AN OVERVIEW OF THE POTENTIAL AND AGRONOMIC UTILIZATION OF BIOLOGICAL N_2-FIXATION IN WETLAND RICE CULTIVATION.

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SUMMARY

The review summarizes quantitative estimates of biological N_2 fixation (BNF) in ricefields and the current status of the utilization of N_2-fixing organisms as biofertilizer in rice cultivation. Heterotrophic bacteria in rice rhizosphere and the bulk of soil, cyanobacteria, either free-living or in symbiosis with *Azolla*, and legume green manures are considered with regard to their potential for increasing rice yield, the current status of their utilization by farmers, and the prospects for use with regard to the identified limiting factors.

BNF has been the most effective system for sustaining production in low-input traditional rice cultivation. On the other hand, the utilisation of N_2 fixing organisms in intensified rice production systems encounter serious limitations. The utilization of free-living microorganisms (heterotrophic bacteria and cyanobacteria) is refrained by their moderate potential and technological problems, especially the non establishment of inoculated strains. *Azolla* and legumes used as green manures have a high potential as N source, but their utilization is severely limited by socio-economic factors.

CONTENTS

1. INTRODUCTION

More than half the world population is dependant upon rice, which occupied 145 millions hectare of land in 1988 for a global production of 468 million tonnes. In about 75% of rice land rice grows in flooded conditions during part or all the cropping period.

Flooding changes the chemistry, microbiological properties, and nutrient supply capacity of soil. It leads to the differentiation of a range of macro- and micro-environments differing by their redox, physical properties, light status, and nutrient sources for the microflora. As a result, all N_2-fixing groups can and do grow in ricefields: indigenous heterotrophic bacteria --in soil and associated with rice--, photosynthetic bacteria, and cyanobacteria; and introduced *Azolla* and legumes for green manure (Fig. 1). This explains why traditional wetland rice cultivation has been extremely sustainable: because of BNF, a moderate but stable yield has been maintained for thousands of years without without N fertilizer addition and without deterioration of the environment (Bray 1986).

