Advances in Crop Science and Technology

Fituma et al., Adv Crop Sci Tech 2017, 5:4
DOI: 10.4172/2329-8863.1000290

Research Article | OMICS International

Effect of Inoculating *Bradyrhizobium* on Phosphorus Use Efficiency and Nutrient Uptake of Soybean Intercropped with Sugarcane in Calcareous Soil of Metahara, Central Rift Valley, Ethiopia

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Received date: June 25, 2017; **Accepted date:** July 06, 2017; **Published date:** July 13, 2017

Abstract

At a soil pH value of above 7.0, inorganic phosphorus (P) is highly susceptible to precipitation as insoluble form that is unavailable to the plants. Hence, a field experiment was conducted at Metahara Sugar Estate under irrigation during the 2014/15 cropping season to evaluate the effect of inoculating *Bradyrhizobium* on P uptake and P use efficiency of soybean intercropped with sugarcane. The treatments consisted of three levels of inoculation (Legumefix, SB6B1 and uninoculated) and four rates of P (0, 23, 46 and 69 kg P_2O_5 ha^{-1}). The experiment was laid out as a randomized complete block design (RCBD) in a factorial arrangement and replicated three times. Analysis of the data indicated that *Bradyrhizobium* inoculation significantly increased plant N concentration and P uptake compared to the uninoculated treatment. But the effect of P rates and its interaction with inoculation was not significant on N concentration and P uptake but significantly increased total P uptake at the application of 69 kg P_2O_5 ha^{-1}. P use efficiency indices were improved in response to inoculating the crop with *Bradyrhizobium*. The higher AE (13.6 kg kg^{-1}), PRE (31.8%) and PUE (10.6 kg kg^{-1}) were obtained by SB6B1 inoculation and higher PE (117.2 kg kg^{-1}) and APE (161.7 kg kg^{-1}) were obtained by Legumefix inoculation all at 23 kg P_2O_5 ha^{-1} except PE which recorded at 69 kg P_2O_5 ha^{-1}. Thus, it can be concluded that SB6B1 isolate will be used as the best inoculant followed by Legumefix isolate with 23 kg P_2O_5 ha^{-1} of P fertilizer rate. However, strategies for increasing P use efficiency by adopting best management practices like co-inoculation of phosphate solubilizing microorganism or mycorrhiza with these *Bradyrhizobium* inoculants should be adopted to enhance P use efficiencies.

Keywords: *Bradyrhizobium*; *Glycine max*; Nutrient uptake; P use efficiency; Inoculant

Introduction

Phosphorus (P) is the most essential element for plant growth and development next to nitrogen (N) [1]. It is one of the most important nutrients limiting crop production on more than 30% of the world's arable land. Some estimates, world resources of inexpensive P may be depleted by 2050 [2]. Phosphorus has significantly positive effect on nodulation nitrogenase activity and the yield of pulse crops [3]. However, more than 80% of the added P gets fixed or precipitated, and only a part of it goes to soil solution which may be taken up by crops (Leytem and Mikkelsen) [4] because it rapidly forms insoluble complexes with cations and is incorporated into organic matter by microbes (von Uexkuill and Mutert; Vance) [5,6] Sustainable management of P in agriculture that enhances P acquisition and exploits these adaptations to make plants more efficient at acquiring the nutrient is very important. Therefore, in areas with low soil fertility, with a poor supply and/or high cost of fertilizers, cultivating legumes with high efficiencies of P uptake and P use would be very useful. A two site experiment conducted at Metahara Sugar Estate showed that P application (0 to 300 kg ha^{-1} double Super Phosphate) had no significant effect on sugarcane yield [7]. This might be ascribed to the high P precipitation caused by presence of high calcium carbonate [8].

Several key processes which affect the availability of P to plants in the P cycle are mediated by different types of microbial processes [9]. Most researches in microbial inoculants to enhance P availability and root uptake have centered on soil microorganisms capable of solubilizing sparingly-available P [10]. Qin et al. [11] demonstrated that soil beneficial microorganisms including rhizobia can solubilize the insoluble form of organic and inorganic P. Increase in productivity of wheat by 30-40% was due to inorganic P application with inoculation, as compared to P alone [12]. In addition, enhancement of P utilization from insoluble P through inoculation of rhizobia has been demonstrated in lettuce [13]. The objective of this study was thus to evaluate the effect of inoculating selected *Bradyrhizobium* isolates on N and P uptake and P use efficiency of soybean intercropped with sugarcane at Metahara Sugar Estate in the Central Rift Valley of Ethiopia.

