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Original article

Inoculant plant growth-promoting microorganisms enhance utilisation of urea-N and grain yield of paddy rice in southern Vietnam

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ABSTRACT

Field experiments were conducted during successive rainy seasons in 2006 in the Chau Thanh district of southern Vietnam to evaluate the effects of an inoculant plant growth promoter product called "BioGro" and N fertiliser rates on yield and N and P nutrition of rice. The results indicated that inoculation with BioGro, containing a pseudomonad, two bacilli and a soil yeast, significantly increased grain and straw yields and total N uptake in both seasons, as well as grain quality in terms of percentage N. Nitrogen fertilisation increased grain and straw yields as well as total N and P uptakes significantly in both cropping seasons. The estimated grain yield response to added N was quadratic in nature with and without added BioGro. In the first crop, BioGro out-yielded the control up to 90 kg urea N ha^{-1} whilst in the second season the beneficial effect of BioGro was observed up to 120 kg urea N ha^{-1}, indicating either an interaction of the inoculant with higher yielding seasonal conditions or a cumulative effect of BioGro application. In the first season, the estimated N rate for maximum grain yield was 103 kg N ha^{-1} with BioGro while it was 143 kg N ha^{-1} without BioGro. The maximum estimated grain yields were 3.21 and 3.18 t ha^{-1} with and without BioGro, respectively. This information indicates that BioGro was able to save 40 kg N ha^{-1} with an additional rice yield of 30 kg ha^{-1} in the season. In the second rainy season, the estimated N rates for maximum grain yields were 94 and 97 kg N ha^{-1} with and without BioGro, respectively. The estimated maximum grain yields were 3.49 and 3.25 t ha^{-1} with and without BioGro, respectively. The two seasons' combined results indicate that application of BioGro improved the efficiency of N use by rice significantly, saving 43 kg N ha^{-1} with an additional rice yield of 270 kg ha^{-1} in two consecutive seasons at the experimental site. The extra efficiency was shown by the fact that the same yield of rice was obtained with about 40 and 60 kg less fertiliser-N that the maximum yields with urea alone in the two successive harvests on the same plots.

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1. Introduction

Most of the rice soils of the world are deficient in N. Biological nitrogen fixation by cyanobacteria and diazotrophic bacteria can only meet a fraction of the N requirement [3,25]. Fertiliser N applications are thus necessary to meet the rice crop's demands. Generally, urea is the most convenient N source for rice. However, the efficiency of use of the urea-N in rice paddies is often very low, generally around 30–40%, in some cases even lower [10,17]. The low N-use efficiency is attributed mainly to ammonia volatilisation, denitrification, leaching, and run-off losses [12,22,41]. However, the magnitude of N loss varies depending on environmental conditions and management practices. Volatilisation and denitrification cause atmospheric pollution through the emission of gases such as nitrous oxide, nitric oxide, and ammonia [1,42]. Nitrous oxide absorbs infrared radiation contributing significantly to greenhouse warming and the depletion of the stratospheric ozone layer [8]. Nitric oxide contributes to the formation of tropospheric ozone, a major atmospheric pollutant that affects human health, agricultural crops, and natural ecosystems [11]. Nitric oxide is also a precursor to nitric acid, a principal component of acid deposition [26]. The deposition of nitric oxide and ammonia in terrestrial and aquatic ecosystems can lead to acidification, eutrophication, shifts in species diversity, and effects on predators and parasite systems [42,47]. Leaching of nitrate causes groundwater toxicity [45]. Excess amounts of nitrate in drinking water and food may cause methemoglobinemia in infants, respiratory illness and decreased content of vitamin A in the liver [8,40].

These problems cannot be alleviated completely. However, the efficiency of fertiliser N use can be increased by reducing the losses by several means including the application of inoculant plant growth-promoting (PGP) bacteria [15,16,27]. Plant growth-promoting microorganisms like *Rhizobium* and *Azospirillum* can reduce the use of urea-N by growth promotion through the production of auxins, cytokinins, gibberellins and ethylene [21]. In principle, the use of plant growth-promoting microorganisms can increase crop plants' capacity to utilise fertiliser-N efficiently. *Azospirillum* inoculation can enhance ammonium uptake by rice plants [33]. *Rhizobium* can also increase N uptake by rice plants as a PGP bacterium [5,6]. Obviously, the increased N uptake by rice plant will result in the reduction of N losses to the environment. Thus, if inoculant PGP microorganisms can be used successfully in rice cultivation, there should be a reduction in the environmental pollution problems from N losses by retaining N in the biological plant–soil cycle.

Previous field experimental results in the northern part of Vietnam near Hanoi indicated that the multi-strain inoculant plant growth-promoting BioGro increased rice grain yield and N uptake significantly [34,36]. However, the bulk of Vietnam's rice crop is produced in the south where farm size is generally larger. With this view in mind a study was undertaken to evaluate the effect of BioGro with variable rates of fertiliser N on yield, N and P nutrition of rice and to test the hypothesis that PGPR strains can significantly improve the efficiency of nutrient use by rice.

