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Effects of *Azospirillum amazonense* and *Bacillus subtilis* on high-yielding rice (cv. Ma Lam 213) cultivated on sandy loam soils of Phu Yen province, Vietnam

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Abstract: Two field experiments were conducted to evaluate effects of two endophytic bacterial strains (*Azospirillum amazonense* SHL70, *Bacillus subtilis*TAL4*)* together with different levels of inorganic nitrogen and phosphorus fertilizers on the growth and grain yield of high-yielding rice (cv. Ma Lam 213) cultivated on silty clay loam soils of Dong Hoa district and Tuy An district, Phu Yen province, Vietnam in Summer-Autumn 2014 cropping-season. The results of two field trials showed that bacterial inoculation in rice plants (either SHL70 or TAL4 and especially mixture of SHL70 and TAL4) plus 60 kg N and 40 kg P_2O_5/ha having yield components and grain yield were not significant difference with those of rice only applying 120 kg N and 80 kg P_2O_5/ha without inoculation therefore both of bacteria strains provided 50% biological nitrogen and available phosphorus quantity for high-yielding rice requirement, improvement of quality grain and soil fertility of Dong Hoa district and Tuy An district, Phu Yen province.

Keywords: *Azospirillum amazonense*, Biological Nitrogen Fixation, *Bacillus subtilis*, Grain Yield, High-Yielding Rice, Phosphate Solubilization

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important crops in the world and paddy soil comprise the largest anthropogenic wetland on earth [1] and chemical fertilizers, substances composed of known quantities of nitrogen, phosphorus and potassium, keep an important role in the growth and yield of crops as high-yielding rice. Nitrogen (N) is one of the most important nutrients in plants and is a limiting in plant growth and development and available phosphorus (P) deficiencies in many parts of the world [2] also is the limiting factor for rice growth and development especially root development, poor flowering and lack of seed etc., consequently causing degradation in quality and quantity [3].

Recently, the potential for microbes to increases nutrient availability and to enhance crop growth has garnered the attention of the researchers, and the increasing reliance on biological processes and plant interactions with microbes through ‘ecological intensification’ may be one of the most promising strategies to overcome these problems [4]. Bio-fertilizers containing efficient microorganisms, improve plant growth in many ways compared to synthetic fertilizers, by way of enhancing crop growth, sustainability of environment and crop productivity and efficient microbes with PGPR and endophytic bacteria have been produced as bio-fertilizer for crop production in sustainable agriculture [5].

Phu Yen, is one of seven coastal provinces of central Vietnam, has 57,100 ha for rice cultivation, with average grain yield (6.036 tons/ha) and rice productivity every year estimating 344,700 tons (Statistics 2012 of Ministry of Vietnam Agriculture and Rural Development) and Phu Yen province needs large amount of chemical fertilizers to produce rice productivity to involve food requirement every year. However this overuse of chemical N and P fertilizers not only is economically expensive but also itiates a cascade of large-scale environmental impacts, including threatening ecosystem sustainability around the world by causing terrestrial and aquatic eutrophication and acidification and by creating large hypoxic zones [6][7]. Our previous reports indicated the endophytic bacteria as *Azospirillum amazonense* SHL70, *Bacillus subtilis*TAL01 strains could produce phytohormone, promotes the growth and rice of yield and significantly reduces the required amount of soil N and P fertilizers [8][9]. The aims of this study (i) to identify the effects of two these strains on high-yielding rice, (ii) evaluate soil fertility of two these sites in Phu Yen province.

2. Materials and Methods

2.1. Bacterial Strains

Azospirillum amazonense SHL70 and *Bacillus subtilis* TAL01were isolated from the rice plants [8]. Two strains were stored at 4^{o}C on Burk N-free agar [10] and NBRIP agar [11] and they also multipled in Burk N-free broth medium and NBRIP broth medium during 2-3 days and population reached to over 10^8 cfu/ml.

2.2. Plant Seeds and Paddy Soils

The rice cultivar used was an indica (*Oryza sativa* L.) "Ma Lam 213", which is a common cultivar grown in Phu Yen province and it originated from Phu Yen Crop Cultivar Center. The rice seeds were immersed with warm water (65-70^{o}C) overnight, and rinsed six times in sterilized water and rice seeds already were used in two field experiments at Dong Hoa district and Tuy An district (Figure 1).

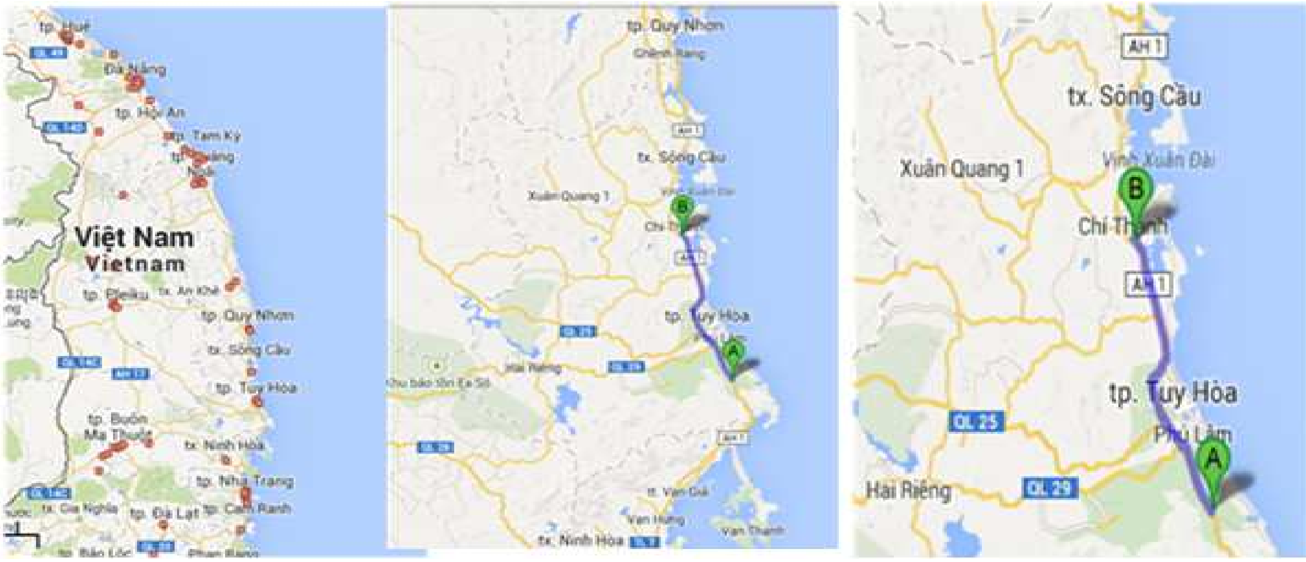


Figure 1. *Two experimental sites with A (Dong Hoa dist.) and B (Tuy An dist.)*

Two experimental rice fields were conducted in two sites: Dong Hoa dist. And Tuy An dist. of Phu Yen province (12°42'36" to 13°41'28"N and from 108°40'40" to 109°27'47"E), Vietnam from July 2014 to October 2014. The region has a typical subtropical climate with an annual precipitation of 1935 mm and a mean annual temperature of 29^{o}8C. The soil for the experiment was presented in Table 1.

