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Effects of bacterial inoculant biofertilizers on growth, yield and nutrition of rice Australia

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ABSTRACT

Inoculant biofertilizer application increased fertilizer nitrogen (N) use efficiency in Vietnam in some previous field experiments. Similar results may be obtained in Australia. With this view in mind, a greenhouse experiment and two field experiments were conducted using a Vietnamese inoculant biofertilizer (BioGro) and several other plant growth promoting (PGP) bacteria. In the greenhouse trial, bacterial inoculations increased shoot and root weights of rice plants significantly. In the field experiments, particularly with *Rhizobium leguminosarum,* similar effects including significant differences in nitrogen uptake in vegetative matter were observed at the panicle initiation (PI) stage. However, these effects were not significant on grain yield at harvest and it is concluded that the much longer period of growth for Australian rice may allow compensation between treatments. Re-inoculation of plants at the PI stage, and lower application rates of N fertilizer in at least two splits are suggested for future field experiments.

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bacterial inoculant; biofertilizer; rice

Introduction

Rice yield is increasing globally due to increased population. While paddy (un-milled rice) yield (t ha^{-1}) in Australia was 6.00 in 1960, it increased to 10.00 in 2015 (Table 1). Consequentially, fertilizer consumption, especially nitrogen (N), phosphorus (P), and potassium (K) are generally increasing gradually over the years (Table 2). Application of nitrogen fertilizer 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 N is lost as a result of ammonia volatilization, denitrification, and leaching causing environmental pollution (Choudhury and Kennedy, 2005; Zhao et al., 2010; Hakeem et al., 2011; Mai et al., 2010). The recovery of fertilizer N in rice culture is very low, generally around 30–40%, in some cases even lower (Choudhury and Khanif, 2001, 2009; Wang et al., 2011). Inoculation with bacterial biofertilizers may reduce the application of fertilizer N by increasing N uptake by plants (Choudhury and Kennedy, 2004; Kennedy et al., 2004; Choudhury et al., 2014). Vigorous seedling growth is important for the successful establishment of rice and other crops. Both single and multistrain biofertilizers are used to inoculate rice seedlings. Previous investigations in several countries have shown that rice seedling growth was enhanced following inoculation with plant growth promoting micro-organisms, leading to increased grain and straw yields, and enhanced efficiency of fertilizer N use (Biswas et al., 2000a, 2000b; Mirza et al., 2000; Malik et al., 2002; Yanni and Dazzo, 2010; Jha et al., 2009).

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Table 1. Rice harvested area, paddy production, paddy yield and estimated amount of N removed per hectare in Australia over the years since 1960.

Year	Rice harvested area (000 ha)	Paddy production (000 t)	Paddy yield (t ha^{-1})	Estimated amount of N removed (kg ha^{-1})
1960	19	114	6.00	96.00
1970	38	299	7.87	125.92
1980	104	729	7.01	112.16
1990	89	787	8.84	141.44
2000	177	1643	9.28	148.48
2010	76	724	9.53	152.48
2015	30	300	10.00	160.00

The data on rice harvested area, paddy (un-milled rice) production and paddy yield have been collected from the USDA database available in the IRRI website (IRRI, 2016). The amount N removed (kg ha^{-1}) was estimated considering that the rice crop generally removes 16 kg N per ton of paddy production (De Datta, 1981).

Table 2. Consumption of N, P and K fertilizer in Australia over the years since 1961.

	Total fertilizer consumption (000 tons)		
Year	N	P_2O_5	K_2O
1961	35.0	582.5	54.0
1970	123.4	756.9	91.2
1980	248.0	853.0	128.0
1990	439.4	578.9	145.4
2000	951.0	1107.0	217.0
2010	982.0	816.8	168.1
2013	1314.8	815.8	215.0

Source: IFA (2016).

Field studies in Vietnam (Nguyen et al., 2002, 2003) showed that 'BioGro', containing three bacterial strains (1N, 3C, and 4P) isolated from Vietnamese rice cropping soils, increased rice yields significantly. The first strain, *Pseudomonas fluorescens* (1N) has the ability to reduce acetylene (C_2H_2) to ethylene (C_2H_4), indicating its potential for N_2 fixation. The second strain, *Citrobacter freundii* (3C) apparently produces extra-cellular compounds that inhibit the growth of some other rhizosphere organisms. The third strain, *Klebsiella pneumoniae* (4P) also a diazotroph, can solubilize precipitated calcium phosphate [$Ca_3(PO_4)_2$] in an agar medium. One greenhouse and two field experiments were conducted to evaluate the effects of bacterial inoculant biofertilizers on growth, yield, and nutrition of rice.

Materials and methods

Greenhouse experiment

Preparation of bacterial cultures

All bacterial strains were obtained from the SUNFix Centre for Nitrogen Fixation culture collection and grown for 24 hours at 25°C in 200 mL of modified nutrient broth (MNb). Whilst shaking at 1500 rpm, the number of colony forming units (CFU) was counted by plating a serial dilution from

Table 3. Treatments used in greenhouse trial, greenhouse experiment at the University of Sydney, October–November 2004.

