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EFFECTS OF RHIZOBIA AND PHOSPHATE-SOLUBILIZING BACTERIA ON SOYBEAN (*Glycine max* L. Merr.) CULTIVATED ON FERRALSOLS OF DAKLAK PROVINCE, VIETNAM

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ABSCTRACT

Two field experiments were conducted during 2015 at DakLak province to study the effects of rhizobia and phosphate-solubilizing bacteria (PSB) on soybean (cv. Cujut) cultivated on ferralsols. In the first experiment was carried out at Centre for Agriculture Research, Western Highland Agricultural Institute (at Buon Ma Thuot city), consisted of five treatments as follows: control (no fertilizer, no inoculant), 400 kg/ha NPK 15-15-15, rhizobial inoculant [with liquid cover seeds] + 20 kg N/ha applied at 10 days after sowing [DAS], PSB inoculant [with liquid cover seeds] + 20 kg N/ha at 10 DAS, rhizobial and PSB inoculant [with liquid cover seeds] + 20 kg N/ha at 10 DAS from April to August, 2015. In the second experiment was carried out at Buonho town, consisted of four treatments as control (no fertilizer, no inoculant), 100 kg/ha thermophosphate (15% P_2O_5) + 25 kg/ha NPK 16-16-8 applied at 20 and 40 at DAS, rhizobial and PSB inoculant [with liquid cover seeds] + 200 kg biofertilizer + 20 kg N/ha at 10 DAS and rhizobial and PSB inoculant [with liquid cover seeds] + 400 kg biofertilizer + 20 kg N/ha at 10 DAS. The results showed that application of rhizobial inoculant and/or PSB inoculant produced significantly higher yield component, grain yield than control and did not differ from 400 kg/ha NPK 15-15-15 in the experiment 1. In the second experiment, using biofertilizer for soybean cultivation supported higher yield component, grain yield and oil, protein in seed than control and equivalent with treatment of 100 kg/ha thermophosphate (15% P_2O_5) + 25 kg/ha NPK 16-16-8. It therefore

seems that biofertilizers can be considered as a replacement for part of chemical fertilizers in soybean cultivation on ferralsols.

KEYWORDS: Highland, Ferralsols, Phosphate-solubilizing bacteria, Rhizobia, Soybean.

INTRODUCTION

Soybean (*Glycine max*) is one of the most important oil seed crop in the world. It contains 18 to 22% oil, highly desirable in diet and have 40 to 42% of good quality protein (Fatima et al. 2006); Soybean protein is rich in valuable amino acid lycine (5%) in which most of the cereals are deficient (Rana et al. 2014). Soybean, like other legumes, fixes atmospheric nitrogen in association with gram-negative soil bacteria of the genera *Bradyrhizobium* and *Sinorhizobium* (Jordan, 1982; Scholla and Elkan, 1984). Many rhizobial inoculant products have been applied for soybean cultivation for along time (FAO, 1984). However there were many researches showed that many PGPR as PSB supported good nodulation and rhizobia-legume symbiosis (Wasule et al. 2007; Rosas et al. 2007), the results led high grain yield and protein content in seeds (Zarei et al. 2012). The biofertilizer (consisted of rhizobia and PSB) was not only as well as soybean grain yield applying with 100 kg N and 60 kg P_2O_5/ha but also quality soybean seed [protein and lipid content in seed] was higher than soybean seed using of chemical fertilizers at Dong Thap province, Mekong Delta, Vietnam (Diep, 2005).

Next to nitrogen, phosphorus is an important plant nutrient involved in several energy transformation and biochemical reactions including biological nitrogen fixation (Devi et al., 2012). P is needed in relatively large amounts by legumes for growth and nitrogen fixation and has been reported to promote leaf area, biomass, yield, nodule number, nodule mass, etc.., in a number of legumes (Berg and Lynd, 1985; Pacovsky, 1986; Kasturikrishna and Ahlawat, 1999). Phosphorus deficiency can limit nodulation by legumes and P fertilizer application can overcome the deficiency (Carsky et al. 2001). Large amount of P applied as fertilizer enters into the immobile pools through precipitation reaction with highly reactive aluminum (Al^{3+}) and iron (Fe^{3+}) in acidic and calcium (Ca^{2+}) in calcareous or normal soils (Gyaneshwar et al.. 2002; Hao et al., 2002). Phosphate-solubilizing bacteria (PSB) are being used as biofertilizer since 1950s (Kudashev, 1956; Krasilinikov, 1957). Release of P by PSB from insoluble and fixed/absorbed forms is an import aspect regarding P availability in soils (Devi et al. 2012).

