Chapter 11
BioGro: A Plant Growth-Promoting Biofertilizer Validated by 15 Years' Research from Laboratory Selection to Rice Farmer's Fields of the Mekong Delta

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Abstract Since their original isolation from rice paddies near Hanoi, the set of microbial strains comprising the biofertilizer BioGro have been subjected to extensive and intensive experimentation in both laboratory and the field. Based on a hypothesis that such strains inoculated onto rice and other plants could significantly reduce the need for chemical fertilizers, this has been successfully tested using numerous procedures, documented in a series of peer-reviewed papers. The BioGro strains have been examined by a range of molecular and biochemical techniques, also providing means of quality control of inoculants. A positive response by rice plants to BioGro strains has been confirmed by proteomics. More than 20 randomized block design field experiments conducted in Vietnam or Australia have confirmed their effectiveness under a range of field conditions, reviewed here. Interactions with different rice cultivars have also been examined. While the response to inoculation is complex, the hypothesis of increased nutrient efficiency has been amply confirmed as consistent with observations. Finally, an extensive participatory research project over 3 years in the Mekong Delta showed reductions in fertilizer needs as high as 52 % as rice farmers learned to apply the technology. This result shows the importance of such adaptive practices for successful application of this biofertilizer technology in field condition.

Keywords PGPR • Pseudomonas • Bacilli • Soil yeast • Rice proteomics

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11.1 Introduction

Nitrogen fertilizer application is essential to maintain growth and yield of rice due to acute N deficiency in the rice soils. However, a substantial portion of the applied fertilizer nitrogen is lost due to ammonia volatilization, denitrification and leaching causing environmental pollution problems (Choudhury and Kennedy 2005). Due to these losses, fertilizer N recovery in rice culture is very low, generally around 30–40 %, in some cases even lower (Choudhury and Khanif 2001, 2009). These losses cannot be completely alleviated. However, the use of bacterial inoculant biofertilizers can lessen the need for the application of fertilizer N by more efficient N uptake by plants (Choudhury and Kennedy 2004; Kennedy et al. 2004, Choudhury et al., 2014).

Seedling growth is important for the successful establishment of rice and other crops. Previous investigations showed that rice seedling growth was enhanced by due to inoculation withof plant growth promoting (PGP) microorganisms, resulting in increased grain and straw yields as well as fertilizer N use efficiency (Biswas et al. 2000a, b; Yanni et al. 1997). Both single- and multi-strain biofertilizers are used to inoculate rice seedlings (Balandreau 2002; Malik et al. 2002). Jacques Balandreau (2002) developed the spermosphere concept in the 1990s suggesting that the role of inoculation was to provide a competitive advantage to beneficial microbes in the high nutrient zone of the rhizosphere. He might equally well have called it the chemosphere, given that carbon and other nutrients excreted by plant roots provide a locally favourable zone for microbial function. Inoculation with significant numbers of beneficial PGP strains is important to ensure that microbes with low benefits to the plant or even antagonists did not preferentially colonize the root zone by being significantly outnumbered. As a result, it is necessary to inoculate with up to 10^{11} viable cells of microbial strains per ha to guarantee an effective result as enhanced plant growth. It is surmised that these microbes predominate forming a biofilm with the surface of the roots.

A multi-strain biofertilizer called BioGro was developed by Nguyen Thanh Hien in Hanoi University of Science using the spermosphere principle during the 1990s (Nguyen et al. 2002). Earlier attempts to assist farmers with limited fertilizer inputs using microbial strains isolated in other countries failed to provide consistent benefits. The strains of this BioGro biofertilizer were isolated from rice rhizosphere near Hanoi and characterized for beneficial traits under laboratory conditions. Then the product was tested in numerous field experiments in Vietnam and Australia under AusAID (Australian Agency for International Development), ACIAR (Australian Centre for International Agricultural Research) and World Bank Development Marketplace funded research projects. This chapter reviews salient findings of those experiments, accumulating the relevant information altogether.

11.2 Isolation and Characterization of Strains of BioGro

Before 2005, BioGro contained three strains of bacteria (1N, 4P and 3C) selected from rice rhizosphere in the Hanoi area of Vietnam. From 2005, a combination of four strains (1N, HY, B9 and E19) has been used. The strains were identified at the University of Sydney using a number of different techniques including morphological, biochemical and genetic methods (Kecskés et al. 2008a). Subsequently, the strain identities were also confirmed at the laboratories of the German Culture Collection of Microbes (DSMZ), Braunschweig, Germany. The strains comprising 'BioGro1I' were identified as *Pseudomonas fluorescens* (1N), *Citrobacter freundii* (3C) and *Klebsiella pneumoniae* (4P), whereas 'BioGro 2' consisted of *P. fluorescens* (1N), *Bacillus subtilis* (B9), *Bacillus amyloliquafaciens* (E19) and a soil yeast, *Candida tropicalis* (HY). Survival of these strains at sufficiently high numbers greater than ca. 107 colony-forming units per g (cfus) in as peat cultures has been evaluated more recently (Rose et al. 2011).