Materials and Methods

Description of the experimental site

A field experiment was conducted at Metahara Sugar Estate under irrigation during 2014/15. The estate is located at 8°53'N latitude and 39°52'E longitude at an altitude of 950 meters above sea level in the Eastern Shewa Administrative Zone, Oromia Regional State, about 200 km south-east of the capital city, Addis Ababa, Ethiopia.

Citation: Fituma T, Tana T, Argaw A (2017) Effect of Inoculating *Bradyrhizobium* on Phosphorus Use Efficiency and Nutrient Uptake of Soybean Intercropped with Sugarcane in Calcareous Soil of Metahara, Central Rift Valley, Ethiopia. Adv Crop Sci Tech 5: 290. doi: 10.4172/2329-8863.1000290

The long term mean (LTM) annual rainfall is 551 mm with the LTM annual maximum and minimum air temperatures of 33.0 and 17.5°C, respectively. According to meteorological information recorded in the last five decades, the rainfall period ranged from April to October albeit the maximum rainfall was recorded in the months of July (127.4 mm) and August (140 mm) in Metahara Sugar Estate.

Most soils of the experimental site are developed under tropical hot condition from alluvium-colluvium parent materials which include basic volcanic rocks such as (basalt, limestone), acidic volcanic rocks such as (granite, sandstone) as well as recent and ancient alluvial soils [8,14]. Soils of Metahara Sugar Estate are classified as Calcaric Cambisols [8].

Description of experimental materials

Carrier based *Bradyrhizobium* inoculants, namely, indigenous isolate (SB6B1) and exotic UK-isolate (Legumefix) were obtained from the Soil Microbiology Laboratory of Holetta Agricultural Research Center and used for seed inoculation at planting. Soybean variety 'Williams' was obtained from Hawassa Agricultural Research Center and intercropped with high yielding and widely cultivated sugar cane variety 'B52-298'.

Experimental procedures and design

The experiment consisted of four rates of phosphorus (0, 23, 46 and 69 kg P_2O_5 ha^{-1}) in the form of triple super phosphate (TSP) (0:46:0%; N: P_2O_5: K_2O) and three types of inoculant inoculation, i.e., SB6B1 (local isolate), Legumefix (UK isolate) and uninoculated control. The experiment was laid out as a randomized complete block design (RBCD) in a factorial arrangement and replicated three times per treatment.

Management of the experiment

Carrier-based inoculants of each isolate were applied at the rate of 10 g inoculant/kg seed [15]. To ensure that the applied inoculants stick to the seed, the required quantity of inoculants was suspended in 1:1 ratio in 10% sugar solution for 10 minutes.

Land preparation was done by a tractor (ripping, uprooting of old cane stools, disking, leveling and furrowing) and a selected portion of land was then divided into blocks and plots for this experiment. Sugarcane was planted on 21st November 2015 in the furrow trench with end-to-end sett position and 145 cm row spacing. Soybean seed was also sown in the following day at one side of the ridge with the spacing of 10 cm between plants and similar row spacing as sugarcane on 8.7 m × 5.0 m (43.5 m^2) gross plot size, which holds 6 rows of both soybean and sugarcane but data were collected from four central rows. There was a 1 m space between each plot and two furrow (2.90 m) path between blocks, in which no cane was planted.

The experiment was carried out using an irrigated field with furrow irrigation method with an irrigation interval of seven days which was recommended for soybean cultivation. Nitrogen fertilizer at the rate of 20 kg N ha^{-1} was applied with Urea (46% N) being a source of the nutrient.

Plant tissue sampling and analysis

At physiological maturity, five randomly taken soybean plants were harvested from the four central rows and partitioned into grain and straw. The grain and straw samples were separately oven-dried at 70°C to a constant weight, ground to pass through 1 mm sieve and saved for tissue analysis of grain and straw. Total N in grain and straw subsamples were quantitatively determined by a kjeldahl procedure [16]. Nitrogen content of the grain and straw was determined by multiplying the N concentrations in the dry matter of the tissues by the respective grain and straw dry yields. Phosphorus in grain and straw subsamples were determined by using Meta vanadate method [17]. Phosphorus uptake in the grain and straw of soybean was determined from the phosphorus content of the respective parts after multiplying with the grain and straw yields, respectively.