DakLak province is situated in the highland of Vietnam, it locates from 107°20’03” to 108°59’43” E and from 12°10’00” to 13°24’59” N. The soils are mainly red latosols (from origin of volcanic mountain) or ferralsols (FAO classification) with a pH range of 5.28 - 5.45. They are considered a good nutrient, with an average organic matter of 3%, a total nitrogen range of 0.18 – 0.20%, but it has concentrations of low available phosphorus, cation exchange capacity, exchangeable K (Trinh, 2012). Many kinds of crop such as rubber, coffee, pepper, upland-rice, corn and soybean have been cultivated on ferralsols permenantly. Soybean is also planted on ferralsols during wet season (April to November in year) but low grain yield (1 – 2 ton(s)/ha). In this study, selected rhizobia strains and PSB were evaluated on yield component, grain yield and soil characteristics [after harvesting] and biofertilizer technology has taken a part to minimize production costs with granule fertilizer which suitable for soybean cultivation mechanise.

MATERIALS AND METHODS

Two field experiments were conducted at Centre for Agricultural Research, Western Highland Agricultural Institute (Buon Ma Thuot city) in Summer-Autumn cropping-season 2015 (April to August) and at Buonho town, DakLak province in Autumn-Winter cropping-season 2015 (September to December).

Soil characteristics

The soil was ferralsols (or red latosol) in pH of 4.64, low in organic matter (3.575%), nitrogen total (0.135%) and available P_2O_5 (8.177 mg/kg) in the first experiment and pH=5.42, organic matter (3.822%), N total (0.137%) and available P_2O_5 (6.322 mg/kg) in the second experiment (Origin: Soil analysis Lab., Institute of Western Agriculture-Forestry Science [WASI]).

Rhizobial and PSB inoculant, biofertilizer

Rhizobia and PSB strains: *Bradyrhizobium japonicum* strain CJ02 (Diep and So, 2016) was produed in YEM broth in 4 days, reached to >10^9 cell/ml and *Burkholderia* sp. S31 strain (Diep et al. 2016) was produced in NBRIP broth in 2 days reached to >10^9 cell/ml.

Biofertilizer consisted of organic matter (35%), thermophosphate (15% P_2O_5)(5%) + Dolomite (0.5% P_2O_5, 50% $CaCO_3$, 10% $MgCl_2$)[dolomite is by-product of cement factory](45%), ground black rice-hull ash (15%) and PSB liquid at moisture 25% and the mixture was made to granule with size 5-7 mm diameter (Figure 1).

Experimental design

Both experiments were arranged with completely block randomized design with four replications, each plot was a treatment with 20 square meter (4x5 m)(Figure 2), total was 24 plots (the 1st experiment) and 16 plots (the 2nd experiment). Weed control two times (20 and 40 DAS), pest control according to the guide of Department of Plant Protection, DakLak province, the experiments were watered by rainy. The first experiment had five treatments as follows control (no fertilizer, no inoculant), 400 kg/ha NPK 15-15-15, rhizobial inoculant [with liquid cover seeds] + 20 kg N/ha applied at 10 days after sowing [DAS], PSB inoculant [with liquid cover seeds] + 20 kg N/ha at 10 DAS, rhizobial and PSB inoculant [with liquid cover seeds] + 20 kg N/ha at 10 DAS and the second experiment had four treatments as control (no fertilizer, no inoculant), 100 kg/ha thermophosphate (15% P_2O_5) + 25 kg/ha NPK 16-16-8 applied at 20 and 40 at DAS, rhizobial and PSB inoculant [with liquid cover seeds] + 200 kg biofertilizer + 20 kg N/ha at 10 DAS and rhizobial and PSB inoculant [with liquid cover seeds] + 400 kg biofertilizer + 20 kg N/ha at 10 DAS.