Table 1. *Chemical and physical properties of experimental soil*

Properties	Dong Hoa site values	Tuy An site values
pH (H_2O)	5.67	6.47
Organic matter (%)	4.21	2.61
Total N (%)	0.225	0.134
Total P (%)	0.072	0.080
Available P (mg 100 g^{-1})	0.184	0.094
Exchangeable K (mg kg^{-1})	71.76	36.66
EC (mS/cm)	0.349	0.134
Bulk Density (kg/dm2)	1.002	1.002
Sand (%)	12.9	14.0
Silt (%)	46.1	51.8
Clay (%)	41.0	34.2

In general, both of experimental soils are silty clay loam soil but soil fertility of Dong Hoa site is better than Tuy An one.

2.3. Bacterial Endophyte Inoculation and Rice Cultivation

Azospirillum amazonense SHL70 and *Bacillus subtilis* TAL4 were first activated in 100 ml of Burk N-free broth medium [10] and NBRIP broth medium [11], respectively in two separated flasks (250 mL) for 3 days at 120 rpm in an orbital shaker at 28^{o}C, after that they were transferred to 10 L of Burk N-free broth medium and NBRIP broth medium in 2 big flasks (10-L) incubated in plants after 21 days sowing. For the inoculated part (A) soak the roots with 6 L the suspension of *Azospirillum amazonense* SHL70, the inoculated part (B) soak the roots with 6 L the suspension of *Bacillus subtilis* TAL04, 3 litres suspension *Azospirillum amazonense* SHL70 and 3 litres *Bacillus subtilis* TAL4 mixed each other in 10 minutes after that this mixture (AB) soak the roots and control (C) soaking 6 litres sterilized water. Rice root get soaked in 6 hours before transplanting with distance 15 x15 cm in the separated plots which were divided by small dikes (40x40 cm).

Plants were regularly watered during the growing season and plant protection was done by recommendations of Phu Yen Plant Protection Service.

2.4. Experimental Design

The experiment was a split-plot design with four replications with main plot (NP fertilizers with three levels: no fertilizer, 60N-40P_2O_5, 120N-80P_2O_5) and subplot (bacterial strains included *Azospirillum amazonense* SHL70 (A), *Bacillus subtilis* TAL4 (B), mixture of *Azospirillum amazonense* SHL70 and *Bacillus subtilis* TAL01 (AB) and control (C)[no inoculation]).

Chemical N fertilizer (urea contains 46%N) was applied in three applications: the first application was made at 10 days after sowing (DAS)(40% of total N), the second application was performed at the tillering stage (20 DAS)(40% of total N) and the third application at the panicle differentiation stage (35-40 DAS)(20% total N); potassium (Potassium chloride with 60% K_2O) the first application was made at 10 days after sowing (DAS)(50% of total K), the second application was performed at the tillering stage (35 DAS)(50% of total K) and Phosphorus (thermophosphate 15% P_2O_5) fertilizers were applied as basal dressing before transplant by broadcasting under surface soil.

2.5. Investigation Items and Sample Collection

Growth parameters such as plant height, panicle number per square meter, spikelet per panicle, percentage of no-seed grain, thousand grain weight were recorded at ripening stage the recommendations of Phu Yen Agriculture Extension Center.

Plant samples (rice straw and grain) were collected randomly from each treatment at the ripening stage, they were dried for analyses of biomass and of total N and others.

In harvesting stage, rice seeds were separated, then placed in an oven at at 70^{o}C and dried at 70^{o}C to a constant weight. The dry weight was recorded and the dried samples were milled to pass through a 1 mm screen and then stored in plastic bags for chemical analysis.

The grain yield was measured after harvesting all plants in 5 m^2/plot, grains were dried at 70^{o}C and weighed when grain reached to a 14% moisture.

2.6. Soil Sampling

Soil samples from the plough layer (0-20 cm) were collected two times: before the experiment and rice harvested. Soil cores (10-15 cm) were randomly sampled from each plot using a soil auger after rice was harvested. These soil samples were air dried and stored in sealed glass containers at room temperature and they were transferred to Soil Analysis Laboratory,Dept. of Soil Science, Agriculture and Applied Biology College, Can Tho University to analyse pH, total N, available P, exchangeable K and organic matter other with physical parameters.

2.7. Statistical Analysis

The experiment was analyzed as a two-way ANOVA with four levels of endophytes (subplot) and with three levels of chemical NP fertilizer. All analyses were conducted using the programme MSTATC, Minitab 16. The data were considered significantly different at $P<0.05$.

3. Results and Discussion

3.1. Effects of Two Strains on Rice at Dong Hoa Site, Phu Yen Province

Using two endophytic bacterial strains inoculated in rice plant 21 day-old supported plant height and yield components of rice cultivated on sandy loam soil, and applied with 50% chemical fertilizers (60N-40P) had the highest agronomic characteristics, this demonstrated that effects of endophytic bacteria are applying 60N-40P for rice cultivation at Dong Hoa site, Phu Yen province (Table 2). Application of two endophytic bacterial strains plus 120N-80P in rice cultivation had the highest grain yield however applying 120N-80P only had the grain yield the same as grain yield of rice inoculated with two strains plus 60N-40P treatment (Figure 3), this showed that application of chemical NP fertilizer have not responsed growth requirement of rice when it has cultivated on sandy loam soil of Dong Hoa site.

Besides, the effects of two endophytic bacterial strains and 50% chemical NP fertilizers increased the grain number/panicle and decreased no-seed ratio (%) were presented the positive regressive between grain yield and grain number/panicle and the negative regressive between grain yield and no-seed ratio (%) very significantly (Figure 2).

