Plant and Soil **194**: 1–10, 1997.

Introduction: Assessing opportunities for nitrogen fixation in rice - a frontier project

J.K. Ladha[1], F.J. de Bruijn[2] and K.A. Malik[3]
[1]*International Rice Research Institute, P.O. Box 933, Manila, Philippines** [2]*DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan, U.S.A. and* [3]*National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan*

Key words: endophyte, legume-*Rhizobium*, *nif* gene, nitrogen fixation, rice

Abstract

Recent advances in understanding symbiotic *Rhizobium*-legume interactions at the molecular level, the discovery of endophytic interactions of nitrogen-fixing organisms with non-legumes, and the ability to introduce genes into rice by transformation have stimulated researchers world wide to harness opportunities for nitrogen fixation and improved N nutrition in rice. In a think-tank workshop organized by IRRI in 1992, the participants reaffirmed that such opportunities do exist for cereals and recommended that rice be used as a model system. Subsequently, IRRI developed a *New Frontier Project* to coordinate the worldwide collaborative efforts among research centers committed to reducing dependency of rice on mineral N resources. An international Biological Nitrogen Fixation (BNF) working group was established to review, share research results/materials and to catalyze research.

The strategies of enabling rice to fix its own N are complex and of a long-term nature. However, if achieved, they could enhance rice productivity, resource conservation, and environmental security. The rate of obtaining success would, of course, benefit tremendously from concerted efforts from a critical mass of committed scientists around the world, as well as a constant and continued funding support from the "donor" community.

Population growth and increasing demand for rice

Rice is the most important staple food for over two billion people in Asia and for hundreds of millions in Africa and Latin America. To feed the ever-increasing population of these regions, the world's annual rice production must increase from the present 460 million to 560 million by the year 2000 and to 760 million by 2020 (IRRI, 1993). If future increase in rice production has to come from the same or even a reduced land area, rice productivity (yield ha^{-1}) must be greatly enhanced to meet these goals.

Inorganic N fertilizer, a key input for rice production

Nitrogen is the nutrient that most frequently limits agricultural production. Global agriculture now relies heavily on N fertilizers derived at the expense of petroleum, which in turn is vulnerable to political and economic fluctuations in the oil markets. Nitrogen fertilizers, therefore, are expensive inputs, costing agriculture more than $45 billion (US) per year.

In the tropics, lowland rice yields 2–3.5 t ha^{-1} utilizing naturally available N derived from biological nitrogen fixation (BNF) by free-living and plant-associated diazotrophs (Watanabe and Roger, 1984; Ladha et al., 1993) and from mineralization of soil N (Bouldin, 1986; Kundu and Ladha, 1994). For higher yields, additional N must be applied. Achieving the 50% higher rice yields needed by 2020 will require at least double the 10 million t of N fertilizer that is currently used each year for rice production (IFA-IFDC-FAO, 1992; IRRI, 1993). Manufacturing the fertilizer for today's needs requires 544×10^9 MJ of fossil fuel energy annually (Mudahar, 1987a,b). Industrially produced N fertilizer depletes non-renewable resource and poses human and environmental hazards. In spite of an unlimited supply of N_2 in the air, manufacturing 1 kg of N fertilizer requires 6 times more energy than that

---

* FAX No: +6328711292. E-mail: J.K.Ladha@cgnet.com

needed to produce either P or K fertilizers (Da Silva et al., 1978). Nevertheless, over the past two and a half decades, farmers have become increasingly dependent on chemical sources of N for obtaining higher grain yields to meet the demands of enlarging population (see Figure 1).

