Biol Fertil Soils (2004) 39:219–227
DOI 10.1007/s00374-003-0706-2

REVIEW ARTICLE

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Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production

Received: 13 November 2002 / Accepted: 10 November 2003 / Published online: 24 January 2004

Abstract The N requirement of rice crops is well known. To overcome acute N deficiency in rice soils, this element is usually supplied to the rice crop as the commercially available fertilizer urea. But unfortunately a substantial amount of the urea-N is lost through different mechanisms causing environmental pollution problems. Utilization of biological N fixation (BNF) technology can decrease the use of urea-N, reducing the environmental problems to a considerable extent. Different BNF systems have different potentials to provide a N supplement, and it is necessary to design appropriate strategies in order to use BNF systems for efficient N supply to a rice crop. Research has been conducted around the world to evaluate the potential of different BNF systems to supply N to rice crops. This paper reviews salient findings of these works to assess all the current information available. This review indicates that the aquatic biota Cyanobacteria and *Azolla* can supplement the N requirements of plants, replacing 30–50% of the required urea-N. BNF by some diazotrophic bacteria like *Azotobacter*, *Clostridium*, *Azospirillum*, *Herbaspirillum* and *Burkholderia* can substitute for urea-N, while *Rhizobium* can promote the growth physiology or improve the root morphology of the rice plant. Green manure crops can also fix substantial amounts of atmospheric N. Among the green manure crops, *Sesbania rostrata* has the highest atmospheric N_2-fixing potential, and it has the potential to completely substitute for urea-N in rice cultivation.

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 total rice-cropped area and rough rice production in the world were 147.14 million hectares and 576.28 million tons, respectively, in 2002 [International Rice Research Institute (IRRI) 2003]. Rice plants require large amounts of mineral nutrients including N for their growth, development and grain production. Rice crops remove around 16–17 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 fertilizer N applications are required to meet a rice crop's N demand. 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; Choudhury et al. 2002a). This low N-use efficiency is mainly due to denitrification, NH_3 volatilization and leaching losses (Ponnamperuma 1972; De Datta and Buresh 1989). NH_3 volatilization and denitrification cause atmospheric pollution through the production of greenhouse gases like N_2O and NH_3 (Reeves et al. 2002). NO_3^- leaching causes groundwater toxicity (Shrestha and Ladha 1998). In addition to these environmental problems, the long-term use of urea depletes the soil organic matter. These problems are of great concern to soil and environmental scientists around the world. Alternate sources of N should be applied to minimize these problems if possible.

Biological N fixation (BNF) technology can play an important role in substituting for commercially available N fertilizer use in rice culture, thus reducing these environmental problems to some extent. Use of biofertilizers can prevent the depletion of the soil organic matter (Jeyabal and Kuppuswamy 2001). Rice crops are grown in both wetland and upland cultures. However 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

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anaerobic bacteria can fix N. Aerobic bacteria like *Azotobacter* can live in the oxygenated rhizosphere of the rice plant and can fix atmospheric N, while anaerobic bacteria like *Clostridium* can live in the reduced soil layer and can fix N. A wetland rice ecosystem is the favourable 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, cyanobacteria, can fix atmospheric N_2 as free-living aquatic biota (Hashem 2001). Other biological N fixers used in rice culture are *Azospirillum*, *Herbaspirillum*, and *Burkholderia* (Baldani et al. 2000; Balandreau 2002; Malik et al. 2002). Green manure crops like *Sesbania*, cowpea, chickpea etc. can fix N in rice cropping patterns (Ladha and Kundu 1997; Choudhury et al. 2002b). The literature on the beneficial effects of BNF technology for rice production is voluminous (Kundu and Ladha 1995; Ladha and Reddy 1995; Peoples et al. 1995; Nguyen et al. 2002). This paper discusses the prospects and potentials of different BNF systems for sustainable rice production.