In BioGro 1, strain 4P was selected for its ability to solubilize insoluble PO_4 in an agar medium. Strain 3C was selected for its ability to produce extracellular compounds which inhibited 50 % of a test group of 100 rhizosphere organisms (Nguyen et al. 2002). Each of the three bacteria (1N, 4P and 3C) were grown in separate broth cultures and added to separate bags of carrier formulated by mixing high organic-clay soil 50 %, rice husks 25 %, sugar 1 %, plus water and broth culture 24 %. These separate cultures were mixed in the field immediately before use in the ratio of 10 parts 1N: 10 parts of 4P: 1 part of 3C (Nguyen et al. 2002).

In BioGro 2, each of the four organisms were grown in separate broth cultures, and separately added to the carrier formulated by mixing peat (clay soil) 75 %, plus water and broth culture 24 % and sugar 1 % and incubated for 48 h at 30 °C. These four carriers were mixed before use in equal proportions. (Nguyen 2008). 1N (*Pseudomonas fluorescens*) was selected for its ability to grow on nitrogen-free medium, although it is not an N_2-fixing organism, and to reduce acetylene to ethylene as an indication of potential N_2 fixation. HY (*Candida tropicalis*) is a soil yeast and was initially selected for its ability to solubilize insoluble PO_4 in an agar medium. It has been shown to have a suite of plant growth promoting properties acting to promote robust root systems in rice plants (Amprayn et al. 2012). Two other strains B9 (*Bacillus subtilis*) and E19 (*Bacillus amyloliquefaciens*) were selected for their ability to break down protein, cellulose and starch (Nguyen 2008; Deaker et al. 2011).

Detailed methods for ensuring quality control of the inoculant strains have been published in a widely distributed laboratory monograph (Deaker et al. 2011; Krishnen et al. 2011). This manual includes a range of molecular and physiological methods applicable to many other PGPR microbes (Krishnen et al. 2011).

Table 11.1 Effects of BioGro on grain yield of rice at Dai Moi, Hanoi during 1999 to 2001

Year	Grain yield (t/ha)		Difference	Least significant difference (LSD) at 5 % level
	Without BioGro	With BioGro (111 kg/ha)		
1999	5.7	6.7	+1.0	0.5
2000	6.3	6.0	−0.3	Non-significant
2001	5.5	6.2	+0.7	0.3

Adapted from Nguyen et al. (2002)

Table 11.2 Effect of BioGro on grain yield of rice at farmers' demonstration during 1999–2001

Year	Grain yield (t/ha)		Difference	% increase
	Without BioGro	With BioGro		
1999	5.4	6.1	0.7	12.96
2000	5.3	6.4	1.1	20.75
2001	4.9	5.6	0.7	14.28

Adapted from Nguyen et al. (2002)

11.3 Initial Field Evaluation of BioGro Potential

Field experiments were conducted at Dai Moi near Hanoi under a research project funded by the Australian Agency for International Development (AusAID). In those experiments, BioGro 1 was used. Demonstrations at farmers' fields were also conducted. Details of the experimental procedures and findings are available in Nguyen et al. (2002, 2003). In the field experiments at Dai Moi, BioGro increased grain yield significantly in 1999 and 2001 whereas the effect of BioGro was non-significant in 2000 (Table 11.1). In the demonstrations at farmers' fields, the positive effect of BioGro in increasing grain yield was observed in all the years (Table 11.2). The percent increases in grain yield were 12.96, 20.75 and 14.28 in 1999, 2000 and 2001, respectively. An economic analysis indicated that BioGro application in rice cultivation was economically and environmentally beneficial for the farmers (Barret and Marsh 2002). A similar beneficial effect of BioGro was predicted in Australian conditions (Williams and Kennedy 2002).

Although the positive effect of BioGro 1 was observed, one of strain *Citrobacter freundii* (3C) is a human enteric organism, thus caution regarding health safety was recommended. Development of a newer version of BioGro excluding *Citrobacter freundii* (3C) and *Klebsiella pneumoniae* (4P) was recommended.