Although the use of N fertilizer has increased substantially, a large number of farmers still use little or no N fertilizer because of several factors: its non-availability at times, lack of cash to buy it, and poor yield response due to adverse conditions. Furthermore, more than half the applied fertilizer N is lost (through denitrification, ammonia volatilization, leaching and runoff) because rice is grown in an environment conducive to N losses (see Figure 2). This not only represents a cash loss to the farmer but may lead to considerable environmental pollution. In addition, large denitrification losses during the transition from aerobic to anaerobic soil conditions may represent an important source of nitrous oxide, a gas linked with the greenhouse effect and the destruction of the stratospheric ozone layer. It is in this context BNF-derived N assumes importance in the lowland soils that provide about 86% of the world's rice.

Alternative sources of nitrogen

Free-living and associative systems

Diverse N_2-fixing microorganisms (aerobes, facultative anaerobes, heterotrophs, phototrophs) grow in wetland rice fields and contribute to soil N pools. The major BNF systems known include cyanobacteria and photosynthetic bacteria that inhabit floodwaters and the soil surface, and heterotrophic bacteria in the root zone (rhizosphere), or in the bulk soil.

The contributions of cyanobacterial BNF are estimated to be 10–80 kg N ha^{-1} crop^{-1}, averaging about 30 kg N ha^{-1} crop^{-1} (Roger and Watanabe, 1986). Since the discovery of the importance of cyanobacteria in N gain under flooded conditions, many inoculation experiments have been conducted using cultured cyanobacteria to improve soil fertility and grain yields of rice. Based on an extensive review of the literature in which gains in grain yields ranged between 0–3.7 t, Roger and Watanabe (1986) calculated that cyanobacterial inoculation increased rice yields only by an average of 337 kg grain ha^{-1} crop^{-1}.

Heterotrophic bacterial BNF averages 7 kg N ha^{-1} (App et al., 1986), ranging from 11–16 kg N ha^{-1} and contributing to 16–21% of total rice N need (Zhu et al., 1984; Shrestha and Ladha, 1996).

Some free-living heterotrophic bacteria form associations with roots and other submerged portions of the rice plant. Varietal differences in supporting rhizospheric N_2 fixation in rice have been shown (Ladha et al., 1993). The latter raises the possibility to select and breed rice genotypes with higher BNF potential, high soil N uptake, and high N use efficiency to obtain high yields (Ladha et al., 1988, 1993, 1997). Recently Wu et al. (1995) mapped several rice loci mediating the variety dependent ability to stimulate N_2 fixation. Their results indicate that the varietal ability of rice to enhance N_2 fixation in the rhizosphere is controlled by multiple genes. The identification of quantitative trait loci underlying this trait provides the first real evidence for the presence of genetic factors which mediate the interaction with diazotrophs in the rice rhizosphere and lays the foundation for increasing N_2 fixation through genetic manipulation of rice plants and their partner microbes.

Green-manure systems

Aquatic plant like the water fern *Azolla*, and the semi-aquatic legumes such as *Sesbania*, *Aeschynomene* or *Astragalus*, are recommended green-manure plants for rice since they fix N_2 symbiotically. In a continuous long-term experiment at IRRI, an average of 16 crops gave an estimate of about 60 kg N ha^{-1} crop-1 of *Azolla* and *Sesbania* BNF in 50-60 days (Ladha et al., 1993). Yields with *Azolla* and *Sesbania* were equivalent to those with 60 kg N ha^{-1} as urea fertilizer in the wet season. In the dry season, the yields were 6.5 t ha-^{1} with *Sesbania* and 7.7 t ha^{-1} with *Azolla* as compared to 5.8 t ha^{-1} in a treatment of 60 kg N ha^{-1} urea. These data demonstrate the potential of *Azolla* and *Sesbania* to produce yields of 6–8 t ha^{-1}, roughly equivalent to an application of 100-200 kg N ha^{-1} as urea. Farmers, however, usually have little economic incentive to chose *Azolla* or *Sesbania* over N fertilizer since additional costs such as labor, land opportunity, irrigation, seed/inoculum, P and pesticides are involved.

