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UTILIZATION OF BNF TECHNOLOGY SUPPLEMENTING UREA N FOR SUSTAINABLE RICE PRODUCTION

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□ *The critical nitrogen (N) requirement of rice crops for maximum yields is well known. To overcome acute N deficiency in rice soils as a result of an increasing yield per unit area in the past 50 years of almost three-fold, this element has been usually supplied to the rice crop as the chemical fertilizer 'urea'. Unfortunately a substantial amount of the urea-N is lost through different mechanisms, causing environmental pollution problems. In principle, utilization of biological nitrogen fixation technology can supplement the use of urea-N, reducing the environmental problems to a considerable extent by improving nitrogen use efficiency. Different biological nitrogen fixation (BNF) systems have different potentials to provide an N supplement. It is necessary to design appropriate strategies to obtain more sustainable N supply to the rice crop. This paper reviews research to evaluate the potential of different BNF systems to supply N for the rice crop, assessing the current information.*

Keywords: biological nitrogen fixation, sustainable rice production

INTRODUCTION

Rice (*Oriza sativa*) is the major food crop of nearly half of the world's population. The production of rice per unit area is increasing over the years due to increasing demands as population is increasing. In 1960, the global average rough rice yield per hectare was only 1.84 tons while it increased to 4.27 tons in 2010 (Table 1). The data presented in Table 2 indicates that the rough rice yield varies among the countries; however, the increasing trend in per hectare yield over the years is noticed in all the eight countries. Rice plants require large amounts of mineral nutrients including nitrogen (N) for its growth, development, and grain production. Rice crops remove

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TABLE 1 World rice cropped area, rough rice production and estimated amounts of N removed per hectare over the years since 1960

Year	Rice cropped area (000 ha)	Rough rice production (000 t)	Yield (t ha^{-1})	Estimated amount N removed (kg ha^{-1})
1960	120138	220612	1.84	29.44
1970	132655	312511	2.36	37.76
1980	144412	396972	2.75	44.00
1990	146974	519403	3.53	56.48
2000	151898	594366	3.91	62.56
2001	150897	595453	3.95	63.20
2002	146367	564221	3.85	61.60
2003	148914	585975	3.93	62.88
2004	151338	597495	3.95	63.20
2005	153395	623831	4.07	65.12
2006	154193	627004	4.07	65.12
2007	155065	647195	4.17	66.72
2008	157835	670525	4.25	68.00
2009	156152	660613	4.23	67.68
2010	158401	676502	4.27	68.32

The data on rice cropped area and rough rice production were obtained from the USDA database available in the IRRI website (IRRI, 2011). The amount of N removed (kg ha^{-1}) was estimated from the observation that rice crops remove 16 kg N per ton of rough rice production (De Datta, 1981).

around 16 kg N for the production of each ton of rough rice including straw (De Datta, 1981; Ponnamperuma and Deturck, 1993; Sahrawat, 2000). However, most of the rice soils of the world are deficient in N, so the fertilizer N applications are required to meet the crop demand. With increasing yield the consumptions of fertilizer N, phosphorus (P) and potassium (K) are generally increasing over the years (Tables 3–5). Generally, urea is applied as the N source for rice production. But the efficiency of added urea-N is very low, often only 30-40%, in some cases even lower (De Datta, 1978; Choudhury and Khanif, 2001, 2009; Choudhury et al., 2002a). This low N-use efficiency is mainly due to denitrification, ammonia volatilization, and leaching losses (Ponnamperuma, 1972; De Datta and Buresh, 1989; Choudhury and Kennedy, 2005). Ammonia volatilization and denitrification cause atmospheric pollution through the production of greenhouse gases like nitrous oxide and ammonia (Reeves et al., 2002). Nitrate leaching causes groundwater toxicity (Shrestha and Ladha, 1998). In addition to these environmental problems, the long-term use of urea depletes soil organic matter content. These problems are of great concern to the soil and environmental scientists around the world. Alternate sources of N should be applied to minimize these problems if possible.

Biological nitrogen fixation (BNF) technology can play an important role to supplement chemical N fertilizer use in rice culture, reducing these environmental problems to some extent. Use of bio-fertilizers can prevent the depletion of soil organic matter content (Jeyabal and Kuppuswamy, 2001). Rice crops are grown in both wetland and upland cultures. However

TABLE 2 Rice cropped area, rough rice production and estimated amount of N removed per hectare in eight rice growing countries over the years since 1960