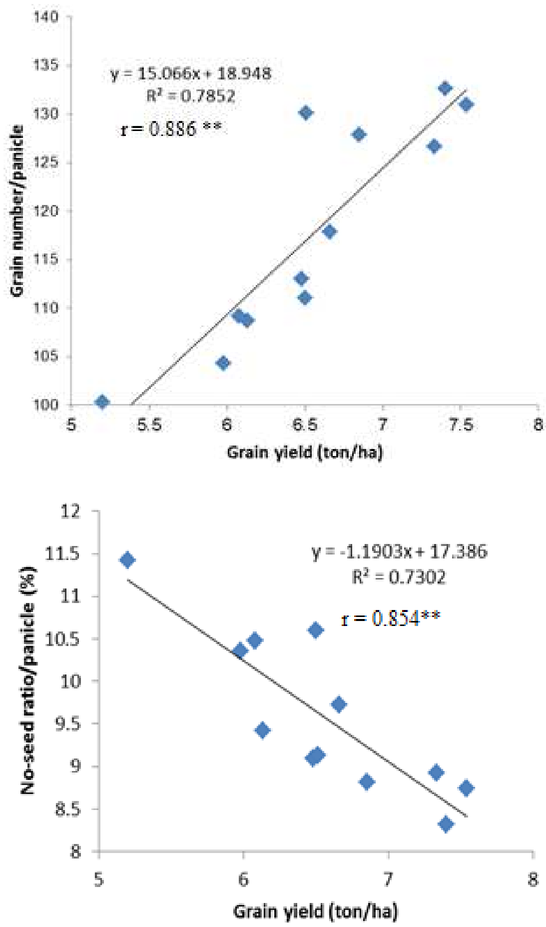
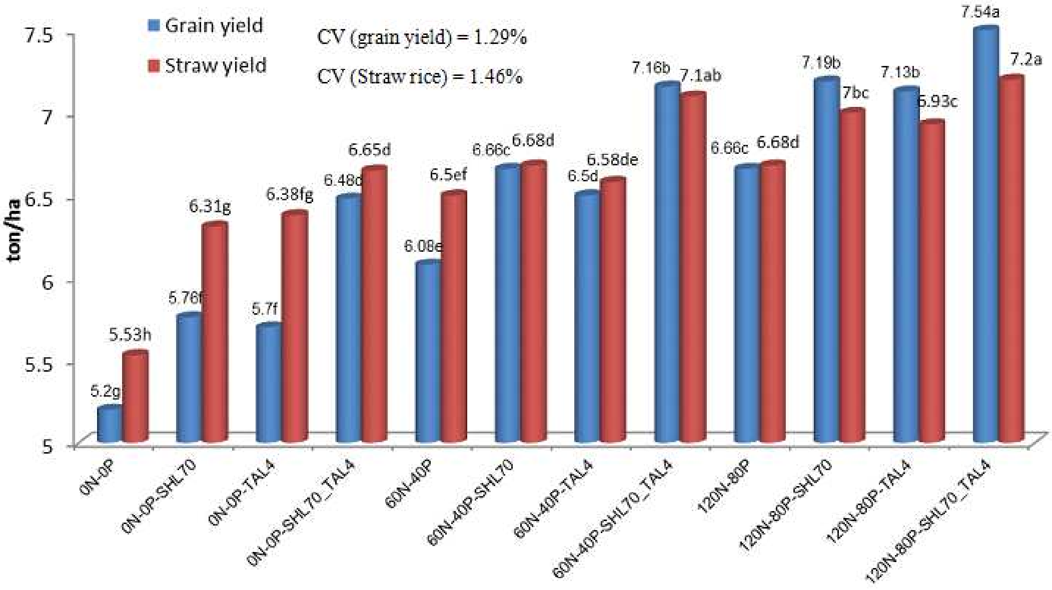


Figure 2. *The correlation between grain yield (ton/ha) and grain number/panicle positively and no-seed ratio (%) negatively in the experiment of Dong Hoa site*



Means within a column followed by the same letter/s are not significantly different at p<0.05

Figure 3. *Effects of two endophytic bacterial strains and chemical fertilizers on grain yield and straw yield of of high-yielding rice (cv. Ma Lam 213) cultivated on sandy loam soil of Dong Hoa site, Phu Yen province, Vietnam in Autumn-Winter 2014 cropping season*

Table 2. *Effects of two endophytic bacterial strains and chemical fertilizerson plant height and yield components of high-yielding rice (cv. Ma Lam 213) cultivated on sandy loam soil of Dong Hoa site, Phu Yen province, Vietnam in Autumn-Winter 2014 cropping season*

Treatment	Plant height (cm)	Panicle number /tiller	Panicle number /m^2	Panicle length (cm)	Grain number / panicle	No-seed ratio (%)	1000- grain weight (gr)
0N-0P-no inoculation	85.90 h	5.58 h	200.7 h	21.50g	100.3 f	11.67 a	23.61 d
0N-0P-SHL70	91.85 f	6.28f	225.9f	22.55 ef	104.0 f	09.81 bc	24.28 ab
0N-0P-TAL4	90.90g	6.13g	220.5g	22.20 f	101.8 f	10.59 b	23.91 bcd
0N-0P-SH70&TAL4	95.00 d	6.95 bcd	250.2bcd	22.88 de	118.3 d	08.73 def	24.23 abc
60N-40P-no inoculation	91.40 fg	6.48 e	233.1 e	22.15 f	109.2 e	10.47 b	23.83 cd
60N-40P-SHL70	96.55 b	6.90 cd	248.4 cd	23.58 c	126.2 c	09.11 cde	24.25 abc
60N-40P-TAL4	93.95 e	6.83 d	245.7 d	23.50 c	122.0 cd	09.73 bc	24.22 abc
60N-40P-SHL70&TAL4	98.15 a	7.30a	262.8a	25.53 a	145.4 a	07.83 f	24.37 a
120N-80P-no inoculation	92.20 f	6.93 cd	249.3 cd	23.20 cd	120.1 d	09.13 cd	23,91 bcd
120N-80P-SHL70	96.00 bc	6.98 bc	251.1 bc	25.75 a	135.7 b	08.16ef	24.44 a
120N-80P-TAL4	95.15cd	7.08 b	254.7 b	24.45 b	133.2 b	08.52 def	24.09 abc
120N-80P-SHL70&TAL4	98,65 a	7.35 a	264.6 a	25.80 a	143.4 a	08.02f	24.48 a
F calculated	**	**	**	**	**	**	**
C.V (%)	0.67	1.49	1.49	1.53	2.71	7.18	1.28
No inoculation	89.83 c	6.33c	227.7c	22.28c	109.9 c	10.42a	23.78 c
SHL70	94.80b	6.72b	241.8b	23.96ab	121.9 b	09.02 ab	24.32ab
TAL4	93.33 b	6.68b	240.3b	23.38b	119.0 bc	09.61ab	24.07b
SHL70&TAL4	97.27 a	7.20 a	259.2 a	24.73 a	135.7 a	08.19c	24.36 a
F calculated	**	**	**	**	**	**	**
C.V (%)	2.44	6.20	6.19	5.07	10.50	10.85	1.26
0N-0P	90.91b	6.23b	224.3b	22.28c	106.1b	10.20a	24.01
60N-40P	95.01a	6.88a	247.5a	23.69b	125.7a	09.28b	24.17
120N-80P	95.50a	7.08a	254.9a	24.80 a	133.1a	08.46c	24.23
F calculated	**	**	**	**	**	**	n.s
C.V (%)	3.08	5.44	5.43	4.49	8.70	11.62	1.53