11.4 Field Evaluation of BioGro

A fortunate follow-up of the findings of AusAID-funded project led to conduct further research in both Australia and Vietnam under an ACIAR (Australian Centre for International Agricultural Research)-funded project. The findings are summarized

Table 11.3 Effects of BioGro on biomass, yield and N uptake of Jarrah rice, field experiment at Yanco, November 2004 to April 2005

Parameter	Treatment		Difference
	50 kg N/ha	50 kg N/ha + BioGro	
Plant dry biomass (t/ha) at panicle initiation (PI) stage	2.38	2.86	+0.48
Grain yield (t/ha)	6.10	6.42	+0.32
Straw yield (t/ha)	5.69	5.83	+0.14
Total N uptake (kg/ha) by whole plant at maturity	75.3	80.0	+4.7

Adapted from Kecskés et al. (2008b)

in the proceedings of ACIAR (Kennedy et al. 2008). Some of the findings were also published in impact factor journals (Phan et al. 2009, 2011; Nguyen et al. 2014; Kecskés et al. 2016). Some salient findings are presented here.

11.5 Field Experiment at Yanco Agricultural Research Institute Australia

A field experiment was conducted during November 2004 to April 2005 to evaluate the beneficial effect of BioGro 1 and other two biofertilizers on rice yield (Kecskés et al. 2008b). A short-duration rice variety Jarrah was used as the test crop. At panicle initiation (PI) stage, BioGro increased plant biomass by 0.48 t/ha, while at maturity grain yield increase by BioGro was only 0.32 t/ha (Table 11.3). This finding indicates that the positive effects of BioGro inoculation decreased at later growth stages of the rice plant. This might be an effect of the much longer growth duration of the rice plants in Australia compared to that of Vietnam. Reinoculation of bacteria at PI stage, and application of nitrogen fertilizer in at least two splits (2/3 at final land preparation and 1/3 at PI stage) are recommended for the next field experiments. Lower N rates are also recommended.

11.6 Field Experiment at Jerilderie Rice Research Institute

A field experiment was conducted during 2005 to 2006 to evaluate the beneficial effect of BioGro 1 and other two biofertilizers on rice yield (Kecskés et al. 2008b). A long-duration rice variety Amaroo was used as the test crop. At PI stage, BioGro inoculation increased plant biomass and N uptake slightly while this positive effect disappeared at maturity stage (Table 11.4). As Amaroo is a long-duration variety, reinoculation of bacteria at PI stage, and application of nitrogen fertilizer in at least two splits (2/3 at final land preparation and 1/3 at PI stage) are recommended for the next field experiments. Lower N rates are also recommended.

Table 11.4 Effects of BioGro on Biomass, N uptake and yield of Amaroo rice, field experiment at Jerilderie, 2005–2006

Parameter	Treatment		Difference
	50 kg N/ha	50 kg N/ha + BioGro	
Plant dry biomass (t/ha) at PI stage	2.66	2.77	+0.11
N uptake (kg/ha) at PI stage	37.3	44.8	+7.5
Grain yield (t/ha)	7.28	6.27	−1.01
Straw yield (t/ha)	6.33	6.11	−0.22

Adapted from Kecskés et al. (2008b)

Table 11.5 The effect of inoculating rice with BioGro for one, two or three seasons on yield and panicle formation in the third season, spring 2006

BioGro inoculation programme	Grain yield (t/ha)	Number of panicles/hill
Uninoculated	5.99	6.25
Inoculated in season 1 only	5.45	6.40
Inoculated in seasons 1 and 2	6.75	6.70
Inoculated in seasons 1, 2 and 3	7.32	7.90
F probability	0.054	0.013
LSD (0.05)	–	0.95
LSD (0.10)	1.10	0.77

Adapted from Nguyen (2008)

11.7 Field Experiments Conducted in Northern Vietnam

A set of replicated field experiments were conducted in the Hanoi area of Vietnam in 2005, 2006 and 2007 to evaluate the beneficial effect of BioGro 2 on rice cultivation. Three types of experiments (successive application of BioGro, rates of BioGro application and timing of BioGro application) were carried out by Hanoi University of Science. Another experiment on varietal difference on BioGro response was conducted at Thanh Tri, near Hanoi in 2006 by the Vietnam Academy of Agricultural Science, Hanoi.