Figure: 1 Biofertilizer (granule)

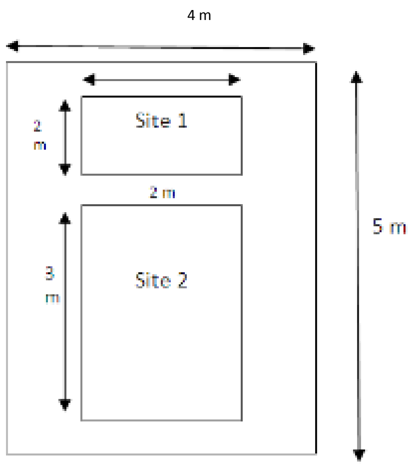

Figure: 2 Layout of 1 plot with site 1 and site 2

Yield component and Grain yield

Plant height and yield component were recorded at the time of maturity. Five randomly plants were taken at site 1 (Figure 2) to measure plant height, number of pods/plant, hundred seed weight. All plants in site 2 (Figure 2) were harvested to calculate grain yield after soybean seeds were oven dried at 70^{o}C for constant weight. Soybean seed was also chosen to ground for determination of total nitrogen and oil contents.

Estimation of protein and oil

Total nitrogen content of soybean seed was determined by Micro-Kjeldahl method as recommended by AOAC, 1975). Nitrogen contents were multiplied by dry matter-based factor 5.71 to determined total protein content (Sadassivam and Manickam, 1996) and Oil content of soybean seeds was estimated by adopting Soxhlet Ether Extraction method (Sadassivam and Manickam, 1996).

Soil analysis

After harvesting, soil samples of each treament were collected to analyse as soil pH with pH meter, N total by Micro-Kjeldahl method, Available P_2O_5 by Colorimetric method (Murphy and Riley, 1962), Organic matter with Walkley-Black method.

Statistical Analysis

All the data pertaining to the present investigation were statiscally analyzed as per the method described by Gomez and Gomez (1984). The stastistically significance of various effects was tested at 5 per cent level of probability.

RESULTS AND DISCUSSION

The first experiment (Centre for Agricultural Research, Ban Me Thuot city)

Plant height, Yield component and Grain Yield

The data from Table 1 showed that no difference between the treatments however application of inorganic fertilizer and rhizobial and PSB inoculants increased number of pods/plant in comparison to control signigicantly and using 400 kg NPK/ha and rhizobial inoculant plus 20 kg N/ha supported the highest number of pod/plant but hundred seed weight of 400 kg NPK/ha and rhizobial and PSB inoculant [with liquid cover seeds] + 20 kg N/ha treatments were the lowest and applying PSB inoculant [with liquid cover seeds] + 20 kg N/ha had the highest number of pods/plant perhaps no-seed/plant ratio was lowest.

Table: 1 Effects of rhizobial, PSB inoculants and inorganic fertilizer on plant height, yield component and grain yield of soybean (cv. Cujut) cultivated on ferralsols (at Centre for Agricultural Research, Western Highland of Agriculture Institute, Ban Me Thuot city)

Treatment	Plant height (cm)	Pods /plant	100-seed weight (g)	No-seed ratio (%)	Grain yield (kg/ha)	Increased Ratio (%)
Control	60.25	21.25	19.09	2.12	1835	0
400 kg NPK 15-15-15	58.75	29.00	18.45	3.10	2102	14.55
Rhizobial inoculant + 20kg N	63.12	30.15	19.99	4.29	2165	17.98
PSB inoculant + 20kg N	66.04	24.25	20.39	1.96	2112	15.09
Rhizobial + PSB inoculant + 20kg N	61.62	27.25	18.50	4.33	1932	05.29
calculated F	**NS**	**	**	*	**	
LSD.05		**2.00**	**1.08**	**0.80**	**83**	
C.V (%)	**8.64**	**10.21**	**3.72**	**33.08**	**5.46**	

NS: not significant

Application of rhizobial inoculant (2165 kg/ha) or PSB inoculant (2112 kg/ha) had the highest grain yield and both did not differ from 400 kg NPK/ha treatment but mixture of rhizobia and PSB inoculant (1932 kg/ha) did not support grain yield. Therefore applying rhizobial inoculant or PSB inoculant plus 20 kg N/ha can save amount of NPK equivalent with approx. 40 kg N, 60 kg P_2O_5, 60 kg K_2O/ha.

