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EFFECTS OF A MULTISTRAIN BIOFERTILIZER AND PHOSPHORUS RATES ON NUTRITION AND GRAIN YIELD OF PADDY RICE ON A SANDY SOIL IN SOUTHERN VIETNAM

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□ *Field experiments during two successive rainy seasons were conducted in southern Vietnam to evaluate the effects of a commercial inoculant biofertilizer ('BioGro') and fused magnesium phosphate (FMP) fertilizer on yield and nitrogen (N) and phosphorus (P) nutrition of rice. Inoculation with BioGro containing a pseudomonad, two bacilli and a soil yeast significantly increased grain yield in the second season and straw yield in both seasons by 3–5%. The FMP fertilizer significantly increased grain yield from 1.72–2.33 t ha^{-1} to 2.99–3.58 t ha^{-1} along with total N and P accumulation at all rates in both cropping seasons. In the first season the difference in grain yield between BioGro treated and untreated plots was marginal but in the second season BioGro out-yielded the control at all the rates of added P. Overall, BioGro application did not compensate for low P fertilizer application to the same extent previously demonstrated for low N fertilizer applications.*

Keywords: rice, plant-growth promotion, fused magnesium phosphate, *Pseudomonas*, *Bacillus*, rhizosphere

INTRODUCTION

Cereal and legume crops especially require large amounts of phosphorus (P) for growth, development and grain production. When native soil P deficiencies limit plant growth, as occurs in many light-textured agricultural soils, P is supplemented by the application of chemical fertilizer. The commonly-used single superphosphate, mono- or diammonium phosphate fertilizers

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are relatively soluble compared to raw rock phosphates. Unfortunately, after their application a substantial portion can be lost in runoff, or become fixed to soil (Choudhury et al., 2007; Sharpley et al., 2001). These problems cannot be alleviated completely. Nevertheless, plant growth-promoting (PGP) micro-organisms enhance the capacity of plants to absorb nutrients like nitrogen (N) and P efficiently, resulting in stronger growth and higher crop yields (Biswas et al., 2000; Choudhury and Kennedy, 2004; Kennedy et al., 2004; Yanni et al., 1997). Specific P-mobilizing bacteria assist directly by converting fixed P into plant available forms (Ahmed et al., 2008).

A number of different bacteria have been isolated and characterized for their P-mobilizing ability from locations around the world, including diverse members of the genera *Rhizobium, Aspergillus, Penicillium, Bacillus, Pseudomonas,* and *Enterobacter* [for recent reviews, see Khan et al. (2007) and Vassilev et al. (2006)]. The mechanisms by which they act include proton (H^+) excretion to dissolve P from acid-soluble soil P fractions and fertilizers; exudation of organic anions that desorb and mobilize hydrous oxide-bound P; and release of enzymes (phosphatases) to liberate organic P (Trolove et al., 2003). Because of these different mechanisms, soil and type of P-fertilizer will have a significant influence on the effectiveness of biofertilizer strains selected on the basis of P mobilization. Other biological factors such as ecological competitiveness, phytohormone production, pathogen repression activity and mutualism with arbuscular mycorrhizal fungi influence the capability of P-solubilizing bacteria to promote plant growth.

Despite the myriad of laboratory isolates, the true field potential of PGP, P-mobilizing bacteria is difficult to predict by laboratory tests (Gyaneshwar et al., 2002). The performance of candidate strains should be validated under field conditions before widespread application. A number of authors have observed substantial differences between glasshouse and field trials, with more variable results occurring under field conditions (Kucey, 1987; Chabot et al., 1996; Whitelaw et al., 1997; Babana and Antoun, 2006). Complex interactions between bacterial strains, soil types, the rate and type of fertilizer applications, seasonal conditions and crop species can influence the effectiveness of inoculants, particularly with respect to P-use efficiency, defined as the percentage of applied P that is recovered in a particular crop (Whitelaw, 2000; Gyaneshwar et al., 2002). To elucidate the contribution of P-solubilization to plant growth-promotion, Whitelaw et al. (1997) emphasised the recommendation of Abbott and Robson (1984) that inoculation experiments be conducted simultaneously with a response curve that incorporates a number of different P rates.

