectares of land with a corresponding gross annual production of approximately 94, 773 metric tons (CSA, 2009).

Despite the importance of the crop in the world as a rich source of protein and oil, the yield is generally very low in Ethiopia, i.e. below 1 ton ha⁻¹ (CSA, 2009) as compared to the USA and Asian soybean producing countries. Poor soil fertility status is considered as one of the factors contributing to low yield. Nitrogen (N) and phosphorus (P), in that order, are the two plants growth limiting soil fertility factors in many soil types including those in Ethiopia. The study monitored by FAO in 38 sub-Saharan African countries, including Ethiopia showed that there are high nutrient depletion rates in N, P and potassium in different farming systems (Stoorvogel *et al.*, 1993). To address this deficiency, farmers in Ethiopia have used the uniform blanket application of 100kg Diammonium phosphate (DAP) ha⁻¹ for all legumes plants including soybean. However, this did not result in any significant difference in soybean yields. Moreover, the utilization efficiency of phosphatic fertilizers by plants is only 20 to 25% largely due to its chemical fixation in soil (Hedley *et al.*, 1995). Unbalanced use of chemical fertilizers had led to a reduction in soil fertility and to environmental degradation (Gyaneshwar *et al.*, 1998) and the cost of chemical fertilizers has increased so that it is unaffordable for developing country farmers such as Ethiopia.

As a consequent, there has recently been a growing level of interest in environmentally friendly sustainable agricultural practices including organic farming systems (Rigby and Caceres, 2001; Lee and Song, 2007). For example, *Rhizobium* and phosphate solubilizing microorganisms would reduce the need for N and P chemical fertilizers and decrease adverse environmental effects. Therefore, in the development and implementation of sustainable agriculture

techniques, biofertilization is of great importance in alleviating environmental pollution and the deterioration of nature (Elkoca *et al.*, 2008).

The microsymbiont of soybean *Bradyrhizobium japonicum* is the highly efficient N fixer forming symbiotic association with soybean. According to Unkovich and Pate (2000), the amounts of N₂-fixed (kg ha⁻¹) by soybean have been up to 450 Kg N ha⁻¹. Thus soybean depends on its symbionts for a large part of its N requirements for effective growth and dry matter production. Phosphate solubilizing bacteria (PSB) have the capacity to convert insoluble phosphates into soluble forms for plant growth. Linu *et al.* (2009) reported that inoculation of PSB improved nodulation, root and shoot biomass, straw and grain yield and P and N uptake of the crop.

Studies carried out by Wasule *et al.* (2007) and Son *et al.* (2007) clearly revealed that co-inoculation of *Bradyrhizobium* and phosphate solubilizing microorganism significantly improved soybean growth and its yield components as compared with the sole application of *Bradyrhizobium* or phosphate solubilizing microorganism. Because in Ethiopia no work has been done on these lines, emphasis has been put on the possibility of the greater utilization of unavailable P forms in soil i.e. about 95-99% of soil P by the action of phosphate solubilizing microorganisms (PSM) and the utilization of huge amounts of atmospheric N through symbiotic N fixing microorganisms. In this regard, an attempt has been made to study the effects of co-inoculation of bradyrhizobia (*Bradyrhizobium japonicum*) and phosphate solubilizing bacteria (*Pseudomonas spp*) on nodulation, yield and yield components of soybean in Assossa area with the following objectives:

To evaluate the effect of dual inoculation of *Bradyrhizobium* and *Pseudomonase sp.* on soybean nodulation, yield and yield components.

To compare the effects of combined application of chemical fertilizers (N and P

fertilizers) and biological fertilizers (Bradyrhizobia and *Pseudomonas* sp.) on yield and yield components of soybean in Assossa area.

MATERIALS AND METHODS

Site Preparation

The experiment was conducted at Assossa Agricultural Research Station (altitude 1,480 m a.s.l., N09°58'41.7", E034°38'09.5"), 670 km far from capital city of Ethiopia, Addis Ababa, located in 'Benishangul Gumuz' region. The location has been under soybean plantation in the past year. The study was performed from June up to October 2008 cropping season.

Source of Isolates

Bradyrhizobium japonicum (TAL 378 and TAL 379) strains for soybean were obtained from NifTal project and Micen, Hawaii, USA type culture collection. Phosphate solubilizing bacterium (PSB) was isolated from Jimma acidic soil, as a superior *Pseudomonas* spp., from among all investigated isolates (Asfaw Hailemariam, 2003).