2. Materials and methods

A field experiment was conducted at Chau Thanh in Tay Ninh province (latitude, 11° 20′ 60 N, longitude, 106° 4′ 0 E) in the first rainy season 2006 (April to August) to evaluate the effects of applying an inoculant plant growth-promoting BioGro and N rates on yield and N and P nutrition of a high quality short duration rice variety Trau Nam. Sowing, transplanting and harvesting dates for this short season rice were 28 April, 20 May and 17 August, respectively. Before setting up the experiment, three soil samples were collected from three corners of the

Table 1 – Identification of microbial strains in BioGro

Strain	Identity	Sequences 16S rDNA	Profile of fatty acids	Gram stain
1N	*Pseudomonas* spp.	Partial sequences up to 100% similarity to *Pseudomonas fluorescens* but type strain similarity is only 94%; low correlation suggests that IN is a member of a new species of *Pseudomonas*	Fatty acid profile typical of the RNA-group I of the genus *Pseudomonas*	Negative
HY	Preliminary tests indicate *Candida* spp.			
B9	*Bacillus subtilis*	The partial sequencing of the 16S rDNA shows a similarity of 100% to *Bacillus subtilis*		Positive
E19	*Bacillus amyloliquefaciens*	Partial sequencing of 16S rDNA shows 99.8% similarity to *Bacillus amyloliquefaciens* and other members of the *Bacillus subtilis* group		Positive

Identification based on in-laboratory studies and confirmed by Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, German Collection of Microorganisms and Cell Cultures, http://www.dsmz.de/).

experimental plot at a depth of 0–15 cm. The samples were air dried, ground, and passed through a 2 mm sieve. The processed soil was analysed for texture (particle size), organic matter content, pH, cation exchange capacity, total N and available N contents. Soil pH (1:5, soil:water ratio) was measured by glass electrode [38]. Organic matter was analysed by the potassium dichromate and H_2SO_4 digestion method [48]. Particle size analysis was carried out by the hydrometer method [7]. Cation exchange capacity was determined by the ammonium acetate extraction method [44]. Total N content was determined by H_2SO_4 digestion followed by steam distillation and estimation of N by titration with dilute HCl [9]. Available N content was determined by KCl extraction followed by steam distillation and estimation of N by titration with dilute HCl. The experiment was repeated soon after harvesting in the second rainy season 2006 (August to December) in the same treatment plots. The sowing, transplanting and harvesting dates were 29 August, 22 September and 16 December 2006, respectively. Rice seedlings were germinated in nurseries and transplanted on hills 15 × 15 cm in both seasons.

There were two main factors in the experiments: BioGro (present or absent) and N rates (0, 30, 60, 90 and 120 kg urea N ha^{-1}). BioGro was applied at 240 kg ha^{-1}, with 40 kg applied to seedling nurseries and 200 kg in the field during transplanting. The microbial composition of BioGro was as indicated in Table 1. The four strains were inoculated from broth into separate batches of peat (74%), augmented with sugar (1%, w/w) plus water and broth culture (25%, w/w) [34] and these separate cultures in peat were mixed in equal proportions and matured for about a week before use. Recovery of the strains using serial dilution onto agar plates indicated viable organisms in the range 10^7–10^8 cells g^{-1}.

The experiment was conducted in a split-plot design with four replications. BioGro was used in the main plots and N rates in the sub-plots. The unit sub-plot size was 5.1 × 3.9 m, about 20 m^2. A blanket dose of phosphorus (P) was added at the P-rate of 30 kg P_2O_5 ha^{-1} from fused magnesium phosphate (FMP) to all the plots at final land preparation. Potassium (K) was added to all the plots at 60 kg K_2O ha^{-1} as muriate of potash (KCl) in three splits (20% at final land preparation, 40% at 15 days after transplanting and 40% at 40 days after transplanting). Nitrogen from urea was also applied at treatment rates in three splits (33.3% at final land preparation, 33.3% at 15 days after transplanting and 33.3% at 40 days after transplanting). The experiment was conducted in irrigated paddy conditions with necessary intercultural operations carried out as required. Grain and straw samples were harvested at maturity. Grain yield was recorded from a 5 m^2 area of each plot while straw yield was recorded from 16 hills from each plot. Grain yield was adjusted at 14% moisture content while straw yield was recorded on an oven-dry basis (80 °C). Grain and straw samples were analysed for total N and P contents. The N content was determined by H_2SO_4 digestion followed by steam distillation and titration procedure [50]. The P content was determined by dry ashing the plant samples at 490 °C in 4 h followed by estimation of P by colorimetric spectrophotometry [32]. The N and P uptakes were calculated by multiplying total N or P contents with the dry weight of grain or straw. Agronomic efficiency of added N was calculated by taking the differences in grain yield of each N application rate with the control and dividing the difference by the amount of N added.

All the data were analysed at the University of Sydney using the GenStat program version seven [37]. Analysis of variance (ANOVA) was carried out for each parameter followed by the least significant difference (LSD) test was done when the *F* probability value was significant. The grain yield responses to N fertilisation were interpreted using the differential calculus following the method of Gomez and Gomez [23].

3. Results and discussion

The soil was determined as a texturally loamy sand having organic matter content of 1.49%, pH value of 5.31 before flooding, with a cation exchange capacity of 4.08 cmol kg^{-1}. Total N was determined as $0.078 \pm 0.004\%$ and available N as 0.13 ± 0.01 mg kg^{-1}.

