PROBLEMS IN APPLICATION OF BIOLOGICAL DINITROGEN FIXATION IN WETLAND RICE*

I. WATANABE, P. A. ROGER, and J. K. LADHA**
Soil Microbiology Department
The International Rice Research Institute
Los Banos, Laguna, Philippines

SUMMARY

Technological constraints and the effects of N fertilizers on main N2-fixing agents for wetland rice -- (1) Azolla-Anabaena symbiosis, (2) free-living blue-green algae (BGA), (3) associative bacteria, and (4) short duration legumes-rhizobia symbiosis -- are described.

None of those agents alone is panacea in solving the shortage of N fertilizer for wetland rice culture. Available technologies for using Azolla and short duration legumes are limited by many technological constraints.

Technologies to consistently increase N2 fixation by free-living BGA and bacteria are not yet available.

INTRODUCTION

Rice is grown in flooded conditions wherever water is available in sufficient quantity. About 75% of ricelands are lowlands (wetlands), where rice grows in flooded fields throughout, or part of, the cropping period. Evidence that flooding favors N2-fixation in soils has been obtained from long-term fertility trials, N-balance studies, and acetylene reduction assays. Although the amount of N2 fixed in ricefields is still debatable, the estimates made by Burns and Hardy (1975) are generally accepted. The value of 30 kg N ha-1 of wetland rice crop corresponds to a yearly global input of 3.2 million ton N. Soybean is grown over an area of 55 million ha (1980 statistics) and the total N it fixes is of the order of 5 million t/annum, the N2-fixation rate being taken as 90 kg N ha-1 per crop.This simple calculation shows that N2 fixation in lowland ricefields in the world is almost approximate to that contributed by soybean. Nevertheless, the amount of research on N2 fixation in flooded ricefields 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 plan to remove or reduce fertilizer subsidies, a situation that may result in, or has brought about, a decrease in Nfertilizer consumption and thus in rice production. Policy makers have raised these questions: 1) can biological nitrogen fixation supply the N required for rice growth, and 2) are techniques to increase biological N2 fixation (BNF) available to farmers?

In some Asian countries, the consumption of chemical fertilizer has increased tremendously in recent years. Policy makers may raise the opposite opinion that BNF is no longer important in industrial or semi-industrial countries. This paper pinpoints the problems of current BNF technology, the factors to manipulate to increase the activity of N2-fixing systems as well as the availability of fixed N to rice, and the effects of N fertilizers on BNF. For additional information, the reader is referred to recent reviews (Watanabe and Roger 1984, Roger and Watanabe 1986, Watanabe 1986).

1. RICEFIELD ECOSYSTEM AND N2-FIXING ORGANISMS

Rice is grown under diverse ecological conditions. Major groups of rice growing environments are (1) irrigated (7.7 x 107 ha), 2) rainfed lowland (3.3 x 107 ha), 3) deep water (1.2 x 107 ha), 4) upland (1.9 x 107 ha), and 5) tidal wetland (5 x 106 ha) (IRRI 1985). The importance of N2-fixation may differ with these conditions. A prolonged dry period between wet season crops would favor N2-fixation of a more aerobic nature. However, N2-fixation under wetland conditions remains the major process.

Flooding the soil creates anaerobic conditions a few millimeters beneath the soil surface. Flooding and rice cultivation lead to the differentiation of five major environments differing in

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**Visiting Scientist, ORSTOM, France.

physicochemical and trophic properties: (l) floodwater, (2) surface oxidized soil, (3) reduced soil, (4) rice plants (including submerged part of shoot, roots, rhizosphere, and phyllosphere), and (5) soil below the puddled layer. Because of the heterogeneous character of flooded rice soils, various groups of N2-fixing organisms -- aerobic and anaerobic, photosynthetic and organotrophic -- contribute to the patchwork of N gains in flooded rice soils (Watanabe et al 1980). From the ecological point of view, the major N2-fixing organisms in ricefields can be classified as (1) three groups of autotrophs, namely photosynthetic bacteria, free-living blue-green algae (BGA), and symbiotic BGA in Azolla; (2) two groups of heterotrophs comprising N2-fixing bacteria in the soil, and N2-fixing bacteria associated with rice; and (3) root-nodule bacteria (Rhizobium). The growth of leguminous crops is possible after or before wet season and allows full use of BNF during the nonflooded period. The recently reported potential of flood-tolerant stem-nodulating legumes opens the possibility of utilizing legume green manures in flood-prone rainfed ricefields during the premonsoon period. This aspect has been included in the paper.