Inoculant Preparation

The bradyrhizobia isolates were grown in the yeast extract mannitol broth medium in 250 ml flasks shaken at 125 rpm at 25°C (Somasegaran and Hoben, 1994). When subculture reached mid log phase, sterile medium was used to dilute the inoculums to an OD₆₂₀ of 0.08 (equivalent to 10⁸ cells ml⁻¹) (Zhang *et al.*, 2003). Then the bradyrhizobial liquid was mixed with peat sterilized at 121°C for one hour, until the inoculums contained 50% moisture and rhizobial inoculant population of >10⁸ cells/g. Using plastic bags, the inoculums were then stored at room temperature.

Similarly, *Pseudomonas* spp. was grown in Pikovskaya's broth medium in 250 ml flasks shaken at 125 rpm at 25°C for 7 to 10 days to reach the bacterial population of >10⁸ cells/ml cultured broth (Kumar and Chandra, 2008). Then the cultured liquid was mixed with sterile peat, at 50% moisture and the inoculant population of >10⁸ cells gm⁻¹ of inoculums. Using plastic bags, the inoculums were stored at room temperature.

Field Layout

Treatments were arranged in a randomized complete block design with three replications, with a gross plot size of 3.6 m×4 m= 14.4 m² with six rows of plants and net plot size of 2.4 m×4 m= 9.6 m² with 4 harvestable rows. There were six treatments as listed below:

T₁= Negative control (without chemical and biofertilizers)

T₂= N (46 Kg of N ha⁻¹)

T₃= P (46 Kg P₂O₅ ha⁻¹)

T₄= Positive control (N (46 Kg of N ha⁻¹)+P (46 Kg P₂O₅ ha⁻¹))

T₅= TAL-378 (Bradyrhizobium japonicum)+PSB (*Pseudomonas* spp.)

T₆= TAL-379 (Bradyrhizobium japonicum)+PSB (*Pseudomonas* spp.).

The fertilizer sources used were trisuper phosphate (46 kg P₂O₅ ha⁻¹) and urea (46 kg ha⁻¹) fertilizers for N and P. The fertilizer levels were adopted from conventional farmers' fertilizer recommendation level. Both fertilizer types were applied and incorporated into the soil before seeding, where applicable as indicated in treatment combination. The spacing between rows and plants was 60 cm and 5 cm, respectively with 1m spacing between each block.

Planting Method

Seeds, surface sterilized by immersion in 70% (v/v) ethanol for one minute followed by three minutes in 4% (v/v) sodium hypochlorite and rinsed six times with



sterile distilled water as indicated in Somasegaran and Hoben (1994) were used. Surface sterilized and healthy seeds were soaked in sterilized distilled water for six hours. Then, the moistened seeds were coated by mixing them with strain inoculated peat carrier and were hand sown in the field.

The final harvestable population of soybean plants was 320 per plot. A sample of the bulk soil was air dried and ground to pass through a 2-mm hole size sieve before determining soil pH (1:2.5, soil:water ratio), electrical conductivity (EC) by conductivity meter, exchangeable Ca, Mg, K and Na extracted by ammonium acetate and determined using atomic absorption spectrophotometer and flame photometer; organic matter by chromic acid digestion, available soil P extracted by 0.5 M NaHCO₃ and determined using turbidimetric method, total soil N by Kjeldahl method and texture class determined by hydrometric procedure. All soil analysis determinations were performed according to methods described

in Sertsu and Bekele (2000). Texture and other soil properties of the sample soil are presented in Table 1.

Harvest and Data Collection

All plant samples in the experiment were taken by randomized uprooting of five plants in both sides of boarder rows from each plot. The first sampling was done at the late flowering and early pod setting for nodulation (nodule number, nodule volume and nodule fresh weight), shoot dry weight, shoot height and root length. Soil adhering to the roots was removed by washing with tap water. Nodules attached to each plant root were removed and separately evaluated for the nodulation parameters. The harvested shoots were oven dried at 70°C for at least 48h and weighed. The N contents of the dried shoots were determined using Kjeldahl analysis (Sertsu and Bekele, 2000). At maturity, all remaining plants from the middle four rows were harvested and number of pods per plant, number of seeds per pod, number of seeds per pods per plant, plant height, 300 seeds weight, and seed yield per hectare were determined. The days required for the emergence of seedlings, bearing flowers and maturity were also recorded.

Statistical Analysis

The collected data were subjected to analysis of variance (ANOVA) using General Linear Models Procedure of SAS software ver. 10 (Hatcher, 2003). Means of all treatments were calculated and the differences were tested for significance using the least significant differences (LSD) test at 0.05 probability (p) level. Correlation coefficients were calculated to study the associative relations among the measured traits using the Pearson correlations (Hatcher, 2003).

Table 1. Chemical, physical and biological characteristics of the soil used in this study.