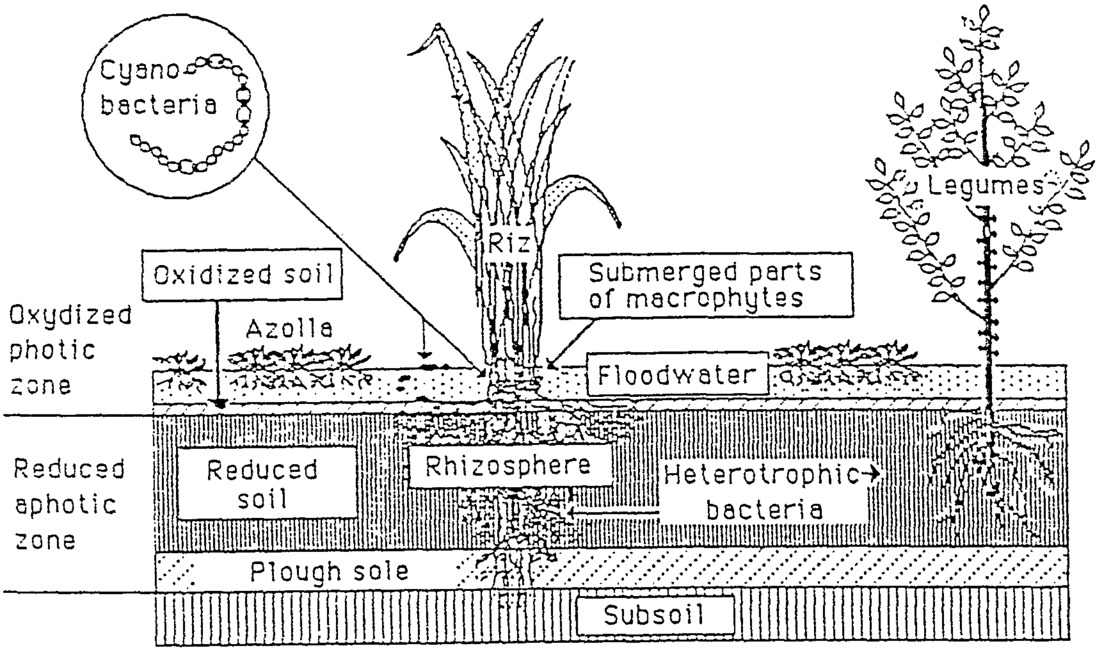


Fig. 1. Macro-environments of the wetland ricefield ecosystem and N_2-fixing organisms.

The oldest technologies utilizing N_2-fixing organisms in ricefields are the incorporation of legumes and *Azolla* as green manures. Legume have been traditionally used in most rice growing countries; the rediscovery of stem nodulating species (Dreyfus and Dommergues 1981) has renewed interest in their potential as green manure in wetlands. The use of *Azolla* dates back to the 11th century in Vietnam and the 14th century in China (Lumpkin and Plucknett 1982). The N_2-fixing symbiont of *Azolla* was identified by Strasburger in 1873 but progress in *Azolla* biotechnology (i.e. recombination and sexual hybridization), is recent (Wei *et al.* 1986, Lin *et al.* 1988, Lin and Watanabe 1988).

The agronomic potential of N_2-fixing cyanobacteria was recognized in 1939 by De, who attributed the natural fertility of wetland ricefields to BNF by these organisms. Research on cyanobacterial inoculation of rice fields was initiated in Japan by Watanabe *et al.* (1951) and then continued in India (Venkataraman 1981). Research on cyanobacteria agroecology in ricefields developed during the last decade (Roger 1991).

Although the presence of N_2-fixing bacteria in rice roots was reported as early as 1929 by Sen, the study of the potential of N_2-fixing heterotrophs started in 1971, when Rinaudo and Dommergues, and Yoshida and Ancajas, using the acetylene reduction assay (ARA), demonstrated that some BNF is associated with wetland rice roots. Early inoculation trials were reported by Dobereiner and Ruschel (1962) with *Beijerinckia*, and by Sundara *et al.* (1962) with *Azotobacter*. Most of the reports on bacterial inoculation of rice fields (40 of 44), however, have been published since 1976. Between 1976 and 1981, most of the trials were with *Azotobacter*. Since 1983, most trials have been with *Azospirillum*. Research on BNF in the rice rhizosphere has also revealed differences in the ability of rice genotypes to stimulate associative BNF and N uptake (Ladha *et al.* 1988c). This suggests that N utilization by rice can be improved by selection and breeding of varieties that can stimulate the development of a more efficient associated microflora.

Reviews related with BNF in ricefields deal with its estimation and contribution to N balance (Roger and Ladha 1992), agronomic use of N_2-fixing biofertilizers (Roger and Watanabe 1986), and the microbial management of wetland ricefields (Roger et al. 1993). Specific reviews on N_2-fixing organisms in ricefields deal with heterotrophs (Yoshida and Rinaudo 1982), BNF associated with straw (Ladha and Bonkerd 1989), rice varietal differences in stimulating BNF (Ladha *et al.* 1988c), cyanobacteria (Roger 1991), *Azolla* (Watanabe 1982), and legume green manures (Ladha *et al.* 1988b, Ladha *et al.* 1992).

This paper summarizes quantitative data on BNF estimates in wetland rice fields and consider for each of the major groups of N_2-fixing organisms, indigenous or introduced in ricefields, the N_2-fixing potential, the potential to increase rice yield, the current status of their utilization, and the prospects with regards to identified limiting factors.