Country	Year	Rice cropped area (000 ha)	Rough rice production (000 t)	Yield (t ha^{-1})	Estimated amount N removed (kg ha^{-1})
Australia	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	95	850	8.95	143.20
Bangladesh	1960	8857	14522	1.64	26.24
	1970	9912	16731	1.69	27.04
	1980	10309	20844	2.02	32.32
	1990	10435	26781	2.57	41.12
	2000	10887	37633	3.46	55.36
	2010	11800	48455	4.11	65.76
China	1960	31500	59730	1.90	30.40
	1970	32358	109990	3.40	54.40
	1980	33878	139906	4.13	66.08
	1990	33064	189331	5.73	91.68
	2000	29962	187909	6.27	100.32
	2010	29820	199000	6.67	106.72
India	1960	34128	52011	1.52	24.32
	1970	37592	63401	1.69	27.04
	1980	40152	80527	2.01	32.16
	1990	42687	111448	2.61	41.76
	2000	44361	127483	2.87	45.92
	2010	44000	142514	3.24	51.84
Malaysia	1960	528	1152	2.18	34.88
	1970	697	1678	2.41	38.56
	1980	696	2059	2.96	47.36
	1990	662	2019	3.05	48.80
	2000	665	2169	3.26	52.16
	2010	665	2462	3.70	59.20
Pakistan	1960	1181	1547	1.31	20.96
	1970	1503	3303	2.20	35.20
	1980	1933	4689	2.43	38.88
	1990	2114	4898	2.32	37.12
	2000	2376	7204	3.03	48.48
	2010	2400	7501	3.13	50.08
Philippines	1960	3198	3705	1.16	18.56
	1970	3195	5235	1.64	26.24
	1980	3459	7723	2.23	35.68
	1990	3433	9885	2.88	46.08
	2000	4030	12515	3.11	49.76
	2010	4450	16429	3.69	59.04
Vietnam	1960	4602	9168	1.99	31.84
	1970	4631	9915	2.14	34.24
	1980	5468	11842	2.17	34.72
	1990	6278	18777	2.99	47.84
	2000	7493	31020	4.14	66.24
	2010	7390	39973	5.41	86.56

The data on rice cropped area and rough rice production have been collected from the USDA database available in the IRRI website (IRRI, 2011). The amount N removed (kg ha^{-1}) was estimated considering that the rice crop generally removes 16 kg N per ton of rough rice production (De Datta, 1981).

TABLE 3 Consumption of fertilizer N globally and in eight rice growing countries over the years since 1961

	Total fertilizer N consumption (000 tons)								
Year	World	Australia	Bangladesh	China	India	Malaysia	Pakistan	Philippines	Vietnam
1961	11784.4	35.0	20.0	880.0	310.0	25.7	62.1	35.8	—
1970	31423.1	123.4	99.2	2987.0	1310.0	60.8	263.5	107.6	166.2
1980	60492.7	248.0	266.2	11787.0	3522.3	139.4	842.8	209.8	129.0
1990	76777.1	439.4	609.2	1923.0	7565.5	262.1	1471.7	400.6	425.4
2000	82069.6	951.0	995.8	22720.0	10910.6	359.3	2264.6	488.2	1332.0
2008	98647.9	835.0	1173.5	33236.7	15090.1	531.7	3035.0	450.0	975.0

Source: IFA, 2011.

about 85% of the total rice cropped area is under wetland culture. In upland culture aerobic bacteria can fix atmospheric N while in wetland culture both aerobic and anaerobic bacteria can fix N. Aerobic bacteria like *Azotobacter* can live in the oxygenated rhizosphere of rice plant and can fix atmospheric N, while anaerobic bacteria like *Clostridium* can live in the reduced soil layer and can fix N. Wetland rice ecosystem is the favorable habitat for aquatic biota like *Azolla* and blue-green algae. *Azolla* can fix a substantial amount of N in symbiotic association with *Anabaena* (Mian, 2002), while blue-green algae, often called cyanobacteria, can fix atmospheric N as a free-living aquatic biota (Hashem, 2001). Other biological nitrogen fixers in rice culture are *Azospirillum*, *Herbaspirillum*, and *Burkholderia* (Baldani et al., 2000; Balandreau, 2002; Malik et al., 2002). Both single and multi-strain inoculant biofertilizers are used in rice cultivation. Recent investigations showed that a multi-strain inoculant bio-fertilizer called BioGro performed well in supplementing urea N for rice production in Vietnam (Pham et al., 2008; Phan et al, 2009). Green manure crops like *Sesbania*, cowpea, chickpea etc. can fix N in rice cropping patterns (Ladha and Kundu, 1997; Choudhury et al., 2002b). Literature on the beneficial effects of BNF technology for rice

TABLE 4 Consumption of fertilizer P globally and in eight rice growing countries over the years since 1961

	Total fertilizer P consumption (000 tons)								
Year	World	Australia	Bangladesh	China	India	Malaysia	Pakistan	Philippines	Vietnam
1961	11037.4	582.5	—	122.0	70.8	27.8	10.6	26.5	61.4
1970	21201.5	756.9	34.0	907.0	305.1	54.7	30.5	44.0	76.9
1980	31912.1	853.0	118.4	2952.0	1091.1	118.8	227.0	53.1	23.0
1990	35920.3	578.9	231.8	5769.6	3124.5	153.7	388.5	104.8	105.7
2000	32812.3	1107.0	250.3	8664.0	4248.0	198.1	675.1	124.1	501.0
2008	34297.0	818.3	80.0	10500.0	6506.2	177.0	629.9	108.1	612.8

Source: IFA, 2011.