Table 2 – Effects of BioGro and N rates on grain and straw yields of Trau Nam rice, and agronomic efficiency of added N, Chau Thanh District, Vietnam, first rainy season 2006

N rate (kg ha^{-1})	BioGro[a]		Mean
	Without	With	
Grain yield (t ha^{-1})			
0	2.19	2.24	2.22 d
30	2.50	2.78	2.64 c
60	2.85	2.98	2.92 b
90	3.11	3.17	3.14 a
120	3.16	3.14	3.15 a
Mean	2.76 B	2.86 A	
Straw yield (t ha^{-1})			
0	2.17	2.44	2.31 d
30	2.36	2.72	2.54 c
60	2.79	2.83	2.81 b
90	2.84	2.99	2.92 ab
120	3.07	2.96	3.02 a
Mean	2.65 B	2.79 A	
Agronomic efficiency of added N (kg grain per kg added N)			
30	10.50 aB	18.08 aA	
60	11.00 aA	12.46 bA	
90	10.23 aA	10.39 bcA	
120	8.09 aA	7.50 cA	

Interaction effects of BioGro and N rate were not significant on grain and straw yields. The effect of BioGro on grain yield was significant at *F* probability level of 0.091 with LSD (0.10) value of 0.0978. The effect of N rate on grain yield was significant at *F* probability level of <0.001 with LSD (0.05) value of 0.1537. The effect of BioGro on straw yield was significant at *F* probability level of 0.077 with LSD (0.10) value of 0.1286. The effect of N rate on straw yield was significant at *F* probability level of <0.001 with LSD (0.05) value of 0.2047. The interaction effect of BioGro and N rate was significant on agronomic efficiency of added N at *F* probability of 0.025 with LSD (0.05) value of 4.179. Within a parameter, values followed by different capital letters in a row or different small letters in a column are significantly different by least significant difference (LSD) at the respective probability level mentioned above.

[a] BioGro (1N + HY + B9 + E19 at the ratio of 1:1:1:1) was applied at 240 kg ha^{-1}. 1N stands for *Pseudomonas fluorescens*. HY is a yeast. B9 and E19 are bacteria capable of breaking down cellulose, protein and starch.

Table 3 – Effects of BioGro and N rates on grain and straw yields of Trau Nam rice, and agronomic efficiency of added N, Chau Thanh District, Vietnam, second rainy season 2006

N rate (kg ha^{-1})	BioGro[a]		Mean
	Without	With	
Grain yield (t ha^{-1})			
0	2.67	2.86	2.77 d
30	3.04	3.27	3.16 c
60	3.11	3.30	3.21 bc
90	3.26	3.60	3.43 a
120	3.21	3.45	3.33 ab
Mean	3.06 B	3.30 A	
Straw yield (t ha^{-1})			
0	2.23	2.35	2.29 d
30	2.37	2.63	2.50 c
60	2.59	2.77	2.68 b
90	2.91	3.17	3.04 a
120	3.01	3.02	3.02 a
Mean	2.62 B	2.79 A	
Agronomic efficiency (kg grain per kg added N)			
30	12.33	13.67	13.00 a
60	7.38	7.29	7.33 b
90	6.56	8.25	7.41 b
120	4.50	4.92	4.71 c
Mean	7.69 A	8.53 A	

Interaction effect of BioGro and N rate was not significant on all the parameters. Effect of BioGro was significant on grain and straw yields at *F* probability levels of 0.049 and 0.070, respectively. LSD (0.05) value for grain yield was 0.23 while LSD (0.10) value for straw yield was 0.14. Effect of BioGro was not significant on agronomic efficiency. Effect of N rate was significant on all the three parameters at *F* probability level of <0.001. LSD (0.05) values were 0.13, 0.14 and 2.67 for grain yield, straw yield and agronomic efficiency, respectively. Within a parameter, values followed by different capital letters in a row or different small letters in a column are significantly different by LSD at the respective probability level mentioned above.

[a] BioGro (1N + HY + B9 + E19 at the ratio of 1:1:1:1) was applied at 240 kg ha^{-1}. 1N stands for *Pseududomonas fluorescens*. HY is a yeast. B9 and E19 are bacteria capable of breaking down cellulose, protein and starch.

The BioGro formulation contained equal quantities of four microbial strains, a Gram negative pseudomonad very similar but not identical to the type strain of *Pseudomonas fluorescens*, a soil yeast still not definitely identified selected for its ability to mobilise insoluble Ca-phosphates, and two Gram positive amylolytic and proteolytic bacilli, identified as *Bacillus subtilis* and *Bacillus amyloliquefaciens* respectively, as shown in Table 1. Experience suggests that maximum plant growth responses are more likely with all strains present [35]. However, all four strains individually showed PGP effects when tested for root proliferation in laboratory tests with rice seedlings. As is now routine in field applications of BioGro, the combined inoculant plant growth-promoting bacteria was confirmed as stimulating rice root development by comparisons with rice plants grown in nurseries uninoculated [35]. In other studies [34] the PGP effect rather than unknown direct effects of the carrier material alone (e.g. its low nutrient content) have been ruled out as a cause of plant growth-promotion.