2. ASSESSMENT OF BIOLOGICAL N_2-FIXATION IN RICEFIELDS

2.1. Methods for measuring BNF in ricefields

They are three major approaches to estimate BNF during a crop cycle:

- balance studies in long-term fertility experiments, or in pot experiments conducted over several crop cycles.
- integrating short term measurements performed at intervals during the crop. This approach can be used with acetylene reducing activity (ARA) and short-term ^{15}N incorporation measurements to determine BNF by specific agents. Only ARA has been used for field studies at the crop cycle level.
- determination of the maximum biomass of the N_2-fixing agent and the % N derived from the air (Ndfa) of this biomass. To avoid underestimating N_2 fixed, this requires that the agent studied buit its maximum biomass with little turnover. Therefore, it has been used only with rice and macrophytic green manures (*Azolla* and legumes).

ARA measurement, despite recognized limitations, is still the most popular method. It was used in about 2/3 of the 38 quantitative BNF studies related to rice published since 1985 (Roger and Ladha 1992). Methods where composite and/or standardized samples collected *in situ* are incubated under controlled laboratory conditions have been developed to overcome limitations due to the heterogeneous (log-normal) distribution of photodependant N_2-fixers and the greenhouse effect that develops in enclosures used for incubation *in situ*. Roger *et al.* (1991) drew general conclusions on sampling strategies for a given accuracy.

^{15}N incorporation has been used for short-term studies to assess BNF by various agents, to identify active sites in soil or rice plant, and to establish the C_2H_2/N_2 conversion factor in cyanobacteria and *Azolla* (Watanabe and Roger 1985b, Eskew 1987).

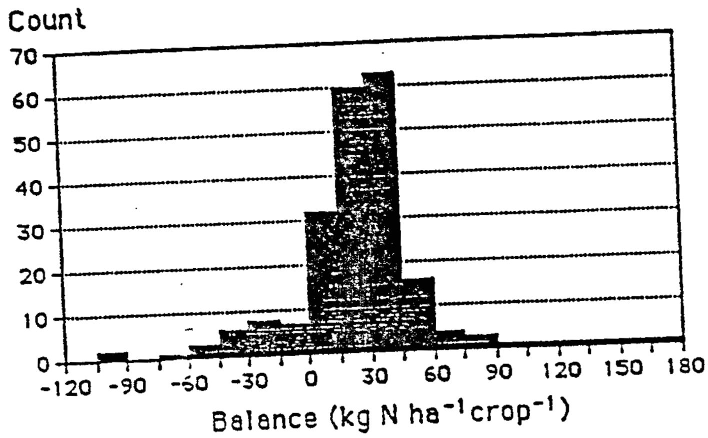
The ^{15}N dilution method is attractive because one sampling can provide an estimate of BNF in plants integrated over time. With aquatic N_2 fixers, fast changes in ^{15}N enrichment of the floodwater over time results in large errors in estimating % N derived from the atmosphere (Ndfa) (Witty 1983). This can be solved by the sequential addition of ^{15}N in water (Kulasooriya *et al.* 1988) but the method is not widely used. This method cannot be used with cyanobacteria because the N level in water sufficient for growth of nonfixing control algae may inhibit cyanobacteria growth directly or through competition (Roger and Ladha 1992). Difference in natural ^{15}N abundance ($\partial^{15}N$) was used to estimate Ndfa in *Azolla* (Yoneyama *et al.* 1987).

Table 1. Bibliographic study of N-balance estimates in wetland ricefields *
(after Roger and Ladha 1992)

1a. Major statistics of the set of data analyzed:

Number of data: 211
Minimum: -102
Mean: 24.2
Standard deviation: 33.1
Unit: kgN ha^{-1} crop $cycle^{-1}$
Maximum: 171
Median: 27.0
Coefficient of variation: 136 %

1 b. Histogram of the data.