11.8 Successive Application of BioGro

These field experiments were conducted in the Hanoi area for three successive crops beginning in spring 2005 at the same site to determine whether repeated inoculation with BioGro would further increase grain yield or affect its components. In the third season (spring 2006), application of BioGro in each of the three seasons increased grain yield significantly over both uninoculated rice and rice inoculated in the first season only at 10 % level of probability, confirming its beneficial effect on rice crops (Table 11.5). When inoculated with BioGro for two seasons the yield of rice

Table 11.6 Effect of the amount of BioGro applied on grain yield, spring 2006

Amount of BioGro applied (kg/ha)	Grain yield (t/ha)	Increase over control (t/ha)
0	5.45 b	–
50	6.91 a	1.46
100	6.83 a	1.38
200	6.25 a	0.80

Adapted from Nguyen (2008)
Values followed by a common letter in a column are not significantly different at 5 % level by least significance difference (LSD)

was 0.57 t/ha less than when applied for three seasons, but this difference was not significantly different (P=0.10). However, inoculation in two seasons did increase yield by 1.30 t/ha compared with a single inoculation in the first season. Application of BioGro in all three seasons increased the number of panicles per hill significantly over all other treatments at 5 % level of probability.

11.9 Rates of BioGro Application

This field trial was established in spring 2006 on plots on six farms in the Hanoi area to investigate the effect of the rate of BioGro applied on grain yield and yield components. Four rates of BioGro (0, 50, 100 and 200 kg/ha) were applied at transplanting. Treatment plots of farmers 1 and 2 received 200 kg/ha of BioGro, treatment plots of farmers 3 and 4 received 100 kg/ha of BioGro and treatment plots for farmers 5 and 6 received 50 kg/ha of BioGro. A control (0 kg/ha of BioGro) was included at all the farmers' plots. BioGro applied at 50, 100 or 200 kg/ha increased grain yield significantly compared with uninoculated rice at 5 % probability level (Table 11.6). Thus it was evident that increasing the amount of BioGro from 50 to 100 or 200 kg/ha did not further increase yield.

11.10 Timing of BioGro Application

This field trial was established in spring 2007 to evaluate the effects of higher amounts of BioGro and the timing of the application on yield and yield components of rice. The experiment was conducted on three farms in Dai Mo village near Hanoi. An uninoculated control was also established on each farm. In farm one, a 150 m^2 plot was used for the uninoculated control and another 150 m^2 plot used for the rice seedlings treated with BioGro at 38 kg/ha in the nursery area. In farm two, a 120 m^2 plot was used for the uninoculated control treatment and another 120 m2 plot was used for rice treated at the seedling stage with 38 kg/ha and at transplanting with BioGro at 278 kg/ha. In farm three, a 203 m^2 plot was used for the uninoculated control treatment and another 203 m^2 plot was used for rice treated at the seedling stage and at transplanting as in Farm 2, plus 139 kg/ha BioGro at 1 month after transplanting.

Details of the experimental findings are available in Nguyen (2008), not presented in this chapter. On the first farm where a single application of BioGro at 38 kg/ha was used in the nursery only, it increased grain yield by 9 %. On the second farm where a total of 316 kg/ha was applied in a split application to the rice paddy, the increased grain yield was 80 %. When three applications of BioGro were made on a third farm, grain yield obtained by two or three applications were similar. However, the variation in % increase over control was due to the variation in the yields in control plots between the farms. This indicates an effect of soil fertility on the effectiveness of biofertilizer application. When averaged over all plots, inoculation increased the number of panicles per m^2 from 240 to 331, and the number of fertile seeds per panicle from 76 to 150. Neither of these parameters appeared to be significantly influenced by the third application of 139 kg/ha at 28 days after transplanting the seedlings.

11.11 Varietal Differences

The experiment was initiated in spring 2006 in Thanh Tri, Hanoi and repeated in the following season (summer 2006) to evaluate the beneficial effects of BioGro2 on six rice varieties (three common varieties: KD18, AYT01, VD8; and three quality varieties: LT2, HT1, BT7). Three treatments used in the experiment were T 1: 100 % NPK + farmyard manure (FYM) as control, T 2: BioGro + 50 % NP +100 % K+ FYM, and T 3: Biogro + 30%NP + 100%K + FYM. N fertilizer rates for spring and summer were 260 kg and 220 kg urea/ha, respectively. In both the seasons, 450 kg triple super phosphate (TSP), 180 kg muriate of potash (KCl), 10 tonnes FYM and 283 kg Biogro/ha were used.

Full amounts of FYM and P was applied at transplanting time in both the seasons. N and K were applied in two splits (50 % at transplanting + 50 % at active tillering stage) in both the seasons. BioGro was applied in two splits (83 kg/ha during seed sowing by mixing with seeds + 200 kg/ha applied on experimental plots during transplanting). The formulation of BioGro was 1N + HY + B9 + E19 at the ratio of 1:1:1:1.