Treatment	Strain	Ratio
T1 (Control)	No strain: sterile modified nutrient broth only	*
T2 (BioGro)	1N (*Pseudomonas fluorescens*), 3C (*Citrobacter freundii*) and 4P (*Klebsiella pneumoniae*)	10:1:10
T3 (modified BioGro)	1N and 4P	1:1
T4 (*Azospirillum*)	*Azospirillum lipoferum 596*	—

*Not applicable.

each individual culture broth. Finally, individual cultures were either mixed or applied separately as follows: T1, Control (sterile MNb); T2, 1N (*Pseudomonas fluorescens*) plus 3C (*Citrobacter freundii*) and 4P (*Klebsiella pneumoniae*), 10:1:10; T3, 1N:4P, 1:1; T4, *Azospirillum lipoferum 596*. The description of the treatments is presented in Table 3.

Growing rice seedlings

Rice seeds of variety Amaroo were soaked in water for 24 hours prior to being soaked for another hour in bacterial suspensions prepared as per treatment described above. Plastic pots filled with 850 g of sand were moistened with 100 mL of water allowing for six replicates of each treatment and control. The pots were completely randomized in the greenhouse where the temperature fluctuated between 20 to 30°C for the duration of the trial. Four rice seeds were transferred to each pot and allowed to germinate. Germination was completed in eight days. The seedlings were allowed to grow for a further 4 weeks in flooded conditions by adding 100 mL of potable Sydney water to each pot on alternate days and were harvested 31 days after germination. Root and shoot lengths were measured. Roots and shoots were separated and placed in paper bags and dried at 70°C for four days. Then, shoot and root weights were recorded.

Field experiment at Yanco Agricultural Research Institute

Soil analysis

The experiment was conducted at Yanco Agricultural Research Institute, New South Wales (NSW), Australia. The cropping pattern of the location is rice-fallow-rice with two years interval between two rice crops. The classification of the soil as per Commonwealth Scientific and Industrial Research Organization (CSIRO) is hypercalcic, subnatric, red sodosol (Isbell, 1996). Prior to commencing the experiment, soil samples were collected from the experimental plots at a depth of 0–15 cm. The samples were air dried, ground, and passed through 2 mm sieve. The processed soil was analyzed for pH, organic matter content, particle size, cation exchange capacity, total N, available P, exchangeable K, calcium (Ca), and magnesium (Mg). Soil pH (soil water ratio 1:5) was measured by a glass electrode (Peech, 1965). Organic matter was analyzed by the potassium dichromate and sulfuric acid (H_2SO_4) digestion method (Walkley and Black, 1934). Particle size was analyzed by the hydrometer method (Black, 1965). Cation exchange capacity, exchangeable K, Mg, and Ca were determined by ammonium acetate extraction (Schollenberger and Simon, 1945). Total N was determined by LECO (Leco Corp., St Joseph, MI, USA) combustion method (Sweeney and Rexroad, 1987) and available P by the ammonium fluoride (NH_4F)-hydrochloric acid (HCl) extraction method (Bray and Kurtz, 1945). The soil analytical results are presented in Table 4. As the soil was deficient in N, the application of fertilizer N was essential to meet the rice crop's demands.

Table 4. Properties of the initial soil, field experiment at Yanco, November 2004 to April 2005.

Soil property	Result
pH	6.35
Organic matter (%)	3.2
Particle size analysis	
% Sand	63.26
% Silt	7.35
% Clay	29.39
Textural class	Sandy clay loam
Cation exchange capacity (cmol kg^{-1})	12.64
Total N (%)	0.10
Available P (mg kg^{-1})	18.06
Exchangeable K (cmol kg^{-1})	0.62
Exchangeable Ca (cmol kg^{-1})	7.15
Exchangeable Mg (cmol kg^{-1})	4.01

Soil classification as per CSIRO: hypercalcic, subnatric, red sodosol (Isbell, 1996).

Table 5. Description of the treatments used in the trial, field experiment at Yanco, November 2004 to April 2005.

Treatment designation	Treatment	Description
T1	Control	Peat containing sterile MN broth + 50 kg N ha^{-1} as urea
T2	Recommended N rate	Peat without broth + 150 kg N ha^{-1} as urea
T3	BioGro	Vietnamese bacterial strains (consist of a 10:1:10 mixture of *Pseudomonas fluorescens*, *Citrobacter freundii* and *Klebsiella pneumoniae*) + 50 kg N ha^{-1} as urea
T4	*Azospirillum* & *Herbaspirillum*	Consist of 1:1:1 mixture *Azospirillum brasilense* Sp7-S, *A. lipoferum* 687 and *Herbaspirillum seropedicae*, + 50 kg N ha^{-1} as urea
T5	*Rhizobium*	*Rhizobium leguminosarum* bv. *trifolii* + 50 kg N ha^{-1} as urea

Experimental design, basal fertilizers and inoculation of rice seeds

The experiment was laid out in a randomized complete block (RCB) design with four replications. Unit plot size was 3 m × 10 m. Description of the treatments is presented in Table 5. The recommended rate of P fertilizer for the area [20 kg P ha^{-1} from triple superphosphate (TSP)] was applied as blanket to all the treatments. Nitrogen fertilizer was applied as urea as described in the treatments (Table 5). Both N and P were applied at final land preparation. Pre-inoculated peat was applied to the experimental plots according to the treatments presented in Table 5 one day before sowing the rice seeds. A short duration rice variety, Jarrah, was used as the test crop. Pre-soaked seeds of Jarrah were sown by broadcasting at a rate of 140 kg ha^{-1}.