Conventional versus novel systems

Estimates of the N supply potential of different BNF systems are provided in Table 1. Among the conventional systems, the free-living/associative diazotrophs have low to moderate potential to supply N to rice because the N_2 fixed outside the plant is subject to

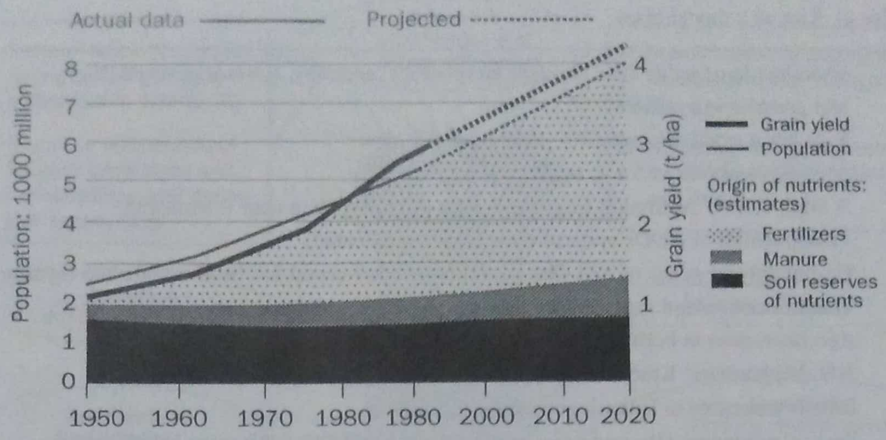

Figure 1. Global trends in population growth, cereal grain yield and origin of plant nutrients (source: Bøckman et al., 1990).

Table 1. Conventional and future biological nitrogen fixation systems for sustainable rice production (from Ladha and Reddy, 1995)

BNF system	N supply potential (kg ha^{-1})	Rice grain yield potential (t ha^{-1})	Essential trait in rice genotype[a]	Technology availability	Economic feasibility and farmers adoption
CONVENTIONAL BNF SYSTEMS					
Free-living/Associative (phototrophs and heterotrophs)	50–100	3–6	ANFS NAE NUE	3–5 yrs	High
Green-manure (*Azolla* and *Sesbania*)	100–200	5–8	NAE NUE	Available	Low
FUTURE BNF SYSTEMS					
Endophytic	?	?	$Endo^{+} fix^{+}$ NUE	3–5 yrs	High
Induced Symbiosis (Rhizobia, *Frankia*, etc)	>200	>8	$nod^{+} fix^{+}$ NUE	>5 yrs	High
nif gene transfer	>200	>8	$nif^{+} fix^{+}$ NUE	>5 yrs	High

[a] ANFS = associative N_2 fixation stimulation; NAE = nitrogen acquisition efficiency; NUE = nitrogen utilization efficiency; Endo = Endophytic; *fix* = N_2 fixation ability; *nif* = N_2 fixation genes; *nod* = nodulation ability.

losses due to various loss processes as described earlier. Therefore, only genotypes having appropriate N use efficiency traits (i.e., large associative N_2 fixation and N acquisition, and efficient N utilization traits) can produce grain yields of 3–5 t ha^{-1} when depending on N supply from free-living/associative diazotrophs (Ladha et al., 1993). However, these yield levels cannot meet the growing demand for rice. Green-manures have high N supply potential to support rice grain yields of 5–8 t ha^{-1}, but due to the reasons discussed above, they do not present an attractive option for the farmer. Green-manures are also known to enhance the emission of methane which contributes to greenhouse effect (see Figure 2). If symbiotic or other nitrogen fixation systems could be incorporated/assembled in rice then the N supply potential could be higher because fixed N

