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SOIL SCIENCE

Influence of different types of Strains of *Bradyrhizobium japonicum* Inoculation and Phosphorus Fertilizer Rates on Nitrogen Uptake Efficiency and N_2 Fixation of Soybean (*Glycine max* L. (Merrill)) in Ethiopia

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ABSTRACT

Soybean production in western Ethiopia particularly at Bako has remained low, partly due to soil nutrient depletion and degradation, which is considered serious threats to agricultural productivity and food security. This study was conducted to assess the effect of Bradyrhizobium japonicum strains and phosphorus rates on nitrogen uptake efficiency, N2 fixation of soybean, seed yield, yield components, yield and quality to enhance soybean production and yeild. Treatments included factorial combinations of four Bradyrhizobium japonicum strains with un-inoculated, (Soybean murodk, Soybean 12 and Soybean MAR-1495) and four phosphorus levels (0, 23, 46 and 69 kg P_2O_5 ha^{-1}) which were laid out in Randomized Complete Block Design (RCBD) with three replications. Interaction of Phosphorus and Bradyrhizobium inoculation, revealed that application of 69 kg P_2O_5 ha^{-1} with Soybean 12 inoculation significantly resulted enhanced leaf area (64.2 cm^2), and above ground biomass (8241 kg ha^{-1}), whereas Soybean MAR-1495 strain inoculation produced highest harvest index (48.77% $plant^{-1}$). Among all treatments, combined application of Soybean 12 along with 46 kg of P_2O_5 ha^{-1} gave better performance of soybean with highest seed yield. While, phosphorus rates at 46 and 69 kg P_2O_5 ha^{-1} with Soybean 12 inoculation resulted in maximum leaf area index of 0.157 $plant^{-1}$. Hence, 46 kg P_2O_5 ha^{-1} phosphorus rate with Soybean 12 inoculation was found to be economical and superior in yield.

Highlights

- Days to 50% flowering and physiological maturity, leaf area, leaf area index, above ground biomass yield, seed yield and harvest index of soybean were increased with combined application of *B. japonicum* and phosphorus rates.
- Combined application of soybean 12 plus 46 kg of P_2O_5 ha^{-1} gave better performance in soybean, with maximum leaf area and seed yield.

Keywords: Bradyrhizobium japonicum, Phosphorus, Soybean, Yield

In the International trade market, soybean ranks number one among the major oil crops with an average protein contents of 40% on dry matter basis, and it is second only to groundnut in terms of oil content 20% (Collombet 2013) and carbohydrates content (20 - 26%) in addition to some nutrients and vitamins among the food legumes (Ahmad *et al.* 2010). The oil obtained from the crop is mostly unsaturated fatty acids which is free from cholesterol. Dugje *et al.* (2009) reported that soybean is richer in protein than any of common vegetable or legume food sources in Africa. It is used as a good source of unsaturated fatty acids, minerals (Ca and P) and vitamins A, B, C and D (Alam *et al.* 2009). Soybean is a crop that can play major role as protein source for resource poor farmers of Ethiopia who cannot afford animal products. Besides, it can also be used as oil crop, animal feed, poultry meal, for soil fertility improvement and more importantly as income for the country (NSRL, 2007).

In Oromia region in Ethiopia, 9,611.04 ha land was cultivated with production of 22,300.63 tons with the productivity of 2.32 tons ha^{-1}; which is low as compared to world average of 2.6 tons ha^{-1} (Akpalu *et al.* 2014). In west Shewa Zone, the crop has good potential particularly at Bako area, however its productivity is low (1.9 tons ha^{-1}). Availability of phosphorus in the soil influences the efficiency of *Rhizobium* that fixes atmospheric nitrogen in association with nodulating legumes as it is directly involved in biological nitrogen fixation via *Bradyrhizobium japonicum*. This has attracted considerable research attention world-wide due to its economic viability for resource poor farmers and environmental friendliness. In fact, soybean is estimated to fix 80% of its nitrogen needs (Smaling *et al*. 2008). Soybean N_2-fixation is beneficial as it provide necessary N to the plant from the atmosphere which otherwise would proceed from soil and manure (Chianu *et al*. 2011). The fixation of soybean as much as 300 kg of N ha^{-1} in addition to the release in the soil, of 20 - 30 kg N ha^{-1} for the following crop had been estimated (Hungria *et al*. 2006). To improve soybean yield, biological nitrogen fixation, contribution to soil fertility restoration, inoculation with efficient strains of *Bradyrhizobia* has already been tested in several countries (Tairo and Ndakidemi 2014). Objectives of the study was to assess the effect of different strains of *Bradyrhizobium japonicum* and to determine optimal phosphorus rates on growth, nodulation, uptake of nitrogen and phosphorus fertilizers, yield components and yield of soybean.