We are interested in the sustainable production of rice using PGP microorganisms to increase nutrient use efficiency, thereby reducing the fertilizer application requirement. Previous field experiments in northern Vietnam indicated that a commercial multi-strain biofertilizer ('BioGro', Biofertilizer Action Research Centre, Hanoi, Vietnam) increased rice grain yield and N uptake significantly (Nguyen et al., 2003). More recent experiments in southern Vietnam, where the bulk of the rice crop is produced and farm size is generally larger, confirmed the ability of BioGro to increase yield and N accumulation (Phan et al., 2009). However, information about the effect of PGP micro-organisms on P-use efficiency of rice is limited. Further, the use of fused-magnesium phosphate (FMP) fertilizer is becoming a preferred source of P over other sources like single superphosphate and di-ammonium phosphate because of its higher acid-neutralising capacity and slower release properties, avoiding fixation in soil (Cekinski and da Silva, 1998). With these views in mind two field experiments were conducted to evaluate the effects of BioGro with variable rates of FMP on yield, and N and P nutrition of rice and to test the hypothesis that PGP strains improve the efficiency of P use by rice.

MATERIALS AND METHODS

Site Description and Soil Characterization

Field experiments were conducted at Chau Thanh district, Tay Ninh province, Vietnam (latitude, 11° 20′ 60 N, longitude, 106° 4′ 0 E) in the first rainy season of 2006 (April to August) and in the second rainy season of 2006 (August to December), with relatively little rain from October to December, repeating all fertilizer treatments in the same plots. Key chemical and physical properties of the topsoil (0–15 cm) are presented in Table 1.

Biofertilizer Preparation

The BioGro inoculant used for both experiments contained four microbial strains: *Pseudomonas fluorescens* (1N), *Bacillus subtilis* (B9), *Bacillus amyloliquefaciens* (E19) and a soil yeast, *Candida tropicalis* (HY) (Phan et al., 2009). The four strains were inoculated from broth into separate batches of peat (74%), augmented with glucose (1%, w/w) plus water and broth culture (25%, w/w) (Nguyen et al., 2003). These separate cultures in peat were

TABLE 1 Chemical and physical properties of field site topsoil

Property	Value	Unit	Method
Texture (particle size)	Loamy sand		Hydrometer method (Black, 1965)
pH	5.31		Glass electrode (1:5 soil:water)
Organic matter content	14.9	$g\ kg^{-1}$	Walkley and Black (1934)
Cation exchange capacity	4.08	$cmol_c\ kg^{-1}$	Schollenberger and Simon (1945)
Total N	780	$mg\ kg^{-1}$	Bremner and Mulvaney (1982)
Available N	0.13	$mg\ kg^{-1}$	Bremner and Mulvaney (1982)
Total P	180	$mg\ kg^{-1}$	Olsen and Dean (1965)
Available P	14.2	$mg\ kg^{-1}$	Olsen et al. (1954)

mixed in equal proportions one week prior to their application. Recovery of the strains by serial dilution plating indicated their concentration was in the range of 10^7–10^8 cells of each strain per g.

Experimental Design

The two-factor experiment was conducted in a split-plot design with inoculant treatment (+/- BioGro) as the main plots and P fertilizer rate (0, 4, 13 and 26 kg P ha^{-1}) as sub-plots, with four replications. The plot size was 5.1 m x 3.9 m. Nitrogen was added at 90 kg N ha^{-1} as urea to all plots in three split applications (33.4% at final land preparation, 33.3% at 15 days after transplanting (DAT) and 33.3% at 40 DAT. Potassium (K) was added to all the plots as muriate of potash (KCl), equivalent to 30 kg K ha^{-1}, in three splits: 20% at final land preparation, 40% at 15 DAT and 40% at 40 DAT. Phosphorus was added as FMP [15–17% phosphorus pentoxide (P_2O_5), 28–32% calcium oxide (CaO), 14–18% magnesium oxide (MgO), 20–25% silicon dioxide (SiO_2)] obtained from the Ninh Binh Fused Magnesium Phosphate Company (NIFERCO, Hanoi, Vietnam) at the treatment rates given, all at final land preparation prior to flooding. The FMP had an acid-neutralizing capacity of 11.5 mol kg^{-1}, as measured by titration against HCl.

BioGro was applied at 240 kg ha^{-1}, where 40 kg ha^{-1} was applied to seedling nurseries and an extra 200 kg ha^{-1} applied in the field during transplanting. Chemical fertilizers and biofertilizers were applied by hand broadcasting. Rice seedlings (*Oryza sativa* cv. 'Trau Nam') were germinated in nurseries and transplanted with the spacing of 15 cm x 15 cm in both seasons. Sowing, transplanting and harvesting dates for the first short-season rice were 28 April, 20 May and 17 August, respectively and those for the second crop were 29 August, 22 September and 16 December 2006, respectively. The experiment was conducted under irrigated conditions with necessary intercultural operations carried out as required.