Table 2. Rice as a model plant

- Excellent knowledge base on plant morphology, anatomy, agronomy, physiology and genetics is available;
- Rice has a small genome (415 million bp/1C) size:
  3 x >*Arabidopsis*; 5.5 x < maize; 39 x < wheat);
- A High density restriction fragment length polymorphism map is available;
- Transposition of Ac/Ds systems have been documented;
- Co-linearity of genes of rice with genomes of other cereal has been established (syntony);
- Transformation and regeneration through protoplast, biolistic and *Agrobacterium* in both indica (i.e., IR43; 58; 64; 74) and japonica (i.e., Taipei 309; Nipponbare; Kinokihari; Yamabiko) rice has been achieved;
- Rice is amenable to heterologous gene expression;
- Several agronomically important genes (disease and pest resistance) have been integrated and expressed;
- Inheritance of foreign gene (i.e., *Bt*, *Cryla(b)*; Chitanase, *Chi11*) in both indicas and japonicas has been studied;
- Rice has been extensively utilized in studying the biochemistry and molecular genetics of host-pathogen (bacterial blight, blast, sheath blight, virus) interaction. Disease resistance (bacterial blight and blast) genes have been cloned and their expression is being studied in rice.

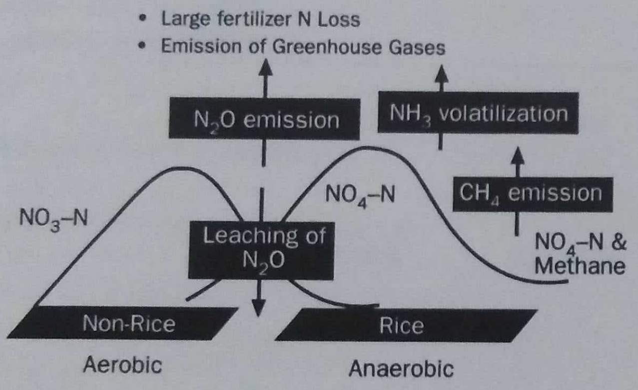

Figure 2. Rice is grown in an environment conducive to significant loss of N.

would be available directly to the plant with little or no loss. This in turn, would promote higher grain yield in rice varieties with efficient N utilization traits.

Assessing opportunities for nitrogen fixation in rice - a frontier project

As pointed out above, more rice must be produced from less land with a minimum of cost. Moreover, environmentally adverse inputs must be reduced to meet the challenge of feeding the world's growing population of rice consumers on a sustainable and equitable basis. Nowhere is this challenge more intense than in supplying N to the crop. IRRI's objective of increasing the yield plateau of rice grown under tropical conditions from 10 to 15 t ha^{-1} for the next 30 yr demands that attention be paid to sustainable methods of N supply. Rice suffers from a mismatch of its N demand and N supplied as fertilizer, resulting in 50–70% loss of applied N fertilizer. Two basic approaches may be used to solve this problem. One is to regulate the timing of N application based on the plant's needs, thus increasing the efficiency of the plant's use of applied N (Cassman et al., 1997). The other is to increase the ability of the rice system to fix its own N (Bennett and Ladha, 1992; Ladha and Reddy, 1995; Reddy and Ladha, 1995; de Bruijn et al., 1995). The latter approach is a long-term strategy, but it has large environmental benefits while helping specifically resource poor farmers. Furthermore, farmers more easily adopt a genotype or variety with useful traits than they do crop and soil management practices that are associated with additional costs. If a BNF system could be assembled in the rice plant itself, it would enhance the N supply potential with little or no loss, besides ensuring no additional economic burden on farmers.

As mentioned above, in 1992, IRRI organized a Think-Tank Workshop to assess the feasibility of (symbiotic) nitrogen fixation in rice (Khush and Bennett, 1992). The experts attending the Think-Tank Meeting agreed to work toward the goal of achieving (symbiotic) associations/nodulation and nitrogen fixation in