2. AZOLLA-ANABAENA SYMBIOSIS

2.1. Current status of utilization.

Because of its high N content, rapid growth in flooded soils, and ability to grow together with rice, Azolla has been used as green manure for centuries in northern Vietnam and southern China.In both countries, techniques for growing Azolla for rice culture became a topic of scientific investigation and systematic dissemination in the late 1950s. Azolla cultivation technology in China and Vietnam did not become known to scientists in the other Asian countries until the mid 1970s. Since then, interest and research in these countries have increased.

Since 1978, IRRI has organized workshops, training courses and network trials on the use of Azolla (International Network on Soil Fertility and Fertilizer Evaluation for Rice [INSFFER]).

Azolla growth trials in various parts of the Philippines were a cooperative project between the Ministry of Agriculture and Food and IRRI. Of these trials, that in South Cotabato, Mindanao Island, was the most successful. The inland area of South Cotabato has a long rainy season (9-ll months), a high level of available soil P, and well-irrigated ricefields free from indigenous Azolla, with many small surrounding ponds. Farmers in the area are using Azolla with little change in farming system and labor input. An economic survey in 1982 (Kikuchi et al 1984) revealed savings of about US$10-37 ha-1 with the use of Azolla. Initially, A. pinnata was used, but the recently introduced A. microphylla has now almost overcome A. pinnata. Stimulated by this success, the Philippine Government is promoting the use of Azolla in other areas of the Philippines (Mabbayad 1987). 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 in it has increased not only in Asia but also in Africa (Van Hove et al 1983) and Latin America (Fiore 1984). In China and Vietnam, Azolla use as green manure is decreasing, but interest in its other possible uses as fish and animal feed, mineral scavenger, and depollutant has increased (Liu 1984).

2.2. Problems in adopting Azolla technology

At optimum conditions (22o), A. pinnata's maximum biomass is 100 kg N ha-1; A. filiculoides, 140 kg N ha-1 (Watanabe and Berja 1983); and A. microphylla, 190 kg N ha-1 (unpublished). Results of INSFFER Azolla trials at 14 sites in 1983 and 1984 gave an average of 36 t ha-1 fresh biomass of Azolla (2 crops), which corresponds to about 60 kg N ha-1. At IRRI, the maximum biomass obtained was 80 kg N ha-1 from A. microphylla for 28 days (unpublished). Discrepancy of biomass between optimum and actual field conditions are due to many constraints in the field. Technical constraints on Azolla use in the tropics are 1) low temperature tolerance, 2) need for phosphate fertilizer application, 3) insect damage, 4) year-round maintenance of inoculum, and 5) need for good water control.

2.2.1.Temperature. Optimum temperature for most Azolla species is below the average temperature in the tropics. Temperature limitations can be reduced by selecting cold- or heat-tolerant strains (Watanabe and Berja 1983). Among strains so far tested at IRRI, Azolla microphylla #418 was most tolerant of high temperature (37o day/29oC night) (unpublished).

2.2.2. Phosphate application.Surveys of N and P contents of Azolla grown in pots with different soils and in the field showed that the threshold values of P deficiency was 0.4% P in Azolla (dry weight basis), 20 ppm P (Olsen) in soil, and 0.15 ppm P in floodwater (Watanabe and Ramirez 1984; Ali et al 1986). Most Azolla grown in the field had P content below 0.4% and N content below 4% (unpublished). Phosphorus application can increase Azolla growth under economically feasible conditions. Split application of superphosphate can increase N gains of Azolla by 4.6 g N.g-1 P (Watanabe et al 1980). Recent data from A. microphylla were 10 g N.g-1 P (unpublished). Animal dung can partly substitute P requirement. P application in the inoculum increases P content of Azolla and permits the P-enriched Azolla to multiply without P application in the main field until it becomes P deficient (P% J(tab)-OM 0.2) (unpublished).

2.2.3. Year-round maintenance.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 village organization is needed. In China, trials have evaluated sporocarps for oversummering, overwintering, or germplasm preservation, because they are more tolerant of adverse conditions than sporophytes (Lumpkin 1985). The growth of the newly germinated sporophytes was, however, still slow to meet the inoculant requirement in the ricefields. More importantly, conditions for sporocarp formation are notknown. Low temperature stimulates sporulation in the tropics (Kannaiyanand Rains 1985).