Azolla biofertilizer

Azolla is a free-floating freshwater fern, which fixes atmospheric N through the symbiotic association with *Anabaena azollae* that lives inside the dorsal lobes of *Azolla* leaves, potentially supplying a substantial amount of N to the rice crop (Moore 1969). *Azolla* can fix 22–40 kg N ha^{-1} within 30 days (Peoples et al. 1995). It can be grown simultaneously with irrigated rice without additional requirements for land and water (Singh and Singh 1990; Mian and Kashem 1995). Rice yields can be increased by 1.4–1.5 t ha^{-1} by *Azolla* application (Mian 2002; Cissé and Vlek 2003). There are now seven recognised species of the family Azollaceae: *Azolla caroliniana*, *A. maxicana*, *A. filiculoides*, *A. microphylla*, *A. rubra*, *A. nilotica and A. pinnata* (Mian 2002). *Azolla* can contribute 40–60 kg N ha^{-1} per rice crop (Kannaiyan 1993). The N accumulated by *Azolla* is derived mostly from the air. It has been established, by using the ^{15}N tracer technique, that rice plants can assimilate around 33% of the N fixed by *Azolla* within 60 days (Mian 1984, 1985). However this recovery of N by rice plants varies with soil conditions (Galal 1997). *Azolla* can also be used directly as a feed for fish, and a technology called "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 an N source for rice crops, it can be used for reclaiming saline soils, reducing evapotranspiration, and to control weed infestations in rice crops (Hove and Lejeune 1996). It can also be used to purify wastewater as it can accumulate P and some heavy metals from water (Sarkar and Jana 1986; Shiomi and Kitoh 1987). However, there are some constraints on the production and use of *Azolla*, like the availability and control of water supplies, P limitations in soils, predators of *Azolla*, and its temperature sensitivity. A high-temperature-tolerant *Azolla* species, *Azolla microphylla*, can be used in tropical countries to overcome the problem of its sensitivity to high temperatures. This species can survive at temperatures of up to 38±1°C and can fix N (Kannaiyan and Somporn 1989). Other problems of *Azolla* production can be alleviated by proper management practices.

Cyanobacterial biofertilizer

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, which accumulate 19–28 kg N ha^{-1} $crop^{-1}$, and can reduce the use of urea fertilizer in rice culture by 25–35% (Hashem 2001). The population of cyanobacteria varies among soils (Begum et al. 1996). Experimental results at the IRRI (Los Baños, Philippines) 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 N accumulated by cyanobacteria is derived from the air (Inubushi and Watanabe 1986). The 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 yields varies depending on soil types. Findings of several field experiments conducted on different types of soils show that cyanobacteria can be used to reduce urea-N inputs by 25–35% for a rice crop in acid, saline and red soils while they are less effective in calcareous and neutral soils (Hashem 2001).

Cyanobacteria can play a major role in improving the soil environment in addition to N fixation. They have the capacity to reclaim saline soils (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. They can benefit the rice plants by producing growth-promoting substances, and by increasing the availability of P by excretion of organic acids (Roger and Kulasooriya 1980). However, there are some limitations for the use of cyanobacterial bio-fertilizer for rice crops. P deficiency, the presence of a high concentration of N in the flood water, a low pH and the presence of grazer populations can limit the growth and N fixation capacity of cyanobacteria (Ladha and Reddy 1995). These constraints can be alleviated by the application of PO_4^{3-}, lime and biocides (like neem), and the deep placement of N fertilizer (Grant et al. 1986; Roger and Watanabe 1986).

Bacterial inoculant biofertilizers

Bacterial inoculant biofertilizers are efficient sources of N used to substitute for 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 the growth physiology or root morphology of the rice plant. In addition, by acting as plant growth-promoting rhizobacteria (PGPR), they can improve the ability of the rice plant to assimilate soil N. The beneficial effects of PGPR have been well demonstrated in both greenhouse and field conditions (Yanni et al. 1997; Biswas et al. 2000a, 2000b). Increases in rice grain yields and estimated amounts of N_2 fixation by different N_2-fixing systems are presented in Table 1.

