Plant and Soil 90, 343–357 (1986).
Ms. NF 27

Nitrogen fixation by non-legumes in tropical agriculture with special reference to wetland rice

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Key words Associative N_2-fixation Azolla Blue-green algae N_2-fixation Paddy soil Rice

Summary Of the 143 million hectares of cultivated rice land in the world, 75% are planted to wetland rice. Wet or flooded conditions favour biological nitrogen fixation by providing (1) photic-oxic floodwater and surface soil for phototrophic, free-living or symbiotic blue-green algae (BGA), and (2) aphotic-anoxic soil for anaerobic or microaerobic, heterotrophic bacteria. The *Azolla-Anabaena* symbiosis can accumulate as much as 200 kg N ha^{-1} in biomass. In tropical flooded fields, biomass production from a single *Azolla* crop is about 15 t fresh weight ha^{-1} or 35 kg N ha^{-1}. Low tolerance for high temperature, insect damage, phosphorus requirement, and maintenance of inoculum, limit application in the tropics. Basic work on taxonomy, sporulation, and breeding of *Azolla* is needed. Although there are many reports of the positive effect of BGA inoculation on rice yield, the mechanisms of yield increase are not known. Efficient ways to increase N_2-fixation by field-grown BGA are not well exploited. Studies on the ecology of floodwater communities are needed to understand the principles of manipulating BGA. Bacteria associated with rice roots and the basal portion of the shoot also fix nitrogen. The system is known as a rhizocoenosis. N_2-fixation in rhizocoenosis in wetland rice is lower than that of *Azolla* or BGA. Ways of manipulating this process are not known. Screening rice varieties that greatly stimulate N_2-fixation may be the most efficient way of manipulating the rhizocoenosis. Stimulation of N_2-fixation by bacterial inoculation needs to be quantified.

Introduction

Rice was selected as a crop that can be grown in flooded conditions. Flooding favours rice growth environments by (1) bringing the soil pH near to neutral, (2) making nutrients like P and Fe more available, (3) depressing soil organic matter decomposition and, thus, maintaining soil N fertility, (4) stimulating N_2-fixation, (5) depressing the outbreak of soil-borne diseases, (6) supplying nutrients from irrigation water, (7) supressing weeds, especially those of C4 type, and (8) acting as a water reservoir and preventing soil erosion.

Thus, rice is grown in flooded conditions wherever water is available in sufficient quantity. About 75% of the 143 million hectares of rice land are lowlands (wetlands), where rice grows in flooded fields during the entire, or part of, the cropping period.

Evidence that flooding favours N_2-fixation in soils have been obtained from long-term fertility trials[48], N balance studies[41], and acetylene reduction assays[56].

Although the amount of N_2 fixed in rice fields is still debatable, estimates made by Burns and Hardy[5] of 30 kg N ha^{-1} yr^{-1}, may be possible. Total N fixed by lowland rice fields is calculated as 3.2 million t N per year. Soybean is grown over an area of 55 million ha (1980 statistics) and the total N fixed by it is of the order of 3.3 million tonnes of N per annum, the N_2-fixation rate being taken as 60 kg N ha^{-1} per crop. Although estimates of N_2-fixing rates are still debatable, this simple calculation shows that N_2-fixation in lowland rice fields in the world is similar to that contributed by soybean. Nevertheless, the amount of research on N_2-fixation in flooded rice field is much less than that devoted to soybean.

Recent price increases in commercial N fertilizer have hit the economy of poor farmers in developing countries. Some governments are planning to remove or reduce fertilizer subsidies, a situation that may result in, or has already brought about, a decrease in N fertilizer consumption. Policy makers have raised the question whether biological nitrogen fixation can supply the N required for rice growth and whether techniques to encourage biological N_2-fixation (BNF) are available to farmers.

In this paper, the author attempts to pinpoint problems of current BNF technology and tries to answer the following:

(1) Can we manipulate BNF systems in flooded rice to increase rice yield?

(2) What are the factors to be manipulated to increase BNF?

Rice field ecosystem and N_2-fixing organisms

Environmental characteristics

The principal environmental characteristics of wetland rice fields are determined by flooding, the presence of rice plants and aquatic weeds, and agricultural practices[49].

Flooding the soil creates anaerobic conditions in the soil a few millimeters beneath the soil surface. Flooding and rice plants together lead to the differentiation of five major environments, differing in physico-chemical and trophic properties: (1) floodwater; (2) surface oxidized soil; (3) reduced soil; (4) rice plants (including submerged part of shoot, roots, rhizosphere and phyllosphere); (5) soil below the puddled layer.