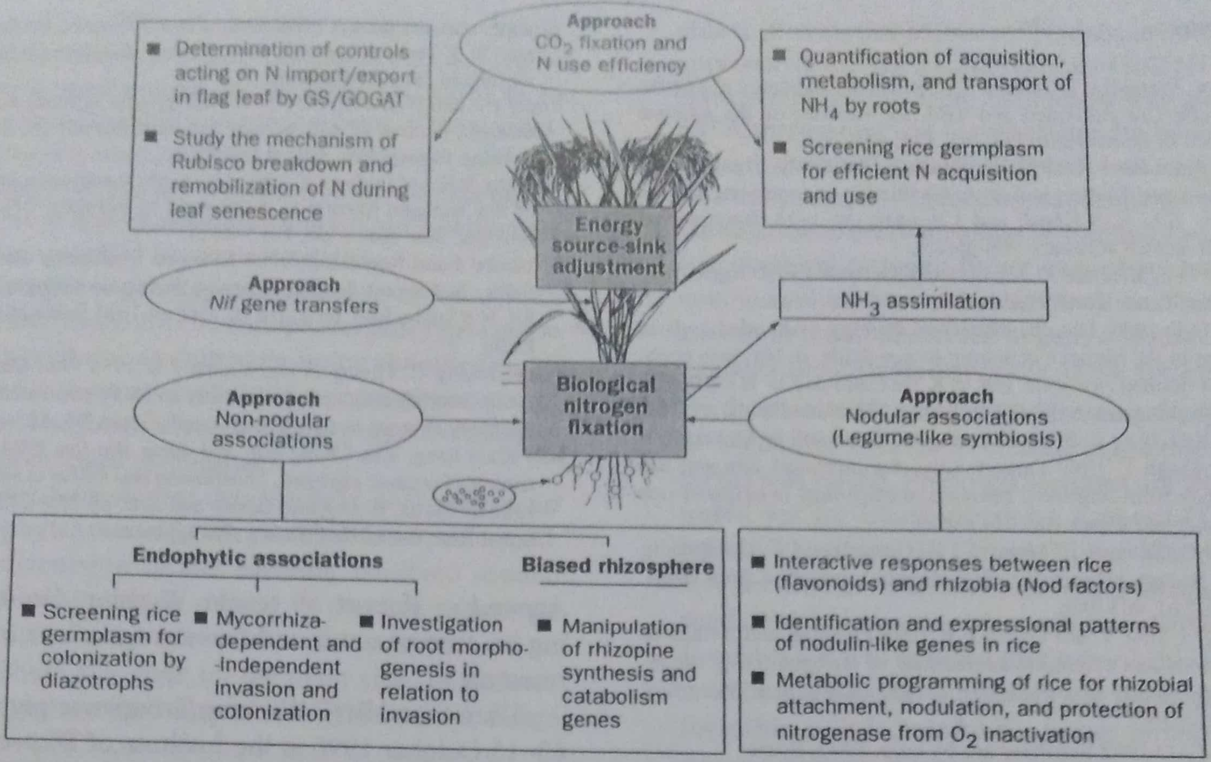

Figure 3. Potential research approaches for achieving improved nitrogen fixation and N use efficiency in rice.

rice. However, they also concluded that exploratory research would clearly be needed to assess the feasibility of novel approaches, that rice was an excellent plant model system (see Table 2), and identified four major short- and long-term approaches (see Figure 3):

- Non-nodular associations - Improve the associations between rice and nitrogen-fixing soil bacteria. This includes achieving colonization and invasion of rice roots by suitable diazotrophs.
- Nodular associations (legume-like symbiosis) - Lay the foundation for the engineering of rice plants capable of “nodulation”. This approach includes identifying compatible rhizobia and varieties of rice, examining the defense response of rice to find ways to avoid responses that would inhibit symbiosis or the nitrogen fixation process.
- Transferring N_2 fixation (*nif*) genes - Transform rice with *nif* genes to ensure expression of nitrogenase, protection of nitrogenase from inactivation by oxygen, and an energy supply for N_2 fixation without compromising yield.
- CO_2 fixation and N use efficiency - Increase the understanding of nitrogen metabolism in rice and impact of N_2 fixation on carbon and energy budgets.