Soil parameter	Assosa soil (value???)
pH(H ₂ O)	5.6
EC(ds m ⁻¹)	0.139
Soil texture class	Loam
Sand (%)	41
Silt (%)	36
Clay (%)	23
Na(Cmol(+) kg ⁻¹)	0.15
K(Cmol(+) kg ⁻¹)	0.7
Ca(Cmol(+) kg ⁻¹)	10.33
Mg(Cmol(+) kg ⁻¹)	5.67
CEC(Cmol(+) kg ⁻¹)	37.84
Base saturation (%)	45
Total nitrogen (%)	0.221
Organic carbon (%)	3.18
C/N ratio	14
Available P (mg kg ⁻¹)	4.3
Available potassium (mg kg ⁻¹)	121.2
MPN (number of rhizobia g ⁻¹ of soil)	<10 ¹

RESULTS AND DISCUSSION

Except mean of days to emergence, days to flowering, days to maturity and root length, all other parameters were significantly ($P \geq 0.05$) affected by the treatment (Table 2). Similar results were reported on soybean by Son, et al. (2006) and Abdalla and Omar (2001). They observed that the dual inoculation of *Bradyrhizobium japonicum* and phosphate solubilizing bacteria significantly increased nodulation, seed and biomass yield, nutrient uptake and symbiotic N fixation. Moreover, combined inoculation with N_2 -fixing and phosphate-solubilizing bacteria was found to be more effective than inoculation with single microorganism by providing a more balanced nutrition for plants (Belimov *et al.*, 1995). Dual inoculation increased yields in sorghum (Algawadi and Gaur, 1992), barley (Belimov *et al.*, 1995), and black gram (Tanwar *et al.*, 2002).

Plant Height at Harvest

As shown in Table 2, plant height (cm) at harvest ranged from 42.53 to 71.1 cm for the negative control, and for the treatment inoculated with both TAL-378 (*Bradyrhizobium japonicum*) and PSB (*Pseudomonas sp.*), respectively. The maximum plant height at harvest was related to dual inoculated plant with TAL-378+PSM. It was significantly ($P \leq 0.05$) higher than the negative control and the N only treated plot. However, it was not significantly different from the following treatments: P, N+P, P and dual inoculation with TAL-379+PSB. Similarly, dual inoculation significantly increased the plant height in wheat by 23.5% over the negative control (Aftab Afzal and Asghari Bano, 2008). Also, El-Azouni (2008) stated that phosphate solubilizing fungi inoculated soybean plants scored significantly higher plant heights which

was 81% over the non-inoculated treatment.

Nodulation

The present study indicated that, inoculation of *Bradyrhizobium japonicum* significantly increased the number of nodule, nodule volume per plant and nodule fresh weight per plant as compared to the uninoculated plants. The highest number of nodules per plant was recorded in dual application of *Bradyrhizobium* (TAL-378) and PSB, which scored 74.67 nodules per plant followed by TAL-379+PSB (20 nodules per plant). On the other hand, treating with N+P, P and N fertilizers did not significantly affect the number of nodules per plant. Except for the P treated plots, all of other plots treated with chemical fertilizers scored less than 2 nodules per plant. The positive effect of inoculation with TAL-378+PSB on nodulation could indicate the lack of compatible *Bradyrhizobium japonicum* strain in the Ethiopian soil, limiting production of soybean in some parts of Ethiopia (Woldemeskel, 2007; Hailemariam, personal communication).

Nevertheless, P treatment, even if there was no significant difference on nodulation compared to the negative control, resulted in relatively higher nodulation than the sole N and dual N and P treated plants. The supply of P to host plant stimulated the multiplication of rhizobia and essentially their movement through the soil towards the root system thereby improving the nodulation (White, 1953; Madhok, 1961). Comparing the co-inoculated treatments, the TAL-378+PSB resulted in significantly higher nodulation than plants treated with TAL-379+PSB. The data indicated that, nodule number was positively correlated with number of pods per plant ($r = 0.50$, $P < 0.05$), seed yield per hectare ($r = 0.58$, $P < 0.05$), plant total N ($r = 0.57$, $P < 0.05$), plant P ($r = 0.50$, $P < 0.05$), nodule volume ($r = 0.99$, $P < 0.0001$), and nodule fresh weight ($r = 0.99$, $P < 0.0001$).



Table 2. Response of soybean to co-inoculation with *Bradyrhizobium japonicum* and phosphate solubilizing bacteria (*Pseudomonas* sp.) investigated at the time of flowering stage.