Floodwater is a photic and aerobic environment, where aquatic communities of producers (algae and aquatic weeds) provide organic matter to the soil, and consumers (bacteria, zooplankton, invertebrates, *etc.*) recycle nutrients.

The reduced soil layer is a nonphotic anaerobic environment, where the Eh is predominantly negative, reduction processes predominate, and microbial activity is concentrated in soil aggregates containing organic debris[42].

The rice plant provides two major sub-environments: submerged plant parts and roots. Roots support the growth of bacteria outside and inside the root. The rhizosphere is oxidized by oxidizing ability or oxygen excretion. When the rhizosphere cannot maintain itself in an oxidized state it becomes reduced and substances such as sulphide accumulate[55].

The soil below the puddled layer generally has low microbial activity. Redox potential is determined mostly by the water regime of the paddy fields. In well-drained soils, this layer is oxidized even when flooded. After drainage or drying, the reduced soil becomes re-oxidized.

N_2-fixing microorganisms in wetland rice fields

Because both aerobic and anaerobic, or both photic and nonphotic, conditions exist in the flooded rice field, almost all major N_2-fixing groups can grow in this ecosystem. These are free living and symbiotic autotrophs, symbiotic heterotrophs, and aerobic, facultative anaerobic, and anaerobic free-living heterotrophs. Anaerobic metabolites such as H_2, CH_4, and sulphide can also support chemolithotrophic N_2-fixation at the aerobic/anaerobic interface. Floodwater, the submerged plants, and the aerobic soil surface are sites for photodependent N_2-fixation. Heterotrophic N_2-fixation occurs preferentially in nonphotic environments: the soil aggregates that contain organic debris, and the rhizosphere[42,50].

From the ecological point of view, the major N_2-fixing organisms in rice fields can be classified as:

(1) three groups of autotrophs, namely photosynthetic bacteria, free living blue-green algae (BGA), and symbiotic BGA in azolla; and

(2) two groups of heterotrophs comprising N_2-fixing bacteria in the soil, and N_2-fixing bacteria associated with rice.

N_2-fixation by rhizobia in symbiosis with green manure legumes has long been used as organic fertilizers in rice culture. This topic is beyond the scope of this symposium. Although green manure is not so widely used now as 30 years ago in many countries for socio-economic reasons, its high N_2-fixing rate and technical feasibility should not be overlooked.

Potential of N_2-fixation by various BNF agents

To determine what BNF technologies are used or will be used, we must consider the potential of various BNF agents or their maximum N_2-fixing rates in ideal conditions.

Table 1. Potentialities of BNF in flooded rice soil

Agents	Theoretical (kg N . ha^{-1})	Measured value (kg N . ha^{-1})	References
Azolla	100–190 per one crop of azolla	30–140 per one crop of azolla	21, 47
Blue-green algae	42–150 per one crop of rice	A few – 80 per one crop of rice	30
Associative	40	1.3–7.7 per one crop of rice	see text

Two kinds of estimates of potential are made. One is based on a theoretical consideration in optimum conditions; the second, on measured values. Results are shown in Table 1.

The following section describes the current status of BNF technologies for the use of major BNF agents in flooded rice soils, problems for adoption in wider areas of different environmental and socioeconomic conditions, and research strategies for achieving better use of BNF technologies.

Azolla-Anabaena symbiosis

Current status of utilization

Because of its high N content and rapid growth in flooded soils, *Azolla* has been used as green manure for centuries in northern Vietnam and southern China. Recognized records date back at least to the 11th century in Vietnam and to the Ming dynasty (1368–1644 AD) in China. In both countries techniques of growing *Azolla* for rice culture became a topic of scientific investigation and systematic dissemination in the late 1950s.

In Vietnam, 8–12% of the country's total harvested rice area (5×10^5 ha) was used for growing *Azolla* (personal communication from Nguyen Vy from Vietnam). Statistics in China show 5% of the spring rice area and 2% of the harvested area were used for growing *Azolla*[24].

In Vietnam, *Azolla* is used only before spring rice which is transplanted in early February. *Azolla* is propagated in the main rice fields in December and January, the coolest months. *Azolla* inoculum for crop production is selected, maintained and propagated in Azolla Multiplication Centres, and sometimes cultivated in farmers' cooperatives. In suitable conditions *Azolla* is grown and incorporated with the soil three times before transplanting rice, amounting to 60 t fresh weight ha^{-1}. *Azolla pinnata* has been used and *A. filiculoides* was recently introduced[14].