MATERIALS AND METHODS

Field experiment was carried out in Bako Agricultural Research Center (BARC) research station, Oromia National Regional State, during 2018 main cropping season. High yielding soybean variety Jalele-AGS-217 was used in the study. Crop specific carrier based *B. japonicum* strains namely, Soybean murodk, Soybean12 and Soybean MAR-1495 were utilized. Triple superphosphate (TSP) consisting 46% P_2O_5 was applied in row basis as per the treatment and mixed with soil just at the time of planting. Treatments consisted two factors namely four levels of phosphorus fertilizer (0, 23, 46 and 69 P_2O_5 kg ha^{-1}), and three different types of *Bradyrhizobium japonicum* strains inoculants and control (Un-inoculated, Soybean murodk, Soybean 12 and Soybean MAR-1495). Treatments were arranged in 4 4 in factorial combination in randomized complete block design (RCBD) replicated thrice.

RESULTS AND DISCUSSION

Root Length

The main effect of *B. japonicum* inoculation and phosphorus rates, and their interaction effects had highly significant (P 0.01) influenced on soybean root length (Table 1). The longest root of soybean crops (29.45 cm $plant^{-1}$) was obtained from application of 69 kg P_2O_5 ha^{-1} plus Soybean 12 strain that statistically at par with seeds inoculated with similar strain with 46 kg P_2O_5 ha^{-1} (28.92 cm $plant^{-1}$) and seeds inoculated with Soybean MAR-1495 strain combined with 46 and 69 kg P_2O_5 ha^{-1} (27.77 and 27.92 cm $plant^{-1}$) respectively (Table 1). The lowest root length (13.13 cm $plant^{-1}$) was recorded on control treatment (Table 1). However, when

Table 1: Interaction effects of *B. japonicum* strains and phosphorus fertilizer rates on root length (cm $plant^{-1}$) of soybean

Phosphorus (P_2O_5 kg ha^{-1})	***Bradyrhizobium japonicum* strains**				
	Un-inoculated	**SB Murodk**	**Soybean 12**	**SB MAR-1495**	**Mean**
0	13.13^{f}	22.28^{cde}	23.73^{bcd}	23.88^{bcd}	20.76
23	19.29^{e}	22.38^{cde}	22.40^{cde}	21.10^{de}	21.29
46	25.72^{abc}	25.27^{abcd}	28.92^{a}	27.77^{ab}	26.92
69	26.3^{7ab}c	25.50^{abc}	29.45^{a}	27.92^{ab}	27.31
Mean	21.13	23.86	26.13	25.17	24.07
LSD (0.05)	3.69				
CV (%)	9.20				

Values with the different letter (s) in a column and row are significantly different (P 0.05).

phosphorus fertilizer at rates of 46 and 69 kg P_2O_5 ha^{-1} either with all strains of *B. japonicum* or without all strains of *B. japonicum* inoculation on all eight treatment parameters of root length was statistically at par each other. Thus, both rates 46 and 69 kg of P_2O_5 ha^{-1} with Soybean 12 strain increased soybean root length by 120 and 124 % respectively, compared to un-treated control treatment. The presence of Phosphorus in the combined application of phosphorus with *B. japonicum* strains inoculation might be improved the length of soybean tap roots. In line with the study, Tahir *et al.* (2009) stated that the combination of *Rhizobium* inoculation, 25 kg N ha^{-1} and 90 kg P_2O_5 ha^{-1} increased soybean root length by 96% over the control treatment. In general application of reduced level of nitrogen as starter fertilizer at planting together with application of phosphorus fertilizer for root growth and development, and *B. japonicum* strains inoculation for nodulation collectively enhanced plant growth and development.