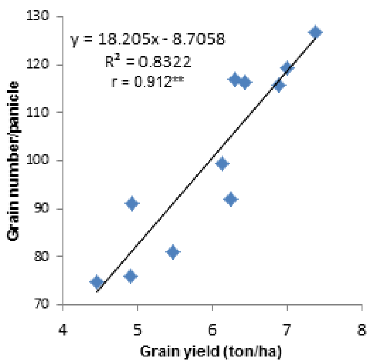
Means within a column followed by the same letter/s are not significantly different at p<0.05
*and ** are significantly different at 0.05 and 0.01 probability respectively

When analysis of multiple-regression among grain yield and two parameters (grain number/panicle and panicle number/m^2) revealed that there was a close correlation very significantly and grain number/panicle affected to grain yield strongly, the effects of two endophytic bacterial strains supported to panicle number/m^2 and grain number/panicle, this led to high grain yield.

The aquation of multiple regression as follows: Y = -1.43 + 0.005 X1 + 0.030 X2
(with X1 = grain number/panicle and X2 = panicle number/m^2) (R^2 = 0.98]. This showed that panicle number/m^2 was important.

3.2. Effects of Two Strains on Rice at Tuy an Site, Phu Yen Province

The results of Tuy An site were the same as of Dong Hoa site, the effects of two endophytic bacterial strains plus 50% chemical fertilizers (60N-40P) had the highest plant height and yield components of rice cultivated on sandy loam soil of Tuy An site, Phu Yen province (Table 3). Application of two endophytic bacterial strains plus 120N-80P in rice cultivation had the highest grain yield however applying 120N-80P only had the grain yield the same as grain yield of rice inoculated with two strains plus 60N-40P treatment (Figure 5), this showed that application of chemical NP fertilizer have not responsed growth requirement of rice when it has cultivated on sandy loam soil of Tuy An site.



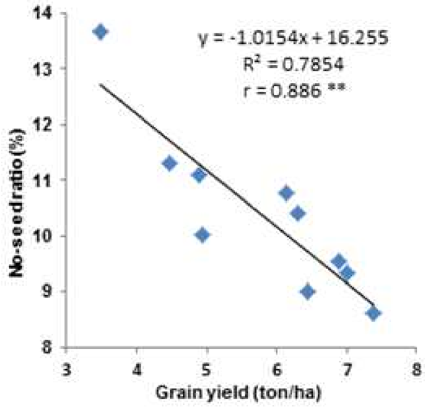


Figure 4. *The correlation between grain yield (ton/ha) and grain number/panicle positively and no-seed ratio (%) negatively in the experiment of Tuy An site*

Endophytic bacterial strains affected to two parameters of yield component (grain number/panicle and no-seed ratio (%)) as bacteria increased grain number/panicle and they reduced no-seed ratio (%), these results were presented in Figure 4. and analysis of multiple regression between grain yield and two parameters (grain number/panicle and panicle number/m^2) showed that there was a close correlation very significantly and grain number/panicle affected to grain yield strongly, the effects of two endophytic bacterial strains supported to panicle number/m^2 and grain number/panicle, this led to high grain yield.

The aquation of multiple regression as follows: Y = 9.06 + 0.06 X1 - 0.037 X2
(with X1 = grain number/panicle and X2 = panicle number/m^2) (R^2 = 0.87)

This showed that grain number/panicle was important. and regression equation as follows:

$$Y = 1673.86 + 47.20\ X1 – 8.89\ X2$$

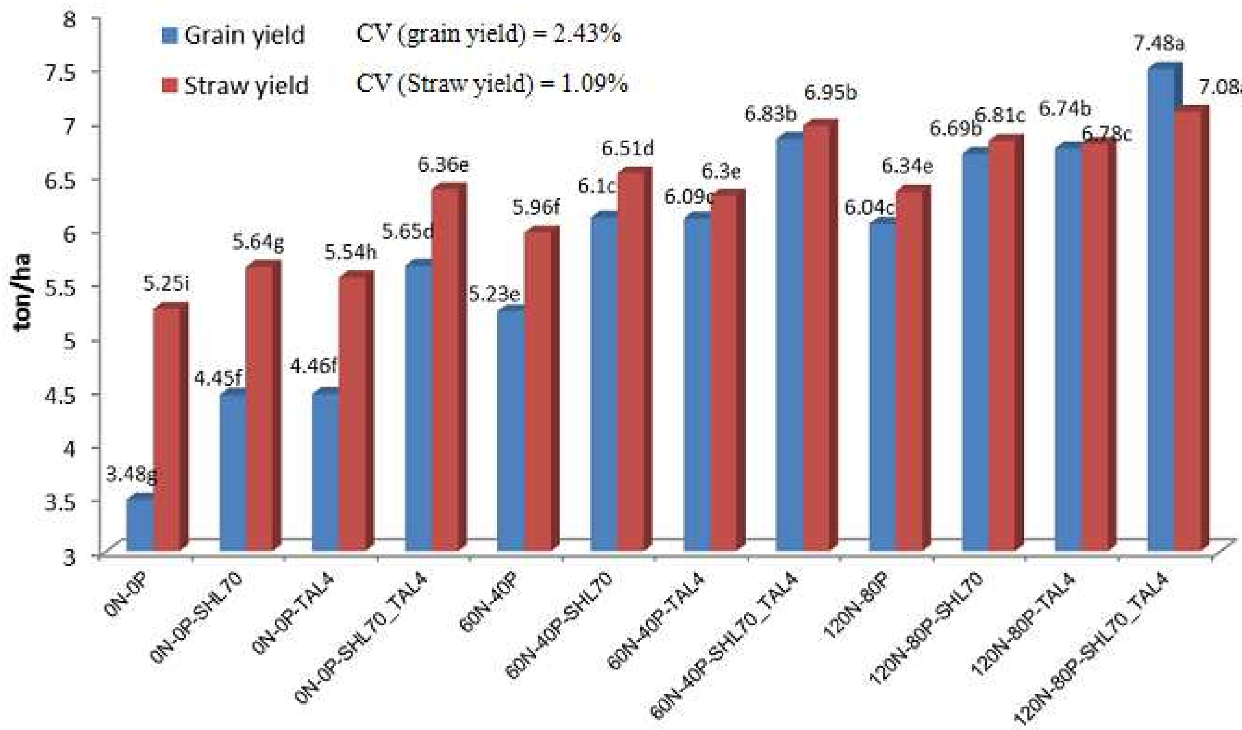
Y = N content in soil, X1 = N content in seed rice and X2 = N content in straw rice (R^2 = 0.855)