In China, *Azolla* is now used from 37 (Sandong) to 19°N (Hainan). Because *A. pinnata* was best at 25°C daily average temperature, the seasons of *Azolla* growth in the main rice fields differs from one place to another. *Azolla* grown before early rice, which is sown in spring, is common. *Azolla* is grown either before or after transplanting rice or at both times. Sophisticated and labour-intensive care of *Azolla* nurseries is taken to overcome high temperature (>30°) or low temperature (< 10°C).

Recently *A. filiculoides,* which is favoured by low temperature and produces more biomass than *A. pinnata,* was introduced. The area growing *A. filliculoides* is now increasing[1,2,24]. *Azolla* cultivation technology in China and Vietnam was not known to scientists in the other Asian countries until the mid 1970s. Since then, interest and research in these countries has increased. Since 1978, IRRI has organized workshops, training courses and network trials on the use of *Azolla* (International Network Soil Fertility Fertilizer Evaluation for Rice, INSFFER). *Azolla* growth trials in various parts of the Philippines were a cooperative project between the Ministry of Agriculture and IRRI. Of these trials, that in South Cotabato, Mindanao Island, was the most successful. The inland area of South Cotabao has a long rainy season (9–11 months), a high level of available soil P, and well-irrigated rice fields free from indigenous *Azolla,* with many small surrounding ponds. Farmers in this area are using *Azolla* with little change in farming system and labour input. An economic survey in 1982[21] revealed savings of about US$ 10–37 ha^{-1} by the use of *Azolla.* Initially *A. pinnata* was used, but recently, *A. microphylla* was introduced; both grow well. Stimulated by this success, the Philippine government is advocating the use of *Azolla* in other areas of the Philippines. So far, in no area has *Azolla* technology been adopted by farmers as widely as in South Cotabato.

In other parts of Asia, *Azolla* technology is not beyond small-scale trials in selected areas, although interest has increased not only in Asia but also in Africa[38] and Latin America[11].

Problems of adopting technology

At optimum conditions (22°), *A. pinnata* can produce 100 kg N ha^{-1} *A. filiculoides* 140 kg N ha^{-1}[47], and *A. microphylla* 190 kg N ha^{-1} (unpublished).

Results of INSFFER *Azolla* trials at 18 sites in 1979 and at 12 sites in 1980 gave an average of 15 t ha^{-1} fresh biomass of *A. pinnata* which corresponds to about 30 kg N ha^{-1}[15]. At IRRI, the maximum biomass obtained was 45 kg N ha^{-1}[16].

The major factors limiting the growth of *Azolla* are, first, high temperature in the tropics. Optimum temperature for growing all *Azolla* species is below 25°C[47]. *Azolla filiculoides* is a cold-loving species and *A. pinnata* and *A. microphylla* are more tolerant of high temperature than other species[17].

Second, insect damage and, frequently, fungus attack become severe at higher temperature. Total development period of webworm (Diptoptera, Pyralidae) at Hanzhou, China[1] was 38 days in April and May (average temperature 17°C) and 18–21 days in July and August (average temperature 28°C). In IRRI, the total development period was about 25 days[17]. Rate of growth of the insect increases with temperature, whereas the rate of growth of the host plant decreases. Without insect damage, *Azolla* can produce biomass corresponding to $30\,\text{kg}\,\text{N}.\text{ha}^{-1}$, which is of reasonable agronomic significance, but with insect damage, growth is much less in the tropics.

Third, the supply of nutrients frequently limits *Azolla* growth in floodwater. *Azolla* biomass production higher than $1\,\text{g}\,\text{N}.\text{m}^{-2}$ was obtained in soils with available P (olsen P) contents higher than 25 ppm[52]. Soils from Koronadal, in South Cotabato, Philippines, have a high available P content and low P sorption capacity. In this area, *Azolla* grows well even without P fertilizer and can be a good substitute for N fertilizer.

Phosphorus application can increase *Azolla* growth under economically feasible conditions. Split application of superphosphate can increase N gains of *Azolla* by $4.6\,\text{g}\,\text{N}.\text{g}^{-1}\,\text{P}$[46] The optimum levels of P application are $5.2\,\text{kg}\,\text{P}.\text{ha}^{-1}$ and $2.2\,\text{kg}\,\text{P}.\text{ha}^{-1}$, at the world market price of superphosphate and the Philippine price in 1980, respectively. Cow dung or other animal wastes can partly substitute for phosphorus fertilizer[1].