Treatment	Days to emergence	Days to flowering	Days to maturity	Plant height at harvest (cm)	Nodule number per plant	Nodule volume per plant (cm ³)	Nodule fresh weight per plant (mg)	Root length (cm)	Shoot height (cm)
N (Urea)	12.7±0.58a	79.3±3.21a	150.0±3.21a	49.6 ±4.99b	0.3±0.58c	0.013 ±0.011c	0.013±0.011c	19.3±2.52a	3.9±0.53b
P (TSP)	13.0±0.57a	81.0±1.00a	150.0±1.54a	67.5 ±4.81a	6.7± 3.06bc	0.667 ±0.306bc	0.500±0.230bc	22.3±1.53a	4.5±0.24ab
N (Urea)+P (TSP)	13.0±1.00a	82.0±4.58a	150.3±0.58a	71.0 ±6.00a	2.0±2.00c	0.100 ±0.100c	0.137±0.172c	21.3±1.53a	5.2±0.31a
TAL-379+PSB	12.7±1.54a	80.7±4.62a	149.3±2.08a	68.6±2.48a	20.0±5.00b	1.866±0.503b	1.950±0.644b	23.7±6.65a	4.8±1.35ab
TAL-378+PSB	13.0±2.00a	82.3±4.61a	150.0±1.00a	71.1±6.929a	74.7±19.30a	6.330±2.081a	6.990±1.943a	23.3±4.04a	5.1±0.34a
Negative control	12.3±0.58a	82.3±3.79a	149.0±1.00a	42.5±2.50b	1.0±0.00c	0.233±0.152c	0.197±0.240c	20.7±1.53a	3.9±0.15b
MSE	1.20	2.68	1.30	5.22	7.85	0.90	0.85	2.73	0.66
CV (%)	9.58	3.30	0.86	8.45	45.01	58.47	52.06	12.54	14.67
LSD	2.18	4.88	2.35	9.50	14.29	1.63	1.54	4.97	1.19

Data are means of three replicates ±SE.

Same letters are not significantly different at LSD $P < 0.05$ level.

Different letters within a column indicate significant difference.

Similar effects were obtained in nodule volume per plant and nodule fresh weight per plant. The highest nodule volume per plant and nodule fresh weight per plant were obtained in TAL-378+PSB (6.33 ml plant⁻¹) followed by TAL-379+PSB (1.866 ml plant⁻¹), but the remaining treatments scored statistically lower nodule volumes than the inoculated treatments. Furthermore, nodule volume per plant was positively correlated with seed yield per hectare ($r= 0.56$, $P< 0.05$), plant total N ($r= 0.58$, $P< 0.05$) and plant P ($r= 0.49$, $P< 0.05$)

TAL-378+PSB resulted in significantly higher nodule fresh weight (6.99 mg plant⁻¹) than all other treatments. Similarly, TAL-379+PSB scored significantly higher nodule fresh weights (1.95 mg/plant) than the N treated, N and P treated and negative controls. Data indicated that inoculation with TAL-379+PSB did not significantly increase nodule number; nodule fresh weight and nodule volume per plant compared with plants fertilized with an optimum amount of P fertilizer alone. This could be due to the fact that P enhanced the formation of higher nodule number, nodule fresh weight and nodule volume and it has direct positive effects on the indigenous rhizobia found in the rhizosphere itself (Linu *et al.*, 2009). It is known that every aspect of N₂ fixing nodule formation is limited by the availability of P (McDermott, 1999). Nodule fresh weight was positively correlated with number of pods per plant ($r= 0.42$, $P< 0.05$), seed yield per hectare ($r= 0.57$, $P< 0.05$), plant total N ($r= 0.59$, $P< 0.05$) and plant P ($r= 0.50$, $P< 0.05$).

Shoot Height at Late Flowering and Early Pod Setting Stage

Inoculation with TAL-378+PSB and fertilization with N and P fertilizers resulted in significantly higher shoot height than the negative control and N fertilized plants. Plants fertilized with N scored statistically similar shoot height to the negative control.

Inoculation with TAL-378+PSB increased shoot height by 30.5% over the negative control. Similarly, in plants fertilized with N and P fertilizers, this was increased by 32.6% as compared to the negative control. Moreover, shoot height was positively correlated with root length ($r= 0.83$, $P< 0.0001$) and plant P ($r= 0.55$, $P< 0.05$).

Yield and Yield Components

The positive effect of dual inoculation was clearly observed on number of pods per plant, number of seeds per plant and seed yield per hectare. Co-inoculation with TAL-378+PSB and dual fertilization of N and P significantly increased ($P\leq 0.05$) number of pods per plant, number of seeds per pod per plant and seed yield per hectare as compared to the uninoculated negative control which can positively contribute to the grain yield of soybean. This might be the result of the microorganisms involved in P solubilization, which can enhance plant growth by increasing the efficiency of biological fixation, enhancing the availability of trace elements and by the production of plant growth promoting substances (Gyaneshwar *et al.*, 1998).