The relationship between Grain yield and number pods/plant and 100-seeds weight closely (Figure 3 and 4). Therefore rhizobia, PSB and/or inorganic fertilizer afftected to number of pods/plant and 100-seeds weight and they influenced to grain yield.

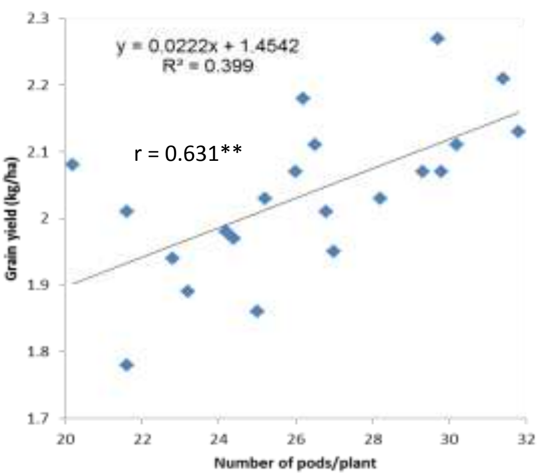

Figure: 3 Relationship between grain yield and number of pods/plant

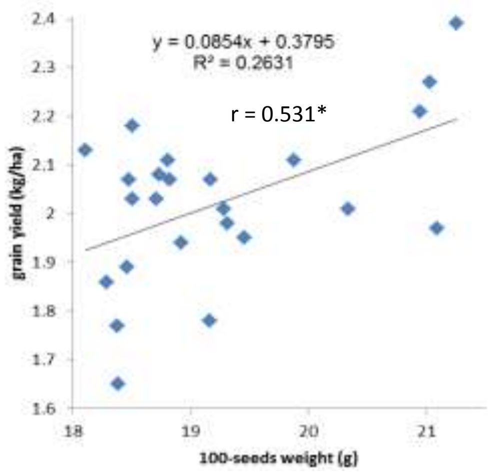

Figure: 4 Relationship between grain yield and 100-seeds weight

Application of mixture of rhizobia and PSB inoculant had the lowest protein content in soybean seed (32.17%) and protein yield (622 kg/ha) while applying rhizobial inoculant had the highest protein content in soybean seed (35.45%) and protein yield (768 kg/ha), and applying 400 kg NPK/ha supported protein content (35.32%) and high protein yield (742 kg/ha), PSB inoculant had low protein content in soybean seed (34.39%) but protein yield (727 kg/ha) did not differ from protein yield of 400 kg NPK/ha treatment (Table 2). Interestingly, mixture of rhizobia and PSB inoculant had the highest oil content in soybean seed, this led high oil yield (414 kg/ha), equivalent with the three treatments (rhizobial, PSB inoculant and 400 kg NPK); oil content in soybean seed of control treatment was the lowest (while high protein content), this led the lowest oil yield of control treatments significantly. Oil yield of two treatments (rhizobial and PSB inoculant) were the highest and they did not differ with oil yield of NPK treament.

Table: 2 Effects of rhizobial, PSB inoculants and inorganic fertilizer on protein content and oil content in soybean seed (cv Cujut) cultivated on ferralsols (at Centre for Agricultural Research, Western Highland of Agriculture Institute, Ban Me Thuot city)

Treatment	Protein content (%)	Protein Yield (kg/ha)	Oil content (%)	Oil yield (kg/ha)
Control	35.23	645	19.43	356
400 kg NPK 15-15-15/ha	35.32	742	20.17	424
Rhizobial inoculant + 20 kg N/ha	35.45	768	20.01	433
PSB inoculant + 20 kg N/ha	34.39	727	20.62	435
Rhizobial+PSB inoculant + 20 kg N/ha	32.17	622	21.43	414
calculated F	**	**	**	**
LSD.05	**0.85**	**32**	**0.12**	**17**
C.V (%)	**1.62**	**6.08**	**0.82**	**5.43**