Phosphorus use efficiency

Based on the results of plant tissue analysis, phosphorus use efficiency indices were computed [18].

Agronomic Efficiency (AE): is defined as the quantity of grain yield per unit of nutrient applied.

AE (kg kg^{-1})=G_f-G_u/N_a (1)

where: G_f is the grain yield of the fertilized plot (kg), GU is the grain yield of the unfertilized plot (kg), and Na is the quantity of P applied (kg).

Physiological Efficiency (PE): is defined as the aboveground biomass yield obtained per unit of nutrient uptake.

PE (kg kg^{-1})=BY_f-BY_u/N_f-N_u (2)

where: BY_f is the aboveground biomass yield (grain plus straw) of the fertilized plot (kg), BY_u is the aboveground biomass yield (grain plus straw) of the unfertilized plot (kg), N_f is the nutrient uptake (grain plus straw) of the fertilized plot (kg) and N_u is the nutrient uptake (grain plus straw) of the unfertilized plot (kg).

Agro-Physiological Efficiency (APE): is defined as the grain yield obtained per unit of nutrient uptake.

APE (kg kg^{-1})=G_f-G_u/N_f-N_u (3)

where: G_f and G_u are grain yields from fertilized and unfertilized plots (kg), respectively; N_f and N_u are P uptakes (grain plus shoot) from fertilized and unfertilized plots (kg), respectively.

Phosphorus Recovery Efficiency (PRE): is defined as the quantity of nutrient uptake per unit of nutrient applied.

PRE (%)=N_f-N_u × 100/N_a (4)

where: N_f and N_u are nutrient uptakes (grain plus straw) from fertilized unfertilized plots (kg), respectively, and Na is the quantity of nutrient applied (kg).

Phosphorus Utilization Efficiency (PUE): is defined as the product of physiological efficiency and recovery efficiency. PUE (kg kg^{-1})=PE × PRE

Results and Discussion

Selected soil physical and chemical properties of the soil

Analysis of the soil of the experimental field indicated a clayey texture with a clay content of 70%. The soil pH could be rated as moderately alkaline according to the rating of Tekalign Tadese [19] (Table 1). The organic matter content of the soil is low according to the rating of Tekalign Tadese [19]. The low organic matter content of the soil might be attributed to the intensive cultivation underway for a long time and continuous removal of crop residues through sugarcane burning. In line with this result, BAI [8] reported soil organic matter of Metahara Sugar Estate ranges from low to very low.

Soil property	Value	Soil property	Value
Depth (cm)	20	OM (%)	1.70
Particle size (%)		EC (dSm^{-1})	0.37
Sand	12	Exchangeable Na (cmol(+)kg^{-1})	1.89
Silt	18	Exchangeable K (cmol(+)kg^{-1})	3.33
Clay	70	Exchangeable Ca (cmol(+)kg^{-1})	49.0
Textural class	clay	Exchangeable Mg (cmol(+)kg^{-1})	11.0
pH (1:2.5 H_2O)	7.70	CEC (cmol(+)kg^{-1})	67.0
TN (%)	0.12	PBS (%)	97.3
Avail P (ppm)	5.60	$CaCO_3$ (%)	7.00

Table 1: Physical and chemical properties of soils of the experimental site before planting. Note: P: Available phoshorus, CEC: Cation exchange capacity, EC: Electrical conductivity, OM: Organic matter, PBS: Percent base saturation, TN: Total nitrogen.

The analysis further indicated that the soil has low contents of total nitrogen and available phosphorus (Table 1) according to the ratings of Tadese and Marx et al. [19,20] respectively. The low nitrogen content could be attributed to the low soil organic matter content. The low available P could be ascribed to the precipitation of phosphorus into unavailable forms of calcium and magnesium carbonates. Consistent with this result, BAI [8] reported low available phosphorus in the Estate because of high P precipitation. Beside, the cation exchange capacity (CEC) of the soil was rated in the range of very high as reported by Landon [21] with the dominant cation being calcium in the exchange site. The high CEC of this soil might be attributed to the high clay content in the soil. Percent calcium carbonate ($CaCO_3$) content (7.0%) was moderate according to the rating of Nachtergaele et al. [22].