3.1. *Grain and straw yields, and agronomic efficiency*

In the first rainy season, BioGro increased grain yield significantly with an *F* probability level of 0.091 (Table 2) while the increase in grain yield with BioGro application was also significant at *F* probability level of 0.049 in the second rainy season (Table 3). This increase in significance could indicate a cumulative effect of successive BioGro applications with each rice crop, although other factors such as irradiance or temperature in the different seasons could have been involved, particularly given the higher yield in the second season. The effect of N rates on grain was significant at *F* probability level less than 0.001 in both seasons. Nitrogen (N) fertilisation increased grain yield significantly up to 90 kg N ha^{-1}, but at the highest rate the effect on yield was not significant (Tables 2 and 3). The significant increases in grain yields with N fertilisation were attributed to low soil fertility status of the experimental site which had low organic matter content (1.49%) as well as low total N content (0.078%). The total N content of the experimental soil was below the critical deficiency level of 0.20% and a N response was expected. The estimated grain yield response from fertilisation was quadratic in nature with and without BioGro in both the seasons (Figs. 1 and 2). In the first season BioGro out yielded the control at up to 90 kg N ha^{-1} while in the second season the beneficial effect of BioGro was noted up to 120 kg N ha^{-1}, possibly indicating a cumulative effect of BioGro applications. In the first season, the estimated N rate for maximum grain yield was 103 kg N ha^{-1} with BioGro while it was 143 kg N ha^{-1} without BioGro. The equations

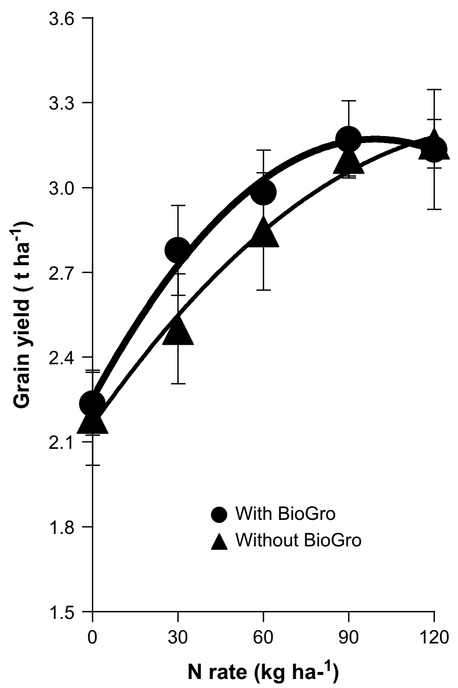

Fig. 1 – Estimated grain yield response of Trau Nam rice to added N with and without BioGro, Chau Thanh District, Vietnam, first rainy season 2006.
$y = 2.2541 + 0.0185x - 0.00009x^2$ (with BioGro). $R^2 = 0.991$. $y = 2.1611 + 0.0143x - 0.00005x^2$ (without BioGro). $R^2 = 0.9915$**. **Significant at 1% level.**

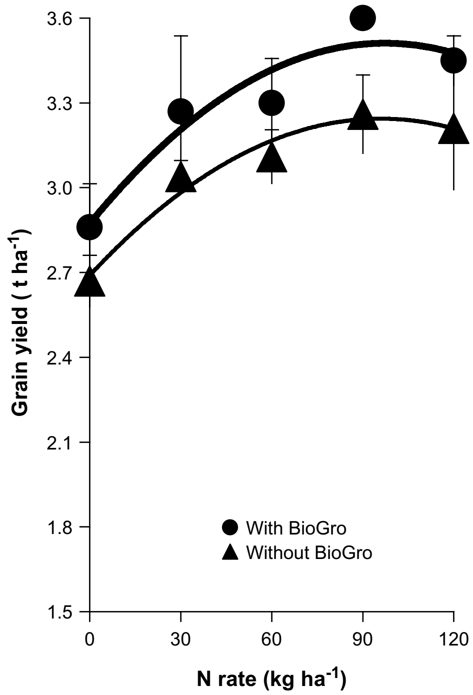

Fig. 2 – Estimated grain yield response of Trau Nam rice to added N with and without BioGro, Chau Thanh District, Vietnam, second rainy season 2006.
$y = 2.8726 + 0.0131x - 0.00007x^2$ (with BioGro). $R^2 = 0.911^*$. $y = 2.6894 + 0.0116x - 0.00006x^2$ (without BioGro). $R^2 = 0.9668^*$. *Significant at 5% level.

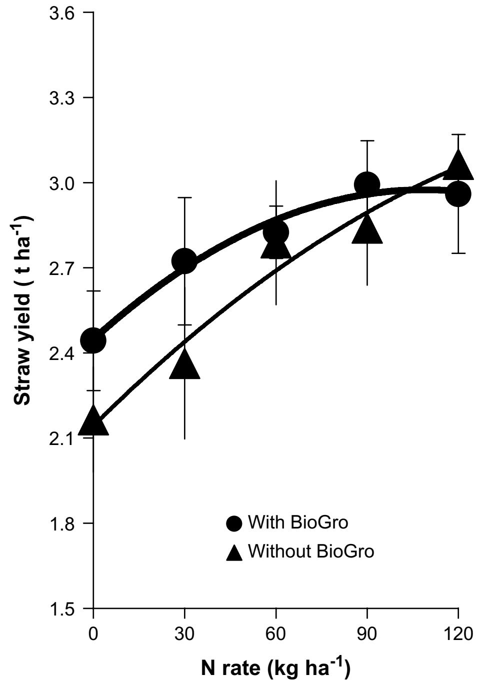

Fig. 3 – Estimated straw yield response of Trau Nam rice to added N with and without BioGro, Chau Thanh District, Vietnam, first rainy season 2006.
$y = 2.448 + 0.0097x - 0.00004x^2$ (with BioGro). $R^2 = 0.9807^{**}$. $y = 2.1432 + 0.0106x - 0.00003x^2$ (without BioGro). $R^2 = 0.965^*$. **Significant at 1% level. *Significant at 5% level.