Some salient findings of the Summer 2006 experiment are presented (Table 11.7). Grain yield with BioGro along with 50 % reduced amount of NP fertilizer gave statistically similar amount of grain with 100 % NP in four varieties (LT2, HT1, KD18 and AYT01). In variety VD8 BioGro application increased grain yield significantly while the opposite result was obtained in the other variety BT7. This finding thus indicates that BioGro application should be related to rice variety.

Table 11.7 Grain yields (t/ha) of rice varieties as affected by BioGro in combination with chemical fertilizers, Thanh Tri, Hanoi, summer 2006

Rice variety	Treatment		Difference
	T1 (Control): 100 % NP	T2 (BioGro): 50%NP + BioGro	
LT2	3.79	3.73	−0.06[ns]
HT1	4.42	4.53	+0.11[ns]
BT7	3.91	3.57	−0.34*
KD18	5.57	5.53	−0.04[ns]
VD8	5.60	5.95	+0.35*
AYT01	4.28	4.27	−0.01[ns]

Adapted from Pham et al. (2008)
ns non-significant
*significant at 5 % level by LSD

Table 11.8 Effects of BioGro on grain and straw yields of Trau Nam rice, Chau Thanh District, Vietnam

Season	Parameter	Without BioGro	With BioGro	Difference
First Rainy Season 2006	Grain yield (t/ha)	2.76	2.86	+0.10*
	Straw yield (t/ha)	2.65	2.79	+0.14*
Second Rainy Season 2006	Grain yield (t/ha)	3.06	3.30	+024**
	Straw yield (t/ha)	2.62	2.79	+0.17*

Adapted from Phan et al. (2009)
*Significant at 10 % level of probability, **Significant at 5 % level of probability

11.12 Field Applications Conducted in Southern Vietnam

The effect of BioGro 2 biofertilizer on nutrient uptake and grain yield of rice was investigated on a grey degraded soil of Thanh Dien village, Chau Thanh district, TayNinh province, Southern Vietnam. Initial soil was loamy sand with pH 5.31, 1.5 % organic matter content, 4.08 cmol/kg cation exchange capacity and 0.11 cmol/kg exchangeable K. Field experiments were carried out in two consecutive seasons (the first and the second rainy seasons). Different application rates of N and P were used to investigate the response of rice crop and the interactions between biofertilizer and nutrients. Trau Nam, a local rice variety with duration of 110 days, was used as the test crop.

Details of the experimental procedures and findings are available (Phan and Tran 2008; Phan et al. 2009, 2011). Some salient findings are presented in Table 11.8 of this chapter. The findings indicate that BioGro increased gain yield significantly at 10 and 5 % levels of probability in the first and second seasons, respectively. This indicates the seasonal impacts on the effectiveness of BioGro in rice crop. The intensity of solar energy, which is a seasonal attribute, might influence the activity of microorganisms, thereby affecting excretion of nutrients into the rhizosphere. The effect of BioGro on significantly increasing yield over a full range of N applications with maximum solar energy (Fig. 11.1). By contrast, BioGro applied in the

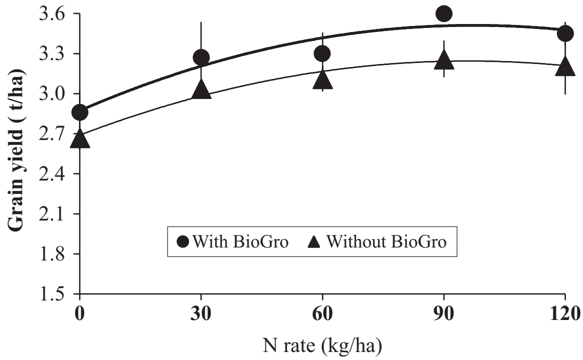

Fig. 11.1 Estimated grain yield response of Trau Nam rice to added N with and without BioGro, Chau Thanh district, Vietnam, second rainy season 2006 (Courtesy of Phan and Tran 2008)

first (heavy) rainy season with less solar insolation gave peak stimulation in grain yield in the range 30–60 kg N per ha, but no stimulation at maximum yield of 120 kg per ha N.

$$y = 2.8726 + 0.0131x - 0.00007x2(\text{with BioGro}), r2 = 0.911\, at\, P < 0.05$$

$$y = 2.6894 + 0.0116x - 0.00006x2(\text{without BioGro}), r2 = 0.9668\, at\, P < 0.05$$

A follow-up study was conducted using the ^{15}N-labelled urea in the Institute of Agricultural Sciences, Ho Chi Minh City, Southern Vietnam. Details of the experimental procedures and findings are available in Phan et al. (2008). A greenhouse experiment was conducted to evaluate the inoculation effect of 1N (*Pseudomonas fluorescens*) bacteria on dry matter production and fertilizer N uptake of rice plants. A grey degraded soil was used for this experiment, which was collected from Chau Thanh district, TayNinh province of Southern Vietnam. Four kilograms of soil were used per pot, and four replicates were used per treatment. Phosphorus and potassium were added to all pots as fused-magnesium phosphate (P_2O_5) and K_2O as muriate of potash (KCl) at a rate equivalent to 60 kg/ha of each fertilizer.