Nitrogen content and p uptake of soybean

Nitrogen contents of grain, straw and total biomass: Inoculation with *Bradyrhizobium* significantly (P<0.01) influenced the N contents of the grain and straw as well as the total biomass of soybean (Table 2). *Bradyrhizobium* inoculation alone improved the whole N content of soybean regardless of P application (Table 2). Both SB6B1 and Legumefix inoculations significantly increased grain, straw and total N contents over the uninoculated control. The highest mean N contents of grain, straw and total biomass yield were obtained from inoculation with SB6B1 isolate albeit no significant variation was observed between the two *Bradyrhizobium* inoculants. SB6B1 increased grain, straw and total biomass contents of N by 6, 14 and 8%, respectively compared to Legumefix while the increase over uninoculated control was higher by 147, 97 and 130%. An increase in N contents due to *Bradyrhizobium* inoculation could be related to the significant increase in nodulation resulting in higher accumulation of N through biological N_2 fixation [23].

Treatment	Nitrogen			Phosphorous		
	Grain N	Straw N	Total N (grain+straw)	P uptake by grain	P uptake by straw	Total P uptake
P rate (kg P_2O_5 ha^{-1})						
0	71	35.2	102.2	11.5	5.3	16.8b
23	82.9	36.6	119.5	13.8	5.9	19.7ab
46	85.2	31.1	116.3	11.5	6	17.4b
69	84.1	37.3	121.4	15.8	6.9	22.7a
Significance	NS	NS	NS	NS	NS	*
LSD (0.05)	NS	NS	NS	NS	NS	3.93
Inoculation						
Un inoculated	41.8b	22.4b	64.2b	10.0b	5.1b	15.0b
SB6B1	103.2a	44.1a	147.4a	16.1a	6.7a	22.8a
Legumefix	97.4a	38.7a	136.1a	13.34a	6.3a	19.6a
Significance	**	**	**	**	*	**
LSD (0.05)	11.7	6.7	11.52	3.31	1.12	3.4

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CV (%)	17	22.5	11.7	29.8	22	21

Table 2: Effect of *Bradyrhizobium* inoculation and P application on N content and P uptake in grain and straw (kg ha^{-1}) of soybean intercropped with sugarcane at Metahara Sugar Estate. Where: NS, * and **: Non significant, significant at 5 and 1%, respectively; CV: Coefficient of variation, LSD: Least significant difference. Means within the same factor and column followed by the same letter are not significantly different at 5% level of significance.

This current result is in agreement with the findings of Tahir et al. [24] who reported that soybean N accumulation in grain, straw and total biomass was increased by 9, 122 and 76% over the control due to inoculation with *Bradyrhizobium*. This result is also in accord with the finding of Tajini et al. [25] who reported that inoculation with rhizobia improved symbiotic N_2 fixation even under phosphorus deficiency. In line with this result, Tufenkci et al. [26] reported that Rhizobium inoculation improved NPK uptake.

Phosphorus fertilizer rate and its interaction with inoculants did not significantly affect the N contents of the grain and straw as well as the total biomass at physiological maturity although soil available P was low (Table 2). This might be due to the high alkalinity of the soil which predisposes the available P in the soil to precipitation into unavailable forms. However, a slight increase in total plant N content was obtained due to small P application (23 kg P_2O_5 ha^{-1}) with SB6B1 and Legumefix inoculation as compared to uninoculated treatment. This shows that application of 23 kg P_2O_5 ha^{-1} is optimum for sufficient uptake of nitrogen by the crop. Consistent with this result, Tekle Yoseph and Walelign Worku [27] reported that P significantly increased the soybean grain N and straw N contents at lower P rate (25 kg P ha^{-1}) than at higher level (50 kg P ha^{-1}).