Root Fresh Weight

Analysis of variance indicated that the main effect of *B. japonicum* strains inoculation and phosphorus rates, and their interaction effects had highly significant (*P 0.01*) influence on soybean root fresh weight. Root fresh weight of soybean was significantly differed in interaction of phosphorus rates and *B. japonicum* strains (Table 2). The largest (7.6 gm $plant^{-1}$) and lowest (2.50 gm $plant^{-1}$) mass of root fresh weight was obtained from combination 69 kg P_2O_5 ha^{-1} with Soybean 12 strain and un-treated control treatment respectively (Table 2). Application of Phosphorus at the rate of 69 kg P_2O_5 ha^{-1} plus Soybean 12 strain increased mean of soybean root fresh weight by 204% compared to control treatment. This is due to the application of *B.japnicum* and phosphorus independently as well as control treatment have no more contribution on mass of soybean root fresh weight as their interaction effects. Root fresh biomass increased with increasing application of phosphorus rates plus *B. japonicum* inoculants. This finding is in line with Badar *et al.* (2015) who observed a significant increase in root fresh weight of peanut plants (*Arachis hypogea* L.) 60 kg DAP ha^{-1} plus *Rhizobial* inoculant gives 1 gm $palnt^{-1}$, whereas the lowest root fresh weight of peanut plant (0.2 gm $plant^{-1}$) was registered on control treatment.

Root Dry Weight

The main effect of *B. japonicum* inoculation and phosphorus rates, and their interaction were significantly (*P* 0.01) affected soybean root dry weight. The maximum (2 gm $plant^{-1}$) root dry weight was observed on the combination of 69 kg P_2O_5 ha^{-1} plus Soybean 12 strain, whereas the least root dry weight (0.42 gm $plant^{-1}$) was recorded from control treatment (Table 3). The highest root dry weight registered where Soybean 12 strain with 69 kg P_2O_5 ha^{-1} increased by 3.76 folds over control treatment. Like root fresh weight, application of phosphorus rates with *B. japonicum* might have lead to increased root growth of the soybean that confirms the improvement of root dry weight of soybean. The growth may be attributed to increase in cell dry matter accumulation with well-developed soybean tap root during growth of the plant. In agreement with this study, Tahir *et al.* (2009) reported that

Table 2: Interaction effects of *B. japonicum* strains and phosphorus fertilizer rates on root fresh weight (gm $plant^{-1}$) of soybean

Phosphorus (P_2O_5 kg ha^{-1})	***Bradyrhizobium japonicum*** **strains**				
	Un-inoculated	**SB Murodk**	**Soybean 12**	**SB MAR-1495**	**Mean**
0	2.50^{e}	5.73^{bcd}	5.40^{cd}	5.13^{cd}	4.69
23	4.03^{de}	5.67^{bcd}	4.90^{cd}	2.87^{e}	4.37
46	6.63^{abc}	5.60^{bcd}	7.37^{ab}	6.33^{abc}	6.48
69	6.30^{cde}	5.73^{cde}	7.60^{a}	6.46^{abc}	6.52
Mean	4.87	5.68	6.32	5.20	5.52
LSD (0.05)	1.53				
CV (%)	16.7				

Values with the different letter (s) in a column and row are significantly different (P 0.05).

Table 3: Interaction effects of *B. japonicum* strains and phosphorus fertilizer rates on root dry weight (gm $plant^{-1}$) of soybean

Phosphorus (P_2O_5 kg ha^{-1})	*Bradyrhizobium japonicum* strains				
	Un-inoculated	**SB Murodk**	**Soybean 12**	**SB MAR-1495**	**Mean**
0	0.42^{d}	1.38^{bc}	1.59^{ab}	1.33^{bc}	1.18
23	1.51^{ab}	1.53^{ab}	1.53^{ab}	0.93^{c}	1.37
46	1.78^{ab}	1.57^{ab}	1.52^{ab}	1.71^{ab}	1.65
69	1.42^{bc}	1.47^{ab}	2.00^{a}	1.83^{ab}	1.68
Mean	1.28	1.49	1.66	1.45	1.47
LSD (0.05)	0.47				
CV (%)	19.3				

Values with the different letter (s) in a column and row are significantly different (P 0.05).