TABLE 5 Consumption of fertilizer K globally and in eight rice growing countries over the years since 1961

	Total fertilizer K consumption (000 tons)								
Year	World	Australia	Bangladesh	China	India	Malaysia	Pakistan	Philippines	Vietnam
1961	8836.4	54.0	1.5	10.0	37.1	13.6	—	19.0	5.3
1970	15764.0	91.2	10.4	25.0	199.0	61.1	1.2	49.6	38.4
1980	23826.4	128.0	28.7	527.0	617.6	250.3	9.6	55.7	39.3
1990	24320.2	145.4	90.0	1761.0	1308.5	493.7	32.7	81.5	29.2
2000	22095.1	217.0	143.0	3364.0	1565.1	650.0	22.8	122.2	450.0
2008	23082.0	214.5	50.0	4685.0	3312.6	903.2	25.0	113.0	378.0

Source: IFA, 2011.

production is voluminous (Kundu and Ladha, 1995; Ladha and Reddy, 1995; Peoples et al., 1995; Nguyen, 2008). This paper discusses on the prospects and potentials of different BNF systems for sustainable rice production.

Azolla Biofertilizer

Azolla is a free-floating fresh water fern, which fixes atmospheric N through the symbiotic association with the cyanobacterium, *Anabaena azollae* lives inside the dorsal lobes of *Azolla* leaves, potentially supplying a substantial amount N to the rice crop (Moore, 1969). *Azolla* can contribute 40-60 kg N ha^{-1} per rice crop (Kannaiyan, 1993). *Azolla* can fix 22–40 kg N ha^{-1} within 30 days (Peoples et al., 1995), and can accumulate up to 64–75 kg N ha^{-1} within 60 days increasing rice yield up to 1.5–2.4 t ha^{-1} (Table 6). It can be grown simultaneously with irrigated rice without additional costs for land and water (Singh and Singh, 1990; Mian and Kashem, 1995). There are now seven existing species of the family Azollaceae – *Azolla caroliniana, A. maxicana, A. filiculoides, A. microphylla, A. rubra, A. nilotica and A. pinnata* (Mian, 2002). The amount of N accumulated by *Azolla* comes mostly from air and it has been established by the ^{15}N tracer technique that rice plants can assimilate around 33% of fixed N by *Azolla* within 60 days (Mian, 1984, 1985).

TABLE 6 Beneficial effects of *Azolla* in increasing rice yield and N_2 fixation in field experiments

		Increase in rice yield			
Country	Season	Amount (t ha^{-1})	(%)	Estimated amount of fixed N_2	Reference
Bangladesh	Dry	1.5	50	64	Mian, 2002
Philippines	Dry	2.4	54	75	Ladha et al., 2000
	Wet	1.2	30	68	
India	Dry	1.6	80	Data not available	Mishra and Mishra, 2007

However this recovery of N by rice plant varies with soil conditions (Galal, 1997). *Azolla* can also be used directly as feed for fish. Based on this concept a technology called as 'Azobiofer' has been developed for the production and use of *Azolla* for irrigated rice and fish cultivation (Mian, 2002).

In addition to the use of *Azolla* as N source for rice crop, it can be used for reclaiming saline soils, reducing evapotranspiration, and controlling weed infestation in rice crops (Hove and Lejeune, 1996). It can also be used to purify wastewater as it can accumulate phosphorus and some heavy metals from water (Sarkar and Jana, 1986; Shiomi and Kitoh, 1987). However, there are some constraints like water availability and control, phosphorus limitations in soils, predators of *Azolla,* and temperature sensitivity of *Azolla* are encountered during *Azolla* production and use. A high temperature tolerant *Azolla* species, *Azolla microphylla,* can be used in tropical countries to encounter the temperature problem. This species can survive at higher temperature (up to 38±°C) and can fix nitrogen (Kannaiyan and Somporn, 1988). Other problems for Azolla production can be alleviated by proper management practices. Provided sufficient labor is available, *Azolla* remains an option for filling the gap in N-supply as the cost of oil for its production augments. Past practices of subsidizing application of urea for food security reasons would be better replaced by developing means of promoting more environment-friendly strategies.