shown in Fig. 1 were used for these estimations. The maximum estimated grain yields were 3.21 and 3.18 t ha^{-1} with and without BioGro respectively, although more significant differences were observed with sub-maximal urea-N applications. This information indicates that, for maximum yield, BioGro saved 40 kg N ha^{-1} with an additional rice yield of 30 kg ha^{-1} in the first rainy season. In the second rainy season, the estimated N rates for maximum grain yields were 94 and 97 kg N ha^{-1} with and without BioGro, respectively. The estimated maximum grain yields were 3.49 and 3.25 t ha^{-1} with and without BioGro, respectively. The equations shown in Fig. 2 were used for these estimations. This indicates that, for maximum yield, BioGro saved only 3 kg N ha^{-1} but with an additional rice yield of 240 kg ha^{-1} in the second rainy season. The two seasons' combined results show that BioGro application saved 43 kg N ha^{-1} with an additional rice yield of 270 kg ha^{-1} in two consecutive crops on the same experimental site. However, simply by inspection of Fig. 2, it is clear that the maximum rice yield possible with urea alone was achieved with 60 kg less of urea-N per ha with BioGro added, indicating a much greater efficiency of utilisation of soil-N as a nutrient for rice with the added PGP microorganisms.

The effect of BioGro on straw yield was significant at F probability levels of 0.077 and 0.070 in the first and second rainy seasons, respectively (Tables 2 and 3). The effect of N fertilisation on straw yield was significant at F probability level less than 0.001 in both seasons. Nitrogen fertilisation increased straw yield significantly up to 120 kg N ha^{-1} in the first season (Table 2) while its effect was significant on straw yield up to 90 kg N ha^{-1} in the second season (Table 3). Estimated straw yield response with N fertilisation was quadratic in nature with and without BioGro in the first season (Fig. 3). BioGro out yielded the control up to 90 kg N ha^{-1}; beyond this rate there was slight decrease in straw yield with BioGro application. In the second season, the estimated straw yield response from N fertilisation was quadratic in nature with BioGro while it was linear without BioGro (Fig. 4). BioGro out yielded the control up to the highest rate of added N indicating the greater effect of BioGro application in the second season.

The interaction effect of BioGro and N rate was significant on agronomic efficiency (kg grain per kg added N) at an F probability level of 0.025 in the first season (Table 2). At 30 kg N ha^{-1}, BioGro increased agronomic efficiency significantly over the control while the effect of BioGro was not significant at other N rates although there were increases in agronomic efficiency with BioGro at 60 and 90 kg N ha^{-1}. At 120 kg N ha^{-1}, BioGro application decreased agronomic efficiency slightly although the difference was not significant. A significant decrease in agronomic efficiency was noticed at 60 kg N ha^{-1} compared to the agronomic efficiency at 30 kg N ha^{-1} with BioGro. Agronomic efficiency again decreased significantly at 120 kg N ha^{-1} compared to the agronomic efficiency at 60 kg N ha^{-1} with BioGro. Agronomic efficiency of added N decreased with increasing N rates without BioGro although the differences were not significant. In the second season,

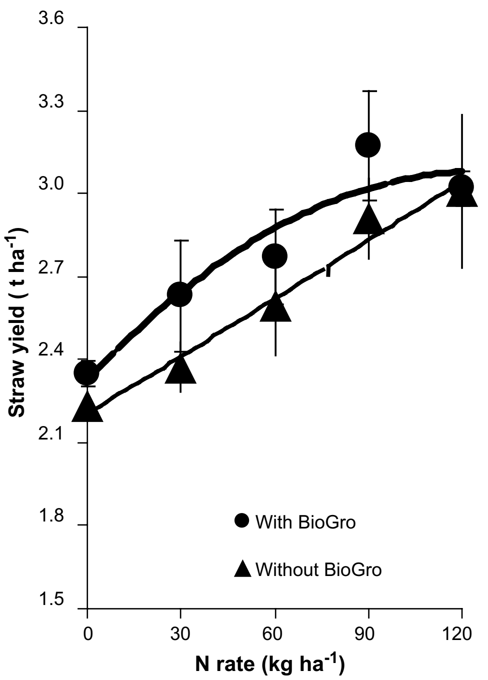

Fig. 4 – Estimated straw yield response of Trau Nam rice to added N with and without BioGro, Chau Thanh District, Vietnam, second rainy season 2006.
$y = 2.3263 + 0.012x - 0.00005x^2$ (with BioGro).
$R^2 = 0.9095$*. $y = 2.202 + 0.007x$ (without BioGro).
$R^2 = 0.9764$. **Significant at 1% level. *Significant at 5% level.**

BioGro application increased agronomic efficiency slightly (Table 3) although the difference was not statistically significant. Agronomic efficiency decreased significantly with increasing N rates up to 120 kg N ha^{-1}. The decrease in agronomic efficiency of added N with increasing N rates is in agreement with some previous findings [14,20].

The beneficial effect of BioGro on rice grain and straw yields are in agreement with the previous findings of the experiments conducted in Vietnam under an Australian Agency for International Development (AusAID) funded research project on inoculant plant growth-promoting bacteria [34,36]. The beneficial effects of plant growth-promoting bacteria on rice yield as well as N fixation were observed in several experiments in Pakistan [28–30]. Similar results were obtained by other researchers in both greenhouse and field conditions [4,24,31,39,43,49].