The number of pods per plant ranged from 61.83 pods per plant in plants fertilized with N and P chemical fertilizers to 27.57 pods per plant (the negative control). Inoculation with TAL-378+PSB increased number of pods per plant by 112.7% over the negative control, 47.6% as compared to N fertilized plants, 89.4% over the plants inoculated with TAL-379+PSB, and 90.1% over the plant fertilized by P fertilizer alone. However, there was no significant difference in pods per plant between the N+P fertilized plants and plants inoculated with TAL-378+PSB. This could be due to the fact that dual inoculation with TAL-378+PSB provided adequate nitrogen N and P nutrients relative to the chemical fertilizers (Akhtar *et al.*, 1988). Number of pods per plant was positively correlated with nodule volume



($r = 0.47$, $P < 0.05$), nodule fresh weight ($r = 0.48$, $P < 0.05$) and plant P ($r = 0.74$, $P < 0.001$). Nodulation showed positive correlation with yield components such as number of pods per plant as a result of *Bradyrhizobium japonicum* inoculation, which in turn reduces the use of P fertilizer in soybean production (Son *et al.*, 2006).

Inoculation with TAL-378+PSB and dual fertilization of N and P fertilizers recorded significantly higher numbers of seeds per pod per plant than the other treatments. Inoculation with TAL-378+PSB increased number of seed per pod per plant by 125.6% over the negative control, 46.2% as compared to N fertilized, 90.45% over co-inoculated with TAL-379+PSB, and 89.4% as compared to P fertilized plants. Number of seeds per pod per plant was positively correlated with plant height at harvest ($r = 0.53$, $P < 0.05$) and plant P ($r = 0.88$, $P < 0.0001$). A positive correlation between seed yield and plant P indicates the beneficial effect of PSB in increasing the yield of soybean and reducing the requirement of phosphatic fertilizers (Dubey, 1996).

Of the treatments tested, only TAL-378+PSB scored significantly higher seed yield per hectare than the negative control and N treated plants. But the data did not reveal significant difference ($P \leq 0.05$) in seed yield per hectare among different treatments such as N and P treated plants, P fertilizer treated plants, TAL-379+PSB and TAL-378+PSB. Inoculation with TAL-378+PSB increased seed yield per hectare by 36.3% over the negative control, 34.7% over N fertilized, 16.7% as compared to TAL-379+PSB, 9.8% over P fertilized; and 16% over N and P fertilized plants. In general, the plots treated with TAL-378+PSB indicated significantly higher yield and yield components as compared to the negative control, similar to the results obtained by Zhang *et al.*, (2003) and Agha *et al.* (2004). Zhang *et al.* (2002) pointed out that the increasing yield was due to the formation of more pods per plant and more seeds per plant.

Plant Total N and P

According to the data presented in Table 3, dual inoculation of *Bradyrhizobium japonicum* and *Pseudomonas* sp. was the most effective in increasing plant total N and plant P as compared to the negative control. The highest plant total N was resulted by TAL-379+PSB (4.047%) followed by TAL-378+PSB (3.863%) whereas the lowest was recorded by the negative control (3.11%). This is as a result of the increased available P content of the soil due to the inoculation of PSB thereby increasing symbiotic effectiveness of *Bradyrhizobium japonicum* and the amount of N fixed (Athul R. Sandeep *et al.*, 2008). The N fixation process is energy demanding, consuming about 16ATP synthesis from orthophosphate absorbed from the soils to fix one molecule of N_2 (Giller, 2001). Dual inoculations with TAL-378+PSB and TAL-379+PSB resulted in significantly higher plant total N than all the other treatments except for plants fertilized with N and P fertilizers.

Similarly, there were significant differences ($P \leq 0.05$) in plant P resulted by different treatments. Inoculation with TAL-378+PSB and dual fertilization of N and P fertilizers resulted in significantly higher plant P than other investigated treatments. P uptake increased in dual inoculation of TAL-378+PSB mainly as a result of P solubilizing microorganisms, which in addition to solubilizing P, produce a necessary phytohormone, indole 3 acetic acid (Datta *et al.*, 1982) thereby enhancing root growth and increasing nutrient uptake (Piccini and Azcon, 1987). Co-inoculation with TAL-379+PSB resulted in significantly higher plant P than the negative control but the data did not indicate any significant differences ($P \leq 0.05$) in plant P among N and P fertilized plants and the negative control.

Table 3. Response of soybean to co-inoculation with *Bradyrhizobium japonicum* and phosphate solubilizing bacteria (*Pseudomonas* sp.) investigated at the time of harvesting.