Cyanobacterial Biofertilizer

Cyanobacterial biofertilizer excludes *Anabaena* in symbiosis with *Azolla.* Cyanobacteria (blue-green algae) are photosynthetic prokaryotic microorganisms capable of fixing atmospheric N_2 using sunlight as the sole energy source (Stewart, 1980). Wetland rice fields can provide an ideal condition for the growth of cyanobacteria, fixing 25–30 kg N ha^{-1} $crop^{-1}$, and reducing the use of urea fertilizer in rice culture by 30% (Hashem, 2001). The population of cyanobacteria varies among soils (Begum et al., 1996, 2008). Experimental results at the International Rice Research Institute revealed that the amount of N accumulation by cyanobacteria varies among soils ranging from a few to 50 kg N ha^{-1} $crop^{-1}$ (Roger and Ladha, 1992). It has been established by the ^{15}N tracer technique that around 90% of the accumulated N by cyanobacteria is derived from the air (Inubushi and Watanabe, 1986). Literature on the beneficial effects of cyanobacteria on the growth and yield of rice is voluminous (Ladha and Reddy, 1995; Kannaiyan et al., 1997; Kennedy and Islam, 2001). However, the efficiency of cyanobacteria in increasing rice yield varies depending on soil types. Findings of several field experiments conducted on different types of soils show that cyanobacteria can supplement 30% of urea N for rice crop in acid, saline and red soils while it can supplement 25% of urea-N in neutral soil (Table 7).

TABLE 7 Beneficial effects of cyanobacteria in increasing rice yield and supplementing urea N in different types of soils

	Increase in rice grain yield		Fertilizer N supplement		
Soil type	Amount (t/ha)	%	Amount (kg/ha)	%	Reference
Neutral	0.6	17	10	25	Sattar et al., 2008
Acid	0.3	5	30	30	Hashem, 2001
Saline	0.5	8	20	30	
Red	0.2	3	33	30	

Cyanobacterial biofertilizer excludes *Anabaena* in symbiosis with *Azolla.*

Cyanobacteria can play a major role in improving the soil environment in addition to N fixation. They have the capacity to reclaim salinity of soil (Hashem et al., 1995, Uma and Kannaiyan, 1999). They can improve the organic matter content and water holding capacity of soil, and can reduce soil erosion. Experimental results in the Bangladesh Institute of Nuclear Agriculture showed that cyanobacteria can increase the rice plant's capacity to use more fertilizer N for grain production (Sattar et al., 2008). They can benefit the rice plants by producing growth-promoting substances, and by increasing the availability of phosphorus by excretion of organic acids (Roger and Kulasooriya, 1980). However, there are some limitations for the use of cyanobacterial bio-fertilizer for rice crops. Phosphorus deficiency, presence of high concentration of N in the flood water, low pH and presence of grazer (various protozoans) populations can limit the growth and N fixation capacity of cyanobacteria (Ladha and Reddy, 1995). These constraints can be alleviated by application of phosphate, lime and biocides (like Neem), and deep placement of N fertilizer (Grant et al., 1986; Roger and Watanabe, 1986).

Bacterial Inoculant Biofertilizers

Bacterial inoculant biofertilizers are efficient sources of N to supplement the use of urea-N in rice production. Some bacteria like *Azotobacter, Clostridium, Azospirillum, Herbaspirillum* and *Burkholderia* can supplement urea-N by BNF while *Rhizobium* can supplement urea-N by promoting growth physiology or root morphology of the rice plant. In addition, acting as plant growth-promoting rhizobacteria (PGPR), they can improve the ability of the rice plant to assimilate soil-N. The beneficial effects of bacterial inoculant biofertilizers are presented in Tables 8 and 9.

Rhizobium

Rhizobium leguminosarum bv. trifolii can colonize rice roots endophytically in the fields where rice is grown in rotation with Egyptian berseem clover (*Trifolium alexandrinum*), and can supplement 25–33% of the recommended

TABLE 8 Effects of *Rhizobium leguminosarum* bv. trifolii inoculation on rice grain yield and agronomic fertilizer-N-use efficiency under variable rates of applied fertilizer N[a]

	Applied fertilizer-N rate (kg ha^{-1})				Applied fertilizer-N rate (kg ha^{-1})		
	0	72	144	Mean	72	144	Mean
Rhizobium strain	Grain yield (t ha^{-1})				Agronomic efficiency[b] fertilizer-N-use		
E24	4.50	6.69	7.36	6.18*	92.9	51.1	72.0*
E27	4.65	6.24	7.36	6.08*	86.7	51.1	68.9*
E37	4.95	6.79	7.04	6.26*	94.3	48.9	71.6*
E39	5.26	6.86	8.91	7.01*	95.2	61.9	78.6*
Non-inoculated	3.97	5.17	6.69	5.28	71.8	46.4	59.1