Yield and Nutrition Measurements

Grain and straw samples were harvested at maturity. Grain yield was recorded from 5 m^2 area in the middle of each plot with straw yield recorded from 0.5 m^2 at four locations within each plot, for a total of 2 m^2. Grain yield was adjusted at 14% moisture content with straw yield recorded on an oven-dry basis (80°C). Grain and straw samples were analyzed for total N and P concentration. The N concentration was determined by sulfuric acid digestion followed by steam distillation and titration procedures (Yoshida, 1976). The P concentration was determined by dry ashing the plant samples at 490°C in 4 h followed by estimation of P by spectrophotometry (Murphy and Riley, 1962). All data were analyzed using the GenStat, version seven (VSN International, Oxford, UK). A two way analysis of variance (ANOVA) was carried out for each agronomic harvest parameter using BioGro application and P fertilizer rates as independent factors. Regression analysis was also performed on these parameters using P fertilizer rate as the independent factor.

RESULTS

Grain and Straw Yield

In the first rainy season, BioGro inoculation increased grain yield slightly compared with the non-inoculated plots although the difference was not statistically significant; however, the increase in grain yield due to BioGro application, 3.7%, was significant in the second rainy season (Table 2). In the first season BioGro-treated rice out-yielded the control at three P rates except the 10 kg P_2O_5 ha^{-1}; in the second season the beneficial effect of BioGro was observed at all the P rates (Figure 1). The stepwise effect of P rates on grain yield was significant at F probability level less than 0.001 in both seasons (Table 3). P fertilization increased grain yield significantly up to 60 kg P_2O_5 ha^{-1}, with the estimated grain yield response due to P fertilization being quadratic in nature with or without BioGro in both seasons (Figure 1).

A similar result was observed in straw yields, with the application of BioGro to rice significantly increasing the dry weight of straw in both the first and second rainy seasons by an average of 4.3% (Table 2). The estimated straw yield response due to P fertilization was also quadratic in nature with

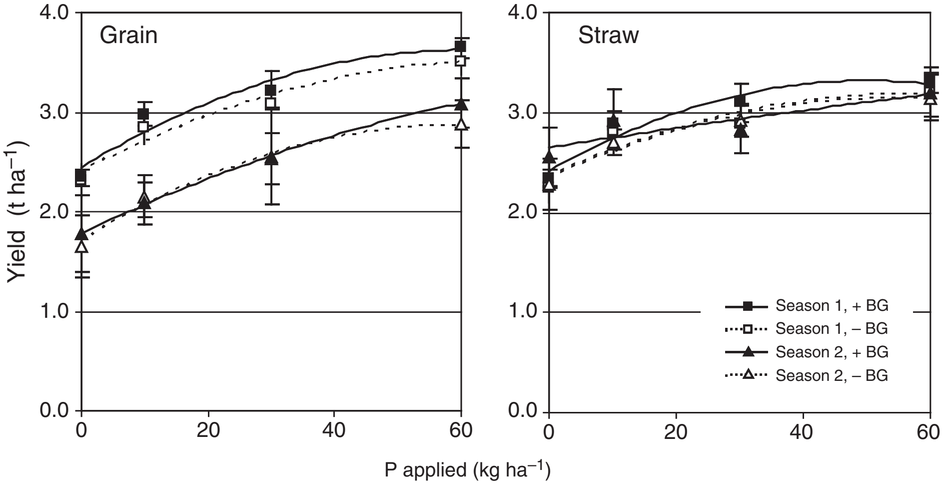

FIGURE 1 Grain and straw yield response of rice to P fertilizer, with (+BG) or without (−BG) BioGro application at 200 kg ha^{-1}, in successive seasons. Data points are means of four replicates; error bars indicate standard deviations.