Treatment	Number of pods per plant	Number of seeds per pod	Number of seeds per pod	Number of seeds per pod	300 seeds weight (g)	Seed yield ha ⁻¹ (Kg)	Plant total Nitrogen (%)	Plant P (mg/kg)
N (Urea)	35.4±7.58b	72.6±17.06b	2a	45.0±2.65a	1653.2±301.1b	1820.0±131.2bc	3.140 ±0.092c	
P (TSP)	27.5±4.73b	56.1±9.29bc	2a	48.7±1.16a	2007.6±211.9ab	1890.0±65.6bc	2.983±0.136c	
N (Urea) + P (TSP)	61.8±9.28a	120.0±20.00a	2a	46.7±1.15a	1912.5±205.1ab	2993.3±212.0a	3.597±0.210b	
TAL-379 + PSB	27.6±1.48b	55.8±2.64bc	2a	47.0±2.65a	1907.4±368.1ab	2100.0±87.2b	4.047±0.306a	
TAL-378 + PSB	52.3±6.52a	106.2±11.03a	2a	48.3±2.52a	2226.7±224.5a	2760.0±415.7a	3.863±0.302ab	
Negative control	24.6±9.99b	47.1±3.30c	2a	44.7±4.16a	1634.2±92.8b	1653.3±140.5c	3.110±0.095c	
MSE	9.10	11.30	0	2.36	271.34	209.30	0.21	
CV (%)	25.10	14.81	0	5.06	14.35	9.50	5.98	
LSD	16.55	20.56	0	4.30	493.63	380.77	0.38	

Data are means of three replicates ±SE.

Same letters are not significantly different at LSD $P < 0.01$ level.

Different letters within a column indicate significant difference.



CONCLUSIONS

The obtained data showed that the application of bradyrhizobia (*Bradyrhizobium japonicum*) and phosphate solubilizing bacteria (*Pseudomonas sp.*) significantly increased the number and dry weight of nodules, yield components, grain yield, plant total N and P uptake of compared to the negative control. Even if there was no significant difference ($P \leq 0.05$) between dual inoculation of TAL-378+PSB (*Pseudomonas sp.* isolated from Jimma soil) and the positive control fertilized with optimum N and P fertilizers, dual inoculation (TAL-378+PSB) resulted in improved performance of seed yield (kg ha^{-1}) as compared to the positive control. Besides the yield advantage, N and P uptake were also improved under TAL-378+PSB as compared to sole application of N or P fertilizers. Therefore, we concluded that, dual inoculation of TAL-378+PSB was found to be beneficial over chemical fertilizers and would be recommended as biological fertilizer for soybean production in Assossa area. In addition, for other parts of the country, similar experiments should be carried out to increase the yield of soybean, protect the environment from chemical pollutants and increase farmers' income through decreasing the production cost of soybean.

REFERENCES

1. Abdalla, M. H. and Omer, S. A. 2001. Survival of Rhizobia/Bradyrhizobia and a Rock Phosphate Solubilizing Fungus *Aspergillus niger* on Various Carriers from Some Agro-Industrial Wastes and their Effect on Nodulation and Growth of Fababean and Soybean. *J. Plant Nurt.*, **24**: 261–272.
2. Aftab, A. and Asghari, B. 2008. *Rhizobium* and Phosphate Solubilizing Bacteria Improve the Yield and P Uptake in Wheat (*Triticum aestivum*). *Inter. J. Agric. Biol.*, **10**: 85-88.
3. Agha, S. K., Oad, F. C. and Buriro, U. A. 2004. Yield and Yield Components of Inoculated and Un-inoculated Soybean under Varying Nitrogen Levels. *Asian J. Plant Sci.*, **3(3)**: 370-371.
4. Akhtar, M., Ahmed, Z. I. and Nazir, N. 1988. Effect of NPK Application on Seed Yield and Quality of Soybean, *J. Agric. Res.*, **26**:129-134.
5. Alagawadi, A. R. and Gaur, A. C. 1992. Inoculation of *Azospirillum brasilense* and Phosphate-Solubilizing Bacteria on Yield of Sorghum (*Sorghum bicolor* L. Moench) in Dry Land. *Tropical Agric.*, **69**: 347–50.
6. Ali, N. 2010. Soybean Processing and Utilization. In: "*The Soybean Botany, Production and Uses*", (Ed.): Singh, G.. CABI International, UK, PP. 345-374.
7. Hailemariam A., 2003. Isolation, Identification and Characterization of Phosphate Solubilizing Microorganisms (PSM) from Ethiopian Soils. *EJAS*, **5(1)**: 57-68.
8. Athul R. Sandeep, Joseph S. and Jisha, M. S. 2008. Yield and Nutrient Uptake of Soybean (*Glycine max* L.) as Influenced by Phosphate Solubilizing Microorganisms. *World J. Agric. Sci.*, **4(S)**: 835-838.
9. Belimov, A. A., Kojemiakov, P. A. and Chuvarliyeva, C. V. 1995. Interaction between Barley and Mixed Cultures of Nitrogen Fixing and Phosphate-Solubilizing Bacteria. *Plant Soil*, **17**: 29–37.
10. Central Statistical Authority (CSA). 2009. *Agricultural Sample Survey. 2008/9 Report on Area and Production for Major Crops (Private Peasant Holdings, Main Season)*. Addis Ababa, Ethiopia, PP.45-47.
11. Datta, M. S., Banik M. and Gupta, R. K. 1982. Studies on the Efficacy of a Phytohormone Producing Phosphate Solubilizing *Bacillus firmis* in Augmenting Paddy Yield in Acid Soils of Nagaland. *Plant Soil*, **69**: 365-373.
12. Dubey, S. K. 1996. Combined Effect of *Bradyrhizobium japonicum* and Phosphate Solubilizing *Pseudomonas striata* on Nodulation Yield Attributes and Yield of Rainfed Soybean (*Glycine max*) Under Different Sources of P in Vertisols. *Indian J. Agric. Sci.*, **66**: 28-32.
13. El-Azouni, I. 2008. Effect of Phosphate Solubilizing Fungi on Growth and Nutrition Uptake of Soybean (*Glycine max* L.) Plants. *J. Appl. Sci. Res.* **4(6)**: 592-598.

