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RESEARCH ARTICLE

APPLICATION OF ENDOPHYTIC *PSEUDOMONAS STUTZERI* AND REDUCE DOSE OF INORGANIC NITROGEN FERTILIZER ON THE GROWTH AND THE YIELD OF RICE CULTIVATED IN CONTINUOUS THREE RICE CROPS SYSTEM IN MEKONG DELTA, VIETNAM.

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Abstract

Mineral fertilizers have been used to increase rice yield, however, excessive use of agrochemicals in rice production caused residue toxicity and environmental pollution through leaching and evaporation. The decreased use of synthetic fertilizers was recommended to maintain the sustainable agriculture system. This article reports on the impact of plant-growth promoting rhizobacteria (PGPR) on rice cultivation in acid sulphate soil in the Mekong Delta, Vietnam to achieve a better harvest index and reduce environmental pollution. A greenhouse and field experiments were carried out in such condition to study the effect of *Pseudomonas stutzeri* on the growth and yield of local rice variety OM1490 cultivated in Bac Lieu province. The results showed that rice inoculated with *P.stutzeri* using 50%N/ha of inorganic fertilizer improved yield components and rice yield equivalent to those of rice grown with 100%N/ha of inorganic fertilizer without inoculation. Root length of inoculated rice with 50%N/ha was significantly longer than those of uninoculated rice without nitrogen fertilizer. This feature was crucial to climate change in drought areas. The findings suggest that *P. stutzeri* with decreased dose of N/ha could promote rice growth and yield compared to the higher dose of N/ha without *P.stutzeri* inoculation.

Introduction:-

In recent years, exporting rice has been one of the most important objectives in Vietnam. To have high quantity of rice for exporting, many techniques have been used such as selection of new high yield rice varieties, appropriate cultural techniques including application of chemical fertilizer especially nitrogen, intensive farming rice in two or three crops per year. In order to get high yield of rice, chemical nitrogen fertilizer has been applied. Consequently, soil fertility becomes exhausted, soil structure compacted, and effective microorganisms also decrease rapidly. This is due to the flooding time in rice fields, low farmer income, environment pollution as well as overuse of chemical nitrogen fertilizers. Several effective nitrogen fixing bacteria have been applied successfully in developing countries such as *Azospirillum brasilense, Azopspirillum lipoferum* (Bao et al. 2013); *Pseudomonas stutzeri* (Vermeiren et al. 1999), *Herbaspirillum seropedicae* (James et al. 2002). The results from these experiments varied due to different

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kinds of soils and various climate conditions. Therefore, the application of *P. stutzeri* and decreased dose of nitrogen fertilizers for rice in the greenhouse and in a field experiment in Bac Lieu province, Vietnam were carried out.

Materials and methods:-

The greenhouse and field trial were at Gia Rai District, Bac Lieu province, Vietnam. Some chemical and mechanical characteristics of soil were shown in Table 2.1.

Chemical nitrogen fertilizer was used in the form of Urea (46%N), phosphorus (Supper phosphate, 16% P_2O_5) and potassium (KCl, 60% K_20). Peat based inoculants *Pseudomonas stutzeri* ($3.2x10^9$CFU/g) was used to coat germinated seeds one hour before sowing at the dose of 5kg of inoculant/ha. Local rice variety OM1490 was used at the dose of 200kg seed per ha. Pesticides were applied when needed.

Table 2.1:- Chemical and mechanical characteristics of soil*.

Characteristics	Chemical analysis	Mechanical analysis (%)
pH (H_2O)	5.31	Clay 54.04
Organic C (%)	4,59	Loam 45.48
Total N (%)	0.2	Sand 0.48
Total P_2O_5(%)	0,099	
Available P_2O_5(%)	39,10	
Exchangeable K_2O	1,23	
EC (mS/cm)	1,802	

*Soil sample was analysed by Department of Soil Science, College of Agriculture and Applied Biology, Cantho University,Vietnam.

Treatments:-

For greenhouse experiment, every pot contained 10kg of soil. Sterile germinated rice seeds were coated with *Pseudomonas stutzeri* produced in peat based inoculant containing $3.2x10^9$ CFU/g. Six treatments with 4 replicates using complete randomized design as follows

Treatment 1: Uninoculated seeds and 0N + 115 P_2O_5 – 90 K_2O
Treatment 2: Uninoculated seeds and applied 100N (100%N) + 115 P_2O_5 – 90 K_2O
Treatment 3: *Pseudomonas stutzeri* +0N +115 P_2O_5 – 90 K_2O
Treatment 4: *Pseudomonas stutzeri* +25N + 115 P_2O_5 – 90 K_2O
Treatment 5: *Pseudomonas stutzeri* +50N + 115 P_2O_5 – 90 K_2O
Treatment 6: *Pseudomonas stutzeri* +75N + 115 P_2O_5 – 90 K_2O

For field experiment, the soil was divided into plots (4m x 5m). The experiment also included 6 treatments with four replicates as mentioned for the greenhouse experiment. Randomized complete block design was used for field experiment.