3.2. *Nitrogen and phosphorus contents in grain and straw*

The interaction effect of BioGro and N rate was significant on N content in grain in the first season (Table 4). BioGro increased N content (%) significantly at 0-N while in other N rates there was no significant difference between with and without BioGro. Nitrogen fertilisation increased N content (%) in grain significantly over control in all the rates of added N without BioGro while the significant effect of N fertilisation on this parameter was noticed at 90 and 120 kg N ha^{-1} when BioGro was applied. In the second season, the interaction effect of BioGro and N rate was not significant on N content in grain (Table 5). BioGro increased N content (%) in grain significantly. The effect of N rate was significant on N content (%) in grain up to 90 kg N ha^{-1}.

Interaction effects of BioGro and N rates were not significant for N content (%) in straw in both the seasons (Tables 4 and 5). The effect of BioGro was not significant on N content (%) in straw in the first season while it increased N content (%) in straw significantly in the second season. Nitrogen fertilisation increased N content (%) in straw significantly over control at 60 kg N ha^{-1} in both the seasons. Beyond this N rate there were slight increases in N content (%) in straw although the differences were not significant.

Table 4 – Effects of BioGro and N rates on N and P contents in grain and straw of Trau Nam rice, Chau Thanh District, Vietnam, first rainy season 2006

N rate (kg ha^{-1})	BioGro		Mean
	Without	With	
N content (%) in grain			
0	0.91 cB	1.00 bA	
30	1.00 bA	1.02 bA	
60	1.06 aA	1.02 bA	
90	1.10 aA	1.09 aA	
120	1.09 aA	1.13 aA	
N content (%) in straw			
0	0.67	0.68	0.68 b
30	0.70	0.74	0.72 ab
60	0.74	0.73	0.74 a
90	0.77	0.73	0.75 a
120	0.74	0.78	0.76 a
Mean	0.72 A	0.73 A	
P content (%) in grain			
0	0.21	0.21	0.21 c
30	0.22	0.22	0.22 b
60	0.23	0.23	0.23 a
90	0.22	0.22	0.22 b
120	0.22	0.21	0.22 b
Mean	0.22 A	0.22 A	
P content (%) in straw			
0	0.08	0.09	0.09 a
30	0.09	0.09	0.09 a
60	0.10	0.09	0.10 a
90	0.09	0.08	0.09 a
120	0.09	0.09	0.09 a
Mean	0.09 A	0.09 A	

Interaction effect of BioGro and N rate was significant on N content (%) in grain at *F* probability level of 0.009 with LSD (0.05) value of 0.047 while the interaction effect was not significant on other three parameters. Effect of BioGro was not significant on N content (%) in straw while the effect of N rate was significant on this parameter at *F* probability level of 0.010 with LSD (0.05) value of 0.048. Effect of BioGro was not significant on P content (%) in grain while the effect of N rate was significant on this parameter at *F* probability level of 0.004 with LSD (0.05) value of 0.008. Effects of both BioGro and N rate were not significant on P content (%) in straw. Within a parameter, values followed by different capital letters in a row or different small letters in a column are significantly different by LSD at 5% probability level.

Table 5 – Effects of BioGro and N rates on N and P contents in grain and straw of Trau Nam rice, Chau Thanh District, Vietnam, second rainy season 2006

N rate (kg ha^{-1})	BioGro		Mean
	Without	With	
N content (%) in grain			
0	1.03	1.12	1.08 c
30	1.10	1.26	1.18 b
60	1.19	1.28	1.24 b
90	1.27	1.38	1.33 a
120	1.29	1.36	1.33 a
Mean	1.18 B	1.28 A	
N content (%) in straw			
0	0.55	0.68	0.62 c
30	0.59	0.69	0.64 bc
60	0.66	0.70	0.68 ab
90	0.68	0.73	0.71 a
120	0.70	0.73	0.72 a
Mean	0.64 B	0.71 A	
P content (%) in grain			
0	0.25	0.26	0.26 a
30	0.24	0.25	0.25 a
60	0.24	0.25	0.25 a
90	0.25	0.25	0.25 a
120	0.23	0.23	0.23 a
Mean	0.24 A	0.25 A	
P content (%) in straw			
0	0.09	0.08	0.09 a
30	0.10	0.09	0.10 a
60	0.12	0.09	0.11 a
90	0.08	0.09	0.09 a
120	0.10	0.09	0.10 a
Mean	0.10 A	0.09 A	

Interaction effect of BioGro and N rate was not significant on all the four parameters. Effect of BioGro was significant on N content (%) in grain and straw with F probability levels of 0.027 and 0.047, respectively with LSD (0.05) values of 0.08 and 0.02, respectively. Effect of BioGro was not significant on P content (%) in grain and straw. Effect of N rate was significant on N content (%) in grain and straw with F probability levels of <0.001 and 0.002, respectively with LSD (0.05) values of 0.07 and 0.05, respectively. Effect of N rate was not significant on P content (%) in grain and straw. Within a parameter, values followed by different capital letters in a row or different small letters in a column are significantly different by LSD at 5% probability level.