Sampling and plant analyses

Plants were sampled at panicle initiation (PI) stage (59 days after seed sowing) and at maturity (130 days after seed sowing). At PI stage, rice plant biomass, tiller number and nutrient [N, P, K, sulfur (S) and Mg] uptakes were measured. At maturity, grain and straw yields, plant height, yield components (panicle number, number of filled grain per panicle, and 1000 grain weight) and N uptake by rice plants were measured. At PI stage, one square meter was sampled from each plot. At maturity, the sampling area for grain yield varied among the plots. However, the minimum area of sampling was 21.2 m^2 $plot^{-1}$. Chemical analyses of plant tissue for nutrient (N, P, K, S, and Mg) contents were done using near infrared reflectance (NIR) spectroscopy (Batten, 1998; Batten et al., 1991).

Field experiment at Jerilderie Rice Research Institute

Soil analysis and experimental design

The experiment was conducted at Jerilderie Rice Research Institute, NSW, Australia. The cropping pattern of the location is rice-fallow-rice with two years interval between two rice crops. The classification of the soil as per CSIRO is gypsic, epipedal, grey vertosol (Isbell, 1996). The soil properties of the experimental plots were measured and determined as described above for the trial at Yanco Agricultural Institute, and the results are presented in Table 6. The experiment was conducted in a RCB design using six replications with a 2.5 m × 15 m unit plot size. A description of the treatments is presented in Table 7.

Inoculation of rice and fertilization

The experimental plots were treated with pre-inoculated peat one day prior to sowing rice seeds. A long duration rice variety, Amaroo, was used for this trial. Seeds were pre-soaked and sown at a rate of 140 kg ha^{-1}. Phosphorus (20 kg P ha^{-1} from mono-ammonium phosphate) was applied to all plots and fertilizer N as urea was applied as per treatment (Table 7). Both N and P were applied all at final land preparation.

Table 6. Properties of the initial soil, field experiment 2006–2006, Jerilderie.

Soil properties	Result
Texture	Clay
pH (water)	5.5
Organic matter (%)	3.1
CEC (meq 100 g^{-1})	16.7
EC (dS m^{-1})	0.09
NO_3-N (ppm)	7.1
NH4-N (ppm)	<1.0
Available P (ppm)	5
Exchangeable K (me 100 g^{-1})	1.12
Exchangeable Ca (me 100 g^{-1})	6.37
Exchangeable Mg (me 100 g^{-1})	7.59
Available S (ppm)	12
Available B (ppm)	0.7
Available Cu (ppm)	1.7
Available Fe (ppm)	133
Available Mn (ppm)	31.8
Available Zn (ppm)	0.5

Soil classification as per CSIRO: gypsic, epipedal, grey vertosol (Isbell, 1996).

Sampling times

Samplings were conducted at PI stage (91 days after sowing) and at maturity (154 days after sowing). At the PI stage, rice plant biomass and N uptake were measured. Chemical analysis of plant tissue for N content was performed using NIR spectroscopy (Batten, 1998; Batten et al., 1991). At maturity, grain and straw yields were recorded.

Statistical analyses

All the data were analyzed by two-way analysis of variance (ANOVA) using the GenStat program version seven (VSN International, Oxford, UK) (Payne et al., 2003). The means were compared using the least significant difference (LSD) test.

Results

Greenhouse experiment

Bacterial counts

The number of colony forming units (CFU) per mL of culture broth for starter inoculant was determined using dilution plating method. The average starter cultures were 4.8×10^8, 1.2×10^9, 1.2×10^9

Table 7. Description of the treatments used in the trial, field experiment at Jerilderie, October 2005 to April 2006.

Treatment designation	Treatment	Description
T1	N0	N control
T2	N50	50 kg N ha^{-1} : 9.1 kg N ha^{-1} as monoammonium phosphate + 40.9 kg N ha^{-1} as urea
T3	N100	100 kg N ha^{-1} : 9.1 kg N ha^{-1} as monoammonium phosphate + 90.9 kg N ha^{-1} as urea
T4	N50 + BioGro*	T2 + 200 kg ha^{-1} of BioGro* (1N:3C:4P = 10:1:10) inoculum in peat
T5	N50 + *Citrobacter freundii*	T2 + 200 kg ha^{-1} of *Citrobacter freundii* inoculum in peat
T6	N50 + *Rhizobium* low rate	T2 + 47.5 kg ha^{-1} of *Rhizobium leguminosarum* bv. *trifolii* inoculum in peat
T7	N50 + *Rhizobium* high rate	T2 + 237.3 kg ha^{-1} of *Rhizobium leguminosarum* bv. *trifolii* inoculum in peat

*1N, 3C and 4P stands for *Pseudomonas fluorescens, Citrobacter freundii* and Klebsiella pneumoniae, respectively.