Plant samples were collected at 50 DAS (both greenhouse and field experiment), color leaf index, plant height, root length, dry weight of root, dry weight of plant were measured. For field experiment, at the harvest time plant height, length of panicle, number of panicle/m^2, 1000- seed weight, dry weight of straw, and rice yield were also determined. Data of the experiment were analysed using Stahgraphics Centuron XV.II software.

Results:-

In the greenhouse:-

Some characteristics of rice plants at 50DAS:-

*Leaf color index***:** Leaf color indexes of rice plant at 50DAS either uninoculated applied 100N or inoculated with *P.stutzeri* but received 75N were not significant defference (Table 3.1). The results showed that rice plants had enough nitrogen for growth. In this case, the nitrogen fixed by bacteria equivalent to about 25N for plant growth. When rice plants received no chemical nitrogen or inoculated with bacteria but received no nitrogen, leaf color was yellow and had the symptom of lacking of nitrogen for growth.This result also proved that bacteria could not fix nitrogen when their host did not have certain amount of nitrogen for growth.

Number of shoot/bush: Rice plants inoculated with bacteria and received 75N had high number of shoots/bush. Rice received 100N had the same number of shoots/bush compared to those received 50N and inoculated with bacteria. This result showed that bacteria could fix nitrogen well and 25N could be saved. Inoculated rice plants received no nitrogen had the same number of shoots/bush of uninoculated with ones received no nitrogen. This meant that when rice plants did not have enough nitrogen for growth, they could not have high amount of shoots/bush.

Plant height: Uninoculated rice received 100N had the highest height. There was not significant difference between plant height of either uninoculated rice applied no nitrogen and inoculated rice received no nitrogen. Increasing nitrogen to inoculated plants from 25N up to 75N helped increasing the plant height. At 50DAS, nitrogen fixing bacteria did not fix enough nitrogen for rice growth of inoculated plants even though 25N up to 75N was applied to inoculated plants.

Dry weight of plants: Even though the height of inoculated plants was lower than those of uninoculated rice received 100N but the biomass accumulated in inoculated plants received 50N and 75N was not significant difference with the uninoculated ones received up to 100N. This meant that nitrogen fixing bacteria played an important role in accumulating biomass for inoculated plants. Uninoculated plants received 0N and inoculated ones received 0N had the same dry weight. This result confirmed the important role of nitrogen for plants growth.

Table 3.1:- Some agricultural characteristics of rice plant grown in greenhouse at 50DAS.

Treatments	Leaf color index	No. of shoots/ bush	Plant height (cm)	Dry weight of plants (g)	Root length (cm)	Dry weight of root (g)
Uninoculated seeds - 0N	2.22d	11.50d	45.25e	1.31c	22.5e	0.74b
Uninoculated seeds-100N	4.00a	15.94b	58.81a	4.84a	28.4c	1.52a
P. stutzeri - 0N	2.44d	11.92d	45.31e	1.68c	25.3d	0.72b
P. stutzeri - 25N	3.18c	15.50c	48.81d	2.96b	27.7c	1.33a
P. stutzeri - 50N	3.54b	17.77ab	52.00c	4.55a	30.4b	1.35a
P. stutzeri - 75N	4.04a	18.05a	56.19b	4.71a	34.9a	1.53a
CV (%)	23.19	18.08	10.51	45.93	14.28	34.82

Means followed by the same letters in the same column was not significantly different (P<.05)

Root length: Root length of inoculated plants received 75N was the longest. The root length of inoculated plants and applied 75N, increased 55.11% compared to those of control ones (uninoculated plants received no nitrogen). This result showed that *P.stutzeri* could produce phytohormones to stimulate the elongation of inoculated rice roots. This characteristic was very important for rice growth in drought condition. Since the long roots helped plants absorb water better.

Dry weight of root: Dry weight of root of inoculated plants received 25N up to 75N and uninoculated ones received 100N was not significant difference. This result showed that bacteria could fix nitrogen equivalent to 25N to 75N and helped plants accumulating dry weight of roots. The result also proved that when plants had not enough nitrogen for growth, the root dry weight was also very low.

Some characteristics of rice plants at harvest time:-

Plant heigth: The plant heigth of inoculated plants received 25N up to 75N and uninoculated plants received 100N was not significant difference (Table 3.2). This means that *P.stutzeri* could fix nitrogen and supported good growth of inoculated plants and saved up to 75N of chemical nitrogen. Thus when plants received not enough nitrogen, plants were short even though they were inoculated with nitrogen fixing bacteria.