Uptake of P in grain, stover and total biomass of soybean: Inoculation with *Bradyrhizobium* had significant (P<0.01) effect on the uptake of P by grain, straw, and total soybean biomass compared to the uninoculated treatment. Phosphorus uptake by grain, straw, and total biomass in response to inoculation with SB6B1 as well as in response to relative to inoculation with Legumefix was significantly higher than the uptake observed in response to no inoculation. Thus, the total P uptake that resulted from inoculation with SB6B1 and Legumefix exceeded the P total uptake obtained in response to no inoculation by about 52 and 31%, respectively (Table 2). The higher P uptake due to inoculation with SB6B1 and Legumefix could be attributed to the fact that some rhizobia have the ability to solubilize precipitated P components, thereby increasing the uptake in plants [11]. Consistent with the results of this study, the finding of Taye Belachew [28] showed that except P uptake in the straw, inoculation of pea by Rhizobium significantly increased both grain and total P uptake. Similarly, Tahir et al. [24] reported that Rhizobium inoculation increased total P uptake by 79%. Havlin et al. [29] also indicated that large quantities of P are found in seed and P is considered to be essential for seed formation.

Improved N status in soybean plants due to better root growth might be the mechanism by which soybean P uptake was increased in plants inoculated with the effective Rhizobium strains on low-P acid soils [30]. Cheng et al. [31] found that inoculating soybean with effective rhizobial inoculants significantly improved root growth as well as N and P contents in low-P acidic soils. In addition, Tang et al. [32] found that total P uptake from sparingly soluble P correlated highly with plant biomass production, N_2 fixation and nodulation, and seed P concentrations. Singh et al. [33] found that inoculation of P solubilizing bacteria increased P content in grain and straw by 10.72 and 31.94%, respectively, over the uninoculated treatment.

In contrast to the main effect of inoculation, P rates and its interaction with inoculation had no significant effect on grain and straw P uptake at physiological maturity (Table 2). However, total P uptake was significantly (P<0.05) increased in response to the increase in the rate of phosphorus application. The maximum total P uptake was recorded by application of (23 and 69 kg P_2O_5 ha^{-1}). Similarly, Egamberdiyeva et al. [34] confirmed that P uptake by soybean increased with increase in the rate of phosphorus application in N-deficient calcareous soils. Apparently, a similar trend was also reported by BAI who found that soils of Metahara Sugar Estate were alkaline and strongly calcareous and that the organic matter and total N contents were low.

Among the tested isolates, SB6B1 inoculation showed significantly higher total P uptake at 69 kg P_2O_5 ha^{-1} than the other rates of P application though no interaction effect was observed.

Phosphorus use efficiency

Agronomic efficiency: The higher the rates of P application, the lower were the agronomic efficiency in all observed treatments. Across P rates, a 2.8, 8.4 and 7.7 kg soybean grain yield was produced per unit of P applied by un-inoculated, SB6B1 and Legumefix inoculation. The highest agronomic efficiency (AE) of 3.9, 13.6 and 11.5 kg kg^{-1} was obtained at 23 kg P_2O_5 ha^{-1} application due to un-inoculated, SB6B1 and Legumefix inoculation (Tables 3-5). However, the least AE value was noted at 69 kg P_2O_5 ha^{-1} in all treatments. Application of P fertilizer above 23 kg P_2O_5 ha^{-1} had no appreciable effect on soybean grain yield. Nonetheless, the AE was greatly influenced by soybean inoculation than un-inoculated. This might be due to the fact that rhizobial symbiosis requires large amounts of P to meet the high energy costs for adenosine triphosphate (ATP) synthesis [32] in order to produced higher grain yield. This agrees with Gifole Gidago et al. [35] who found a declining trend of AE from 69.8 to 9.3 kg kg^{-1} at the P rates ranging from 10 to 60 kg P ha^{-1} on haricot bean. This might be due to small amounts of applied fertilizer optimized nutrient use efficiency [36]. Similar to this result, combined application of phosphorus and inoculation enhanced agronomic efficiency of soybean and common bean over the un-inoculated control [37,38].

Un-Inoculated					
P rate P_2O_5 (kg ha^{-1})	**AE (kg kg^{-1})**	**PE (kg kg^{-1})**	**APE (kg kg^{-1})**	**PRE (%)**	**PUE (kg kg^{-1})**

0	-	-	-	-	-
23	3.9	21.2	21.2	3.1	0.7
46	2.6	25.7	25.7	3.2	0.8
69	2	28.8	28.9	2.6	0.8
Mean	2.8	25.2	25.3	3	0.8

Table 3: Phosphorus use efficiencies of soybean intercropped with sugarcane as affected by P application alone. Note: AE: Agronomic efficiency, PE: Physiological efficiency, APE: Agro-physiological efficiency, PRE: Phosphorus recovery efficiency and PUE: Phosphorus utilization efficiency.