14. Elkoca, E., Kantar, F. and Fiahin, F. 2008. Influence of Nitrogen Fixing and P Solubilizing Bacteria on the Nodulation, Plant Growth, and Yield of Chickpea. *J. Plant Nutr.*, **31**: 157-171.
15. Wolde-meskel, E. 2007. Genetic Diversity of Rhizobia in Ethiopian Soil: their Potential to Enhance Biological Nitrogen Fixation (BNF) and Soil Fertility for Sustainable Agriculture. *Ethi. J. Biol. Sci.*, **6(1)**: 77-95
16. Giller, K. E. 2001. *Nitrogen Fixation in Tropical Cropping Systems*. 2nd Edition, CABI Publishing, Wallingford, UK, PP. 150-151.
17. Gyanerswar, P., Nareshkumar, G. and Parckh, L. D. 1998. Effect of Buffering on the Phosphate Solubilizing Ability of Microorganisms. *World J. Microbiol. Biotechnol.*, **14**: 669-673.
18. Hatcher, L. 2003. *Step by Step Basic Statistics Using SAS: Exercises*. SAS Institute INC., Cary, NC, USA.
19. Hedley, M. J., Mortvedt, J. J., Bolan, N. S. and Syers, J. K. 1995. P Fertility Management in Agroecosystems. In: "*P in the Global Environment, Transfers, Cycles and Management*", (Ed.): Tiessen, H. pp. 59-92.
20. Kumar, R. and Chandra, R. 2008. Influence of PGPR and PSB on *Rhizobium leguminosarum* bv. *Viciae* Strain Competition and Symbiotic Performance in Lentil. *World J. Agric. Sci.*, **4(3)**: 297-301.
21. Lee, J. and Song, S. H. 2007. Evaluation of Groundwater Quality in Coastal Areas: Implications for Sustainable Agriculture. *Environ. Geol.*, **52**: 1231-1242.
22. Linu, M. S., Stephen, J. and Jisha, M. S. 2009. Phosphate Solubilizing *Gluconacetobacter* sp., *Burkholdia* sp. and their Potential Interaction with Cowpea (*Vigna unguiculata* (L.). *Intern. J. Agric. Res.*, **4(2)**: 79-87.
23. Madhok, M. R. 1961. Mineral Nutrition of Legumes. Proc. of the Symposium on Radioisotopes, Fertilizers and Cowdung Gas Plant, ICAR. PP.115-121
24. McDermott, T. R. 1999. P Assimilation and Regulation in Rhizobia. In: "*Nitrogen fixation in Prokaryotes; Molecular and cellular Biology*", (Ed.): Triplett Hurizon, E. W.. Scientific Press, USA, PP.75-81.
25. Piccini, D. and Azcon, R. 1987. Effect of Phosphate Solubilizing Bacteria and Vesicular Arbuscular Mycorrhizal Fungi on the Utilization of Bayovar Rockphosphate by Alfalfa Plants Using a Sand Vermiculite Medium. *Plant Soil*, **101**: 45-50.
26. Rigby, D. and Caceres, D. 2001. Organic Farming and the Sustainability of Agricultural Systems. *Agric. Sys.*, **68**: 21-40.
27. Sertsu S. and Bekele T. 2000. *Procedures for Soil and Plant Analysis*. National Soil Research Center. EARO, Addis Ababa, Ethiopia, PP.15-23.
28. Somasegaran, P. and Hoben, H. J. 1994. *Handbook for Rhizobia—Methods in Legume Rhizobium Technology*. Springer-Verlag, Heidelberg, Germany, PP.10-52.
29. Stoorvogel, J. J., Smaling, E. M. A. and Jansen, B. H. 1993. Calculating Soil Nutrient Balances in Africa at Different Scales. I. Supra-National Scale. *Fert. Res.*, **35**: 227-235.
30. Tanwar, S. P. S., Sharma, G. L. and Chahar, M. S. 2002. Effect of P and Biofertilizers on the Growth and Productivity of Black Gram. *Ann. Agric. Res.*, **23**: 491-493.
31. Son, T. T. N., Diep, C. N. and Giang. T. T. M. 2006. Effect of Bradyrhizobia and Phosphate Solubilizing Bacteria Application on Soybean in Rotational system in the Mekong Delta. *Omonrice*, **14**: 48-57.
32. Unkovich, M. J. and Pate, J. S. 2000. An Appraisal of Recent Field Measurements of Symbiotic N₂ Fixation by Annual Legumes. *Field Crop Res.*, **65**: 211-228.
33. Wasule, D. L., Wadyalkar, S. R., and Buldeo, A. N. 2007. Effect of Phosphate Solubilizing Bacteria on Role of *Rhizobium* on Nodulation by Soybean. In: "First International Meeting on Microbial Phosphate Solubilization", (Eds.): Velazquez, E. and Rodriguez-Barrueco, C. PP.139-142.
34. White, R. O. 1953. *Legumes in Agriculture*. FAO, Rome, Italy, PP: 183-185.
35. Zhang, H. C., Charles, B. T. Driscoll, B., Prithiviraj, H. and Smith, D. L. 2002. Low Temperature-Tolerant *Bradyrhizobium japonicum* Strains Allowing Improved Soybean Yield in Short-Season Areas. *Agron. J.*, **94**: 870-875.
36. Zhang, H., Prithiviraj, B., Charles, T. C., Driscoll, B. T., and Smith, D. L. 2003. Low Temperature Tolerant *Bradyrhizobium japonicum* Strains Allowing Improved Nodulation and Nitrogen Fixation of Soybean in a Short Season (cool spring) Area. *European J. Agron.*, **19**: 205-213.