2.2.4. Insect damage. Surveys of Azolla insect pests in the Philippines and Asian countries revealed two major insect genera: Elophila (caseworm) and Ephestiopsis (spinning worm) (O. Mochida, personal communication). At 27oC, Elophila finishes its life cycle in about 31-32 days and Ephestiopsis in about 20 days. Yield loss of field-grown Azolla due to these insects ranged from 5.8 to 64% with A. microphylla and from 13 to 57% with A. pinnata. Although application of pesticide is effective, no method of insect control is yet economically feasible (IRRI 1986).

2.3. Effect of N fertilizer

In the absence of competing organisms, N2 fixation by symbiotic Anabaena is more tolerant of combined N than that by free-living microorganisms. Acetylene reduction activities (ARA) were reduced by about one-half by 10 mM urea N or ammonium N and 25 mM of nitrate whereas free-living BGA represses ARA completely after 24-hr exposure to 1 mM ammonium salt (Ito et al 1983). In floodwater, however, the growth of competing aquatic plants may hinder the growth of Azolla. In the the presence of green algae, the growth of A. pinnata was inhibited by 60% by 1.4 mM ammonium-N (Watanabe et al 1977).

Azolla canopy prevents light penetration in the floodwater, inhibits the growth of other phototrophs, and depresses photodependent CO2 uptake. Thus, under azolla canopy, the floodwater pH is maintained much lower than in Azolla-free conditions. The presence of Azolla may therefore be expected to significantly decrease N losses due to ammonium volatilization.

3. FREE-LIVING BLUE-GREEN ALGAE (BGA)

3.1. Use of BGA

Experimental inoculation of ricefields with BGA was initiated in Japan in the early 1950s by Watanabe et al (1951). Whereas research on BGA inoculation was completely abandoned in Japan, it was further pursued in India, Burma, Egypt, and China. A similar technique of growing BGA inocula in open-air soil culture is used in the first three countries (Venkataraman, 1981). The method is simple and inexpensive. In countries that recommend inoculation, the area inoculated with BGA is still a small fraction of the total ricefield area. Subba Rao (1982) wrote that the production capacity of BGA flakes in India was only 0.01% of the total inoculum requirement for the country. In 1985, Roger et al reported that algalization was adopted in only two states of India (Tamil Nadu and Uttar Pradesh) where inoculated fields comprised a few percent of the total area under rice. In 1982, only 500 ha of Egyptian ricefields were inoculated with BGA (Alaa El-Din, personal communication). It is, therefore, appropriate to consider this technology as more 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 (1980). Results of field experiments conducted mainly in India report an average 14% yield increase over the control, corresponding to 450 kg grains ha-1 per crop, where algal inoculation was successful. Actual yield increase may be lower than this value, because negative results were seldom reported. The need for algal inoculation arose from an earlier belief that N2-fixing BGA strains were not widely present in ricefields. Surveys of more than 120 soil samples from 5 Asian countries showed that all rice soils contained N2-fixing BGA at densities ranging from a few dozen to 8 x 106 colony forming units (CFU)/cm2 (median 6 x 104 CFU/cm2). Density of N2-fixing BGA in 20 soil-based inocula, either collected from India, Egypt, and Burma or produced at IRRI, ranged from 105 to 3 x 107 CFU/g dry weight. Combining data on BGA abundance in the abovementioned rice soils and the soil-based inocula showed that, most frequently, the number of CFU of N2-fixing BGA in the quantity of inoculum recommended for application (10 kg/ha) is considerably smaller than that of BGA indigenous in soils. It is, therefore, inferred that inoculation may not be the only way to stimulate N2-fixing BGA (Roger et al 1985, IRRI 1986).

It is often observed that BGA occupy a large surface of flooded rice soils. Surveys of BGA biomass in the field or in small inoculated plots showed that 1) a visible growth of BGA usually corresponds to less than 10 kg N/ha, 2) a dense bloom may correspond to 10-20 kg N/ha (Roger et al 1986), and 3) higher values were recorded only after artificial conditions such as experimental microplots on soil-based inoculum production plots (Roger et al 1985).