SB6B1					
P rate P_2O_5 (kg ha^{-1})	AE (kg kg^{-1})	PE (kg kg^{-1})	APE (kg kg^{-1})	PRE (%)	PUE (kg kg^{-1})
0	-	-	-	-	-
23	13.6	33.2	42.8	31.83	10.58
46	6.7	35.5	37.99	17.74	6.3
69	4.8	40.9	27.83	17.36	7.1
Mean	8.4	36.6	36.2	22.3	8

Table 4: Phosphorus use efficiencies of soybean intercropped with sugarcane as affected by SB6B1 inoculation.

Legumefix					
P rate P_2O_5 (kg ha^{-1})	AE (kg kg^{-1})	PE (kg kg^{-1})	APE (kg kg^{-1})	PRE (%)	PUE (kg kg^{-1})
0	-	-	-	-	-
23	11.5	98.2	161.7	7.1	7
46	7.1	114.5	140.2	5.1	5.8
69	4.5	117.2	75	6.1	7.1
Mean	7.7	110	125.6	6.1	6.6

Table 5: Phosphorus use efficiencies of soybean intercropped with sugarcane as affected by Legumefix inoculation.

Physiological efficiency: The physiological efficiency (PE) indicates that biological yield obtained per unit of nutrient uptake. Along P rates slight increase in biomass accumulation was observed and maximum biomass yield was obtained at application rate of 69 kg P_2O_5 ha^{-1} in all treatments (Tables 3-5). Across P rates on average 25.2, 36.6 and 110.0 kg biomass yield were noted per 1 kg of applied P with respect to un-inoculated, SB6B1 and Legumefix. Legumefix inoculation led to higher biomass yield and P uptake than SB6B1 throughout P application rates, whereas, the lowest PE was recorded by un-inoculated control. The higher PE fraction obtained due to Legumefix inoculation might indicate its tendency to accumulate relatively higher biomass yield as P fertilizer rates increase with small amounts of increase in total P uptake. This could also be due to better symbiotic N_2 fixation with Legumefix inoculation thereby increasing the response of soybean to P application [39]. Moreover, it might have produced hormones and solubilizing insoluble P from the soil [33,40]. The slight increase in dry biomass yield at higher P fertilizer application rate indicated that the plants grown at the lowest P level were the most efficient in using P for production of dry matter [41].

Agro-physiological efficiency: Agro-physiological efficiency (APE) is the economic production (grain yield) obtained per unit of nutrient uptake. Along P application rates APE drastically decreased in the inoculated treatments but showed slight increment in the un-inoculated albeit it scored the lowest APE compared to the inoculated. Across P application rates on average 25.3, 36.2 and 125.6 kg grain yield was obtained per unit of nutrient absorbed in un-inoculated, SB6B1 and Legumefix, respectively. The highest agro-physiological efficiency of 42.8 and 161.7 kg kg^{-1} was noted at the lowest P rate of 23 kg P_2O_5 ha^{-1} by SB6B1 and Legumefix, respectively (Tables 3-5). The higher APE by Legumefix inoculation might be due to the presence of plant growth promoting characteristics beside N_2 fixation which enabled to produce relatively higher nutrient uptake at lower P rate fertilizer. Similar results were reported by Singh et al. in lentil. Contrary to this result, Abbasi et al. [42] found the highest (51 kg kg^{-1}) APE for soybean at lower P fertilizer application rate (50 kg P_2O_5 ha^{-1}) than at higher rate of 100 kg P_2O_5 ha^{-1} which produced APE of 42 kg kg^{-1}. In alkaline soil pH the availability of some essential nutrients for plant is reduced [43].

Phosphorus recovery efficiency: Phosphorus recovery efficiency (PRE) provides the quantity of nutrient uptake per unit of nutrient applied. The mean recovery efficiency of P by soybean treated with un-inoculated, SB6B1 and Legumefix were 3.0, 22.3 and 6.1%, respectively. The highest recovery efficiency of 31.8 and 7.1% were noted due to SB6B1 and Legumefix inoculation at the lowest P rate of 23 kg P_2O_5 ha^{-1}. However, the lowest recovery efficiency was noted at 69 kg P_2O_5 ha^{-1} by SB6B1 and un-inoculated and at 46 kg P_2O_5 ha^{-1} by Legumefix inoculation (Tables 3-5). The higher recovery efficiency by SB6B1 inoculation might be due to the fact that large number of strains of Rhizobium and *Bradyrhizobium* could solubilize inorganic phosphate through the enzymatic action of acid and alkaline phosphatase [44] and assimilate the soluble P in plants and prevent it from adsorption or fixation [45].