Table 3. *Effects of two endophytic bacterial strains and chemical fertilizers on plant height and yield components of high-yielding rice (cv. Ma Lam 213) cultivated on sandy loam soil of Tuy An site, Phu Yen province, Vietnam in Autumn-Winter 2014 cropping season*

Treatment	Plant height (cm)	Panicle number /tiller	Panicle number /m2	Panicle length (cm)	Grain number / panicle	No-seed ratio (%)	1000-grain weight (gr)
0N-0P-no inoculation	79.61g	5.83 i	209.7 i	19.44 f	71.65 f	13.66 a	22.54 d
0N-0P-SHL70	85.86e	6.55 fg	235.8 fg	20.44 e	75.75 ef	11.09 cde	23.34 bc
0N-0P-TAL4	85.43 e	6.45 g	232.2 g	20.64 e	74.80 f	11.31 cd	23.28 c
0N-0P-SH70&TAL4	89.75 abc	6.85 cde	246.6 cde	21.75 e	92.13 d	10.02 ef	23.63 abc
60N-40P-no inoculation	83.23f	6.23 h	224.1 h	21.48 e	80.95 e	12.63 ab	23.58 abc
60N-40P-SHL70	88.35 cd	6.75 de	243.0 de	22.40 c	99.83 c	11.94 bc	23.89 abc
60N-40P-TAL4	87.85 d	6.70 ef	241.2 ef	21.65 d	99.30 c	10.77 de	23.93 ab
60N-40P-SHL70&TAL4	90.93 a	7.03 ab	252.9 ab	23.59 a	116.35 b	09.00 fg	24.01 a

Treatment	Plant height (cm)	Panicle number /tiller	Panicle number /m2	Panicle length (cm)	Grain number / panicle	No-seed ratio (%)	1000-grain weight (gr)
120N-80P-no inoculation	88.85 bcd	6.90 bcd	248.4 bcd	21.75 d	91.90 d	11.87 bcd	23,71 abc
120N-80P-SHL70	90.30 ab	6.93 bc	249.3 bc	23.00 b	119.90 b	09.34 fg	23.92 ab
120N-80P-TAL4	90.58 a	6.73 e	242.1 e	22,84 b	115.70 b	09.53 f	23.96 ab
120N-80P-SHL70&TAL4	91.23 a	7.10 a	255.6 a	23.95 a	130.35 a	08.40 g	23.94 ab
F calculated	**	**	**	**	**	**	**
C.V (%)	1.26	1.66	1.66	1.32	4.20	7.29	1.89
No inoculation	82.89 c	6.32 c	227.4 c	20.55 c	81.50 c	12.72 a	23.28 b
SHL70	88.17b	6.74 b	242.7 b	21.95 ab	98.49 b	10.79 b	23.72 ab
TAL4	87.95 b	6.63 b	238.5 b	21.71 b	96.60 b	10.54 b	23.72 ab
SHL70&TAL4	90.63 a	6.99 a	251.7 a	22.76 a	112.94 a	9.14 c	23.86 a
F calculated	**	**	**	**	**	**	**
C.V (%)	3.05	4.17	4.61	5.48	16.75	10.35	2.28
0N-0P	85.16 c	6.42 c	231.1 c	20.32 c	78.58 c	11.52 a	23.20 b
60N-40P	87.59 b	6.68 b	240.3 b	22.03 b	99.11 b	11.08 a	23.85 a
120N-80P	90.24 a	6.91 a	248.9 a	22.88 a	114.46 a	09.78 b	23.88 a
F calculated	**	**	**	**	**	**	**
C.V (%)	3.36	4.62	4.62	4.20	13.07	14.25	2.03

Means within a column followed by the same letter/s are not significantly different at p<0.05
*and ** are significantly different at 0.05 and 0.01 probability respectively



Means within a column followed by the same letter/s are not significantly different at p<0.05

Figure 5. *Effects of two endophytic bacterial strains and chemical fertilizers on grain yield of of high-yielding rice (cv. Ma Lam 213) cultivated on sandy loam soil of Tuy An site, Phu Yen province, Vietnam in Autumn-Winter 2014 cropping season*

Table 4. *Effects of endophytic bacteria and chemical fertilizers on N contents of rice straw, rice seed and soil of Dong Hoa site*