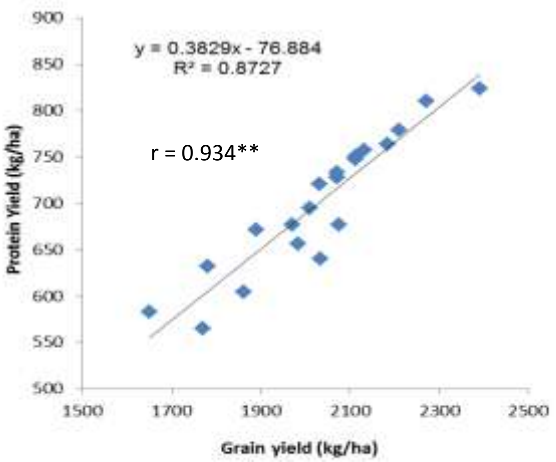

Figure: 5 The relationship between grain yield and protein yield closely

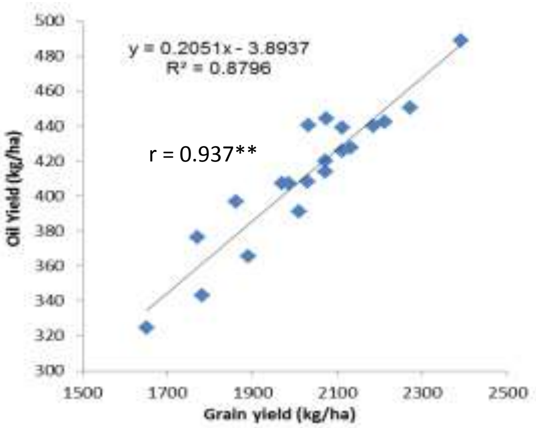

Figure: 6 The relationship between grain yield and oil yield closely

However, application of rhizobial inoculant or PSB inoculant increased grain yield in comparison to control (no fertilizer, no inoculant) and increased grain yield also helped increased protein yield and oil yield, these results were demonstrated the relationship between grain yield and protein yield (Figure 5) and oil yield (Figure 6).

The data from Table 3 showed that no difference about soil pH between treatments and initial treatment; soil N total of control treament reduced sigficantly in comparison to initial while N total of rhizobial and PSB inoculant treatment increased (compared to initial) especially PSB supported the highest soil N total and available P_2O_5 in soil had also the same results as soil N total. Application of rhizobia and PSB improved organic matter concentration in soil after harvesting in comparison to initial treatment while others decreased organic matter concentration in soil.

Table: 3 Effects of rhizobial, PSB inoculants and inorganic fertilizer on soil pH and chemical characteristics of ferralsols (at Centre for Agricultural Research, Western Highland of Agriculture Institute, Ban Me Thuot city)

Treatment	**Soil pH**	**N total (%)**	**Available P_2O_5 (mg/kg dry soil)**	**Organic matter (%)**
Initial	5.64	0.135	40.35	3.575
Control	5.55	0.113	34.78	4.056
400 kg NPK 15-15-15/ha	5.61	0.080	33.60	4.077
Rhizobial inoculant + 20 kg N/ha	5.48	0.117	35.19	4.162
PSB inoculant + 20 kg N/ha	5.47	0.143	36.10	4.137
Rhizobial+PSB inoculant + 20 kg N/ha	5.50	0.091	35.87	4.038
calculated F	**NS**	**	**	**
LSD.05		**0.003**	**4.9**	**0.031**
C.V (%)	**2.33**	**1.77**	**1.86**	**1.01**

NS: not significant

The second experiment (Buonho town)

Plant height, Yield component and Grain Yield

The data from Table 4 showed that no difference between treatments about plant height however application of biofertilizer and inorganic fertilizer increased number of pods/plant and hundred seeds weight significantly but these treatments reduced no-seed pod/plant ratio and these results supported soybean plants having grain yield was higher control significantly.