Considerable interest and support for this project was generated since the Think-Tank Workshop. A New Frontier Project, Assessing Opportunities for Nitrogen Fixation in Rice, was developed in 1994 and included in IRRI’s 1994-1997 and 1998-2006 Medium-Term Plans. The long term objective of this project is to enable rice plants to fix their own nitrogen. This research project involves a committed group of scientists from research disciplines and several institutes around the world. The project has a Working Group (WG), through which IRRI facilitates communication among scientists all over the world with active research interests in nitrogen fixation in rice and other cereals (see appendix for a recent WG meeting report on current status and recommendations for future research). The second BNF working group meeting was organized. As a satellite to the meeting on BNF in non-legumes, held in Faisalabad in November of 1996 (see also accompanying volume by Malik et al., 1997). The exciting papers presented at this satellite meeting are included in this volume, and represent a comprehensive and current picture of our knowledge in the area of assessing opportunities for N_2 fixation in rice and other non-legumes.

References

App A A, Watanabe I, Ventura T S, Bravo M and Jurey C D 1986 The effect of cultivated and wild rice varieties on the nitrogen balance of flooded soil. Soil Sci. 141, 448–452.

Bennett J, Ladha J K 1992 Introduction: feasibility of nodulation and nitrogen fixation in rice. *In* Nodulation and nitrogen fixation in rice. Eds. G S Khush and J Bennett. pp 1–14. International Rice Research Institute, Philippines.

Bockman O C, Kaarstad O, Lie O H and Richards I 1990 Agriculture and Fertilizers. Norsk Hydro, Oslo.

Bouldin D R 1986 The chemistry and biology of flooded soils in relation to the nitrogen economy in rice fields. *In* Nitrogen economy of flooded rice soils. Eds. S K De Datta and W H Patrick Jr. pp 1–14. Martinus Nijhoff Publishers, The Netherlands.

Cassman K G, Peng S, Olk D C, Ladha J K, Reichardt W, Dobermann A and Singh U 1997 Opportunities for increased nitrogen use efficiency from improved resource management in irrigated rice systems. Field Crops Res. *(In press).*

Da Silva J G, Serra G E, Moreira J R, Goncalves J C, Goldenberg J 1978 Energy balance for ethyl alcohol production from crops. Science 210, 903–906.

de Bruijn F J, Jing Y and Dazzo F B 1995 Potential and pitfalls of trying to extend symbiotic interactions of nitrogen-fixing organisms to presently non-nodulated plants, such as rice. Plant Soil 172, 207–219.

IFA-IFDC-FAO 1992 Fertilizer use by crop. FAO, Rome.

IRRI 1993 Rice Research in a time of change. International Rice Research Institute's medium-term plan for 1994–1998.

Khush G S and Bennett J (eds) 1992 Nodulation and nitrogen fixation in rice: Potential and prospects. International Rice Research Institute, Philippines.

Kundu D K and Ladha J K 1994 Efficient management of soil and biologically fixed nitrogen in intensively cultivated rice fields. Soil Biol. Biochem. *(In press).*

Ladha J K, Kirk G J D, Bennett J, Peng S, Reddy C K, Reddy P M and Singh U 1997 Opportunities for increased nitrogen use efficiency from improved lowland rice germplasm. Field Crops Res. *(In press).*

Ladha J K and Reddy P M 1995 Extension of nitrogen fixation to rice - Necessity and possibilities. Geojournal 35, 363–372.

Ladha J K, Tirol-Padre A, Punzalan G C, Watanabe I and De Datta S K 1988 Ability of wetland rice to stimulate biological nitrogen fixation and utilize soil nitrogen. *In* Nitrogen fixation: Hundred years after. Eds. H Bothe, F J de Bruijn and W E Newton. pp 747–752. Gustav Fischer, Stuttgart, New York.