Table 8. Effects of bacterial inoculant biofertilizers on shoot and root growth of rice seedlings, greenhouse experiment at the University of Sydney, October–November 2004.

Treatment	Shoot weight seedling^{-1} (mg)	Root weight seedling^{-1} (mg)	Shoot length (cm)	Root length (cm)
Control	12.5 b	19.6 c	12.5	20.1
BioGro	19.6 a	23.9 bc	12.6	19.9
Modified BioGro	22.9 a	35.8 a	12.1	19.3
Azospirillum	19.3 a	32.1 ab	12.6	20.7
F value	0.027	0.029	0.670	0.461
LSD (0.05)	6.64	11.41	—	—
CV (%)	29.7	34.0	6.6	7.0

Values followed by a common letter in a column are not significantly different by LSD (least significant difference) at 5% probability level.

and 3.3×10^7 for 1N, 3C, 4P, and SCO4, respectively. The CFU per treatment per pot ranged from 6.6×10^7 (SCO4, T4) to 1.2×10^9 (4P, T2, and T3).

Shoot and root growth

While shoot and root lengths were not affected by the inoculations, shoot weight increased significantly (Table 8). There was no significant difference between the bacterial treatments. Shoot weight seedling^{-1} ranged from 12.5 to 22.9 mg. All the bacterial treatments, except one Vietnamese multistrain (1N, 3C, and 4P), increased root weight significantly compared to the control. Root weight per seedling ranged from 19.6 to 35.8 mg.

Field experiment at Yanco Agricultural Research Institute

Plant biomass, tiller and height

Treatment effects were not significant on plant dry biomass (t ha^{-1}) at the PI stage (Table 9). However, inoculation with *Rhizobium* and BioGro out-yielded the control by 0.61 and 0.48 t ha^{-1}, respectively. Tillering of the rice plants was not significantly affected by the treatments, although there were increases in tiller number per square meter due to inoculation with *Rhizobium* and BioGro (Table 9). Correlation between tiller number and plant biomass at PI stage was significant (Figure 1). Recommended fertilizer N rate increased plant height at maturity significantly compared with the bacterial treatments (Table 9).

Yield components, grain and straw yields

Treatments did not affect any of the yield components (panicle number, number of filled grains, and 1000 grain weight); however, estimated grain yield was increased notably ($P < 0.1$) by the recommended N rate over all other treatments except the *Rhizobium* one (Table 9). This significant increase

Table 9. Effects of chemical and biofertilizers on some agronomic parameters and estimated grain yield of Jarrah rice, field experiment at Yanco, November 2004 to April 2005.

Treatment	Plant biomass (t ha^{-1}) at panicle initiation stage	Tiller no. m^{-2} at panicle initiation stage	Plant height (cm) at maturity	Panicle no. m^{-2} at maturity	Number of filled grains panicle^{-1} at maturity	1000 grain weight (g)	Estimated grain yield (t ha^{-1})
Control	2.38	613	73.46	580.0	52.4	22.81	6.38
Recommended N rate	2.28	609	76.26	536.5	69.3	22.65	9.28
BioGro	2.86	642	68.74	546.5	53.3	23.40	6.57
Azospirillum and *Herbaspirillum*	1.78	504	68.94	521.0	46.2	22.98	5.58
Rhizobium	2.99	737	70.20	490.0	60.5	23.42	8.20
F probability	0.103	0.273	0.041	0.373	0.14	0.67	0.083
LSD (0.05)	—	—	5.23	—	—	—	—
LSD (0.10)							2.21
CV (%)	22.75	18.68	4.63	9.50	20.90	3.52	24.27

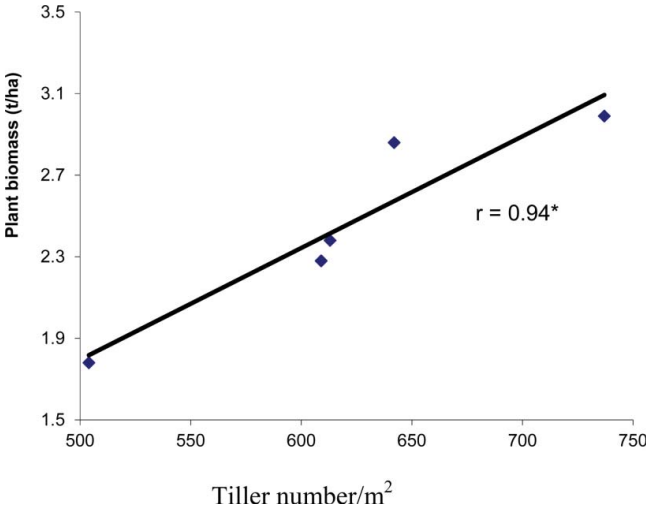

Figure 1. Correlation between tiller number and plant biomass at panicle initiation stage of Jarrah rice in the field experiment conducted at Yanco during 2004–2005. *Significant at $P < 0.05$.

was attributed to higher number of filled grains per panicle in this treatment. There was no significant treatment effect on grain and straw yields, total biomass and harvest index (Table 10). Grain yield increased by 1.07, 0.36, and 0.32 t ha^{-1} over the control in the recommended N rate, *Rhizobium*, and BioGro-treated plots, respectively, but these differences were not statistically significant. Correlation between straw and grain yields was significant (Figure 2).