Number of panicle /bush: The same amount of number of panicle /bush was found in either inoculated plants applied 25N to 75N or uninoculated ones applied 100N. The result of the experiment showed that when plants received not enough nitrogen to grow they could not produce panicle well.

Length of panicle: panicle length of inoculated plants received 75N and those of uninoculated ones were the highest. Inoculated plants received no nitrogen and uninoculated plants had the shortest flower length. So, nitrogen affected the length of panicle.
Dry weight of straw: The result from this study confirmed the effect of nitrogen in producing biomass of rice plants. The dry weight of straw of inoculated plants received no nitrogen and those of uninoculated applied no nitrogen were the lowest. Dry weight of rice straw of inoculated plants received 75N and uninoculated ones received 100N were the highest. Since nitrogen is an important element for plant growth. There was a close correlation between the amount of nitrogen applied to inoculated plants and the dry weight of rice straw in this experiment.

Table 3.2:- Some agricultural characteristics of rice plant grown in greenhouse at harvest time.

Treatments	Plant height (cm)	Number of panicle /bush	Length of panicle (cm)	Dry weight of straw (g)
Uninoculated seeds - 0N	76.75c	10.25b	18.15c	37.65d
Uninoculated seeds-100N	83.50a	16.75a	22.45ab	66.70a
P. stutzeri - 0N	77.25bc	10.75b	18.70c	38.54d
P. stutzeri - 25N	80.50ab	15.25a	21.53b	48.07c
P. stutzeri - 50N	82.75a	16.25a	22.25b	55.89b
P. stutzeri - 75N	83.50a	17.25a	23.40a	66.69a
CV (%)	4.38	25.21	9.97	24.46

Means followed by the same letters in the same column was not significantly different (P<.05)

In the field:-

Some characteristics of rice plants at 50DAS:-

*Leaf color index***:** Leaf color indexes of rice plant at 50DAS either uninoculated or inoculated with *P.stutzeri* but received only 25N were lower than those of either inoculated rice plants applied 25N up to 75N or unioculated rice applied 100N (Table 3.3). Again, the results confirmed that the rice plants needed enough nitrogen for the plant growth and PGPR played an important role in supporting nitrogen to rice plants.

*Plant height***:** Plant height of rice either applied 100N or inoculated with *P.stuzeri* supplemented with 75N were significant higher than those of other treatments (Table 3.3). Plant height of uninoculated rice and inoculated rice applied no nitrogen fertilizer were not significant difference. This means that plants did not have enough nitrogen for their growth even though rice plants were inoculated with bacteria. Then, the plants needed the starter of nitrogen amount for their growth.

*Root length***:** There was not a significant difference between the root length of rice applied 100N and inoculated rice applied 50N and 75N (Table 3.3). Roots of inoculated rice were significant longer than those of the uninoculated rice plants. This might be explained that *P. stutzeri* could synthesize plant growth hormones stimulating the rice roots.

Table 3.3:- Some agricultural characteristics of rice plant at 50DAS.

Treatments	Leaf color index	Plant height (cm)	Root length (cm)	Dry weight of root (g)	Dry weigth of plant (g)
Uninoculated seeds - 0N	2.20c	37.15c	13.63d	0.46c	1.13e
Uninoculated seeds - 100N	3.88ab	55.20a	16.10ab	0.98ab	2.61b
P. stutzeri - 0N	2.45c	38.95c	14.70c	0.58c	1.57d
P. stutzeri - 25N	3.68b	44.75b	15.55bc	0.81b	2.36c
P. stutzeri - 50N	3.80ab	48.30b	16.85a	1.02a	2.66ab
P. stutzeri - 75N	4.05a	52.80a	15.95ab	1.04a	2.84a
CV (%)	23.04	15.56	7.76	30.84	29.59

Means followed by the same letters in the same column was not significantly different (P<.05)

*Dry weight of rice roots***:** The dry weight of roots of inoculated plants applied 50N and 75N was not significantly different with uninoculated rice plants applied 100N. When inoculated plants applied 50N, dry weight of roots increased 121.73% compared to the control plants (uninoculation) and received no nitrogen. The root growth development over the control showed that the inoculated rice plants could absorb water and nutrients in soil effectively and enhance the early growth primary stages of plants. The elongation of root might play an important role in case of drought conditions in many regions because of climate change nowadays.

*Dry weight of rice plants***:** There was not a significant difference between inoculated plants applied either 50N or 75N. When rice plants inoculated with *P.stutzeri* and applied 50N, dry weight of plants increased 135.3% compared to the uninoculated plants and no nitrogen applied (Table 3.3). Again, *P.stutzeri* could support good growth of inoculated plants and provide the plants about 50%N needed.