Table: 4 Effects of biofertilizer and inorganic fertilizer on plant height, yield component and grain yield of soybean (cv Cujut) cultivated on ferralsols at Buonho town, DakLak province

Treatment	Plant height (cm)	Pods /plant	100-seeds weight (g)	No-seed ratio (%)	Grain yield (kg/ha)	Increased Ratio (%)
Control	40.01	20.68	16.42	14.33	1320	0
Inorganic fertilizer	43.35	30.25	17.77	11.32	1660	25.75
Biofertilizer (200 kg) + 20 kg N	43.65	30.38	17.81	8.90	1570	18.94
Biofertilizer (400 kg) + 20 kg N	41.15	28.05	17.03	7.53	1500	13.64
calculated F	**NS**	**	**	**	*	
LSD.05		**0.41**	**0.54**	**2.74**	**232**	
C.V (%)	**5.36**	**7.80**	**2.09**	**16.55**	**10.21**	

NS : not significant

The relationship between Grain yield and number pods/plant and 100-seeds weight closely (Figure 7 and Figure 8). Therefore rhizobia, PSB and/or inorganic fertilizer afftected to number of pods/plant and 100-seeds weight and they influenced to grain yield.

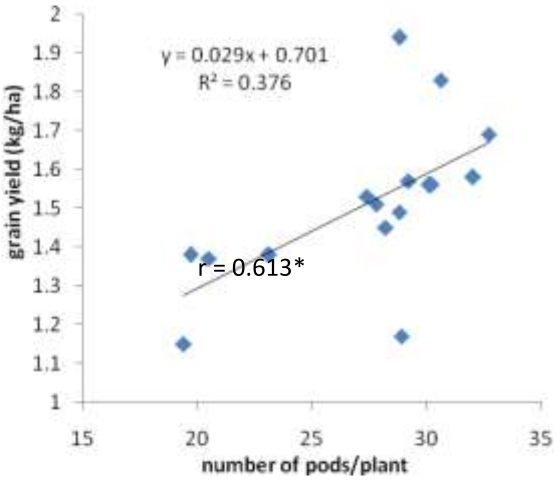

Figure: 7 Relationship between grain yield and number of pods/plant

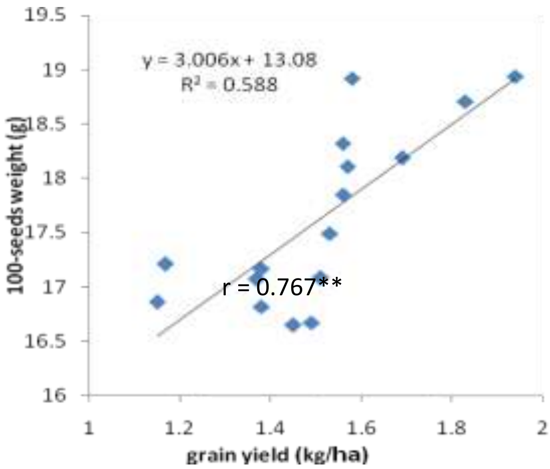

Figure: 8 Relationship between grain yield and 100-seeds weight

Application of biofertilizer significantly produced high protein content (36.99%) and oil content in soybean seed (19.13%) while inorganic fertilizer treatment had the lowest oil content in soybean seed however low grain yield of control treatment, this led lowest protein yield and oil yield of control treatment significantly in comparison to other treatments (Table 5).

Table: 5 Effects of rhizobial, PSB inoculants and inorganic fertilizer on protein content and oil content in soybean seed (cv Cujut) cultivated on ferralsols (Buonho town, DakLak province)

Treatment	Protein content (%)	Protein Yield (kg/ha)	Oil content (%)	Oil yield (kg/ha)
Control	35.51	468	18.52	244
Inorganic fertilizer	35.99	597	17.00	282
Biofertilizer (200 kg) + 20 kg N/ha	36.99	579	18.90	296
Biofertilizer (400 kg) + 20 kg N/ha	35.17	526	19.13	286
F calculated	*	*	**	*
LSD.05	**1.33**	**95**	**0.70**	**32**
C.V (%)	**2.35**	**11.09**	**2.40**	**7.28**