In the first set, N was added as ^{15}N-urea (4.634 % ^{15}N atom excess) at two rates equivalent to 0 and 20 kg N/ha. Twenty five pregerminated seeds, inoculated with 1N bacteria at106 colony-forming units (CFU) per g, were placed in each pot, which is equivalent to 100 kg seed sown per hectare. There were two inoculation treatments—with 1N and without 1N. Seedlings were harvested at 20 days after sowing (DAS) by cutting rice plants at ground level. In the second set, N was added from the same source at three rates equivalent to 0, 20 and 40 kg N/ha. Inoculated pregerminated seeds were placed in the pots and five seedlings were left in each pot. There were two inoculation treatments—with 1N (108 CFU/g) and without 1N. Seedlings were harvested at 45 DAS by cutting rice plants at ground level.

Plant samples were cut at ground level, air and oven dried at 70 °C and ground. Total N contents of the plant samples were determined by the micro-Kjeldahl procedure (Yoshida et al. 1976), and subsequently the ^{15}N abundance was estimated by

Table 11.9 Effects of inoculation of 1N (Pseudomonas fluorescens) bacteria and fertilizer N rate on plant dry weight, total N content and uptake of rice seedlings at 20 DAS

	Fertilizer N rate (kg N/ha)		
Inoculation	0	20	Mean
Dry weight (g/pot)			
Without 1N	8.73	9.02	8.88 b
With	9.02	9.24	9.13 a
Mean	8.88 B	9.13 A	
Total N content (%)			
Without 1N	3.19	3.66	3.43 a
With	3.24	3.57	3.41 a
Mean	3.22 B	3.62 A	
Total N uptake (mg/pot)			
Without 1N	278.4	330.3	304.4 a
With	292.6	330.0	311.3 a
Mean	285.5 B	330.2 A	

Adapted from Phan et al. (2008)
Interaction effect of inoculation and N rate was not significant among the three parameters. Within a parameter, values followed different capital letters in a row or different small letters in a column are significantly (P<0.05) different by least significant difference (LSD)

emission spectrometry (Hauck 1982) at the Nuclear Centre of Ho Chi Minh City using emission spectrometer NOISE-7. The percent ^{15}N atom excess (AE) was calculated by subtracting the natural abundance of ^{15}N (0.3663) from the abundance data of plant samples (Axman and Zapata 1990; Panda et al. 1995).

The calculations for estimating recovery in the plant from ^{15}N-labelled fertilizer were made according to procedures described by Axman and Zapata (1990). The percentage of *N* derived from fertilizer (NdfF) was calculated as follows:

$$\text{NdfF (\%)} = \frac{^{15}\text{N atom \% excess in plant sample}}{^{15}\text{N atom \% excess in labelled fertilizer}} \times 100.$$

The following computations were done as described by Choudhury and Khanif (2001) as follows:

1. Total N uptake (mg/pot) = total N in plant samples (%) × plant dry matter weight (g) × 1000/100
2. Fertilizer N uptake by rice plants = NdfF (%) × total N uptake by rice plants/100
3. Non-fertilizer N uptake = total N uptake − fertilizer N uptake.

At 20 days after sowing (DAS), the interaction effect of inoculation and N rate applied was not significant. However, inoculation individually increased plant dry weight significantly (Table 11.9). On the other hand, ^{15}N AE (%), NdfF (%) and fertilizer N uptake were decreased significantly (P<0.05) as a result of inoculation (Table 11.10). These were attributed to the increase in non-fertilizer N uptake from some source dependent on inoculation. The effect of N rate was significant on dry weight, total N content (%) and total N uptake (Table 11.9). Inoculation decreased

Table 11.10 Effects of inoculation of 1N bacteria on ^{15}N atom excess (AE), %N derived from fertilizer (NdfF), fertilizer N and non-fertilizer N uptakes of rice seedlings at 20 DAS

Inoculation	^{15}N atom excess (%)	NdfF (%)	Fertilizer N uptake (mg/pot)	Non-fertilizer N uptake (mg/pot)
Without	0.84 a	18.01 a	59.2 a	271.0 a
With	0.47 b	10.14 b	33.4 b	296.5 a