Ladha J K, Tirol-Padre A, Reddy C K and Ventura W 1993 Prospects and problems of biological nitrogen fixation in rice production: A critical assessment. *In* New horizons in nitrogen fixation. Eds. R Palacios, J Mora and W E Newton. pp 677–682. Kluwer Academic Publishers, The Netherlands.

Malik K A, Sajjad Mirza M and Ladha J K 1997 Nitrogen fixation with non-legumes. Proceedings of the 7th International Symposium on nitrogen fixation with non-legumes. Kluwer Academic Publishers, Dordrecht. (*In press*).

Mudahar M S 1987a Energy requirements, technology and resources in fertilizer sector. *In* Energy in plant nutrition and pest control. Ed. Z R Helsel. pp 25–62. Elsevier Publications, New York.

Mudahar M S 1987b Energy, efficiency, economics and policy in the fertilizer sector. *In* Energy in plant nutrition and pest control. Ed. Z R Helsel. pp 133–163. Elsevier Publications, New York.

Reddy P M and Ladha J K 1995 Can symbiotic nitrogen fixation be extended to rice? *In* Nitrogen fixation: Fundamentals and applications. Eds. I A Tikhonovich, N A Provorov, V I Romanov and W E Newton. pp 629–633. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Roger P A and Watanabe I 1986 Technologies for utilizing biological nitrogen fixation in wetland rice: Potentialities current usage and limiting factors. Fert. Res. 9, 39–77.

Shrestha R K and Ladha J K 1996 Genotypic variation in promotion of rice nitrogen fixation as determined by nitrogen-^{15}N dilution. Soil Sci. Soc. Am. J. 60, 1815–1821.

Watanabe I and Roger P A 1984 Nitrogen fixation in wetland rice fields. *In* Current developments in biological nitrogen fixation. Ed. N S Subba Rao. pp 237–276. Oxford-IBM Publications, New Delhi.

Wu P, Zhang G, Huang N and Ladha J K 1995 Non-allelic interaction conditioning spikelet sterility in an F_2 population of indica/japonica cross in rice. Theor. Appl. Genet. 91, 825–829.

Zhu Zhao-liang, Liu Chong-qun and Jiang Bai-fan 1984 Mineralization of organic nitrogen, phosphorus and sulfur in some paddy soils of China. *In* Organic matter and rice. pp 259–272. International Rice Research Institute, Philippines.

Appendix: Report of recent Working Group meeting on current status and recommendations for future research.

The Second BNF Working Group meeting was held 13–15 October 1996 at the Institute of Biotechnology and Genetic Engineering (NIBGE) in Faisalabad, Pakistan. It took place in conjunction with the 7th International Symposium on Nitrogen Fixation with Non-legumes on 16–21 October at Faisalabad. Participants shared their results and prioritised the areas of research in rice endophytic/rhizobial interactions and nitrogen metabolism in rice. Following is the summary of the group discussions.

Rice-endophyte interactions

Three approaches to obtain additional N inputs from biological nitrogen fixation in rice were adopted in the 1992 think-tank meeting at IRRI. One of these approaches was to force a tight association between rice and diazotroph to which the sugarcane-*Acetobacter diazotrophicus* interaction could serve as an example. Due to their strategic and advantageous location and the possibility of rapid exchange of nutrients including fixed N, endophytic diazotrophs such as *A. diazotrophicus* are held responsible for the substantial supply of biologically fixed N to the plant.

In the First Working Group meeting held at IRRI in 1995, participating scientists made recommendations for future research in rice-endophyte interaction. Although more than half of these recommendations have been satisfied as shown by the data presented at the Second Working Group meeting held at NIBGE, Faisalabad, much work remains to be done. While some of the studies presently underway may need to be continued, results currently available will allow us to move forward to other important aspects of the project. The following were reported at the meeting:

- Method for surface sterilization of rice tissues to enable proper enumeration and isolation of putative endophytes had been standardized.
- Different rice genotypes had been screened to find a potential diazotrophic endophyte-genotype couple and a predominant nitrogen-fixing endophyte.
- Genetic diversity of putative endophytic population had been studied using polymerase chain reaction-based techniques.
- Colonization studies using mostly non-indigenous endophytes marked with *gusA* had been performed.