Azotobacter

Azotobacter is an aerobic free-living, heterotrophic N_2 fixer. *Azotobacter* depends on C for energy. Its activity in rice culture can be increased by straw application (Kanungo et al. 1997). Rice yields in field trials increased by 0.4–0.9 t ha^{-1} (7–20% increase) 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 the nursery or main field, top dressing or foliar application (Kannaiyan et al. 1980; Chandra and Singh 1996; Kannaiyan 1999; Singh et al. 1999). The estimated N accumulation by rice plants increased by 11–15 kg ha^{-1} due to *Azotobacter* inoculation (Yanni and El-Fattah 1999).

Clostridium

Clostridium is an anaerobic heterotrophic bacterium capable of fixing N_2 in the absence of O_2 (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 the application of straw in rice fields (Mishustin et al. 1983). *Clostridium* is able to fix 5–10 mg N g^{-1} C compound consumed (Mulder 1975), so it is advisable to apply rice straw in soil to stimulate N fixation by *Clostridium.*

Azospirillum

Azospirillum is a heterotrophic bacterium capable of fixing atmospheric N (Roper and Ladha 1995). This organism grows in the rhizosphere of graminaceous 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 IRRI revealed that *Azospirillum* constitutes about 1% 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. 1987a). *Azospirillum* inoculation increased the 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 field conditions, the estimated yield increase was around 1.8 t ha^{-1} (22% increase) as reported by (Balandreau 2002). This species can also increase the height and tiller number of rice plants (Nayak et al. 1986). *Azospirillum* inoculation can increase $PO_4{}^{3-}$ and $NH_4{}^{+}$ uptake by rice plants (Murty and Ladha 1988). It can be applied by using one of three methods: (1) seed dipping in a bacterial suspension for 5 min followed by drying in the shade for 2–4 h; (3) root dipping of rice seedlings in bacterial suspensions overnight; or (3) application of bacterial suspensions to the rhizosphere of rice plants (Islam and Bora 1998). Its inoculation can reduce the bacterial leaf blight of rice with a subsequent increase in yield parameters (Islam and Bora 1998). Mirza et al. (2000) quantified the N fixation by both *A. lipoferum* and *A. brasilense* in a rice crop using the ^{15}N isotope dilution method under greenhouse conditions. They found that the percent N derived from the 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 meet at least 19 and 47% of the N requirement of basmati

Table 1 Increases in rice grain yield and estimated amounts of fixed N_2 by different N_2-fixing systems

N_2-fixing system	Experiment type	Increase in rice grain yield		Estimated amount of fixed N_2	Reference
		Amount	(%)		
Azolla-Anabaena symbiosis	Field	1.5 t ha^{-1}	50	48.2 kg ha^{-1}	Mian (2002)
Cyanobacteria	Field	1.4 t ha^{-1}	29	24.2 kg ha^{-1}	Hashem (2001)
Bacterial inoculant biofertilizers					
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	
Burkholderia vietnamiensis	Field	0.5–0.8 t ha^{-1}	13–22	Data not available	Tran 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)

and super basmati rice, respectively, through BNF. This is obviously evidence of bacterial genotype and crop variety interaction. Before using BNF technology for crops, this interaction should be considered for the efficient utilisation of BNF.

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 generic name *Herbaspirillum* refers to the habitat of the organism (the roots of cereals, which are herbaceous seed-bearing plants) while the specific epithet *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 Ndfa under gnotobiotic conditions (Baldani et al. 2000). Inoculation of *H. seropedicae* in field conditions increased shoot and root length, 1,000-grain weight and grain yield of rice (Arangarasan et al. 1998). Rice seeds can be inoculated with *H. seropedicae* during sowing. Its inoculation can enhance seed germination significantly (Pereira et al. 1988). It can also increase the 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} (44–90% 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 fixed by *H. seropedicae* varies between rice varieties. This information clearly demonstrates that the amount of N_2 fixed 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* capable of fixing N_2 (Estrada-de los Santos et al. 2001; Vandamne et al. 2002). *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 (Tran 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 (Tran Van et al. 2000). In these field trials this strain was capable of reducing fertilizer-N inputs by 25–30 kg N ha^{-1}. Baldani et al. (2000) established by using the ^{15}N tracer technique that *B. vietnamiensis* can fix 19% of the rice plant Ndfa (152 μg N plant^{-1}) under gnotobiotic conditions. 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 Ndfa (273 –372 μg N plant^{-1}), and its inoculation increased rice plant biomass by up to 22 mg plant^{-1} (69% increase) under gnotobiotic conditions (Baldani et al. 2000). *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 should be taken and risk-reducing techniques should be employed while isolating and culturing *Burkholderia* to avoid these problems.