[a]Source: Adapted from Yanni et al., 2001. [b][kg grain (kg applied fertilizer-N)$^{-1}$].
*Mean values differ from the corresponding controls at 95% confidence level.

rate of N fertilizer for rice (Yanni et al., 1997). This bacterium is also able to colonize the interior of the rice roots grown under gnotobiotic conditions. It can increase shoot and root growth, grain yield and agronomic fertilizer N use efficiency significantly. Rice plants can be inoculated with this bacterium by coating seeds in bacterial suspension or by dipping roots of seedlings. Studies conducted at the International Rice Research Institute showed that its inoculation increased growth and yield of rice, and N, P & K uptake by rice plants significantly (Biswas et al., 2000a, 2000b). Nitrogen-15 based studies showed that the increased N uptake was not due to biological nitrogen fixation (BNF). It is evidenced from the results of those studies that certain strains of rhizobia can enhance rice growth and yield through the changes in growth physiology or root morphology rather than BNF. The beneficial effects of some *Rhizobium* strains in increasing

TABLE 9 Increases in rice grain yield and estimated amounts of fixed N_2 by different diazotrophic bacteria

		Increase in grain yield			
Bactria	Experiment type	Amount	%	Estimated amount of fixed N_2	Reference
Azotobacter sp.	Field	0.4–0.9 t ha^{-1}	7–20	11–15 kg ha^{-1}	Yanni and El-Fattah, 1999
Azospirillum lipoferum	Greenhouse	6.7 g plant^{-1}	81	58.9% Ndfa	Mirza et al., 2000
Herbaspirillum spp.	Greenhouse	3.7–7.5 g plant^{-1}	45–90	38.1–58.2% Ndfa	Mirza et al., 2000
Burkholderia vietnamiensis	Field	0.5–0.8 t ha^{-1}	13–22	Data not available	Trân Van et al., 2000
Rhizobium leguminosarum	Greenhouse	0.6–7.9 g pot^{-1}	2–22	23–31 mg pot^{-1}	Biswas et al., 2000a

rice yield and agronomic fertilizer N use efficiency are presented in Table 8. Although it is not contributing much in BNF, its application can save a substantial amount of chemical N fertilizer for rice production, can thereby reduce the risk of environmental pollution to some extent.

Azotobacter

Azotobacter is an aerobic free living, heterotrophic N_2 fixer. *Azotobacter* depends on carbon for energy. It can fix N_2 in the oxygenated rhizosphere of rice plant. Its activity in rice culture can be increased by straw application (Kanungo et al., 1997). Rice yields in field experiments increased by 0.4–0.9 t ha^{-1} due to *Azotobacter* application (Yanni and El-Fattah, 1999). It can be applied in rice culture by different methods like seed dipping, seedling root dipping, soil application at nursery or main field, top dressing or foliar application (Kannaiyan, 1999; Singh et al., 1999; Sattar et al., 2008). The estimated N accumulation by rice plants increased by 11–15 kg ha^{-1} due to *Azotobacter* inoculation (Yanni and El-Fattah, 1999). Experimental results at Bangladesh Institute of Nuclear Agriculture showed that *Azotobacter* inoculation increased rice grain yield significantly with 60, 80, and 100 kg urea N ha^{-1} while its effect was not significant at 0 and 120 kg urea N ha^{-1} (Sattar et al., 2008). These results indicated that *Azotobacter* can supplement urea N, but cannot substitute it completely.

Azospirillum

Azospirillum is a heterotrophic bacterium capable of fixing atmospheric N (Roper and Ladha, 1995; Rodrigues et al., 2008). This organism grows in the rhizosphere of gramineous plants. It can also penetrate the root to grow intercellularly (Baldani and Döbereiner, 1980). Both *Azospirillum lipoferum* and *A. brasilense* have been isolated from roots and stems of rice plants (Ladha et al., 1982) while *A. amazonease* has been isolated from the roots (Pereira et al., 1988). Investigations at the International Rice Research Institute revealed that *Azospirillum* constitutes about one percent of the total aerobic heterotrophs, and about 85% of the *Azospirillum* isolates belong to *A. lipoferum* indicating its preferential colonization for rice plants (Ladha et al., 1987). *Azospirillum* inoculation increased rice yield significantly by 1.6–10.5 g $plant^{-1}$ (32–81% increase) in greenhouse conditions (Mirza et al., 2000; Malik et al., 2002). However, in the field condition, the estimated yield increase was around 1.8 t ha^{-1} (22% increase) as reported by (Balandreau, 2002). This species can also increase plant height and tiller number of rice plant (Nayak et al., 1986). Field experimental results in Hokkaido, Japan also showed rice yield increase (17%) due to *Azospirillum* inoculation (Isawa et al., 2010). In Argentia, *Azospirillum brasilense* inoculation increased grain yield of rainfed rice by 19% in field experiments (Pedraza et al., 2009).