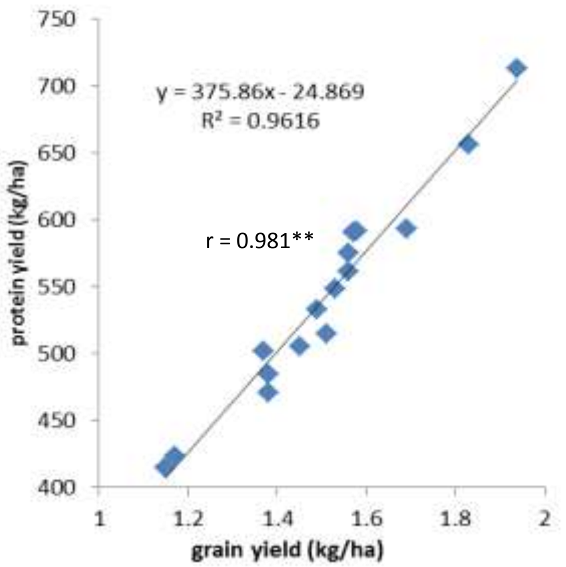

Figure: 9 The relationship between grain yield and protein yield very closely

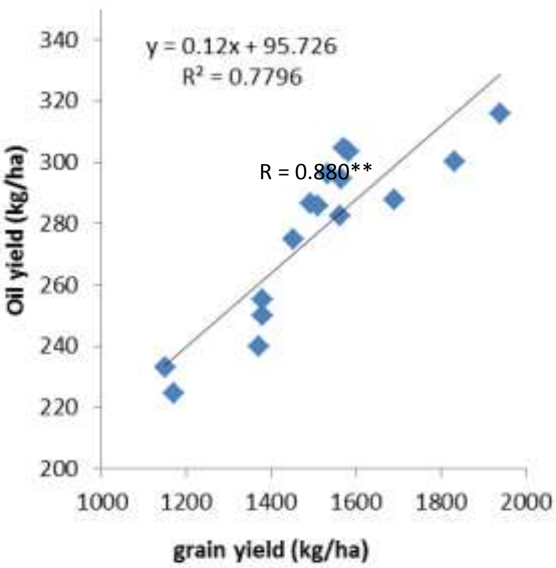

Figure:10 The relationship between grain yield and oil yield very closely

Furthermore, application of biofertilizer for soybean cultivation enhanced protein yield and oil yield, these were demonstrated the relationship between grian yield and protein yield and oil yield (Figure 9 and Figure 10).

After soybean cultivation, soil pH decreased but soil N total increased in comparison to initial however available P_2O_5 in soil reduced. Low organic matter concentration in soil (<2%) even through after a soybean cropping-season, organic matter improved a little (compared to initial)(Table 6).

Table: 6 Effects of rhizobial, PSB inoculants and inorganic fertilizer on soil pH and chemical characteristics of ferralsols (Buonho town, DakLak province)

Treatment	Soil pH	N total (%)	Available P_2O_5 (mg/kg dry soil)	Organic matter (%)
Initial	5.42	0.137	33.22	1.822
Control	5.24	0.162	26.56	1.751
Inorganic fertilizer	5.10	0.151	28.35	1.853
Biofertilizer (200 kg) + 20 kg N/ha	5.25	0.152	27.88	1.902
Biofertilizer (400 kg) + 20 kg N/ha	5.26	0.176	28.32	1.888
calculated F	*	**	**	**
LSD.05	**0.11**	**0.008**	**0.85**	**0.071**
C.V (%)	**1.28**	**3.05**	**1.95**	**2.32**

Low soil pH and N total are charcateristics of ferralsols (Trinh, 2012); while available P_2O_5 and organic matter in ferralsols depended on cultutal practices. However concentrations of available P_2O_5 and organic matter in ferralsols of Cujut site were higher than those of Buonho town site and grain yield of soybean cultivated on ferralsols of Cujut site was higher than Buonho town site. However all of four soil characteristics from two field experiments showed that the soil fertility of both experiments was low therefore Cujut and Buonho' soil need to be improved to increase grain yield and quality seed of soybean when soybean has been cultivated on the ferralsols.