Treatment	**Seed rice Yield (kg/ha)+**	**N in seed rice (%)**	**Protein content in seed rice (%)**	**N content in rice straw (%)**	**Total N in rice straw (kg/ha)**	**Total N in seed rice (kg/ha)**	**Total protein in seed rice (kg/ha)**	**N content In soil (%)**	**Total N in soil (kg/ha)**
0N-0P-No inoculation	4212 g	0.833 f	4.830 f	0.360 g	19.92 h	35.08 h	203.47 h	0.154 f	3088 f
0N-0P-SHL70	4668 f	0.925 d	5.367 d	0.407 f	25.71 g	43.19 f	250.50 f	0.174 e	3476 e
0N-0P-TAL4	4617 f	0.901 e	5.225 e	0.444 de	28.29 f	41.59 g	241.22 g	0.174 e	3475 e
0N-0P-SHL70&TAL4	5245 d	0.955 c	5.540 c	0.509 c	33.87 cd	50.09 d	290.53 d	0.200 bc	4003 bc
60N-40P-No inoculation	4921 e	0.906 e	5.257 e	0.438 e	28.45 f	44.60 e	258.69 e	0.175 e	3491 e
60N-40P-SHL70	5397 c	0.956 c	5.544 c	0.490 c	32.68 d	51.59 c	299.20 c	0.190 d	3804 d
60N-40P-TAL4	5265 d	0.958 c	5.555 c	0.462 d	30.40 e	50.43 cd	292.49 cd	0.190 d	3810 d
60N-40P-SHL70&TAL4	5802 b	0.979 ab	5.678 ab	0.588 a	41.79 a	56.80 b	329.42 b	0.192 cd	3834 cd
120N-80P-No inoculation	5397 c	0.932 d	5.407 d	0.453 de	30.21 e	50.30 cd	291.75 cd	0.181 e	3622 e
120N-80P-SHL70	5822 b	0.959 c	5.560 c	0.537 b	37.55 b	55.81 b	323.67 b	0.193 cd	3850 cd
120N-80P-TAL4	5771 b	0.966 bc	5.605 bc	0.506 c	35.01 c	55.77 b	323.43 b	0.204 ab	4089 ab
120N-80P-SHL70&TAL4	6105 a	0.986 a	5.719 a	0.539 b	38.80 b	60.20 a	349.17 a	0.212 a	4246 a
Calculated F	**	**	**	**	**	**	**	**	**
C.V (%)	1.29	1.35	1.35	3.06	8.25	1.82	1.85	3.22	3.23
No inoculation	4843 c	0.890 c	5.165 c	0.417 c	26.19 c	43.33 c	251.30 c	0.170 c	3400 c
SHL70	5295 b	0.947 b	5.490 b	0.478 b	31.98 b	50.19 b	291.13 b	0.185 b	3710 b
TAL4	5218 bc	0.942 b	5.462 b	0.471 b	31.23 b	49.26 b	285.71 b	0.190 b	3791 b
SHL70&TAL4	5717 a	0.973 a	5.646 a	0.546 a	38.15 a	55.70 a	323.04 a	0.201 a	4028 a
Calculated F	**	**	**	**	**	**	**	**	**
C.V (%)	9.02	3.30	3.30	9.05	13.3	11.59	11.59	6.48	6.48
0N-0P	4685 c	0.903 b	5.240 b	0.430 b	26.95 b	42.49 c	246.43 c	0.176 c	3511 c
60N-40P	5346 b	0.950 a	5.509 a	0.495 a	33.33 a	50.85 b	294.95 b	0.187 b	3734 b
120N-80P	5774 a	0.961 a	5.573 a	0.508 a	35.39 a	55.52 a	322.01 a	0.198 a	3952 a
Calculated F	**	**	**	**	**	**	**	**	**
C.V (%)	6.29	5.29	3.75	11.12	15.04	9.43	9.42	7.41	7.40

+Seed yield = grain yield x 0.81
Means within a column followed by the same letter/s are not significantly different at p<0.05
*and ** are significantly different at 0.05 and 0.01 probability respectively

Table 5. *Effects of endophytic bacteria and chemical fertilizers on P contents of rice straw, rice seed and soil of Dong Hoa site*

Treatment	**Seed rice Yield (kg/ha)+**	**P in seed rice (%)**	**P content in rice straw (%)**	**Total P in rice straw (kg/ha)**	**Total P in seed rice (kg/ha)**	**Total P in plant rice++ (kg/ha)**	**P content In soil (mg/kg)**	**Total P in soil (kg/ha)**
0N-0P-No inoculation	4212 g	0.222 i	0.063 f	3.496 h	9.479 g	12.976 g	4.163 j	8.327 j
0N-0P-SHL70	4668 f	0.232 gh	0.068 ef	4.273 g	10.850 f	15.122 f	5.093 i	10.187 i
0N-0P-TAL4	4617 f	0.236 fg	0.076 de	4.846 efg	10.843 f	15.689 f	5.677 hi	11.353 hi
0N-0P-SHL70&TAL4	5245 d	0.244 e	0.091 c	6.030 d	12.765 e	18.796 d	6.880 ef	13.760 ef
60N-40P-No inoculation	4921 e	0.228 hi	0.068 ef	4.407 fg	11.198 f	15.605 f	5.990 gh	11.980 gh
60N-40P-SHL70	5397 c	0.240 ef	0.076 de	5.075 ef	12.870 e	17.945 e	6.403 fg	12.807 fg
60N-40P-TAL4	5265 d	0.253 d	0.084 cd	5.527 de	13.362 d	18.889 d	7.490 de	14.980 de
60N-40P-SHL70&TAL4	5802 b	0.280 b	0.109 b	7.795 bc	16.203 b	23.999 b	8.663 c	17.327 c
120N-80P-No inoculation	5397 c	0.238 efg	0.078 d	5.224 e	12.889 e	18.112 de	8.067 cd	16.133 cd
120N-80P-SHL70	5822 b	0.226 c	0.105 b	7.297 c	15.466 c	22.763 c	9.613 b	19.227 b
120N-80P-TAL4	5771 b	0.277 b	0.120 a	8.285 b	15.958 b	24.243 b	10.267 b	20.533 b
120N-80P-SHL70&TAL4	6105 a	0.302 a	0.127 a	9.212 a	18.453 a	27.665 a	11.633 a	23.267 a
Calculated F	**	**	**	**	**	**	**	**
C.V (%)	1.29	1.73	5.95	6.92	1.84	2.54	5.47	5.46
No inoculation	4843 c	0.229 c	0.070 c	4.376 c	11.189 c	15.564 c	6.073 b	12.147 b
SHL70	5295 b	0.246 bc	0.083 bc	5.548 bc	13.062 bc	18.610 bc	7.037 b	14.073 b
TAL4	5218 bc	0.255 b	0.093 b	6.219 b	13.388 b	19.607 b	7.811 ab	15.622 ab
SHL70&TAL4	5717 a	0.275 a	0.109 a	7.679 a	15.807 a	23.486 a	9.059 a	18.118 a
Calculated F	**	**	**	**	**	**	*	**
C.V (%)	9.02	7.26	18.33	22.7	11.57	17.48	26.48	26.52
0N-0P	4685 c	0.233 c	0.075 b	4.661 b	10.984 c	15.646 c	5.453 c	10.907 c
60N-40P	5346 b	0.250 b	0.084 b	5.701 b	13.408 b	19.109 b	7.173 b	14.273 b
120N-80P	5774 a	0.271 a	0.108 a	7.504 a	15.691 a	23.196 a	9.895 a	19.790 a
Calculated F	**	**	**	**	**	**	**	**
C.V (%)	6.29	7.62	18.70	22.64	13.26	15.91	16.12	16.15

+Seed yield = grain yield x 0.81
++ P in seed and P in straw rice; Means within a column followed by the same letter/s are not significantly different at p<0.05
*and ** are significantly different at 0.05 and 0.01 probability respectively

Table 6. *Effects of endophytic bacteria and chemical fertilizers on N contents of rice straw, rice seed and soil of Tuy An site*