Table 4: Interaction effects of *B. japonicum* strains and phosphorus fertilizer rates on nitrogen uptake efficiency (kg ha^{-1}) of soybean

Phosphorus (P_2O_5 kg ha^{-1})	*Bradyrhizobium japonicum* strains				
	Un-inoculated	**SB Murodk**	**Soybean 12**	**SB MAR-1495**	**Mean**
0	83.60^{h}	130.40^{fg}	124.70^{g}	118.80^{g}	114.38
23	119.00^{g}	148.90^{ef}	192.30^{bc}	165.40^{de}	156.40
46	141.30^{fg}	213.00^{b}	258.40^{a}	178.00^{cd}	197.68
69	130.20^{fg}	185.50^{fg}	262.30^{a}	194.70^{bc}	193.18
Mean	118.53	169.45	209.43	164.23	165.41
LSD (0.05)	21.55				
CV (%)	7.8				

Values with the different letter (s) in a column and row are significantly different (P 0.05).

the combination of *Rhizobium* inoculation, 25 kg N ha^{-1} and 90 kg P_2O_5 ha^{-1} increased soybean root dry weight by 108% over the control treatment. Similar observation was made by Akpalu *et al.* (2014) reported that a significant increase in root dry weight at increased Phosphorus rates plus *B. japonicum* inoculation strains over un-inoculated treatment.

Nitrogen Uptake Efficiency

The main effect of *B. japonicum* strains inoculation and levels of phosphorus rates, and their interaction effect were highly significantly (*P* 0.01) influenced nitrogen uptake efficiency of soybean. The largest (262.3 kg ha^{-1}) nitrogen uptake efficiency was recorded from combination of 69 P_2O_5 kg ha^{-1} plus Soybean 12 strain, which is statistically at par with similar strain combined with 46 P_2O_5 kg ha^{-1} (258.40 kg ha^{-1}) (Table 4). The smallest (83.6 kg ha^{-1}) nitrogen uptake efficiency of soybean was observed on un-treated control treatment (Table 4). Mean of nitrogen uptake efficiency was increased about 2.1 and 2.14 folds in the treatment combination of soybean seeds inoculated with Soybean 12 strain with 46 and 69 kg P_2O_5 ha^{-1} treatment, respectively over control treatment (Table 4). The increase in nitrogen uptake efficiency of soybean could be due to *B. japonicum* strains inoculation and application of phosphorus rates which increased nitrogenease activity, and nodule mass that ultimately increased plant nitrogen uptake efficiency. In agreement with this study Tahir *et al.*, (2009) reported that total nitrogen uptake efficiency of soybean increase by 123% over the control treatment with the interaction of phosphorus and *Bradyrhizobium* inoculation.

Percent of Nitrogen Derived from Air (%Ndfa)

Analysis of variance showed that the main effects of *B. japonicum* strains and phosphorus rates, and their interaction were significantly (*P* 0.01) influenced percent of nitrogen derived from air (%Ndfa). Moreover, huge (123.9 %) percent of

Table 5: Interaction effects of *B. japonicum* strains and phosphorus fertilizer rates on %Ndfa (%) of soybean

Phosphorus (P_2O_5 kg ha^{-1})	***Bradyrhizobium japonicum* strains**				
	Un-inoculated	**SB Murodk**	**Soybean 12**	**SB MAR-1495**	**Mean**
0	0.00^{g}	71.60^{de}	84.80^{cde}	81.70cde	59.53
23	50.10^{f}	90.30^{cd}	88.70^{cde}	100.70^{bc}	82.45
46	68.10^{ef}	119.40^{ab}	123.90^{a}	99.40^{bc}	102.70
69	48.90^{f}	94.10^{c}	96.10^{c}	103.60^{bc}	85.68
Mean	41.78	93.85	98.38	96.35	82.59
LSD (0.05)	18.95				
CV (%)	13.8				

Values with the different letter (s) in a column and row are significantly different (P 0.05).