1 c. Effect of various factors on N-balance

Factor	Number of data	Mean (kgN ha^{-1} crop $cycle^{-1}$)	Standard error	Level of significance of the difference
N-fertilizer application				1%
-	166	29.7	25.4	
+	45	4.0	47.6	
Planted versus unplanted				1%
+	193	26.5	30.7	
-	18	-0.5	46.2	
Effect of soil exposure to light (all data)				not significant
+	197	25.0	33.9	
-	14	13.2	13.8	
Effect of soil exposure to light (treatments wher no N-fertilizer was applied)				1%
+	152	31.2	25.7	
-	14	13.2	13.8	

1 d. Correlation between N-fertilizer applied and N-balance(kg N ha^{-1}:

Inorganic N-fertilizer:	r = - 0.320 (p> 1%)
Organic N-ferilizer:	r = - 0.157 (p> 1%)
Inorganic and organic N-fertilizer::	r = - 0.365 (p> 1%)

* The set of data is temptatively exhaustive. Data originate from pot and field experiments. Data from pot experiments are extrapolated in kg N ha^{-1} crop $cycle^{-1}$ based on the surface of the pots.

2.2. Estimation of total BNF in wetland ricefields from N balances

Nitrogen balances is currently the only method that provides an estimate of total BNF in ricefields, but the value is underestimated because N losses cannot be taken estimated. Balance studies in the field encounter additional difficulties, as compared with pot experiments, because of sampling errors, unaccounted subsoil contribution, and losses by leaching. Therefore, after early measurements in long-term field experiments (summarized by Greenland and Watanabe 1982), there has been an increased interest in pot studies (App *et al.* 1986, Santiago-Ventura *et al.* 1986, Singh and Singh 1987, Trolldenier 1987).

Table 1 analyses 211 N-balance estimates compiled from the literature (Roger and Ladha 1992). Values range from -102 and +171 kg N ha^{-1} crop $cycle^{-1}$ and average 24 kg N ha^{-1} (Table 1a). Ninety per cent of the values are comprised between -60 and +90 kg N ha^{-1} (Table 1b). Extreme values are from pot experiments conducted over a single crop (Willis and Green 1948) whereas other values are from experiments conducted for at least three crop cycles. N-balance is influenced by N-fertilizer application, the presence of rice, and light availability (Table 1c).

An average positive balance of about 30 kg N ha^{-1} crop $cycle^{-1}$ was obtained when no N-fertilizer was used. This shows that the average potential of BNF in nonfertilized fields can ensure, on a long-term basis, a yield of about 1.5 t ha^{-1} (assuming that, on the long-term, all N_2 fixed is absorbed by the rice plant and 50 kg grain is produced per kg N absorbed).

Balance becomes negligible when N-fertilizer is applied (4kg N ha^{-1} crop $cycle^{-1}$). This results from the two known processes of BNF inhibition by N-fertilizer and N losses by NH_3 volatilization and/or nitrification/denitrification (Roger *et al.* 1987a). A highly significant negative correlation is found between balance value and the quantity of N-fertilizer applied (Table 1d). A lower significance of the correlation observed whith organic manures ($p = 0.05$) as compared with inorganic manures ($p = 0.01$) is in agreement with the observation that N from organic manures is lesss susceptible to losses than N from inorganic fertilizers.

Mean balance values estimated in the presence and in the absence of light (Table 1c) indicates that, on an average, photodependant BNF contributes 2/3 of the balance.

Table 2. Ranges of estimates of N_2 fixed by various agents in wetland ricefields (kgN ha^{-1} $crop^{-1}$) and theoretical maximum potential (after Roger and Ladha 1992).