Treatment	Seed rice Yield (kg/ha)+	N content in seed rice (%)	Protein content in seed rice (%)	N content in rice straw (%)	Total N in rice straw (kg/ha)	Total N in seed rice (kg/ha)	Total protein in seed rice (kg/ha)	N content in soil (%)	Total N in soil (kg/ha)
0N-0P-No inoculation	2815 g	0.764 g	4.431 g	0.371 h	19.50 g	21.50 i	124.71 i	0.101 g	2028 g
0N-0P-SHL70	3905 f	0.853 e	4.948 e	0.429 g	24.19 f	30.75 g	178.32 g	0.121 e	2412 e
0N-0P-TAL4	3615 f	0.806 f	4.676 f	0.453 f	25.09 f	29.13 h	168.98 h	0.126 d	2520 d
0N-0P-SHL70&TAL4	4577 d	0.943 cd	5.471 cd	0.531 cd	33.75 d	43.16 e	250.35 e	0.134 c	2687 c
60N-40P-No inoculation	4232 e	0.838 ef	4.861 ef	0.479 e	28.57 e	35.37 f	205.15 f	0.113 f	2266 f
60N-40P-SHL70	4941 c	0.913 d	5.293 d	0.542 c	35.32 c	45.09 d	261.52 d	0.123 de	2452 de
60N-40P-TAL4	4931 c	0.939 cd	5.449 cd	0.513 d	32.32 d	46.31 d	268.62 d	0.125 de	2504 de
60N-40P-SHL70&TAL4	5528 b	1.072 b	6.219 b	0.587 b	40.78 b	59.26 bc	343.73 bc	0.145 b	2898 b
120N-80P-No inoculation	4890 c	0.991 c	5.515 c	0.471 ef	29.82 e	46.50 d	269.68 d	0.135 c	2698 c
120N-80P-SHL70	5417 b	1.082 b	6.277 b	0.598 b	40.76 b	58.62 c	340.02 c	0.144 b	2878 b
120N-80P-TAL4	5457 b	1.104 b	6.403 b	0.583 b	39.48 b	60.25 b	349.44 b	0.143 b	2856 b
120N-80P-SHL70&TAL4	6055 a	1.263 a	7.323 a	0.670 a	47.44 a	76.45 a	443.38 a	0.153 a	3056 a
Calculated F	**	**	**	**	**	**	**	**	**
C.V (%)	2.43	2.33	2.33	2.62	3.31	2.19	2.20	2.80	2.85
No inoculation	3979 c	0.851 c	4.936 c	0.440 c	25.96 c	34.46 c	199.85 c	0.117 c	2331 c
SHL70	4654 b	0.949 b	5.506 b	0.523 b	33.43 b	44.82 bc	259.96 bc	0.129 b	2580 b
TAL4	4668 b	0.950 b	5.509 b	0.516 b	32.30 b	45.23 b	262.35 b	0.131 b	2627 b
SHL70&TAL4	5387 a	1.093 a	6.338 a	0.596 a	40.66 a	59.62 a	345.82 a	0.144 a	2880 a
Calculated F	**	**	**	**	**	**	**	**	**
C.V (%)	17.14	12.00	12.01	11.91	18.56	27.39	27.41	8.66	8.67
0N-0P	3653 c	0.842 c	4.882 c	0.446 c	25.63 c	31.14 c	180.59 c	0.121 b	2412 b
60N-40P	4908 b	0.941 b	5.455 b	0.530 b	34.25 b	46.51 b	269.76 b	0.126 b	2530 b
120N-80P	5455 a	1.100 a	6.380 a	0.580 a	39.38 a	60.45 a	350.63 a	0.144 a	2872 a
Calculated F	**	**	**	**	**	**	**	**	**
C.V (%)	11.41	9.81	9.31	11.69	16.96	20.41	20.42	8.59	8.67

+Seed yield = grain yield x 0.81

Means within a column followed by the same letter/s are not significantly different at $p<0.05$

*and ** are significantly different at 0.05 and 0.01 probability respectively

Table 7. *Effects of endophytic bacteria and chemical fertilizers on P contents of rice straw, rice seed and soil of Tuy An site*

Treatment	Seed rice Yield (kg/ha)+	P in seed rice (%)	P content in rice straw (%)	Total P in rice straw (kg/ha)	Total P in seed rice (kg/ha)	Total P in plant rice++ (kg/ha)	P content In soil (mg/kg)	Total P in soil (kg/ha)
0N-0P-No inoculation	2815 g	0.214 f	0.049 g	2.584 f	5.942 h	8.527 i	4.163 h	8.327 h
0N-0P-SHL70	3905 f	0.231 e	0.051 g	2.837 f	8.351 g	11.188 h	5.157 g	10.313 g
0N-0P-TAL4	3615 f	0.263 cd	0.052 fg	2.882 f	9.475 f	12.357 g	5.510 fg	11.020 fg
0N-0P-SHL70&TAL4	4577 d	0.272 bc	0.083 c	5.241 c	12.423 d	17.664 e	6.427 ef	12.853 ef
60N-40P-No inoculation	4232 e	0.232 e	0.061 ef	3.608 e	9.654 f	13.261 g	6.443 e	12.887 e
60N-40P-SHL70	4941 c	0.253 d	0.068 de	4.420 d	12.545 d	16.965 e	7.483 d	14.967 d
60N-40P-TAL4	4931 c	0.258 d	0.071 d	4.456 d	12.732 d	17.188 e	7.287 de	14.573 de
60N-40P-SHL70&TAL4	5528 b	0.282 b	0.093 b	6.464 b	15.700 b	22.164 b	8.767 c	17.533 c
120N-80P-No inoculation	4890 c	0.240 e	0.061 ef	3.849 e	11.673 e	15.522 f	8.483 c	16.967 c
120N-80P-SHL70	5417 b	0.275 b	0.072 d	4.931 cd	14.910 c	19.841 d	8.700 c	17.400 c
120N-80P-TAL4	5457 b	0.263 cd	0.094 b	6.440 b	14.391 c	20.831 c	10.633 b	21.267 b
120N-80P-SHL70&TAL4	6055 a	0.299 a	0.111 a	7.850 a	17.977 a	25.828 a	13.167 a	26.333 a
Calculated F	**	**	**	**	**	**	**	**
C.V (%)	2.43	2.57	7.22	7.00	3.32	3.41	7.14	7.13
No inoculation	3979 c	0.228 c	0.057 c	3.347 c	9.090 c	12.437 c	6.363 b	12.727 b
SHL70	4654 b	0.253 b	0.063 bc	4.063 bc	11.936 b	15.998 b	7.113 b	14.227 b
TAL4	4668 b	0.262 b	0.072 b	4.592 b	12.200 b	16.792 b	7.810 ab	15.620 ab
SHL70&TAL4	5387 a	0.284 a	0.095 a	6.518 a	15.367 a	21.885 a	9.453 a	18.907 a
Calculated F	**	**	**	**	**	**	*	*
C.V (%)	17.14	5.42	18.37	24.68	20.84	21.32	29.59	29.60
0N-0P	3653 c	0.245 b	0.059 b	3.386 b	9.048 c	12.434 c	5.314 c	10.628 c
60N-40P	4908 b	0.256 ab	0.073 a	4.737 a	12.658 b	17.395 b	7.495 b	14.990 b
120N-80P	5455 a	0.269 a	0.048 a	5.767 a	14.738 a	20.506 a	10.264 a	20.492 a
Calculated F	**	**	**	**	**	**	**	**
C.V (%)	11.41	8.86	23.20	28.49	19.45	21.32	18.40	18.39