Nitrogen content and uptake

The N contents as a percentage of the whole plant (mixture of grain and straw) at harvest were significantly lower in all the treatments compared with the fully fertilized one (Table 11). Total N uptake (kg ha^{-1}) was significantly higher in the fully fertilized treatment compared to all other treatments. This was attributed to significantly higher N content in plant tissue in this treatment. Total N uptake for the production of one ton of rough rice ranged from 12.05 to 18.72.

Nitrogen content (%) in plant tissue at PI stage was significantly higher in the fully fertilized treatment compared to other treatments ($P < 0.05$, Table 12), but other treatments showed similar values. The N uptake (kg ha^{-1}) at PI stage was similar in the fully fertilized and *Rhizobium* inoculation treatments (Table 12). In the control as well as in the *Azospirillum* + *Herbaspirillum*-treated plants, the N uptake values were significantly lower compared to fully fertilized and *Rhizobium* inoculation treatments. Although the N content in plant tissues was significantly lower in the *Rhizobium*-treated plants compared to fully fertilized ones, higher plant biomass due to *Rhizobium* inoculation contributed to similar amounts of N uptake as in fully fertilized plants.

Table 10. Effects of chemical and biofertilizers on grain and straw yields, total biomass and harvest index of Jarrah rice, field experiment at Yanco, November 2004 to April 2005.

Treatment	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	Total biomass (t ha^{-1})	Harvest index (%)
Control	6.10	5.69	11.79	51.73
Recommended N rate	7.17	6.50	13.67	52.45
BioGro	6.42	5.83	12.25	52.41
Azospirillum and *Herbaspirillum*	5.57	5.48	11.05	50.41
Rhizobium	6.46	5.71	12.17	53.08
F probability	0.185	0.576	0.310	0.699
CV (%)	13.28	15.06	13.36	4.05

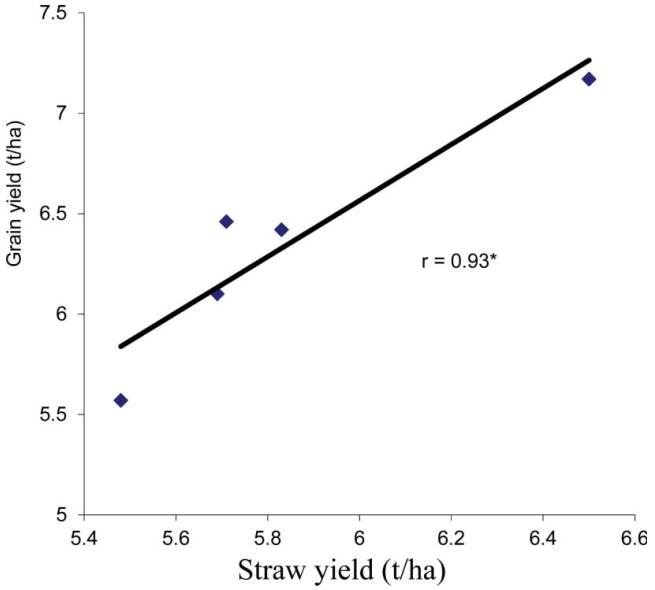

FIgure 2. Correlation between straw and grain yields of Jarrah rice in the field experiment at Yanco during 2004–2005. *Significant at $P < 0.05$.

Other nutrient contents and uptake at PI stage

None of the treatments significantly affected the P, K, and Mg contents (%) in plant tissue at PI stage, whereas the recommended N rate increased S content (%) compared with BioGro-treated plants, but this difference did not reach great significance ($P < 0.1$, Table 12). There was an indication that *Rhizobium* inoculation increased S and Mg uptakes significantly over the control and *Azospirillum* + *Herbaspirillum*-treated plants. BioGro and fully fertilized treatments increased S and Mg uptakes compared to *Azospirillum* + *Herbaspirillum*-treated, but again this difference was not very significant ($P < 0.1$).

Field experiment at Jerilderie Rice Research Institute

Plant biomass and N uptake at PI stage

Plant dry biomass increased significantly ($p < 0.05$) over the control (N0) with all the treatments except T5 (N50 + *Citrobacter freundii*) (Table 13). Addition of a higher N rate (100 kg N ha^{-1}) also increased plant biomass over N50 ($P < 0.1$). Bacterial inoculants did not increase plant biomass significantly over N50 although there was an increase of 0.49 t ha^{-1} with the high rate for *Rhizobium*. N

Table 11. Effects of chemical and biofertilizers on total N content of and uptake by Jarrah rice, and internal N efficiency, field experiment at Yanco, November 2004 to April 2005.