Microorganisms through secretion of different types of organic acids like carboxylic acid (Deubel and Merbach, 2005) and rhizosphere pH lowering mechanisms (He and Zhu, 1988) dissociate the bound forms of phosphate like $Ca_3(PO_4)2$ (Devi et al., 2012), these information explained soil pH of our experiments decreased slightly at harvesting. Besides N requirement for soybean growth through nitrogen biological fixation, soybean plants require many other nutrients as phosphorus and P had quite prominent effects on nodulation, growth and yield parameters (Kumaga and Ofori, 2004). Many soils throughtout the world are P-deficient because the free phosphorus concentration (the form available to plants) even the fertile soil is generally not higher than 10 µM even at pH 6.5 where it is most soluble (Gyneshwar et al. 2002). To circumvent the problem of P deficiency, chemical fertilizers are added to the soils but cost of chemical phosphatic fertilizers is high (Golstein et al., 1993) and low efficiency (<0.1%)(Scheffer and Schachtschabel, 1992). Phosphorus biofertilizers in the form of microorganisms, especially phosphate-solubilizing bacteria in rhizosphere, can help in increasing the availability of accumulated phosphates for plant growth by solubilization (Richardson, 1994; Nautiyal et al., 2000).

Application of phosphorus along with PSB improved phosphorus uptake by plants and yields indicating that the PSB are able to solubilize phosphates and to mobilize phosphorus in crop plants (Rogers and Wolfram, 1993). Devi et al (2012) recognized that mixture single super phosphate + phosphate solubilizing bacteria (SSP+PSB) produced significantly higher number of nodules per plant, dry weight of nodules per plant, number of pods per plant and 100-seed weight than the other treatments, especially maximum grain yield and total phosphorus uptake were also recorded.

PSB enhance the phosphorus availability to plants by mineralizing organic P in soil and by solubilizing precipitated phosphate (Chen et al. 2006; Kang et al. 2002; Pradhan and Sukla, 2005). Dubey et al (1997) have also reported significant increases in grain yield of soybean due to co-inoculation of phosphorus solubilizers. Our results showed that application biofertilizer (mixture of thermophosphate and PSB) for soybean cultivation had high grain yield, protein yield and oil yield as applying NPK or inorganic fertilizer without inoculation.

PSB also are capable of transforming soil phosphorus to the forms available to plant and oil and protein yield were also maximum with PSB inoculant or biofertilizer. It is reported that soybean inoculated by *Bradyrhizobium* bacteria and phosphate solubilizing bacteria increased the seed yield (Singh, 1994; Jat and Ahlawat, 2006).

Phosphate solubilizing bacteria led to increased absortion of other elements by increasing the ability to access phosphorus and thereby can increase crop yield (Mahfouz and Sharaf-Eldin, 2007). Rana et al. (2014) recognized that application of mixture of PSB + *Rhizobium* and 45 kg P_2O_5 on soybean cultivation in India had the highest effect in comparison to applying 60 kg P_2O_5 which results in net saving of 15 kg P_2O_5/ha.

In the Mekong Delta, Son et al (2006) reported that application of *Bradyrhizobium japonicum* and PSB *Pseudomonas* spp. can enhance the number of nodules, dry weight of nodules, yield components, grain yield, soil nutrient availability and uptake of soybean crop. In this study, rhizobia with *Bradyrhizobium japonicum* CJ02 (Diep and So, 2016) and PSB with *Burkholderia* sp. (Diep et al. 2016) were used as inoculant for soybean cultivation; soybean plants were also supplemented with biofertilizer (consisted of thermophosphate + ash + dolomite and PSB) as phosphorus fertilizer.

CONCLUSION

The application of rhizobial inoculant and/or PSB inoculant did not have significant difference with chemical fertilizer, they increased grain yield from 15 to 19% in comparison to control, especially protein yield and oil yield were high. The best cultural practice was (i) inoculant with rhizobia and/or PSB inoculant, and (ii) supplemented with small quantity nitrogen (20 kg N/ha) and thermophosphate (10-20 kg/ha) for soybean cultivation in ferralsols.

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