Azolla inoculum should be kept vegetatively throughout the year. Unless land conditions are suitable for year-round growth, *Azolla* must be grown in nurseries for distribution to farmers. For this purpose, some organization is needed.

In China, trials have been made to evaluate sporocarps for oversummering, overwintering or germplasm preservation, because they are more tolerant of adverse conditions than sporophytes[23]. The growth of the newly germinated sporophytes was, however, too slow to meet the inoculant requirement in the rice fields. More importantly, conditions for sporocarp formation are not known.

Research needs

Azolla is still a wild plant. No attempt has been made to cross it sexually for breeding. At the moment, therefore, selection by naturally grown populations is the only way to get improved strains of *Azolla*. Desirable gronomic traits would be: (1) heat tolerance; (2) tolerance to insect and fungus attack; (3) easier decomposition of the plant N[35] and (4) higher biomass production.

Interspecific crosses may be useful in developing desirable traits. Somatic cell hybridization has been suggested to achieve this goal[37].

Re-inoculating the host with *Anabaena azollae* and producing sporocarps artificially are the greatest problems in the research of the biology of the *Azolla-Anabaena* symbiosis.

Free-living blue-green algae (BGA)

Current status of utilization

Inoculation of rice fields with BGA was initiated in Japan in the early 1950s by Watanabe[43]. Whereas BGA inoculation was completely abandoned in Japan, it was further developed in India, Burma, Egypt, and China. The technique of growing BGA inocula in open-air soil culture in the first three countries is similar[39]. The method is simple, inexpensive, and easily adopted by farmers. The inoculum consists of several species of the genera (*Aulosira, Tolypothrix, Scytonema, Nostoc, Anabaena* and *Plectonema*) provided by research or extension organizations. It is propagated by farmers in shallow trays or tanks with 5–15 cm water, about 4 kg soil/m^2, 100 g triple superphosphate/m^2, and insecticide. In one to three weeks, a thick mat develops on the soil or water surface. The trays are dried, and algal flakes are scraped off and stored in bags for use in the fields. Within 2–3 months, a 2 m^2 tray can produce enough material (10 kg ha^{-1}) to inoculate a hectare. A similar method is used in Egypt, but only the floating algal flake, relatively free of soil, is collected and dried. About 250 g ha^{-1} of dried algal flake is inoculated a week after transplanting rice (Alaa El-Din, personal communication). In Burma, the amount of soil in soil culture is larger than that used in India. In IRRI trials, the Burmese method produced more BGA biomass than the Indian method (unpublished).

The total area inoculated with BGA is still a small fraction of the rice field area in those countries that practise inoculation. Subba Rao[36] wrote that the production capacity of BGA flakes in India was only 0.01% of the total inoculum requirement for the country. In 1982, only 500 ha of Egyptian rice fields were inoculated with BGA (Alaa El-Din, personal communcation). It is, therefore, appropriate to consider the technology at an experimental level of large-scale field testing rather than at a production stage.

The effects of BGA inoculation on rice yield were summarized by Roger and Kulasooriya[30]. Results of field experiments conducted mainly in India report an average 14% yield increase over the control, corresponding to 450 kg grain ha^{-1} per crop, where algal inoculation was used. A similar increase was observed with and without N fertilizer. Because N fertilizer may inhibit growth and N_2-fixing activity of BGA, the effect of algal inoculation in the presence of N fertilizers was usually interpreted as an additional positive effect in addition to stimulation of crop growth by BNF. The author also suggests that the BGA inoculation effect in the presence of N fertilizer is partly due to loss of applied N in floodwater, because ammonia in floodwater is easily lost in conditions that allow algal growth[9].

The need for algal inoculation arose from an earlier belief that N_2-fixing BGA strains were not widely present in rice fields; thus BGA occurred in soil in only 5% of 911 soil samples[44], 33% of 2213 samples[40], and 71% of Japanese soils[27]. In ongoing surveys of Philippine rice soils, we found N_2-fixing strains in all 79 samples collected[31]. N_2-fixing strains are most probably more common in rice fields than was previously thought. Therefore, research should emphasize both inoculation and enhancement of indigenous BGA.

Potential and limiting factors

In rice fields, the N_2-fixing rate of BGA is determined by the area available for their growth, not by water volume. Recent evaluation of blooms in open-air culture indicated a standing biomass of N_2-fixing strains of as much as 150–250 kg dry weight ha^{-1} on an ash-free basis, equivalent to 10–20 kg N ha^{-1}[17]. These values may be considered the maximum standing biomass that can be expected in a rice field at blooming. These underestimates the rate of N_2-fixation, which is the result of the activity of a standing biomass and its turnover.