TABLE 2 Seasonal effects of BioGro on agronomic harvest parameters of Trau Nam rice, Tay Ninh province, southern Vietnam, 2006

	First rainy season				Second rainy season			
Agronomic parameter	Without BioGro	With BioGro	% Change[a]	Stat. signif.[b]	Without BioGro	With BioGro	% Change[a]	Stat. signif.[b]
Grain yield (t ha^{-1})	2.30	2.38	3.5	NS	2.94	3.05	3.7	*
Straw yield (t ha^{-1})	2.76	2.88	4.3	*	2.79	2.91	4.3	*
Grain P concentration (mg kg^{-1})	2.5	2.6	4.0	NS	2.3	2.4	4.3	NS
Grain P accumulation (kg ha^{-1})	5.73	6.12	6.8	NS	6.95	7.40	6.5	NS
Straw P concentration (mg kg^{-1})	0.9	0.9	0.0	NS	0.8	0.8	0.0	NS
Straw P accumulation (kg ha^{-1})	2.4	2.7	10.8	NS	2.1	2.2	7.7	NS
Grain N concentration (mg kg^{-1})	11.5	11.9	3.5	NS	13.4	13.4	0.0	NS
Grain N accumulation (kg ha^{-1})	26.6	28.2	5.7	NS	39.3	40.7	3.6	NS
Straw N concentration (mg kg^{-1})	7.1	7.2	1.4	NS	6.6	6.6	0.0	NS
Straw N accumulation (kg ha^{-1})	19.6	20.7	5.8	**	18.4	18.9	3.2	NS

[a] The percentage change in parameter value afforded by application of BioGro relative to no application of BioGro.

[b] Statistical significance of means differences, averaged over all P rates, as measured by 2-way ANOVA F-test: $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), $P > 0.05$.

(NS). Note: no interaction effects between BioGro application and P fertilizer rates were observed.

TABLE 3 The effects of P fertilization rates on agronomic harvest parameters of Trau Nam rice, Tay Ninh province, southern Vietnam, 2006

Agronomic parameter	First rainy season[a]	Second rainy season[a]
Grain yield (t ha^{-1})	***	***
Straw yield (t ha^{-1})	***	***
Grain P concentration (mg kg^{-1})	NS	***
Grain P accumulation (kg ha^{-1})	***	***
Straw P concentration (mg kg^{-1})	NS	NS
Straw P accumulation (kg ha^{-1})	***	***
Grain N concentration (mg kg^{-1})	NS	NS
Grain N accumulation (kg ha^{-1})	***	***
Straw N concentration (mg kg^{-1})	NS	NS
Straw N accumulation (kg ha^{-1})	***	**

[a]Statistical significance of means differences, averaged over BioGro treatments, as measured by 2-way ANOVA F-test: $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), $P > 0.05$ (NS).

Note: no interaction effects between BioGro application and P fertilizer rates were observed.

or without BioGro in both the seasons. However, unlike grain yield, straw yields were not noticeably different between seasons (Figure 1).

Phosphorus and Nitrogen Concentration and Uptake

BioGro application had no significant effect on any of the P nutritional parameters in either season (Table 2). Similarly, BioGro application had little effect on the N nutrition of rice, with only the N accumulation by straw being significantly greater after BioGro application in the first season (Table 2). In contrast, P fertilization significantly increased the P concentration in grain in the second season and also the total P accumulation of grain and straw in both the seasons (Table 3; Figure 2). Phosphorus fertilization also increased the total N accumulation significantly in grain and straw in both seasons (Figure 3).

DISCUSSION

The purpose of this research was to determine the effects of a multi-strain biofertilizer, containing a known P-solubilizer (*Candida tropicalis* HY; Rose et al., 2008), combined with FMP chemical fertilizer, on rice nutrition and yield. Accordingly, the experimental site was chosen on the basis of a low total P concentration (0.018%) and relatively low available Olsen P concentration (14.2 mg kg^{-1}). This available P concentration falls within the range of 4 to 29 mg P kg^{-1} given as the critical P deficiency level using the Olsen method (Dobermann et al., 1996). In our experiments a significant yield response was observed at all increasing levels of applied FMP fertilizer. Rice yields were over 1 t ha^{-1} more in plots receiving 26 kg P ha^{-1} than in plots receiving