Azospirillum inoculation can increase phosphate and ammonium uptake by rice plants (Murty and Ladha, 1988). It can increase nitrate reductase activity, total protein, and chlorophyll contents in the leaves of rice plants (Roy and Srivastava, 2010). It can be applied using three methods: seed dipping in bacterial suspension for five minutes followed by drying in the shade for 2–4 hours; root dipping of rice seedlings in bacterial suspensions overnight; or application of bacterial suspensions to the rhizosphere of rice plants (Islam and Bora, 1998). Its inoculation can reduce bacterial leaf blight of rice with subsequent increase in yield parameters (Islam and Bora, 1998). Experimental results at Bangladesh Institute of Nuclear Agriculture showed that *Azospirillum* inoculation increased rice grain yield significantly with 60, 80 and 100 kg urea N ha^{-1} while its effect was not significant at 0 and 120 kg urea N ha^{-1} (Sattar et al., 2008). These results indicated that *Azospirillum* can supplement urea N, but cannot substitute it completely. Mirza et al. (2000) quantified the N fixation by both *A. lipoferum* and *A. brasilense* in rice crop using the ^{15}N isotope dilution method under greenhouse condition. They found that the nitrogen derived from atmosphere (%Ndfa) values were 20.0 and 19.9 for *A. lipoferum* and *A. brasilense*, respectively in basmati rice while values were 58.9 and 47.1, respectively in super basmati rice. This information clearly demonstrates that *Azospirillum* inoculation can met at least 19 and 47% of the required N of basmati and super basmati rice, respectively, through BNF. This is obviously an evidence of bacterial genotype and crop variety interaction. Before going for the utilization BNF technology in crops, this interaction should be considered for efficient utilization of BNF.

Clostridium

Clostridium is an anaerobic heterotrophic bacterium capable of fixing N_2 in absence of oxygen (Saralov and Babanazarov, 1983; Kennedy and Tchan, 1992). It is often found in rice soils (Khamas et al., 1994; Elbadry et al., 1999). Its activity increases in response to application of straw in rice fields. It can increase rice yield significantly in favorable condition (Mishustin et al., 1983). *Clostridium* is able to fix 5–10 mg N per gram of carbon compound consumed (Mulder, 1975), so it is advisable to apply rice straw in soil to stimulate N fixation by *Clostridium.* Experimental results at University of Tokyo, Japan, indicated that *Chlostridium bifermentans* was the dominant species in the rice rhizosphere (Doi et al., 2007).

Herbaspirillum

Herbaspirillum is an endophytic diazotroph, which colonizes sugarcane, rice, maize, sorghum and other cereals (Baldani et al., 1986; Pimentel et al., 1991). The species that colonizes rice roots is *Herbaspirillum seropedicae* (Baldani et al., 1986). The genus *Herbaspirillum* refers to the habitat of the organism (the roots of cereals, which are Harbaceous seed-bearing plants) while the species *seropedicae* refers to the place (Seropédica, Rio de Janeiro, Brazil) where it was first isolated (Baldani et al., 1986). This species can fix 31–54% of total rice plant N from atmosphere under gnotobiotic condition (Baldani et al., 2000). Inoculation of *Herbaspirillum seropedicae* in field conditions increased shoot and root length, 1000-grain weight and grain yield of rice (Arangarasan et al., 1998). Rice seeds can be inoculated with *Herbaspirillum seropedicae* during sowing. Its inoculation can enhance seed germination significantly (Pereira et al., 1988). It can also increase shoot and root dry weight of rice plants significantly (James et al., 2002; Roncato-Maccari et al., 2003). Mirza et al., (2000) working with super basmati rice (an aromatic rice) found that rice grain yield increased by 3.7–7.5 g plant^{-1} (45–90% increase) with *Herbaspirillum spp.* inoculation under greenhouse conditions. They also quantified the N_2 fixation by different strains of *Herbaspirillum* in both basmati and super basmati rice using the ^{15}N isotope dilution technique. The%Ndfa values were 19.5–38.7, and 38.1–58.2 in basmati and super basmati, respectively. Gyaneshwar et al., (2002) also reported that the amount of N_2 fixation by *Herbaspirillum seropedicae* varies between rice varieties. This information clearly demonstrates that the amount of N_2 fixation by *Herbaspirillum* varies depending on rice variety.