Table 6: Interaction effects of *B. japonicum* strains and phosphorus fertilizer rates on phosphorus uptake efficiency (kg ha^{-1}) of soybean

Phosphorus (P_2O_5 kg ha^{-1})	***Bradyrhizobium japonicum* strains**				
	Un-inoculated	**SB Murodk**	**Soybean 12**	**SB MAR-1495**	**Mean**
0	6.83^{e}	9.17^{de}	8.71^{de}	9.14^{de}	8.46
23	9.26^{de}	10.70^{cd}	14.07^{b}	12.88^{bc}	11.73
46	10.58^{cd}	15.27^{b}	19.86^{a}	15.14^{b}	15.21
69	11.07^{cd}	13.96^{b}	18.97^{a}	14.64^{b}	14.66
Mean	9.44	12.28	15.40	12.95	12.52
LSD (0.05)	2.26				
CV (%)	10.8				

Values with the different letter (s) in a column and row are significantly different (P 0.05).

nitrogen derived from air (%Ndfa) was recorded by the interaction of 46 kg P_2O_5 ha^{-1} with Soybean 12 strain inoculation, while the lowest % of Ndfa was recorded from control treatment (Table 5). Compared to un-treated control treatment, in all treatments the nitrogen derived from air (%Ndfa) was increased (Table 5). The results are supported by Okito *et al.*, (2004) who reported that combined application of *B. japonicum* strains and phosphorus rates showed increased percent of nitrogen derived from air compared to control treatment.

Phosphorus Uptake Efficiency

The ANOVA showed that Phosphorus uptake efficiency of soybean was significantly (*P* 0.01) influenced by the main factors of phosphorus fertilizer and *B. japonicum* strains and their interaction. The largest amount of phosphorus uptake efficiency 19.86 kg ha^{-1} was obtained from the combination of seed inoculated with Soybean 12 strain with application of 46 kg P_2O_5 ha^{-1}, which is statistically at par with the same strain combined with application of 69 kg P_2O_5 ha^{-1} (18.97 kg ha^{-1}), whereas the least amount of phosphorus uptake efficiency of Soybean (6.83 kg ha^{-1}) was observed on control treatment (Table 6). In contrast with the control treatment, Soybean 12 strain inoculated soybean mean Phosphorus uptake efficiency was increased by 191 and 176 % in the 46 and 69 kg P_2O_5 ha^{-1} treatments, respectively (Table 6). The higher phosphorus concentration in plant benefits the bacterial symbiont and the functioning of its nitrogenease activity, leading to increased nitrogen fixation. In conformity with this study Tahir *et al.*, (2009) reported that Phosphorus uptake efficiency of soybean increased by 208% with the interaction of rhizobium inoculation and phosphorus over the control treatment.

Seed Yield

Seed yield is the result of different inputs, agronomic practices, environment effects and genetic differences. Analysis of variance (Table 7) showed that the main effect of *B. japonicum* strain

Table 7: Interaction effects of *B. japonicum* strains and phosphorus fertilizer rates on seed yield (kg ha^{-1}) of soybean

Phosphorus (P_2O_5 kg ha^{-1})	*Bradyrhizobium Japonicum* strains				
	Un-inoculated	SB Murodk	Soybean 12	SB Mar 1495	Mean
0	1451.00^{h}	1650.00^{gh}	1651.00^{gh}	1649.00^{gh}	1600.25
23	1708.00^{fg}	2065.00^{de}	2539.00^{c}	2272.00^{d}	2146.00
46	1859.00^{efg}	2832.00^{b}	3597.00^{a}	2515.00^{c}	2700.75
69	1939.00^{ef}	2637.00^{bc}	3590.00^{a}	2639.00^{bc}	2701.25
Mean	1739.25	2296.00	2844.25	2268.75	2287.06
LSD (0.05)		227.4			
CV (%)		6.0			

Values with the different letter (s) in a column and row are significantly different (P 0.05).