The discussion at the meeting revolved around many different aspects related to the rice-endophyte interactions although more discussion was focused on the endophyte side than on the plant side. In summary, following are the important suggestions for future research:

- A need to extend molecular tools in comparison to microbiological methods in trying to find the "right diazotrophic endophyte" and examine the genetic diversity of the diazotrophic endophyte population.
- The *Azoarcus*-Kallar grass system is presently the most studied diazotrophic endophyte-plant system. With this in mind, there is a need to continue finding an endophyte-rice genotype couple, and examine the host genes that are specifically expressed during the development of association with endophytes. Suggestion was made to examine perennial rices to find a "right diazotrophic endophyte" because these rices would provide a greater chance for selecting a more beneficial association than annual rices. Studies should also concentrate on a few chosen endophytes and rice genotypes to establish facts regarding infection, distribution, location, growth stimulation, and nitrogen fixation within the plant. About 200 diazotrophic putative endophytes that have been isolated from rice so far could be used for this purpose to screen for the most potential, stable, and aggressive colonizer.
- There is a need to examine and correlate in planta numbers of endophytes and the level of fixed nitrogen required (aimed at about 50% of the N required by the plant) to produce a real impact on plant growth and yield. It has been calculated that the maximum demand for N is 150 kg ha^{-1} for 7–8 t rice yield levels. If the endophyte system is not able to provide this amount of N, then an integrated approach involving a combination of soil N, added fertilizer N, and N from endophyte was suggested. A three-point test for assessing the benefits an endophyte could give to the plant was suggested. The test includes ^{15}N feeding experiments, growth stimulation to the plant under N-limiting conditions (no potential nitrogen-fixing endophyte has been conclusively shown to benefit plant via fixed nitrogen alone), and use of *nif* negative mutant strains to check the growth responses of the system.
- On the plant side, a need for addressing the question of which rice cultivar to be used in the test experiments was raised. Proposed varieties are IR42 (not tested yet for transformation), Lamont (a hybrid japonica variety), IR72 and Taipei 309 (responsive to transformation), and Nipponbare (small seed, small biomass, photoperiod sensitive, transformable). It was stressed that a rice cultivar which is to be used in the studies should be easily transformable as it is helpful for future molecular and genetic studies. It was, however, pointed out that, at present, the use of cultivar is more important regardless of whether the variety is transformable.

Rice-rhizobial interactions and genetic manipulation of rice for nitrogen fixation/ symbiosis

Of the various naturally occurring nitrogen-fixing plant-microbe associations, the symbiosis between rhizobia and legume plants is the best understood scientifically. Moreover, this association is also the most successful agriculturally, providing fixed nitrogen for many of the world's major crop species (e.g., soybean). Therefore, rhizobia-legume symbioses form natural models for efforts to develop a sustainable nitrogen-fixing system in rice. However, it is recognized that this is likely a long-term approach.

The first working group meeting in 1995 itemized in its report a number of research areas likely to yield important information. Most of these research areas have been addressed since 1995 with some notable success. Following are some of the highlights of the research results reported:

- As outlined in 1995, efforts have been made to understand how rhizobia colonize rice, wheat, and *Arabidopsis* roots using *Azorhizobium caulinodans*. These experiments have revealed a con-

- Identification of niches inside the rice root for the most effective and mutually beneficial exchange of C and N between diazotroph and plant.
- Broadening the scope of research on N uptake beyond ammonium and nitrate to include amino acids and other nitrogenous molecules secreted by diazotrophs.
- Inclusion of diazotrophs in system modeling o C/N interactions in plants.
- Focusing both N metabolism research and BNF research on the rice plant at flowering rather than at the seedling stage.

Guest editors: J K Ladha, F J de Bruijn and K A Malik