Burkholderia

The genus *Burkholderia* comprises 29 species, with several of these including *Burkholderia vietnamiensis, B. kururiensis*, *B. tuberum* and *B. phynatum* are capable to fix N_2 (Estrada-de los Santos et al., 2001; Vandamme et al., 2002). The species *B. vietnamiensis* was described by Gillis et al., (1995). It was first isolated from the rhizosphere of young rice plants cultivated on a Vietnamese soil in a phytotron (Trân Van et al., 1994). When used to inoculate rice in field trials it increased grain yields by 0.5–0.8 t ha^{-1} (13–22% increase) demonstrating its potential to enhance rice production (Trân Van et al., 2000). In these field trials this strain was capable of saving 25–30 kg N ha^{-1} from fertilizer. Baldani et al. (2000) established by the ^{15}N tracer technique that *B. vietnamiensis* can fix 19% of the rice plant N (152 μg N plant^{-1}) from the atmosphere under gnotobiotic condition. As this species was isolated from the rice roots and adhering soil, it should not be described as an endophyte (Baldani et al., 1997). An endophytic species (*Burkholderia sp*) has been isolated from the interior of roots, stems and leaves of rice in Brazil. It can fix 31% of rice plant N (273–372 μg N plant^{-1}) from the atmosphere, and its inoculation increased rice plant biomass up to 22 mg plant^{-1} (69% increase) under gnotobiotic condition (Baldani et al., 2000). The species, *Burkholderia glumae* causes grain and seedling rot of rice (Nakata, 2002). Another species, *Burkholderia cepacia,* can be hazardous to human health (Balandreau, 2002). So appropriate care and risk-reducing techniques should be taken while isolating and culturing *Burkholderia* to avoid these problems.

TABLE 10 Beneficial effect of BioGro in increasing grain yield of rice at variable fertilizer N rates in two locations of Vietnam

		Grain yield (t ha^{-1})		Increase in grain yield		
Location	N rate (kg ha^{-1})	Without BioGro	With BioGro	t ha^{-1}	%	Reference
Nam Dinh	30	2.33	2.50	0.17	7.3	Pham et al., 2008
	60	2.47	2.78	0.31	12.6	
	90	2.90	2.93	0.03	1.0	
Chau Thanh	30	2.50	2.78	0.28	11.2	Phan et al., 2009
	60	2.85	2.98	0.13	4.6	
	90	3.11	3.17	0.06	1.9	

Recent diagnostic advances make it possible to separate non-pathogenic and pathogenic species of *Burkholderia* (Suarez-Moreno et al., 2008).

Multi-Strain Inoculant Biofertilizers

Because of the diversity of strains found in rice rhizosphere, multi-strain inoculant biofertilizers may be effective for increasing grain yield of rice reducing its dependence on urea-N. In Vietnam, a multi-strain Biofertilizer called 'BioGro' has been developed at Hanoi University of Science (Nguyen, 2008). The BioGro formulation contained equal quantities of four microbial strains, a Gram negative pseudomonad very similar but not identical to the type strain of *Pseudomonas fluorescens*, a soil yeast *Candida tropicalis* selected for its ability to mobilize insoluble calcium phosphates as well as promoting plant growth and two Gram-positive amylolytic and proteolytic bacilli, identified as respectively *Bacillus subtilis* and *Bacillus amyloliquefaciens*. Field experimental results indicated that this biofertilizer is beneficial in increasing rice yield (Table 10). It has also been established by the ^{15}N tracer technique that one strain of BioGro *Pseudomonas fluorescens* has the ability to increase both fertilizer and non-fertilizer N uptake by the rice plants (Phan et al., 2008). Research results also showed that BioGro is both economically and environmentally beneficial for rice production in Vietnam (Marsh, 2008; Be, 2008). This formulation of BioGro has not been evaluated in Australian conditions, yet. However another formulation (the previous formulation) of BioGro was tested in field experiments at Yanco Agricultural Research Institute and at Australian Rice Research Institute at Jerilderie (Kecskés et al., 2008). The results indicated that BioGro increased grain yield to some extent although the difference was not statistically significant. These results inferred to conduct further research using the modified formulation of BioGro that was successful in increasing rice yield in Vietnam. Recent extensive trials on rice farms conducted in four successive seasons in three provinces of the Mekong Delta

TABLE 11 Potentials of some leguminous green manure crops to fix atmospheric nitrogen

Legume species	Growth duration (days)	N_2 fixation Ndfa (%)	 Amount (kg N ha^{-1})	Reference
Crotalaria juncea	190–195	72–81	199–223	Ladha et al., 1996
Clitoria ternatea	190–195	78–79	200–240	Ladha et al., 1996
Desmanthus virgatus	190–195	78–80	196–226	Ladha et al., 1996
Indigofera tinctoria	225	70	79	Peoples et al., 1995
Aeschynomene afraspera	56	68–76	105–145	Ladha et al., 1992
Aeschynomene indica	116	93–100	75–127	Peoples et al., 1995
Sesbania rostrata	45–65	68–94	70–458	Ladha et al., 1992
Sesbania cannabina	45–55	93	119–188	Pareek et al., 1990
Sesbania sesban	60	13–18	7–18	Peoples et al., 1995

Ndfa = N_2 derived from atmosphere.

with World Bank Development Marketplace funding by the Gates Foundation (Kennedy and Phan, 2011) have confirmed the beneficial effects of inoculation with BioGro, multivariate analysis indicating optimum means of maximizing yield increases and farmers' incomes. Similarly in the combined field inoculation with *Gluconacetobacter diazotrophicus, Herbaspirillum seropedicae, Azospirillum lipoferum* and *B. vietnamiensis strains* in South India has shown up to 23.6% yield increase over the control. (Govindarajan et al., 2008).