Table 6 – Effects of BioGro and N rates on N uptake by Trau Nam rice, Chau Thanh District, Vietnam, first rainy season 2006

N rate (kg ha^{-1})	BioGro		Mean
	Without	With	
N uptake (kg ha^{-1}) by grain			
0	19.80	22.30	21.05 d
30	24.82	28.36	26.59 c
60	30.27	30.25	30.26 b
90	34.29	34.71	34.50 a
120	34.24	35.27	34.76 a
Mean	28.68 B	30.18 A	
N uptake (kg ha^{-1}) by straw			
0	14.36	16.46	15.41 c
30	16.40	20.27	18.34 b
60	20.55	20.61	20.58 ab
90	21.81	21.96	21.89 a
120	22.54	22.94	22.74 a
Mean	19.13 B	20.45 A	
Total N uptake (kg ha^{-1})			
0	34.16	38.76	36.46 d
30	41.22	48.63	44.93 c
60	50.82	50.86	50.84 b
90	56.10	56.67	56.39 a
120	56.78	58.21	57.50 a
Mean	47.82 B	50.63 A	

Interaction effect of BioGro and N was not significant on all the three parameters. Effect of BioGro was significant on N uptake by grain at F probability level of 0.077 with LSD (0.10) value of 1.328. Effect of BioGro was significant on N uptake by straw and total N uptake at F probability levels of 0.004 and 0.021, respectively with LSD (0.05) values of 0.506 and 1.994, respectively. Effect of N rate was significant at F probability level of <0.001 on all the parameters with LSD (0.05) values of 2.143, 2.264 and 3.815 for N uptake by grain, N uptake by straw and total N uptake, respectively. Within a parameter, values followed by different capital letters in a row or different small letters in a column are significantly different by LSD at the respective probability level mentioned above.

Effects of BioGro were not significant on P content (%) in grain and straw in both the seasons (Tables 4 and 5). In the first season, N fertilisation increased P content (%) in grain significantly up to 60 kg N ha^{-1} while in the second the effect of N fertilisation was not significant on this parameter. Effects of both BioGro and N rates were not significant on P content (%) in straw.

The increases in N contents in grain and straw as a result of N fertilisation are in agreement with some previous findings [13,18,19]. These increases in N content in plant tissue are attributed to acute N deficiency in experimental soil which has low organic matter content (1.49%) and low total N content (0.078%) as well as low cation exchange capacity (4.08 cmol kg^{-1}). In fact most of the rice soils of Asia are deficient in N, and rice crops respond sharply to added fertiliser N.

3.3. *Nitrogen and phosphorus uptake*

Interaction effect of BioGro and N rate was not significant on total N uptake in both the seasons (Tables 6 and 7). However, individual effects of both BioGro and N rates were significant on total N uptake. BioGro increased total N uptake significantly in both seasons. These increases were attributed to higher grain and straw yields (Tables 2 and 3) as well as higher N content in grain and straw (Tables 4 and 5) with BioGro application. Total N uptake increased significantly with N fertilisation up to 90 kg N ha^{-1} in both seasons. These increases were attributed to increases in grain and straw yields as well as N content in grain and straw with N fertilisation.

Interaction effect of BioGro and N rate was not significant on total P uptake in both seasons (Tables 8 and 9). Single effect of BioGro was not significant on total P uptake in the first season although there was slight increase in P uptake with BioGro application. However, BioGro increased total P uptake significantly in the second season. These results indicate the cumulative beneficial effect of BioGro on P uptake by rice. Total P uptake increased significantly with N fertilisation up to 60 kg N ha^{-1} in the first season. Beyond this N rate, there were

Table 7 – Effects of BioGro and N rates on N uptake by Trau Nam rice, Chau Thanh District, Vietnam, second rainy season 2006

N rate (kg ha^{-1})	BioGro		Mean
	Without	With	
N uptake (kg ha^{-1}) by grain			
0	27.37	31.95	29.66 c
30	33.47	41.07	37.27 b
60	37.02	42.05	39.54 b
90	41.20	49.60	45.40 a
120	41.27	46.82	44.05 a
Mean	36.07 B	42.30 A	
N uptake (kg ha^{-1}) by straw			
0	12.23	15.90	14.07 d
30	13.83	18.15	15.99 c
60	17.05	19.28	18.17 b
90	19.65	23.00	21.33 a
120	21.08	22.05	21.57 a
Mean	16.77 B	19.68 A	
Total N uptake (kg ha^{-1})			
0	39.60	47.85	43.73 d
30	47.30	59.22	53.26 c
60	54.07	61.33	57.70 b
90	60.85	72.60	66.73 a
120	62.35	68.87	65.61 a
Mean	52.83 B	61.97 A	

Interaction effect of BioGro and N rate was not significant on all the three parameters. Effect of BioGro was significant on all the parameters with *F* probability levels of 0.027, 0.051 and 0.021 for N uptake by grain, N uptake by straw and total N uptake, respectively. LSD (0.05) values were 4.87 and 6.53 N uptake by grain and total N uptake, respectively. LSD (0.10) value was 2.18 for N uptake by straw. Effect of N rate was significant on all the parameters at *F* probability level of <0.001 with LSD (0.05) values of 2.83, 1.75 and 3.69 for N uptake by grain, N uptake by straw and total N uptake, respectively. Within a parameter, values followed by different capital letters in a row or different small letters in a column are significantly different by LSD at the respective probability level mentioned above.