Unless nutrient supply is limiting, the growth of N_2-fixation of BGA is determined by the availability of light and carbon dioxide in the floodwater and at the soil/water interface. A way to estimate BGA potential is, therefore, to assume that all C input in the floodwater and surface soil is through BGA (which is an overestimation). Saito and Watanabe[32] estimated an input of 0.6 tonnes C in phytoplankton . ha^{-1} during one crop of rice. Using this estimate and assuming the C/N ratio of BGA to be between 4 and 16[16], the potential N_2-fixation would be 42–150 kg N . ha^{-1} per crop.

The practical implication of this calculation is the importance of BGA species high in N content (Table 1). Measured N_2-fixing rates biomasses are much lower than these theoretical maxima. Both biotic and abiotic factors limit the growth and N_2-fixation of BGA. In a rice field, a bloom of BGA does not persist long. Since De's experiment[7], many laboratory experiments have demonstrated the stimulative effect of phosphate application. This effect was more pronounced in acid soils where algal N_2-fixation (acetylene reduction) was lower without phosphate application[53]. Cholitkul *et al.*[6] made ARA surveys in long-term fertility plots in Thailand and found a significant effect of phosphate application on the ARA of blue-green algae in acid sulphate soils. To make phosphate application economically feasible, the cost of phosphate fertilizer must be lower than the cost of N fertilizer equivalent to the increase of N fixation by phosphate. The world market cost of 1 kg P_2O_5 as superphosphate approximately equals that of 1 kg N of urea. Laboratory experiments using neutral or slightly neutral soils[8, 25] gave a ratio (N increase per P_2O_5 applied) smaller than one. As demonstrated of Azolla[46], split application of phosphate may be more efficient in stimulating BGA growth than basal application.

Early experiments on BGA inoculation often failed because of the action of grazers[54]. It was often observed that insecticide application stimulated algal growth in floodwater[28]. Field measurements of ARA, algal biomass and number of grazers revealed that suppression of ostracods (one of the potent grazers in floodwater) by commercial pesticides or neem (*Azadirachta indica*) seeds stimulated algal growth and N_2-fixation by BGA[12]. But when other more effective grazers such as snails were present, suppression of ostracods by insecticidal material was not sufficient[17]. Insecticidal actions were not so persistent as to suppress grazers during a whole crop cycle of rice.

BGA species differ in their tolerance to grazing by ostracods; mucilagenous strains are more tolerant of grazing than nonmucilagenous ones. Because mucilagenous species have lower N and dry matter content, less efficient BGA develop under high pressure for grazers.

Research needs

Most of the inoculation experiments simply reported grain yield increase by algal inoculation. Was the inoculum successfully established? What were the biomass and N_2-fixing rate in the inoculated plots? Could the increase in N_2-fixation by inoculation explain the yield increase? How much of the fixed nitrogen was absorbed by plants?

To answer these questions, a study on the ecology of BGA in flooded rice soils was initiated. We encountered difficulty at first due to the great variability of BGA growth and N_2-fixation, both in space and time? It is felt, therefore, that it is absolutely necessary to standardize assay methods.

Quantitative relationship beween BGA and their grazers (ostracods, snails, daphnids, *etc.*) should be studied. IRRI experiments and other results, however, are limited to irrigated rice fields under intensive cultural practice. Studies in various rice growing conditions are needed. Knowledge of floodwater chemistry is too limited to understand the ecology in floodwater and the complex interactions among floodwater communities.

To see if the observed grain yield difference by algal inoculation is due to enhanced BNF, ^{15}N dilution technique would be useful[41]. As mentioned earlier, cultural methods to stimulate indigenous BGA are as important as algal inoculation. Economically feasible ways of stimulating BGA growth or eliminating grazing pressure should be sought.

Associative N_2-fixation

Recent studies using the ^{15}N technique confirmed that bacteria in association with rice roots and submerged portions of shoots can fix N_2 and provide at least a part of this fixed N to the rice plant[10,18,57]. This system is active only in N_2-fixing wetland rice, not in dryland rice[3]. Studies of bacteria associated with rice roots revealed a wide spectrum of bacteria. Through acetylene reduction assays, differences in supporting ARA among various rice varieties were observed by many researchers[13,19,22,45]. In pot experiments inoculation of azospirilla increased rice yield[29,51]. Using ^{15}N dilution, Watanabe and Lin[51] concluded that stimulation of rice growth was not due to enhanced BNF.