Leguminous Green Manures

Green manures can be used in the rice-cropping systems either in pre-rice or post rice phase, depending upon climatic conditions. The potentials of different green manure crops for atmospheric N_2 fixation are shown in Table 11. Among the plant species *Sesbania rostrata* has the highest N_2 fixing potential within 45–65 days of planting. It is grown as a pre-rice green manure. It can grow in both upland and lowland conditions, and can produce nodules in both root and stem. It can be used as green manure to improve soil fertility and to increase nutrient supply for the crop. Its use increases soils capacity to absorb nutrients and improve soil structure and microbial activities (Zaman et al., 1994, 1997). Due to its extensive and deep root systems, it can extract nutrients from the deep soil layers, use insoluble or fixed forms of phosphorus, and make them available to the succeeding rice crop. *Sesbania* is salt-tolerant, and its incorporation improves the physico-chemical properties of saline-alkali soils, thereby providing the facility for better rice growth and yields (Ladha and Kundu, 1997).

Literature on the N-accumulating capacity of *Sesbania* and its beneficial effects on rice cultivation is voluminous (Dargen et al., 1975; Bin, 1983; Roger and Watanabe, 1986; Ventura et al., 1987; Ghai et al., 1988). Despite available information on the beneficial effects of *Sesbania,* it is not yet adopted by the

TABLE 12 Effect of N fertilization and planting arrangements of *Sesbania rostrata* green manuring (stem cutting) on grain yield and total N uptake of rice

Treatment	Grain yield (t ha^{-1})	Total N uptake (kg ha^{-1})
Control (no nitrogen)	3.2	43
80 kg urea-N ha^{-1}	4.0	65
Sesbania rostrata stem cutting at 10 cm x 5 cm spacing	4.5	76
Sesbania rostrata stem cutting at 10 cm x 10 cm spacing	4.0	64
Sesbania rostrata stem cutting at 10 cm x 15 cm spacing	3.9	60
LSD (0.05)	0.41	—

(Source: Choudhury et al., 1996).

farmers on a large-scale because of its competition with the rice crop for land. It is necessary to develop the best techniques to introduce it into the rice cropping systems without sacrificing the rice crop. Research conducted at Bangladesh Rice Research Institute showed that *Sesbania* can be established in the rice cropping patterns by different planting practices (Choudhury et al., 1996, 2002b). Planting of stem cutting with a spacing of 10 cm x 5 cm in the fallow period of the dry season-fallow-rainy season rice cropping pattern has been identified as the best technique to fit *Sesbania* in this pattern for complete substitution of urea-N for the rainy season rice. This practice can even give significantly higher rice grain yield compared to the recommended rate of N fertilizer (Table 12). Intercropping *Sesbania* with the summer rice in the summer season-rainy season rice cropping pattern can completely substitute urea-N for the rainy season rice (Zaman et al., 1996). Although this practice reduces rice yield in the summer season, it is beneficial for sustaining soil fertility and productivity in the long run.

CONCLUSIONS

Based on this review, the following conclusions can be drawn:

1. Urea-N application can be supplemented by 50 and 30% by *Azolla* and Cyanobacteria, respectively.
2. Some bacteria like *Azotobacter, Clostridium, Azospirillum, Hebaspirillum* and *Burkholderia* can supplement urea-N by BNF while *Rhizobium* can supplement urea-N by promoting growth physiology or root morphology of the rice plant.
3. Multi-strain inoculant biofertilizers like ‘BioGro’ have the potential to increase rice yield as well to save chemical N fertilizer use.

4. Green manure crops can fix substantial amounts of atmospheric N. Among the green manure crops, *Sesbania rostrata* has the highest potential of fixing atmospheric N. It can substitute urea-N completely for rice crop.

Although there are limitations to widespread application of BNF technology in rice cultivation, it clearly has the potential to supplement the N supplied by urea-N substantially, improving N-use efficiency and reducing environmental pollution problems of potential value in limiting global warming (Baggs et al., 2002; Bohlool et al., 1992; Xing, 1998). Future research must aim to provide better strategies to overcome not only technological, but also economic and social limitations to the adoption of the various means of achieving BNF at the farm level.

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