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 rate of N fertilizer for rice (Yanni et al. 1997). This bacterium is also able to colonize the interior of rice roots grown under gnotobiotic conditions. It can increase shoot and root growth, grain yield and agronomic fertilizer-N use efficiency significantly (Yanni et al. 1997). Rice plants can be inoculated with this bacterium by coating seeds in a bacterial suspension (Biswas et al. 2000a, 2000b) or by applying a bacterial suspension to transplanted rice 5 days after transplanting (Yanni et al. 1997). Studies conducted at the IRRI showed that its inoculation increased growth and yield of rice, and N, P and K uptake by rice plants significantly (Biswas et al. 2000a, 2000b). ^{15}N-based studies showed that the increased N uptake was not due to BNF (Biswas et al. 2000a). There is evidence from these studies that certain strains of rhizobia can enhance rice growth and yield through the accompanying changes in growth physiology or root morphology rather than BNF. Although *R. leguminosarum* bv. *trifolii* does not contribute much in terms of BNF, its application can save a substantial amount of commercially available N fertilizer for rice production, and can thereby reduce the risk of environmental pollution to some extent.

Limitations of bacterial inoculant biofertilizers and possible solutions

The efficiency of plant-associated N_2 fixation by diazotrophic bacteria may be hampered by a limited supply of energy and substrates. Plant residues and straw incorporated into the soil have been reported as potential sources of C and energy for the growth of, and N_2 fixation by, microorganisms (Matsuguchi 1979). The major fraction of straw is cellulose, which can be a good source of C and energy for the soil and rhizosphere microflora for N_2 fixation and other metabolic activities (Ladha et al 1986). Field experimental results at the IRRI showed that the incorporation of straw in the soil increased soil acetylene reduction activity (ARA), plant ARA, and N_2-fixing bacterial population resulting in an increase in grain and straw yields of rice (Ladha et al 1986). The beneficial effects of rice straw in N fixation is higher in flooded soils than non-flooded soils (Rao 1976). Other organic sources like cellulose, glucose, sucrose, succinate, acetate, butyrate, pyruvate and *n* -propanol can also enhance N_2 fixation in rice soils (Rao 1976, 1978). Kanungo et al (1997) evaluated the effects of placing organic sources (cellulose and rice straw) at different depths on nitrogenase activity associated with four tropical rice soils. They found that placing organic sources in the top profile (1–2 cm) produced a higher nitrogenase activity; while placing them at the other depths (2-4 cm and 4–6 cm) significantly decreased the activity irrespective of soil type. This information clearly demonstrates that asymbiotic N_2 fixation can be enhanced by placing rice straw and cellulose in the upper soil layer.

Rice genotypic differences in BNF

The activity of N_2-fixing bacteria is generally higher in cultivated rice strains than wild strains (Sano et al. 1981). This information suggests that the association between rice plants and N_2-fixing bacteria is controlled by genotypes of the rice plants. Shrestha and Ladha (1996) evaluated the variations among 70 rice genotypes for N_2 fixation using the ^{15}N isotope dilution technique. They found that the %Ndfa varied from 1.5 to 21.0 among the rice varieties. The magnitude of variation in %Ndfa among the rice genotypes varies depending on soil N status as reported by Malarvizhi and Ladha (1999). They found that genotypic differences in %Ndfa were significant and more pronounced at low soil NH_4^+-N (11 mg kg^{-1}) than at higher soil NH_4^+-N (79 and 92 mg kg^{-1}); the %Ndfa ranges were 14.9–35.9 at low soil N and 10.8–23.6 at high soil N. This information indicates that rice plants can utilise more biologically fixed N in soils with a relatively low fertility compared to soils with a high fertility status. Varietal differences in N_2-fixing activity were also found in some other investigations at IRRI (Ladha et al. 1986, 1987b). If rice genotypes with a high ability to stimulate N_2 fixation can be developed while breeding for improved rice varieties, the new genotypes will help in reducing the use of commercially available N fertilizers. This should be kept in mind when planning a rice breeding program. A rice genotype, IR42, developed at the IRRI was reported to have a high BNF trait among several genotypes evaluated (App et al. 1986; Wu et al. 1995).