The lower recovery efficiency was also reported by Abbasi et al. [42] who found PRE of 12.1% in soybean at lower P rate (50 kg P_2O_5 ha^{-1}) and 10.2% when dose increased to 100 kg P_2O_5 ha^{-1}. Syers et al. [46] also reported that in the year of fertilizer application P fertilizer used by plants ranged from 10-30%. The low P recovery efficiency in the present study might be associated with high P fixation property of the soil due to the presence of Ca compounds and clay minerals. Besides, P sorption increases at higher fertilizer rates than at lower application [47]. Kumar and Kairon [48] also determined an apparent P recovery of 4.7% by field grown cotton in alkaline soils. Beside this, Fixen [49] concluded that first year recovery of P is low, not only because the P is immediately "fixed" into plant unavailable forms but also because it moves so little in soils that crop roots are too far from much of the fertilizer-soil reaction zones to be accessed.

Phosphorus utilization efficiency: As shown in Tables 3-5 the efficiency of soybean in P utilization inconsistently decreased as the P fertilizer rate increased. On the average, every kilogram of P applied to the un-inoculated, SB6B1 and Legumefix treated soybean produced 0.8, 8.0 and 6.6 kg of grain yield respectively. The highest P utilization efficiency was observed at 23 kg P_2O_5 ha^{-1} due to SB6B1 and at 69 kg P_2O_5 ha^{-1} by Legumefix inoculation. However, the lowest PUE was noted at 46 kg P_2O_5 ha^{-1} in SB6B1 and Legumefix whereas at 23 kg P_2O_5 ha^{-1} in un-inoculated treatments. It is evident from the result that inoculation enhanced PUE of soybean where better numerical value is attained in SB6B1 followed by Legumefix. This could be due to the fact that symbiotic N_2 fixation is an energy consuming process with a high (16) ATP demand for the reduction of one molecule of N_2 into $2NH_3$ [50]. Phosphorus application also enhanced growth of rhizobial strains and growth of host plants [51,52]. In agreement the declining trend in PUE was reported by Win et al. [41] as P rate increased from 0.5 to 2 mMP. The highest PUE at the lowest P rate with inoculation of P solubilizing bacteria were also reported by Singh et al. [33]. However, P application did not improve PUE significantly in uninoculated treatment.

Conclusion

In this study it was observed that *Bradyrhizobium* inoculation significantly influenced the uptake of N and P in soybean. In addition, inoculation with *Bradyrhizobium* increased use efficiency of P from high pH or calcareous nature of the soil of the study area. But increased application of P fertilizer beyond 23 kg P_2O_5 ha^{-1} did not improve the P use efficiencies except PE. Phosphorus use efficiency indices also varied between inoculants of *Bradyrhizobium* that higher AE (13.6 kg kg^{-1}), PRE (31.8%) and PUE (10.6 kg kg^{-1}) were obtained by SB6B1 inoculation. On the contrary, higher PE (117.2 kg kg^{-1}) and APE (161.7 kg kg^{-1}) were obtained by Legumefix inoculation. Thus, it can be concluded that SB6B1 isolate would be used as the best inoculant followed by Legumefix isolate at Metahara Sugar Estate in order to increase P use efficiency of soybean at lower P fertilizer rate (23 kg P_2O_5 ha^{-1}). Furthermore, strategies for increasing P use efficiency by adopting best management practices should be adopted either through co-inoculation of phosphate solubilizing microorganism or mycorrhiza with these *Bradyrhizobium* strains which might alleviate the high P sorption of the soil of the study area.

Acknowledgements

The authors are grateful to Ethiopian Sugar Corporation for research funding. The authors also thank Tsegaye Temesgen, Rorisa Gudissa, Solomon Chakiso and Abayneh Siyoum for their assistance in the field work. Staff of Metahara Research Station is also acknowledged for their all-round support during the time of the research work.

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