Treatment	N content (%) in whole plant at maturity	N uptake (kg ha^{-1}) by whole plant at maturity	Total N uptake (kg) t^{-1} of rough rice production	Internal N efficiency (kg grain kg^{-1} absorbed N)
Control	0.62	75.3	12.05	83.5
Recommended N rate	0.97	131.6	18.72	54.2
BioGro	0.64	80.0	12.27	83.1
Azospirillum and *Herbaspirillum*	0.62	69.6	12.35	88.2
Rhizobium	0.65	81.0	12.23	84.0
F probability	0.009	<0.001	0.020	0.087
LSD (0.05)	0.19	20.99	4.54	–
LSD (0.10)	—	—	—	21.03
CV (%)	17.17	15.10	19.00	21.03

Table 12. Effects of chemical and biofertilizers on N, P, K, S and Mg contents of and uptake by Jarrah rice at panicle initiation stage, field experiment at Yanco, November 2004 to April 2005.

	Nutrient content (%)					Nutrient uptake (kg ha^{-1})				
Treatment	N	P	K	S	Mg	N	P	K	S	Mg
Control	2.29	0.29	1.71	0.20	0.21	50.29	7.16	41.56	4.50	4.77
Recommended N rate	2.95	0.29	1.55	0.23	0.24	67.33	6.91	35.37	5.27	5.37
BioGro	2.01	0.29	1.83	0.19	0.21	56.19	8.30	51.95	5.30	5.90
Azospirillum and *Herbaspirillum*	2.33	0.28	1.72	0.20	0.21	41.21	5.03	30.27	3.58	3.85
Rhizobium	2.27	0.26	1.51	0.20	0.22	67.92	8.13	46.28	5.92	6.59
F probability	0.03	0.84	0.32	0.09	0.50	0.09	0.103	0.078	0.09	0.08
LSD (0.05)	0.53	—	—	—	—	—	—	—	—	—
LSD (0.10)				0.03		16.19		12.54	1.25	1.37
CV (%)	14.22	11.77	12.15	10.07	11.09	22.83	22.16	24.16	20.32	20.76

content (%) in plant tissue increased significantly ($P < 0.05$) over control (N0) by addition of 100 kg N ha^{-1} alone. All the treatments except N50 increased N uptake (kg ha^{-1}) significantly over control ($P < 0.05$). *Rhizobium* inoculant applied at 237 kg ha^{-1} with 50 kg N increased N uptake (kg ha^{-1}) significantly over N50. *Rhizobium* low rate + N50 increased N uptake over N_{50} alone but the difference failed to be really significant ($P < 0.05$).

Grain and straw yields

Grain yield increased significantly over control (N0) due to application of 50 kg N ha^{-1} (Table 14). Addition of higher N rate or inoculation with bacteria did not increase grain yield over N50 at all. Rather, bacterial inoculation treatments decreased grain yield from N50, although such differences were not statistically significant. Straw yield increased significantly over control due to application of 50 kg N ha^{-1}. Addition of higher N rate or inoculation with bacteria did not increase or decrease straw yield significantly over N50 although there was an increase of 0.21 t ha^{-1} with N100.

Discussion

In the field experiment at Yanco, a high rate of N (150 kg ha^{-1}) was proposed for the long duration (160 days growth) variety Amaroo in two splits, but later the short duration (140 days growth) variety Jarrah was used, due to a late planting schedule. However, N fertilizer rates were not reduced. No N control (without N) treatment was included, so it was not possible to calculate agronomic efficiency of N (kg grain per kg added N), and apparent recovery (%) of added N. Actual fertilizer N recovery could

Table 13. Effects N fertilizer and bacterial inoculant biofertilizer treatments on biomass and N uptake of Amaroo rice at panicle initiation (PI) stage, field experiment at Jerilderie, 2005–2006.

Treatment	Dry plant biomass (t ha^{-1})	N content (%)	N uptake (kg ha^{-1})
N0	1.83	1.51	25.3
N50	2.66	1.61	37.3
N100	3.37	2.14	65.9
N50 + BioGro	2.77	1.78	44.8
N50 + *Citrobacter freundii*	2.56	1.74	41.1
N50 + *Rhizobium* low rate	2.99	1.84	52.1
N50 + *Rhizobium* high rate	3.15	1.81	55.8
F value	0.019	0.028	<0.001
LSD (0.05)	0.82	0.34	14.85
LSD (0.10)	0.69	0.28	12.34
CV (%)	25.3	16.3	27.4

Table 14. Effects N fertilizer and bacterial inoculant biofertilizer treatments on grain and straw yields, and total biomass of Amaroo rice, field experiment at Jerilderie, 2005–2006.

Treatment	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	Total biomass (t ha^{-1})
N0	4.20	4.00	8.20
N50	7.28	6.33	13.61
N100	7.08	6.54	13.62
N50 + BioGro	6.27	6.11	12.38
N50 + *Citrobacter freundii*	6.56	5.68	12.24
N50 + *Rhizobium* low rate	6.57	6.11	12.68
N50 + *Rhizobium* high rate	6.78	5.95	12.73
F value	0.001	0.002	<0.001
LSD (0.05)	1.30	1.09	2.26
LSD (0.10)	1.08	0.90	1.87
CV (%)	15.6	14.3	14.2

be calculated if ^{15}N labelled N fertilizer was applied. The ^{15}N tracer technique is widely used as the precise method to quantify fertilizer N actual recovery (Chen et al., 2010; Wang et al., 2008).