Organism	Reported values	Maximal theoretical values and hypothesis
BNF associated with rice rhizosphere	1-7 kg N ha^{-1} crop $^{-1}$	40 kg N ha^{-1} crop $^{-1}$ If all rhizospheric bacteria are N_2 fixers, C flow through the rhizosphere is 1 t ha^{-1} $crop^{-1}$, and C efficiency is 40 mg N fixed g C^{-1}
BNF associated with straw	2-4 kg N t^{-1} straw applied	35 kg N ha^{-1} crop $^{-1}$ If 5 t of straw is applied and 7 mg N are fixed g^{-1} of straw
heterotrophic BNF (total)	1-31 kg N ha^{-1} crop $^{-1}$	60 kg N ha^{-1} crop $^{-1}$ If all C input (2 t $crop^{-1}$) is used by N_2-fixers
Cyanobacteria	0-80 kg N ha^{-1} crop $^{-1}$	70 kg N ha^{-1} crop $^{-1}$ If the photosynthetic aquatic biomass is composed exclusively of N_2-fixing cyanobacteria (C/N = 7) and primary production is 0.5 t C ha^{-1} $crop^{-1}$
Azolla	20-150 kgNha^{-1} $crop^{-1}$ in experimental plots, 10-50 in field trials	224 kg N ha^{-1} crop $^{-1}$ If *Azolla* maximum standing crop is 140 kg N ha^{-1}, two *Azolla* crops are grown per rice crop, and Ndfa is 80%
Legume green manures	20-190 kgNha^{-1} $crop^{-1}$	212 kg N ha^{-1} crop $^{-1}$ If 265 kg N ha^{-1} is accumulated in 50-60 d and Ndfa is 80%

3. HETEROTROPHIC N_2-FIXATION

31. Estimations of heterotrophic BNF

Total heterotrophic BNF estimated from N balance in unfertilized planted pots covered with black cloth averaged 7 kg N ha^{-1} (App *et al.* 1980). In similar trials, Trolldenier (1987) found balances negatively correlated with the amount of N applied. Extrapolated values averaged 19 kg N ha^{-1} $crop^{-1}$ with 65 kg N ha^{-1}, - 0.3 with 112 kg N, and -14 with 146 kg N. Using available N of a stabilized ^{15}N-labelled soil as control, Zhu *et al.* (1986) estimated that, when no N-fertilizer was applied and photodependent BNF was refrained, heterotrophic BNF contributed 16-21 % of rice N, or 11-16 kg N ha^{-1} $crop^{-1}$.

BNF associated with rice rhizosphere is usually highest at or near heading stage. ARA ranges from 0.3 µmol C_2H_4 $plant^{-1}$ h^{-1} in temperate regions to 2 µmol C_2H_4 $plant^{-1}$ h^{-1} in the tropics (Roger and Watanabe 1986). Assuming (1) that ARA measured at heading lasts for 50 days, (2) an ethylene/ N_2 ratio of 4:1, and (3) a plant density of 25 m^{-2}, the estimated N_2-fixing rate would be 0.8-6 kg N ha^{-1} crop $cycle^{-1}$. The theoretical maximum associative BNF can be calculated by assuming that all rhizospheric bacteria are N_2 fixers and they use all C flux in rhizosphere (1t ha^{-1} crop $cycle^{-1}$) with a high efficiency of 40mg N g^{-1} C. This would be equivalent to 40 kg N ha^{-1} $crop^{-1}$ (Table 2). But bacterial enumerations in rice rhizosphere often show a ratio higher than 10 between N_2-fixing and total bacteria.

BNF associated with straw. Early estimates of BNF after straw incorporation range from 0.1 to 7 (mean 2.1) mg N g^{-1} straw added, in 30 days (Roger and Watanabe 1986). Most data originate from laboratory incubations in darkness of soil enriched with 1 to 100% straw (average 22%) which simulates composting rather than the field situation where straw left is always less than 1% soil dry weight. Moreover, dark incubation allows heterotrophic BNF only, whereas straw incorporation also may significantly increase populations and N_2-fixing activity of photosynthetic bacteria and cyanobacteria (Ladha and Bonkerd 1988). Estimates of BNF in field experiments with straw are not available, but a few semi-quantitative data and laboratory data suggest that straw might increase BNF by 2-4 kg N t^{-1} applied (Ladha and Bonkerd 1988).

These data suggesting that the N-potential of associative BNF is the lowest among the N_2-fixing agents discussed in this review (Table 2).