ارزیابی اثر تلقیح همزمان *Bradyrhizobium japonicum* و باکتری حل کننده فسفات *Pseudomonas spp.* بر گیاه سویا (*Glycine max L. (Merr.)*) در ناحیه آسوسا

۱. آرگاو

چکیده

به منظور مطالعه اثرات تلقیح همزمان (*Bradyrhizobium japonicum* (TAL-378 and TAL-379) و باکتری حل کننده فسفات *Pseudomonas spp.* و میزان کود رایج کشاورزی (کاربرد جداگانه و ترکیبی 46N kg ha^{-1} و $46\text{P}_2\text{O}_5\text{ kg ha}^{-1}$) بر غده سازی، عملکرد دانه و اجزای عملکرد گیاه سویا (*Glycine max L. (Merr.)*) آزمایشی در مرکز تحقیقات کشاورزی آسوسا (AARC) به انجام رسید. تجزیه تحلیل واریانس نشان داد که بیشتر پارامترهای اندازه گیری شده به صورت معنی داری ($P \geq 0.05$) تحت تاثیر تیمارها واقع شدند. تلقیح دوگانه با TAL-378 و PSB، ارتفاع گیاه در زمان برداشت، تعداد غده ها در هر گیاه، حجم غده در هر گیاه، وزن غده تازه در هر گیاه و ارتفاع جوانه در پایان گلدهی و ابتدای تشکیل غلاف را نسبت به دیگر تیمارها بیشتر افزایش داد. همچنین، تلقیح همزمان با TAL-378 و PSB و کوددهی دوگانه با کودهای نیتروژن (N) و فسفر (P) تعداد غلاف در هر گیاه، تعداد دانه در هر غلاف در هر گیاه و عملکرد دانه در هکتار را نسبت به دیگر تیمارها بیشتر افزایش دادند. بررسی بافت گیاه نیز نشان داد که تلقیح همزمان با TAL-378 و PSB بیشترین نیتروژن کل را باعث شد در حالی که کمترین مقدار آن در تیمار شاهد منفی مشاهده گردید. همینطور، تلقیح همزمان با TAL-378 و PSB تیمار شاهد مثبت در مقایسه با سایر تیمارها بیشترین فسفر گیاه را موجب شدند. داده ها نشان دادند که بین تیمارهای بررسی شده از نظر روزهای لازم برای جوانه زنی، گلدهی و بلوغ، تعداد دانه در هر غلاف، وزن ۳۰۰ دانه و طول ریشه تفاوت معنی داری ($P \geq 0.05$) وجود ندارد. بنابراین، تلقیح همزمان با TAL-378 و PSB جهت دستیابی به حداکثر عملکرد دانه سویا در ناحیه آسوسا توصیه می شود.