Adapted from Phan et al. (2008)
Values followed by different letters in a column are significantly (P<0.05) different by LSD

Table 11.11 Effects of inoculation of 1N bacteria and fertilizer N rates on plant dry weight, total N content and uptake of rice seedlings at 45 DAS

	Fertilizer N rate (kg/ha)			
Inoculation	0	20	40	Mean
Dry weight (g/pot)				
Without	19.24 b C	22.43 a B	24.71 aA	
With	21.79 a B	22.55 a B	24.55 aA	
Total N content (%)				
Without	1.51 aA	1.48 b A	1.51 b A	
With	1.44 b C	1.74 a B	1.95 aA	
Total N uptake (mg/pot)				
Without	290.1	331.3	371.8	331.1 b
With	314.2	392.3	479.2	395.2 a
Mean	302.2 C	361.8 B	425.5 A	

Adapted from Phan et al. (2008)
Interaction effect of inoculation and N rate was significant (P<0.05) on dry weight and total N content while it was not significant on total N uptake. Within a parameter, values followed different capital letters in a row or different small letters in a column are significantly (P<0.05) different by LSD

fertilizer N uptake significantly while the non-fertilizer N uptake increased from inoculation (Table 11.10) although the difference was not significant, but it contributed on the total N uptake which was not affected significantly due to inoculation (Table 11.9). The increase in non-fertilizer N uptake due to inoculation might be due to Biological Nitrogen Fixation (BNF). This can be estimated in future studies by using a non-fixing reference crop. These results indicated the benefit on growth of inoculating 1N bacteria, which significantly increased plant dry matter at 20 DAS.

At 45 DAS, the interaction effect of N rates and inoculation was significant (P<0.05) between plant dry weight and total N content (%) and between weight non-fertilizer N uptake (Tables 11.11 and 11.12). With no N applied inoculation increased dry matter yield significantly while at other two N rates the effect of inoculation was not significant. N fertilization increased dry matter yield significantly with increasing N rates without inoculation while the effect of N rate was significant only at N rates of 40 kg/ha with inoculation. Total N uptake increased significantly with inoculation and with N rates. Nitrogen fertilization increased ^{15}N AE (%), NdfF (%) and fertilizer N uptake significantly. Inoculation increased

Table 11.12 Effects of inoculation of 1N bacteria and fertilizer N rates on ^{15}N AE, NdfF (%), fertilizer N and non-fertilizer N uptakes of rice seedlings at 45 DAS

Inoculation	Fertilizer N rate (kg/ha)		Mean
	20	40	
^{15}N AE (%)			
Without	0.55	1.14	0.85 a
With	0.54	1.09	0.82 a
Mean	0.55 B	1.12 A	
NdfF (%)			
Without	11.86	24.51	18.19 a
With	11.69	23.53	17.61 a
Mean	11.78 B	24.02 A	
Fertilizer N uptake (mg/pot)			
Without	39.3	91.1	65.2 b
With	45.9	112.7	79.3 a
Mean	42.6 B	101.9 A	
Non-fertilizer N uptake (mg/pot)			
Without	292.0 b A	280.7 b A	
With	346.4 a B	366.4 aA	

Adapted from Phan et al. (2008)
Interaction effect of inoculation and N rate was significant only on no-fertilizer N uptake
Within a parameter, values followed different capital letters in a row or different small letters in a column are significantly ($P<0.05$) different by LSD

fertilizer N uptake significantly, while its effect was not significant on ^{15}N AE (%) and NdfF (%). This was a result of increased fertilizer N uptake in inoculated plants.

The interaction effect of N rate and inoculation was significant on non-fertilizer N uptake. Inoculation increased non-fertilizer N uptake significantly at both N rates while the N fertilizer effect was variable between inoculation treatments. The effect of N rate was not significant on non-fertilizer N uptake without inoculation, while N fertilization increased non-fertilizer N uptake significantly with inoculation. The possible reason might be the increase in root mass due to 1N inoculation which increased rice plants' capacity to absorb more soil N with increase in fertilizer N application rate. The increased root mass of rice seedlings were observed resulting from inoculation in a greenhouse experiment at University of Sydney (Kecskés et al. 2008b). Similar results were reported by Nguyen (2008) in these proceedings.