This showed that N content in seed rice was important and regression equation as follows:

$$Y = - 5.124 + 1.262\ X1 + 0.542\ X2$$

Y = P content in soil, X1 = P content in seed rice and X2 = P content in straw rice ($R^2 = 0.973$)

This showed that P content in seed rice was important and regression equation as follows:

$$Y = 1812.44 + 17.37\ X1 – 0.23\ X2$$

Y = N content in soil, X1 = N content in seed rice and X2 = N content in straw rice ($R^2 = 0.832$)

This showed that N content in seed rice was important with regression equation as follows:

$$Y = 0.291 + 0.728\ X1 + 1.345\ X2$$

Y = P content in soil, X1 = P content in seed rice and X2 = P content in straw rice (R2 = 0.851)

This showed that P content in straw rice controbuted remarbly

The highest grain yield in 120N80P plus inoculation (both strains) treatment and grain yield of 60N-40P plus inoculation with SHL70 strain treatment did not differ with grain yield of 120N80P without inoculation, therefore inoculation reduced or saved 50% N and P fertilizers (60N40P) in Dong Hoa site and Tuy An site. Application of the mixtute of two strains in rice cultivation increased 87.06 kg/ha protein and 125.64 kg/ha protein in seed rice in comparison to control (without inoculation) at Dong Hoa site and Tuy An site, respectively. Applying 120 kg N + 80 kg P_2O_5/ha plus inoculation (both strains) enhanced 145.70 kg/ha and 318.67 kg/ha protein in seed rice in comparison with control (without inoculation) at Dong Hoa and Tuy An site, respectively (Table 2 and Figure 3)(Table 3 and Figure 5).

When analysis of N and P content in seed rice and straw rice showed that N and P accumulation in rice plant (seed and straw) of mixture of two bacterial strains treatment were higher than N and P content in rice plant of 120N-80P without inoculation, this demonstrated that the high effectiveness of both strains contributed in the accumulation of N and P in rice plant and in cultivated soil in comparison to rice only applying chemical nitrogen and phosphorus without inoculation (Table 4, 5, 6, 7). Besides, with 0N-0P rice plants were inoculated with two strains before planting increased 15.01 kg N from seed rice, 13.950 kg N/ha from straw rice, 915 kg N/ha from biological nitrogen fixation, 3.286 kg P/ha from seed rice, 2.534 kg P/ha from straw rice, 5.433 kg P/ha (12.55 kg P_2O_5/ha) in soil with total P was 11.253 kg P/ha from phosphate solubilization and 21.662 kg N/ha from seed rice, 14.255 kg N/ha from straw rice, 659 kg N/ha from biological nitrogen fixation, 6.481 kg P from seed rice, 2.657 kg P/ha from straw rice, 4.526 kg P/ha (10.455 kg P_2O_5/ha) in soil with total P was 13.664 kg P/ha from phosphate solubilization at Dong Hoa site and Tuy An site, respectively. Therefore, application of endophytic bacterial strains in rice cultivation increased soil fertility in both sites but soil fertility at Dong Hoa site was higher than Tuy An site perhaps high soil fertility at Dong Hoa site.

Rice is the world's most important food crop and a primary source of food for more than half the world's population. More than 90% of rice is grown and consumed in Asia where 60% of the people on earth live [12]. In accordance to Ladha and Reddy [13], nitrogen is the major limiting nutrient for rice production. Nitrogen is used to synthesize plant proteins and nucleic acids, including DNA. Response of rice varieties, especially high-yielding rice, to N is generally recognized, but crop recovery of applied N is only 50% due to the losses in several ways. In this context, biological N_2 fixation (BNF) has been considered an alternative, which may prove to be a better solution to supply nitrogen to the cropping systems of the future [14]. It is widely recognized that BNF can benefit the rice crop [12]. However, rice production is largely limited by soil phosphorus (P) deficiencies in many parts of the world [15][2].

Especially the use of nitrogen fertilizer is of great importance in rice production [3] and N_2 fixation is a prime requisite for plant growth particularly in crops like rice. The results of Rodrigues et al. [12] inoculation of strains of *Azospirillum amazonense* increased grain dry matter accumulation, the number of panicles and nitrogen accumulation at grain maturation, especially BNF contributed up to 27%.

With grain yield from 5 to 8 tons/ha, rice plant required 60 to 100 kg N/ha [16] and biological nitrogen only provided 50 kg N/ha [17]. Therefore using biofertilizer for rice cultivation contributed approx 50% N quantity and it improved soil fertility through phosphate solubilization, IAA biosynthesis [18]. Many endophytic bacterial strains were isolated and identified in soil and rice plant in the Mekong Delta as *Azospirillum lipoferum* [19], *Burkholderia vietnamiensis* [20][21] with good characteristics as high nitrogen fixation, phosphate solubilization and IAA biosynthesis. They have been used in biofertilizer for rice production [22][23].

Azospirillum amazonense SHL70 and *Burkholderia kururiensis* PHL87 were isolated fron rice cultivated on soil of Phu Yen province, Vietnam [8][9] and using two strains as biofertilizer for rice cultivation on sandy loam soil effectively. Oliveira *et al*. [24] application of *Azospirilum amazonense* and *Burkholderia* sp., increased rice biomass to 39% in comparison to control (without inoculation) in pot experiment (Brasil).

4. Conclusion

Using the mixture of *Azospirillum amazonense* and *Bacillus subtilis* increased grain yield and improvement of quality seed rice and soil fertility of cultivated sandy loam soil of Phu Yen province, Vietnam. Application of two bacterial strains in rice plant before transplanting save 50% N and P fertiizers (60 kg N and 40 kg P_2O_5/ha) and the highest grain yield in applying 120 – 80 P_2O_5/ha and inoculated with two strains treatment.

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