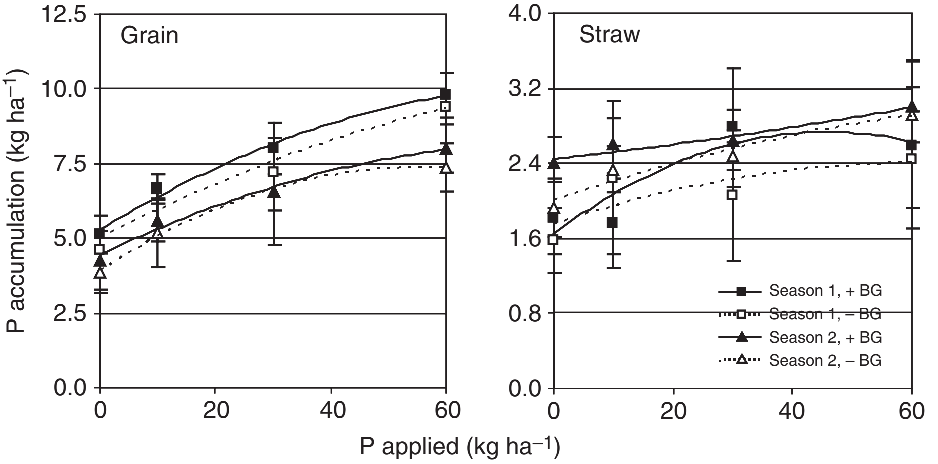

FIGURE 2 Rice grain and straw P accumulation at different rates of P fertilizer, with (+BG) or without (−BG) BioGro application at 200 kg ha^{-1}, in successive seasons. Data points are means of four replicates; error bars indicate standard deviations.

no P fertilizer, and the response curve had not reached a maximum at this fertilizer application rate. This significant yield response warrants promotion of FMP as a suitable P fertilizer for rice in this type of soil. Its high calcium (Ca) and magnesium (Mg) content may have a beneficial liming effect on this acidic soil of pH value around 5.

However, no yield interaction was observed between P rates and BioGro application, and furthermore, no substantial yield increases resulted from BioGro application in these experiments with adequate N fertilization.

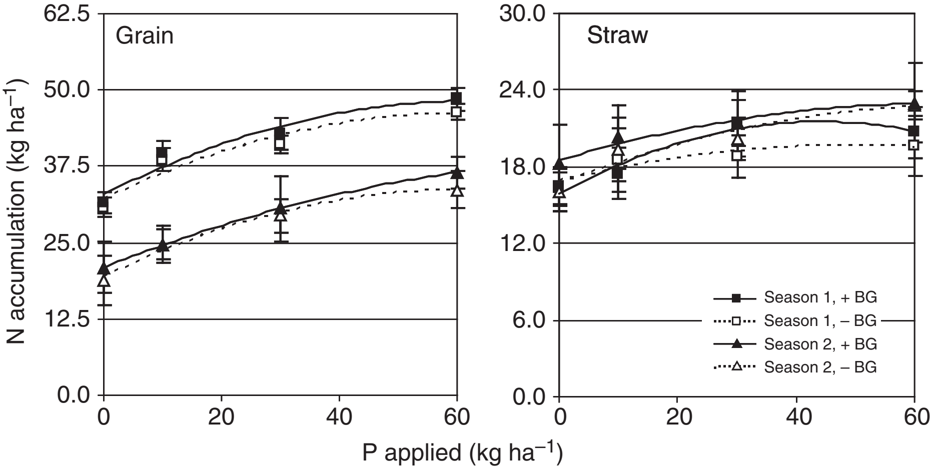

FIGURE 3 Rice grain and straw N accumulation at different rates of P fertilizer, with (+BG) or without (−BG) BioGro application at 200 kg ha^{-1}, in successive seasons. Data points are means of four replicates; error bars indicate standard deviations.

Although plus BioGro treatments gave greater yields than the minus BioGro control at three P rates (0, 13 and 26 kg P_2O_5 ha^{-1}) in the first season, the difference was not statistically significant ($P > 0.10$). In contrast, there was a significant ($P = 0.04$) beneficial effect of BioGro in the second season over all the P rates, but it only increased mean yields by about 3.7%. The effect of BioGro on straw yields, although significant, was also less than a 5% increase in both seasons. *C. tropicalis* strain HY solubilizes calcium phosphates (Rose et al., 2008), so an interaction between BioGro and FMP rates was expected. In contrast, the field results suggest that the major PGP response caused by BioGro is not related directly to increased P-mobilization by the yeast strain HY or by any of the other constituent strains. These results confirm the difficulties in translating promising results from laboratory and glasshouse trials to the field, a fact reflected in the variable performance of P-mobilizing microorganisms applied to other cereal crops (Chabot et al., 1996, Whitelaw et al., 1997; Babana and Antoun, 2006). Indeed, work by Wissuwa (2003) suggests that the major factors acting to increase P-efficiency in certain rice cultivars are related more to root structure and surface area than to increased rates of proton or anion excretion.