These results indicate the positive effects of 1N inoculation in increasing plant biomass, and N uptake from both fertilizer and non-fertilizer sources at 45 DAS. Although this experiment was not continued up to maturity stage, evidences from the other field experiments showed the beneficial effect of BioGro inoculation on grain and straw yields as well as N and P uptakes at maturity (Phan and Tran 2008). Increase in N uptake might be due to increase in fertilizer N uptake, BNF or increase in soil N uptake due to thicker and longer root system. The results of this experiment demonstrated the beneficial effect of 1N inoculation in increasing fertilizer N uptake by the rice plants. This study clearly demonstrates the significant effect on the growth of rice of the plant growth promoting (PGP) organism, the 1N strain of *Pseudomonas fluorescens*, isolated by Professor Nguyen Thanh Hien near Hanoi as a N_2-fixing organism (Nguyen 2008). The use of labelled urea has indicated that the bacterium allows the plant to access significant alternative sources of nitrogen to urea after 3 weeks, more than 40 mg per plant. Once a more extensive root system has developed aided by inoculation as discussed above, the plant's access to fertilizer nitrogen is also enhanced.

11.13 Field Evaluation of BioGro by Participating Rice Farmers

Field experiments were conducted on 20 rice cropping farms in the Mekong Delta, Vietnam with funding of World Bank's Development Marketplace project to evaluate the effectiveness of BioGro2 at farm level with farmers' participatory research. Ten farms were selected from each of two localities: Cai Lay District and Phung Hiep District. All farms had a history of high-yielding rice production for at least 20 years prior to these field experiments. In this area of the Mekong Delta, three crops of rice are regularly grown per year, including a dry season crop planted after the annual flood in September, an early wet season crop, and a late wet season crop. Field experiments were conducted in four successive seasons from summer 2009 to winter 2011, commencing with a late wet season crop prior to the flood. Details of the experimental procedure and findings are available in Rose et al. (2014).

The general experimental design consisted of paired plots at each of the farms. For each farm, a plot of 2000 m^2 was devoted to the trials conducted by farmers. The plot was equally split into two subplots, one for biofertilizer treatment and another one for farmer's normal practice as the control. The subplots were separated with small bunds to ensure isolation from each other and no mobility of water and applied fertilizers. One plot received conventional farmer fertilizer application and the other plot received a biofertilizer application at a rate of100 kg/ha combined with a reduction in chemical fertilizer rate. Fertilizer application rates in the control plots and the percent reduction in the biofertilizer plots differed at each farm depending on individual farmer practice.

The salient findings of these experiments (Rose et al. 2014) are as follows:

- The efficacy of a commercial biofertilizer is strongly dependent on seasonal and site-specific environmental conditions.
- Up to 45 % of the variation in the biofertilizer effect could be ascribed to differences in the timing and magnitude of chemical fertilizers applied simultaneously to the growing crop. Such variation can therefore be managed in order to minimize farmers' risk in adopting the technology.
- The biofertilizer BioGro2 could replace between 23 and 52 % of N fertilizer without loss of yield, but did not appear to be able to replace P or K fertilizer.

- A farmer participatory approach to the application of biofertilizers enabled rapid optimization under field conditions, which in turn increased farmer confidence and the reproducibility of agronomic benefits.

The information will accelerate the practical adoption of biofertilizers into cropping practices by addressing current knowledge gaps that exist between laboratory and field scales. The outcome will be a more sustainable rice production system through a reduced reliance on high inputs of chemical fertilizers.

11.14 Conclusions

The beneficial effect of BioGro in both Australia and Vietnam was observed in field conditions in several experiments conducted under AusAID and ACIAR projects. There are varietal and seasonal differences on the effectiveness of BioGro. Rice crops in Vietnam are grown with short season varieties, allowing up to three crops a year. By contrast, Australian rice crops are long season (Kecskés et al. 2008a, b), with only one crop a year, although the total annual yields in dry matter are similar to those from three crops in Vietnam. A ^{15}N tracer study under greenhouse controlled condition confirmed that the BioGro strain 1N (*Pseudomonas fluorescens*) bacteria is capable of increasing both fertilizer and non-fertilizer N uptake by the rice plant significantly. Apart from reducing the cost of inputs, this technology will also help in reducing environmental pollution, helping satisfy the 'three reductions' sought for inputs of seed, fertilizers and other chemicals (Tran 2008) and safening crop production.

There are still many issues regarding uptake of this beneficial technology by farmers despite its scientific basis being proven. Farmers' participatory research under the World Bank Development Marketplace project showed that application of biofertilizer BioGro is effective in reducing the use of chemical N fertilizer up to 52 % at farm level without decreasing rice yield. Reduced nitrogen inputs can be optimized for BioGro application (Marsh 2008). However, in Vietnam chemical fertilizers are subsidized by government authorities and farmers may be reluctant to take the risk of using biofertilizer products like BioGro when government extension services recommend chemicals. Widespread application of this biofertilizer technology will need financial incentives, perhaps from credits resulting from international policy on climate change.

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