Rhizobium inoculation clearly increased N uptake (kg ha^{-1}) at PI stage over control (visibly to the human eye as extra greenness) (Table 12). This significant effect of *Rhizobium* on N uptake had disappeared at maturity stage as grain yield (Table 10). Nevertheless, *Rhizobium* inoculation increased plant biomass by 0.61 t ha^{-1} over control at PI stage (Table 9), while it increased grain yield by 0.36 t ha^{-1} (Table 10). These results indicate that the positive effects of *Rhizobium* inoculation decreased at the later growth stages of the rice plants. This might be a result of the longer growth durations of the rice plants in Australia compared to that of Vietnam. Re-inoculation 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. The use of ^{15}N labeled N fertilizer is also recommended to quantify the effects of bacterial inoculants on fertilizer N actual recovery.

In all the treatments except the fully fertilized one, the estimated amount of N removed per ton of rough rice production was low (Table 11). Generally, the estimated N removal for the production of one ton of rough rice is 16 to 17 kg (Sahrawat, 2000; Choudhury and Kennedy, 2005). The lower amount of N removal in this study indicates that the N estimation by NIR was not accurate. In the original program, it was proposed to analyze grain and straw separately for total N, but analysis of the whole plant was finally done without separating grain and straw. This could not give the total N uptake accurately. Moreover, it was not possible to calculate the N harvest index, i.e. percentage of N uptake by grain compared to total N uptake by the whole plant. Separate analyses of grain and straw for total N content is recommended for future studies.

In the field experiment at Jerilderie, the grain yield response due to added N was significant at 50 kg N ha^{-1}. Addition of higher N rate did not increase grain yield over N50. Lower N rates (25 and 50 kg N ha^{-1}) are proposed for the future experiments in this site. Bacterial inoculants can be used with 25 kg N ha^{-1}.

Conclusions

The experiments showed that bacterial inoculations increased shoot and root weights significantly in the greenhouse. Similar effects were noticed in the field experiments at panicle initiation stage although the effects disappeared at the harvesting stage. Re-inoculation 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. The use of ^{15}N labelled N fertilizer is also recommended to quantify the effects of bacterial inoculants on fertilizer N actual recovery.

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References

Batten, G. D. 1998. Plant analysis using near infrared reflectance spectroscopy: The potential and the limitations. *Australian Journal of Experimental Agriculture* 38: 697–706.

Batten, G. D., A. B. Blakeney, M. G. Glennie-Holmes, R. J. Henry, A. C. McCaffery, P. E. Bacon, and D. P. Heenan. 1991. Rapid determination of shoot nitrogen status in rice using infrared reflectance spectroscopy. *Journal of the Science of Food and Agriculture* 54: 191–197.

Biswas, J. C., J. K. Ladha, and F. B. Dazzo. 2000a. Rhizobia inoculation improves nutrient uptake and growth of lowland rice. *Soil Science Society of America Journal* 64: 1644–1650.

Biswas, J. C., J. K. Ladha, F. B. Dazzo, Y. G. Yanni, and B. G. Rolfe. 2000b. Rhizobial inoculation influences seedling vigour and yield of rice. *Agronomy Journal* 92: 880–886.

Black, C. A.. 1965. *Methods of Soil Analysis, Parts I and II*. Madison, WI: American Society of Agronomy.

Bray, R. H., and L. T. Kurtz. 1945. Determination of total, organic and available forms of phosphorus in soils. *Soil Science* 59: 39–45.

Chen, Y., C. Wu, X. Tang, S. Yang, and J. Wang. 2010. Fate of nitrogen from organic and inorganic sources in rice-wheat rotation cropping system. *Agricultural Sciences in China* 9: 1017–1025.

Choudhury, A. T. M. A., M. L. Kecskés, and I. R. Kennedy. 2014. Utilization of BNF technology supplementing urea N for sustainable rice production. *Journal of Plant Nutrition* 37:1627–1647.

Choudhury, A. T. M. A., and I. R. Kennedy. 2004. Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production. *Biology and Fertility of Soils* 39: 219–227.

Choudhury, A. T. M. A., and I. R. Kennedy. 2005. Nitrogen fertilizer losses from rice soils and control of environmental pollution problems. *Communications in Soil Science and Plant Analysis* 36: 1625–1639.

Choudhury, A. T. M. A., and Y. M. Khanif. 2001. Evaluation of effects of nitrogen and magnesium fertilization on rice yield and fertilizer nitrogen efficiency using ^{15}N tracer technique. *Journal of Plant Nutrition* 24: 855–871.

Choudhury, A. T. M. A., and Y. M. Khanif. 2009. A ^{15}N tracer study to evaluate the effects of nitrogen and copper fertilization on fertilizer nitrogen efficiency in rice production. *Pakistan Journal Scientific and Industrial Research* 52: 53–58.

De Datta, S. K. 1981. *Principles and Practices of Rice Production*. New York: John Wiley and Sons Inc.