Our previous field experience with BioGro suggests that it has a major effect on the ability of rice to access N sources at low levels of N-application (Phan et al., 2008, 2009). Notably, yield increases caused by BioGro at optimum N fertilizer levels were variable from season to season and appeared to be related to climatic differences. BioGro appears more likely to increase yields during seasons of higher insolation than seasons with lower insolation (Pham et al., 2008; Phan et al., 2009). Thus, in the current experiments where N was applied at a relatively high level, overall PGP effects could have been restricted, particularly if the putatively N_2-fixing pseudomonad in BioGro (Nguyen et al., 2003) was repressed and overall PGP effects were thus reduced. Nevertheless, grain yields, N uptake by grain and P uptake by grain in the second rainy season were all higher than in the first season. Part of this response could be a result of P accumulation and carryover from fertilization in the first season, as total P at the same fertilizer rates was higher in the second season. However, the internal P-use efficiency at all P rates also increased in the second season, indicated by the vertical shift in the grain yield response to P-uptake in Figure 4. This shift implies that seasonal climatic differences played a stronger role in the higher yields in the second season. Of the climatic factors responsible for rice yield differences investigated by Yu et al. (2001), the number of hours of sunshine during the tillering stage and the heading to milk stage particularly affected the yield. Such an effect results in higher grain yields in the tropics from dry season crops compared to wet season crops because of higher irradiance (Yang et al., 2008). Significant interactions between irradiance and nutrient availability occur (Evans and De Datta, 1978). Altogether, based on the results of this and our previous experiments (Phan et al., 2008, 2009), the interactions of N rates, P rates,

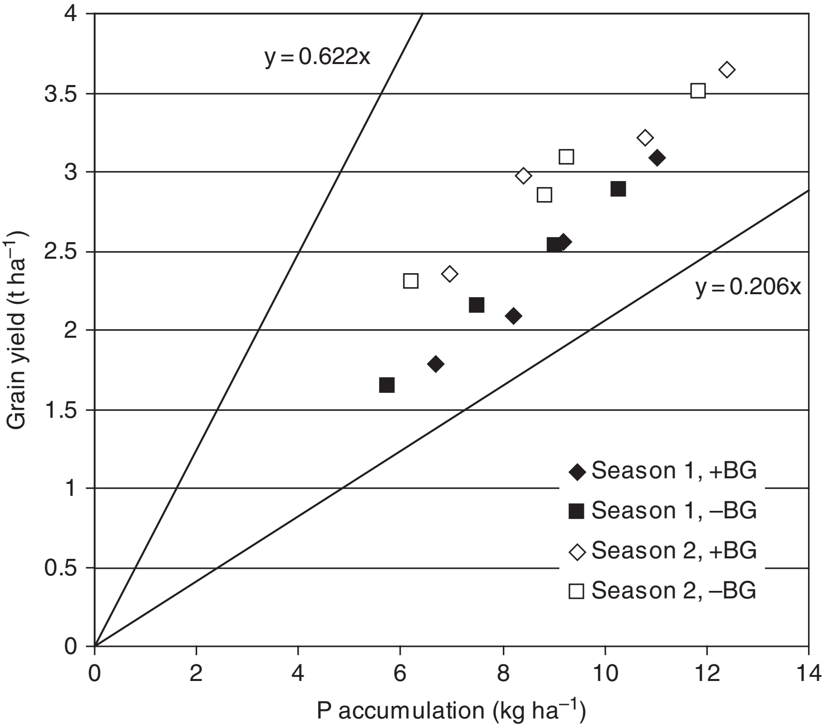

FIGURE 4 The relationship between total P accumulation (kg ha^{-1}) and grain yield (t ha^{-1}) of rice grown with (+BG) or without (−BG) BioGro inoculation, in two consecutive growing seasons. Data points are means of four replicates; error bars have been omitted for clarity. The line $y = 0.622x$ indicates the maximum internal P efficiency while the line $y = 0.206x$ indicates the minimum plant internal P efficiency, derived from 2500 data sets from experiments with irrigated rice throughout S and SE Asia (Witt et al., 1999).

and BioGro needs to be assessed together to determine optimum fertilizer rates under variable seasonal conditions.

The inoculant biofertilizer used in this research was formulated after extensive testing of PGP isolates from rice rhizospheres in laboratory trials. Balandreau (2002) pointed out that the basic rationale for inoculation with specific PGP microbial strains is to ensure early root colonization with higher numbers of beneficial strains already adapted to the rice paddy environment. Because of the rapid increase in the number of biofertilizer products on the market, quality control of the product and the field effect will be crucial for any future success for the role of non-endophytic inoculant biofertilizers. The research presented here demonstrated that, for BioGro at least, the enhancement of P nutrient use efficiency is minimal.

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