Potential

A rough estimation of the maximum value of heterotrophic N_2-fixation in the rhizosphere can be calculated using estimated C flow from the roots, but no data are available for rice. Sauerbeck and Johnen[34], who grew wheat under $^{14}CO_2$ from the seedling stage to maturity estimated that C respired by microorganisms in the rhizosphere and converted to microbial biomass accounted for 4–5 times the remaining root C at harvest. Using this value and 0.2 g C . ha^{-1} of roots at harvest[32], 1.0 t C . ha^{-1} is estimated to pass through the microbial biomass in the rhizosphere. Assuming that all C is used for N_2-fixation (which does not happen) and 40 mg N is fixed/g C consumed, 40 kg N . ha^{-1} would be the theoretical maximum of associative BNF. In many assays, ARA was highest at or near rice heading stage[4,22,34,45] and ranged from 0.3 μmol C_2H_4/plant h^{-1} in temperate regions[4,58] to 2 μmol C_2H_4/plant h^{-1} in the tropics[4,6,45].

Assuming: (1) that ARA measured at the heading stage continues for 50 d, (2) an acetylene/n conversion rate of 3 : 1, and (3) a plant density of $25/m^2$, the estimated N_2-fixing rate would be 0.8–6 kg N ha^{-1} per cropping season. Extrapolation from $^{15}N_2$ incorporation experiments ranges from 1.3 to 7.2 kg N ha^{-1} per cropping season[10,18,57]. Based on measured and potential estimates on N_2-fixation, it may be said that the potential of associative BNF is the least among N_2-fixing systems discussed in the paper.

Nevertheless, this system would have an advantage if rice varieties that could greatly stimulate this process were grown, because simple adoption of such varieties requires least effort by farmers.

Research needs

To screen varieties that could greatly stimulate N_2-fixation, a rapid and accurate method to identify differences needs to be established. The ARA method reveals the activity only at a given point in time. To identify differences throughout the rice crop growing cycle, several assays are needed. Secondly, variation, particularly in the field, is high. Sometimes, CV is as high as 80%[6]. Some modifications to overcome these difficulties of ARA methods are presented by Ladha in this symposium. We used N balance studied to identify differences among varieties in stimulating N gains and found them to be considerable[16]. The N balance technique requires too much time and labour, however, to be used for routine screening assays. We are developing a ^{15}N dilution technique and found that varieties which showed greater ability in stimulating N gains by N balance studies tended to have lower ^{15}N enrichment[17]. One advantage of the ^{15}N dilution method is that it is a non-destructive measurement. For breeding purposes, destructive methods are not appropriate. Analysis of a single leaf could identify differences but the ^{15}N dilution method requires the 'reference' variety to have little N_2-fixation. The identification of such varieties is necessary.

Inoculation of N_2-fixing bacteria may be another way to stimulate associative BNF. Despite an enormous number of inoculation trials, there are few reports that increased plant growth by inoculation was due to enhanced N_2-fixation except in experiments with gnotobiotic culture. To see the effect of bacterial inoculation on N_2-fixation associated with rice, use of the ^{15}N dilution technique[51] and inoculation of Nif^- mutants[26] are useful.

Of course, little is known about the mechanism of bacteria-plant interaction in rhizocoensis. This topic will be discussed by Balandreau in this symposium.

Conclusion

The use of Azolla to promote BNF in flooded rice is already practised and BGA is at a development stage between experimental station and farm. BNF associated with rice is far from being an established practice. Although inoculation of N_2-fixing bacteria gave some success, there are no convincing data to support the positive effects of N_2-fixation[20].

A common characteristic of technologies currently adopted by farmers is intensive labour use and a requirement for inputs. It is unlikely that BNF could be an exclusive N source for producing high yield in economically feasible conditions. Most probably the future of BNF technologies in rice cultivation is integrated with other technologies, including the proper use of N fertilizers, *i.e.* N fertilizers that will give the least interference to BNF.

Basic research is needed to develop these technologies. This need is not for academic research in advanced laboratories but for an accurate description of phenomena in the field, supported by quantitative measurements and a solving of problems encountered during the application of known technologies.

In developing countries, the adoption of BNF technologies in rice culture is needed quickly. Ironically, although researchers repeatedly claim that BNF research is necessary 'to solve world hunger and poverty', recent developments of BNF research in advanced laboratories have constantly bypassed these countries.

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