Hakeem, K. R., A. Ahmad, M. Iqbal, S. Gucel, and M. Ozturk. 2011. Nitrogen-efficient rice cultivars can reduce nitrate pollution. *Environmental Science and Pollution Research* 18: 1184–1193.

IFA (International Fertiliser Industry Association). 2016. IFA Data. Available at: http://ifadata.fertilizer.org/ucSearch.aspx (accessed 9 February 2016).

IRRI (International Rice Research Institute). 2016. World Rice Statistics. Available at: http://ricestat.irri.org:8080/wrs2/entrypoint.htm (accessed 9 February 2016).

Isbell, R. F. 1996. *The Australian Soil Classification*. Collingwood, Australia: Commonwealth Scientific and Industrial Research Organization (CSIRO) Publishing.

Jha, B., M. C. Thakur, I. Gontia, V. Albrecht, M. Stoffels, M. Schmid, and A. Hartmann. 2009. Isolation, partial identification and application of diazotrophic rhizobacteria from traditional Indian rice cultivars. *European Journal of Soil Biology* 45: 62–72.

Kennedy, I. R., A. T. M. A. Choudhury, and M. L. Kecskés. 2004. Non-symbiotic bacterial diazotrophs in crop-farming systems: Can their potential for plant growth promotion be better exploited?. *Soil Biology and Biochemistry* 36: 1229–1244.

Mai, V. T., H. V. Keulen, and R. Roetter. 2010. Nitrogen leaching in intensive cropping systems in Tam Duong District, Red River Delta of Vietnam. *Water, Air and Soil Pollution* 210: 15–31.

Malik, K. A., M. S. Mirza, U. Hassan, S. Mehnaz, G. Rasul, J. Haurat, R. Bally, and P. Normand. 2002. The role of plant-associated beneficial bacteria in rice-wheat cropping system. In: *Biofertilisers in Action*, eds. I. R. Kennedy and A. T. M. A. Choudhury, pp. 73–83. Canberra, Australia: Rural Industries Research and Development Corporation.

Mirza, M. S., G. Rasul, S. Mehnaz, J. K. Ladha, R. B. So, S. Ali, and K. A. Malik. 2000. Beneficial effects of inoculated nitrogen-fixing bacteria on rice. In: *The Quest for Nitrogen Fixation in Rice*, eds. J. K. Ladha and P. M. Reddy, pp. 191–204. Los Baños, the Philippines: International Rice Research Institute.

Nguyen, T. H., R. Deaker, I. R. Kennedy, and R. J. Roughley. 2003. The positive yield response of field-grown rice to inoculation with a multi-strain biofertiliser in the Hanoi area, Vietnam. *Symbiosis* 35: 231–245.

Nguyen, T. H., I. R. Kennedy, and R. J. Roughley. 2002. The response of field-grown rice to inoculation with a multi-strain biofertiliser in the Hanoi district, Vietnam. In: *Biofertilisers in Action*, eds. I. R. Kennedy and A. T. M. A. Choudhury, pp. 37–44. Canberra, Australia: Rural Industries Research and Development Corporation.

Payne, R., D. Murray, S. Harding, D. Baird, D. Soutar, and P. Lane. 2003. *GenStat for Windows*, 7th ed. Oxford: VSN International.

Peech, M. 1965. Hydrogen ion activity. In: *Methods of Soil Analysis, Part 2*, ed. C. A. Black, pp. 914–926. Madison, WI: American Society of Agronomy Inc.

Sahrawat, K. L. 2000. Macro- and micronutrients removed by upland and lowland rice cultivars in West Africa. *Communications in Soil science and Plant Analysis* 31: 717–723.

Schollenberger, C. J., and R. H. Simon. 1945. Determination of exchange capacity and exchangeable bases in soil-ammonium acetate method. *Soil Science* 59: 13–23.

Sweeney, R. A., and P. R. Rexroad. 1987. Comparison of LECO FP-228 "Nitrogen Determinator" with AOAC copper catalyst Kjeldahl method for crude protein. *Journal of Association of Official Analytical Chemists* 70: 1028–1030.

Walkley, A., and I. A. Black. 1934. An examination of Degtjaref method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science* 37: 29–38.

Wang, X., Y. Suo, Y. Feng, M. J. I. Shohag, J. Gao, Q. Zhang, S. Xie, and X. Lin. 2011. Recovery of ^{15}N-labeled urea and soil nitrogen dynamics as affected by irrigation management and nitrogen application rate in a double rice cropping system. *Plant and Soil* 343: 195–2008.

Wang, Y., B. Zhu, Y. Shi, and C. Hu. 2008. Effects of nitrogen fertilization on upland rice based on pot experiments. *Communications in Soil Science and Plant Analysis* 39: 1733–1749.

Yanni, Y. G., and F. B. Dazzo. 2010. Enhancement of rice production using endophytic strains of *Rhizobium leguminosarum* bv. *trifolii* in extensive field inoculation trials within the Egypt Nile delta. *Plant and Soil* 336: 129–142.

Zhao, L., L. Wu, C. Dong, and Y. Li. 2010. Rice yield, nitrogen utilization and ammonia volatilization as influenced by modified rice cultivation at varying nitrogen rates. *Agricultural Sciences* 1: 10–16.