3.2. Stimulation of BGA

Factors which stimulate BGA growth and their N2-fixing activity are 1) phosphorus application, 2) grazer control, 3) deep placement of
fertilizers and, 4) straw application.

3.2.1. P-application. Since De's experiment (1936), many laboratory experiments have demonstrated the stimulative effect of phosphate application. This effect was more pronounced in acid soils where algal N2 fixation (acetylene reduction) was lower without phosphate application (Wilson and Alexander 1979). Cholitkul et al (1980) 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 sulfate soils. However, N increase by P application was below 2.3 g N g-1 P, far lower than that obtained with Azolla. The effect of split application needs examination.

3.2.2. Grazer control. Early experiments on BGA inoculation often failed because of the action of grazers (Yamaguchi 1979). Insecticide application was oftenobserved to stimulate algal growth in floodwater (Raghu and MacRae 1967). 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 N2 fixation by BGA (Grant et al 1983, 1985). But when other more effective grazers such as snails were present, the suppression of ostracods by insecticidal material was not sufficient. Insecticidal actions were not so persistent as to suppress grazers during a whole crop cycle (Grant et al 1985). BGA species differ in their tolerance for 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 grazer pressure.

3.2.3. Deep placement of N fertilizer In pot experiments, deep placement of N fertilizer did not inhibit the growth and N2-fixing activity of BGA (Roger et al 1981). Recent field experiments (Reddy and Roger,unpublished) showed that deep placed N fertilizer partially inhibited (30-70%) photodependent BNF.

3.2.4. Surface application of straw. In pot and laboratory experiments, surface application of straw stimulated BGA growth (Roger et al 1982, Barthakur et al 1983, Yoo et al 1984).

While the effectiveness of these practices in increasing BGA growth and/or ARA has been established, no field experiment has yet quantified the relative contribution of the increased BGA activity and the direct effect of the practice to the increase in yield, when some was observed.

3.3. Effect of N fertilizer

Surveys in long-term fertility plots showed N fertilizer application strongly depresses BGA population and their bloom (Watanabe et al 1977). However, deep placement of N fertilizer could reduce the inhibitory effect of N fertilizers on BGA in floodwater as mentioned above.

4. ASSOCIATIVE N2 FIXATION

4.1. Importance of the process

Recent studies using the 15N technique confirmed that bacteria in association with rice roots and submerged portions of shoots can fix N2 and provide part of the fixed N to the rice plant (Eskew et al 1981, Ito et al 1980, Yoshida and Yoneyama 1980). This system is active in wetland rice, but not in dryland rice (Barraquio et al 1982).

Studies of bacteria associated with rice roots revealed a wide spectrum of bacteria (Ladha et al 1986). Through acetylene reduction assays, differences in supporting ARA among various rice varieties were observed by many researchers (Habte and Alexander 1980, Iyama et al 1983, Lee et al 1977, Watanabe and Barraquio 1979). In pot experiments, inoculation of azospirilla increased rice yield (Rao et al 1983, Watanabe and Lin 1984). Nayak et al (1986) employing 15N incorporation and dilution, and Watanabe and Lin (1984) using 15N dilution concluded that stimulation of rice growth by bacterial inoculation was unlikely because of enhanced BNF.

In many assays, ARA was highest at or near rice heading stage, and ranged from 0.3 J-mMmol C2H4/pla nt h-1 in temperate regions (Yoshida et al 1983) to 2 J(tab)-mMmol C2H4 /plant h-1 in the tropics (Watanabe et al 1978, Ladha et al 1986a, b, Boddey and Ahmed 1981). Assuming (l) that ARA measured at heading stage continues for 50 d, (2) an acetylene/N conversion rate of 3:l, and (3) a plant density of 25/m2, the estimated N2-fixing rate would be 0.8-6 kg N ha-1 per cropping season. Extrapolation from 15N2 incorporation experiments ranges from 1.3 to 7.2 kg N ha-1 per cropping season, while N-balance data obtained by Kjeldahl method from wetland rice crops grown in pots covered with black cloth indicated a contribution of N equivalent to 14-18% of total crop N by rhizospheric heterotrophic bacteria (App et al 1980). Based on measured and potential estimates of N2 fixation, it may be said that the extent of associative BNF is the least among N2-fixing systems discussed in the paper. Furthermore, no cultural practices to encourage associative N2 fixation are known. Nevertheless, this system would be of agronomic significance if rice varieties that could stimulate this process were grown, because its moderate potential would be balanced by a wide adaptability by farmers.