Table 8: Interaction effects of *B. japonicum* strains and phosphorus fertilizer rates on harvest index (% $plant^{-1}$) of soybean

Phosphorus (P_2O_5 kg ha^{-1})	*Bradyrhizobium japonicum* strains				
	Un-inoculated	SB Murodk	Soybean 12	SB MAR-1495	Mean
0	44.29^{de}	40.23^{f}	45.05^{cde}	47.13^{abc}	44.18
23	47.06^{abc}	47.49^{abc}	47.20^{abc}	47.87^{ab}	47.41
46	46.86^{abcd}	47.63^{abc}	45.73^{bcde}	47.92^{ab}	47.04
69	48.45^{ab}	48.53^{ab}	43.60^{e}	48.77^{a}	47.34
Mean	46.67	45.97	45.40	47.92	46.49
LSD (0.05)		2.42			
CV (%)		3.1			

Values with the different letter (s) in a column and row are significantly different (P 0.05).

inoculations and application of phosphorus rates, and their interaction significantly (*P*0.01) influenced seed yield of soybean.

In the present study, results showed that combined application of *B.japnicum* strains with phosphorus rates gave higher seed yields than that of *B. japonicum* strain or phosphorus rates alone (Table 7). The highest (3597 kg ha^{-1}) seed yield was recorded at the rate of 46 kg P_2O_5 ha^{-1} and Soybean 12 strain which was statistically at par with inoculation of similar strain plus 69 kg P_2O_5 ha^{-1} (3590 kg ha^{-1}) (Table 7). The lowest seed yield (1451 kg ha^{-1}) was recorded at control treatment (Table 7). The combination of *B. japonicum* of Soybean 12 strain with phosphorus rate at 46 kg P_2O_5 ha^{-1}, resulted in 148% increased seed yield over control treatment. This result agrees with Shahid *et al.*, (2009) who reported that seed production in soybean can increase by 70-75% when the proper bacterial strains were used to inoculate soybean seeds.

Harvest Index

Harvest index reflects the division of photosynthesis between the seeds and the vegetative plant part, and improvement in harvest index emphasizes the importance of carbon allocation in grain production. The main effect of *B. japonicum* strains and phosphorus rates, and their interaction revealed highly significant (*P* 0.01) effect on harvest index of soybean (Table 8). Treatment with 46 kg of P_2O_5 ha^{-1} and Soybean MAR-1495 strain gave the highest (48.77 %) harvest index, whereas lowest harvest index (40.23%) was recorded on inoculated with strain of Soybean murodk (Table 8). The lowest mean harvest index per plant, even less than the control was recorded from the plots that received Soybean murodk strain. Although, Soybean murodk strain showed significantly differed mean harvest index from the interaction of 46 kg P_2O_5 ha^{-1} plus Soybean MAR-1495 strains, it resulted in 19.12% increase in mean harvest index per plant (Table 8). In the present experiment the decreased mean harvest index per plant recorded from un-inoculated treatment, 69 kg P_2O_5 ha^{-1} phosphorus rate combined to inoculation with strain of Soybean 12, and sole inoculated with strain of Soybean murodk might be due to the influence of vegetative growth and increased above ground biomass yield, which reduced the harvest index. However, the increased mean harvest index per plant with the increase of P fertilizer up to 46 kg P_2O_5 ha^{-1} rate might be due to the influence of greater fruit and seed setting than above ground biomass yield. The result found in this study is in agreement with the results of Malik *et al.* (2006) who reported that harvest index was significantly influenced by applied phosphorus in soybean.

CONCLUSION

The results of the research showed that Days to 50% flowering and physiological maturity, leaf area, leaf area index, above ground biomass yield, seed yield and harvest index of soybean were increased with combined application of *B. japonicum* and Phosphorus rates. On the other hand, plant height and number of primary branches, were increased with alone application both *B. japonicum* strains and phosphorus rates. Among all treatments, combined application of Soybean 12 plus 46 kg of P_2O_5 ha^{-1} gave better performance in soybean, *i.e.* maximum leaf area and seed yield. For soybean production in the western part of Ethiopia, increasing soybean yield with acceptable quality from different combined treatments with good agronomic practices is crucial in the future. The combinations of 46 kg P_2O_5 ha^{-1} fertilizer rates and Soybean 12 strain of *B. japonicum* gave optimum seed yield.

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