Leguminous green manures

Green manures can be used in the rice-cropping systems either at a 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 2. Among the plant species presented in Table 2, *Sesbania rostrata* has the highest N_2-fixing potential within 45–65 days of planting (Ladha et al. 1992b). 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 a green manure to improve soil fertility and to increase the nutrient supply for the crop. Its use increases a soil's capacity to absorb nutrients, and leads to improvements in the 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, and make them available to the succeeding rice crop. *Sesbania* is salt-tolerant, and its incorporation improves the physico-chemical properties of saline-alkaline soils, thereby providing for better rice growth and yields (Ladha and Kundu 1997).

The organism responsible for both stem and root nodulation in *S. rostrata*, and the fixation of N_2 from the atmosphere is *Azorhizobium caulinodans* (Dreyfus et al. 1988). Stem nodules provide a better N_2-fixing system, particularly under flooded conditions (Ladha et al. 1992a). So *S. rostrata* is a suitable green manure for wetland rice crops. The 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

Table 2 Potentials of some leguminous green manure crops to fix atmospheric N_2

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

Table 3 Effect of N fertilization and planting arrangements of *S. rostrata* green manure (stem cutting) on grain yield and total N uptake of rice (Source: Choudhury et al. 1996)

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

al. 1988). Despite available information on the beneficial effects of *Sesbania*, it has not yet been adopted by the farmers for large-scale systems because of its competition with the rice crop for land. It is necessary to develop the best possible techniques to introduce it into the rice cropping systems without sacrificing the rice crop. Research conducted at the 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 stem cuttings with a spacing of 10 cm×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* into this pattern for the complete substitution of urea-N for the rainy season rice. This practice can even give a significantly higher rice grain yield compared to the recommended rate of N fertilizer (Table 3). 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. Although this practice reduces the rice yield in the summer season, it is beneficial as it sustains soil fertility and productivity in the long run.

Conclusions

Cyanobacteria, and *Azolla* can be used to reduce applied urea-N by 30% and 50%, respectively. Some bacteria like *Azotobacter*, *Clostridium*, *Azospirillum*, *Hebaspirillum* and *Burkholderia* can be used to substitute for urea-N by means of BNF while *Rhizobium* can supplement urea-N by promoting growth physiology or root morphology of the rice plant. Green manure crops can fix substantial amounts of atmospheric N. Among the green manure crops, *Sesbania rostrata* has the highest potential to fix atmospheric N. It can completely substitute for urea-N for a rice crop.

There are some limitations of using BNF technology in rice cultivation. The most important limitation of *Azolla* production is its high-temperature sensitivity. A high-temperature-tolerant *Azolla* species, *Azolla microphylla*, can be used in tropical countries to overcome this problem. The growth and N_2 fixation capacity of cyanobacteria may be hampered due to P deficiency, the presence of a high concentration of N in the flood water, a low pH and the presence of grazer populations. The application of $PO_4{}^{3-}$, lime and biocides (like neem), and deep placement of N fertilizer can alleviate these problems. A limited supply of energy and substrates may hamper the efficiency of plant-associated N_2 fixation by diazotrophic bacteria. This problem can be overcome by incorporating plant residues and straw into the soil. Although the green manure crop, *S. rostrata* can completely substitute for urea-N in rice cultivation, it has not yet been adopted by farmers because of its competition with the rice crop. This problem of adoption can be solved by fitting *S. rostrata* into the rice cropping patterns by planting practices like using stem cuttings in the fallow period or intercropping it with rice.

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