Many researchers have expected that there may be varieties which are better stimulators of N2 fixation. Assays by acetylene reduction and total N balance revealed the varietal differences (Ladha et al 1986a, b, IRRI 1983). Generally, long duration varieties, like IR42 are good stimulators of N fixation and/or gains. However, IR50, a short duration variety, also has this trait (Ladha et al 1986a, b, Ladha et al 1987). Natural abundance of 15N in grain slightly differed among varieties (unpublished). It may be possible to use the 15N natural abundance of grain N for screening varieties which may be higher in dependence of biological N2-fixation. However, there is no simple and rapid method for studying a large number of varieties or crossed lines. It is still premature to expect some practical applications from the knowledge of associative N2 fixation.

4.2. Effect of N fertilizer

The inhibitory effect of inorganic N fertilizer on associative N2 fixation is minimal if N fertilizer is applied before transplanting or at an early stage of rice growth (Watanabe et al 1981). In fact, it has recently been found that the application of a low level of inorganic N fertilizer stimulates plant-associative N2 fixation measured by AR assay (Ladha 1986). Associative N2 fixation is most active near heading stage, and at this stage, ammonium concentration at the rhizosphere is negligible. Hence, the effect of N fertilizer on this process is minimum. This is a significant advantage of associative N2 fixation compared with other N2-fixing systems.

5. N2-FIXATION BY SHORT DURATION LEGUMES

If a farmer can use idle land before the wet season rice crop to grow agreen manure for a short period, he can save chemical N fertilizer. Table 1 shows N accumulation of various legumes, determined by the Multiple Cropping Department at IRRI. Vigna can accumulate appreciable amounts of N for short periods (less than 40 days) (Morris et al 1986), but this plant growth was depressed by temporary flooding.

Because the fields are frequently waterlogged before the wet season rice crop in rainfed fields, tolerance for waterlogging is an important character to consider in adopting a green manure. Species of Sesbania have some tolerance for flooding. A N accumulation in the aboveground part of 60-100 kg N/ha for 40-50 days before rice transplanting meets the N fertilizer requirement for rice growth in the tropics. Among all Sesbania spp., S. rostrata has received more attention because of its 1) high N accumulation, 2) flood tolerance, and 3) less inhibition by combined N (Rinaudo et al 1983, Dommergues 1982). S. rostrata can avoid the inhibitive action of flooding and combined N in the root nodules by forming stem nodules (Dommergues 1982).

The green manure crop's ability to scavenge nitrate accumulated during the dry period before flooding is quite important, because otherwise nitrate is lost after flooding. The contribution of N2 fixation
in Sesbania grown in the field is not yet quantified. Constraints in growing fast-growing legumes before wetland rice are 1) high photoperiod sensitivity, 2) need for proper timing of incorporation, and 3) labor requirement for incorporation. At 60 days after sowing, Sesbania rostrata could accumulate 200 kg N/ha, but dry matter was 7 t/ha. It is difficult to incorporate such bulky and partly woody biomass. The rhizobium which forms "stem nodules" (anatomically speaking, these are root nodules) was reported to be specific to stem nodule formation (Dreyfus and Dommergues 1981). The kimeric nature of fast and slow growing rhizobium was reported by (Donald et al 1986). In our experience, the rhizobium forming stem nodules on S. rostrata is widely present in soils and is not so specific. This problem needs further investigation.

6. CONCLUSION

Agronomic significance, technological potentialities, effect of N fertilizer, and environmental constraint on N2-fixing agents discussed in this paper are summarized in Table 2. None of N2-fixing agents alone is panacea for solving the shortage of chemical N fertilizer. They require subtile environmental controls for optimizing their N contribution. The effects of combined N on wet N2-fixing systems in soil are not so simple as those on pure cultures. Competition between N2-fixing and non-N2-fixing organisms which proliferate by using combined N, as well as N adsorption to soil colloids and N absorption by rice complicates the effects of combined N on N2 fixation in flooded soil. Nitrogen fertilizer does not completely depress BNF processes. In the conditions where N fertilizers are abundantly used, rice is still very much dependent on soil N. BNF plays an important role in compensating for N in soil. Basic research is needed to develop BNF 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 the solving of problems encountered during the application of known technologies. In developing countries, the adoption of BNF technologies for 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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