Table 8 – Effects of BioGro and N rates on P uptake by Trau Nam rice, Chau Thanh District, Vietnam, first rainy season 2006

N rate (kg ha^{-1})	BioGro		Mean
	Without	With	
P uptake (kg ha^{-1}) by grain			
0	4.56	4.69	4.63 c
30	5.43	6.16	5.80 b
60	6.36	6.81	6.59 a
90	6.68	6.93	6.81 a
120	6.93	6.73	6.83 a
Mean	5.99 A	6.26 A	
P uptake (kg ha^{-1}) by straw			
0	1.82	2.09	1.96 c
30	2.11	2.49	2.30 b
60	2.64	2.53	2.59 a
90	2.56	2.50	2.53 ab
120	2.82	2.79	2.81 a
Mean	2.39 A	2.48 A	
Total P uptake (kg ha^{-1})			
0	6.38	6.78	6.58 c
30	7.54	8.65	8.10 b
60	9.00	9.34	9.17 a
90	9.24	9.43	9.34 a
120	9.75	9.52	9.64 a
Mean	8.38 A	8.74 A	

Interaction effect of BioGro and N rate, and single effect of BioGro were not significant on all the three parameters. Effect of N rate was significant at *F* probability level of <0.001 on all the three parameters with LSD (0.05) values of 0.3572, 0.3281 and 0.5845 for P uptake by grain, P uptake by straw and total P uptake, respectively. Within a parameter, values followed by a common capital letter in a row are not significantly different. Within a parameter, values followed by different small letters in a column are significantly different by LSD at 5% probability level.

also some increases in total P uptake although the differences were not statistically significant. In the second season, total P uptake increased significantly with N fertilisation up to 90 kg N ha^{-1}. The increases in total P uptake as a result of N fertilisation were attributed to increases in grain and straw yields at higher N rates (Tables 2 and 3).

The increases in N uptake by rice crops from BioGro application are in agreement with some previous findings [34,36]. Other researchers also reported the beneficial effects of inoculant plant growth-promoting bacteria in increasing nutrient uptake by rice including N and P uptake [5,49]. The higher nutrient uptake might be attributed to morphological changes in rice roots, especially increased root number, length and thickness [6,49]. The increases in N uptake as a result of N fertilisation are in agreement with some previous findings [14,17].

Balandreau [2] has pointed out the basic rationale for inoculation with specific plant growth-promoting microbial strains as ensuring early colonisation with beneficial strains already adapted to the rice paddy environment. There should be no need to introduce alien bacteria given that strains such as those in BioGro have all been isolated from rice paddies near Hanoi [34]. Diazotrophs such as strain 1N used in BioGro may have a competitive advantage because of their ability to use carbon-rich exudates from rice roots by fixing their own N, and may contribute to a minor extent to the N-nutrition of rice by biological N_2 fixation. It is possible that the greater effectiveness of BioGro in the second season over the full range of N-fertilisation may be directly connected to greater irradiance or temperature and higher photosyntethic rates of rice. Under such circumstances, the inoculant PGP microorganisms may proliferate more effectively in the rhizosphere as a result of higher rates of energy substrate exudation from rice roots. We intend to investigate such possibilities in future research.

This field study has provided more convincing results for the effectiveness of inoculation with PGP microorganisms by varying the applied N levels. Such studies are rare, although similar results have been obtained using a single strain of N_2-fixing *Burkholderia vietnamensis* [46]. The ability of the inoculated strains to improve the efficiency of utilisation of fertiliser-N, reducing the need for high levels of application is clear from these results. The effectiveness of inoculation is now beyond dispute and the challenge is to further optimise the use of such commercial products so that economic benefits as well as biological benefits are obtained.

Table 9 – Effects of BioGro and N rates on P uptake by Trau Nam rice, Chau Thanh District, Vietnam, second rainy season 2006

N rate (kg ha^{-1})	BioGro		Mean
	Without	With	
P uptake (kg ha^{-1}) by grain			
0	6.64	7.29	6.97 c
30	7.36	8.19	7.78 b
60	7.48	8.16	7.82 b
90	8.06	9.06	8.56 a
120	7.42	7.86	7.64 b
Mean	7.39 B	8.11 A	
P uptake (kg ha^{-1}) by straw			
0	2.05	2.01	2.03 b
30	2.23	2.24	2.24 ab
60	3.12	2.36	2.74 a
90	2.35	2.82	2.59 a
120	2.84	2.65	2.75 a
Mean	2.52 A	2.42 A	
Total P uptake (kg ha^{-1})			
0	8.69	9.30	9.00 c
30	9.59	10.43	10.01 b
60	10.60	10.52	10.56 ab
90	10.41	11.88	11.15 a
120	10.26	10.51	10.39 ab
Mean	9.91 B	10.53 A	

Interaction effect of BioGro and N rate was not significant on all the three parameters. Effect of BioGro was not significant on P uptake by straw while its effect was significant on P uptake by grain and total P uptake at *F* probability levels of 0.003 and 0.037 with LSD (0.05) values of 0.26 and 0.55, respectively. Effect of N rate was significant on all the three parameters at *F* probability levels of <0.001, 0.030 and <0.001 with LSD (0.05) values of 0.57, 0.52 and 0.85 for P uptake by grain, P uptake by straw and total P uptake, respectively. Within a parameter, values followed by different capital letters in a row or different small letters in a column are significantly different by LSD at 5% probability level.

4. Conclusions

BioGro increased grain and straw yields as well as N and P uptake by rice crops in the field experiment conducted in the first and second rainy seasons of 2006 in the Chau Thanh District, Vietnam. Estimated grain and straw yield responses to added N were quadratic in nature with and without BioGro in both seasons. The two seasons' combined results indicate that BioGro application saved 43 kg N ha^{-1} with an additional rice yield of 270 kg ha^{-1} in two consecutive seasons in the experimental site of Chau Thanh District, Vietnam. Furthermore, in the second season the maximum yield of rice possible with urea-N alone was achieved with about 60 kg less urea-N per ha when inoculant PGP microorganisms were added.

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