Some characteristics of rice plants at harvest time:-

*Plant height at harvest time***:** At harvesting, plant height of innoculated plants applied 75N was not significantly different with uninoculated plants applied 100N. Plant height of inoculated rice was higher than the control (Table 3.4). This result showed that *P.stutzeri* could support growth of inoculated plants and help to save inorganic nitrogen fertilizers. Nitrogen fixing *P.stutzeri* used in this research could increase plant height of inoculated plants received 50N 97.2% compared to the control without inoculation.

Length of panicle and number of panicle/m^2: Length of panicle and number of panicle/m^2 of inoculated rice plants applied 50N and 75N were not significant difference compared to the uninoculated plants applied 100N (Table 3.4). The length of panicle and number of panicle/m^2 of inoculated plants applied 50N could increase 16.6% and 21.84% compared to those of the control, respectively.

*Dry weigth of seed and dry weight of straw***:** Inoculated plants applied 25N up to 75N had the 1000-seed weigth higher than those of the control (Table 3.4). Dry weight of straw of inoculated plants was also significant higher than those of the control. Interestingly, *P. stutzeri* could increase shoot dry weight of inoculated straw 298.14% compared to the control.

Yield of rice: The yield of inoculated rice was found more significant than those of the uninoculated plants applied no nitrogen (Table 3.4). High rice yield could receive either when *P.stutzeri* was used to inoculate to rice plants and applied 50N or 75N or uninoculated rice applied up to 100N. In this study *P.stuzeri* could increase 60% of rice yield when inoculated plants applied 50N compared to the control.

Table 3.4:- Effects of inorganic nitrogen and *P.stutzeri* inoculants on rice yield components.

Treatments	**Plant height (cm)**	**Length of panicle (cm)**	**Number of panicle/m^2**	**1000-seed weight (g)**	**Dry weigth of straw (tons/ha)**	**Grain yield (tons/ha)**
Uninoculated seeds - 0N	74.40d	16.94c	531d	23.57c	2.70e	5.05c
Uninoculated seeds - 100N	87.60a	19.69a	640ab	25.57b	8.30b	8.35a
P. stutzeri - 0N	77.10c	17.05c	550cd	23.94c	4.85d	5.43c
P. stutzeri - 25N	79.80b	18.16b	591bc	25.90ab	7.59c	7.15b
P. stutzeri - 50N	81.64b	19.87a	647a	26.09ab	8.77ab	8.03a
P. stutzeri - 75N	87.40a	20.46a	651a	26.61a	9.12a	8.35a
CV (%)	6.36	8.07	9.63	5.08	34.91	21.49

Means followed by the same letters in the same collum was not significantly different ($P<.05$)

Discussion:-

Nitrogen is an important element for plant growth. Plant growth promoting bacteria can promote plant growth because they can fix nitrogen from the atmosphere and supply this nitrogen source to plants. In this research, PGPR *Pseudomonas stutzeri* could enhance the plant height of rice, number of shoot/bush, dry weight of plants, dry weight of roots, number of panicle/bush, length of panicle and dry weight of straw. Tan and his colleagues (2014) found the same result when he applied local variety of PGPR for rice grown in Malaysia. Bao and his colleagues (2013) also found that *Azospirillum* sp. B510 could augment rice growth. Phytohormone such as auxin synthesized by microorganisms has been known for a long time because this substance can proliferate root elongation. Interestingly, *P.stutzeri* also stimulates the root length of inoculated rice plants. Evident is that Kapunik and Okon (1983) found that inoculated wheat with Azospirillum helped increase root length of inoculated 20.6%. According to Fulchieri and his colleagues (1991) and Mia and his fellow workers (2012), PGPR could help drive the root length of inoculate plants. Roots of inoculated rice were significantly longer than those of uninoculated rice plants. It appears that *P.stutzeri* could synthesize plant growth hormones that stimulate rice roots (Hall et al. 1996).The root growth over the control indicated that inoculated rice plants could absorb water and nutrients in soil effectively and enhance the early stages of plant growth (Glick 1995). Isawa and his colleagues (2010) indicated that *Azospirillum* sp. strain 510 increased the plant height of inoculated plants 3.1% compared to the control without inoculation. In this experiment, nitrogen fixing *P.stutzeri* could increase the plant height of inoculated plants received 50N 97.2% compared to the control without inoculation.

Conclusion:-

P.stuzeri could support plant growth and root length, length of panicle, number of panicle/m^2, support rice biomass, and rice yield. Thus, it can be concluded that the NPK uptake and management can be improved by the use of PGPR in rice cultivation, and their application therefore may be more effective in the agricultural field in the Mekong Delta, Vietnam.

The findings of our study contribute to the knowledge of the remarkable effects of PGPR on rice growth and yield.

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