32. Potential of heterotrophic BNF for agronomic utilization

No method adoptable by farmers has been yet designed to enhance on purpose heterotrophic BNF in ricefields. Indeed the incorporation of straw or organic matter favors heterotrophic BNF associated with organic debris, but the purpose of this management is to replenish organic matter and nutrients in soil, BNF promotion being only an additional effect.

Research has mostly aimed at promoting associative BNF by inoculating of selected strains of N_2-fixing bacteria. More recently it was found that there is a potential for selecting and breeding rice varieties more efficient in stimulating associative BNF (Ladha*et al.* 1988c).

321. Rice inoculation with N_2-fixing heterotrophs.

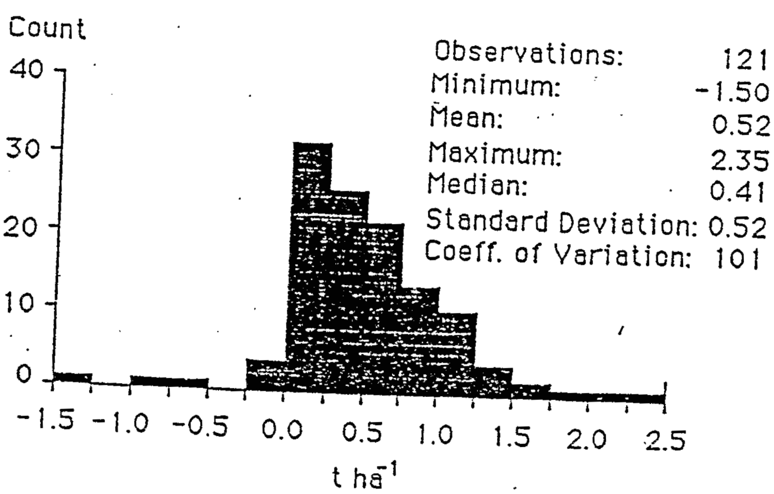
Genera of N_2-fixing bacteria isolated from the rice rhizosphere include *Agromonas, Alcaligenes, Aquaspirillum, Azospirillum, Beijerinckia, Citrobacter, Enterobacter, Flavobacterium, Klebsiella*, and *Pseudomonas* (Roger and Watanabe 1986). Strains most frequently isolated using exudates of rice seedlings as carbon source were *Enterobacteriaceae, Azospirillum* spp., and *Pseudomonas paucimobilis* (Bally *et al.* 1983, Omar *et al.* 1989, Thomas-Bauzon *et al.* 1982). Since N_2-fixing heterotrophs were isolated from rice rhizosphere many trials have been conducted to increase yield by dipping seeds in bacterial cultures or coating them with various carriers, dipping seedlings in cultures, inoculating nursery soil and/or the field, and foliar application.

Table 3 analyses data from 23 articles reporting 210 trials of bacterial inoculation of rice. Most studies reported grain (and sometimes straw) yield. But yield data are difficult to interpret without information on inoculum establishment and N_2-fixing activity by inoculated plants.

Table 3. Bibliographic study of the effect of bacterial inoculation on rice yield.[a] (adapted from Roger *et al.* 1993)

	Grain yield control t/ha	Grain yield difference t/ha	Grain yield difference %	Straw yield difference %	Harvest index (grain/straw) Control	Harvest index (grain/straw) Inoc.	N efficiency (kg grain/kg N) Control	N efficiency (kg grain/kg N) Inoc.
All data (123 field experiments, 87 pot experiments)								
Mean	4.0	0.5	19.8	15.9	0.66	0.69	18.7	19.1
Stdev	1.6	0.5	25.0	16.2	0.20	0.22	17.1	13.7
Maxi	11.1	2.4	125.0	66.7	1.12	1.20	78.0	54.7
Mini	1.0	-1.5	-32.6	-20.9	0.10	0.10	-20.0	-12.0
nb. of data	121	121	210	130	130	130	60	59
Field experiments (123)								
Mean	3.99	0.52	14.35	15.06	0.57	0.57	18.7	19.1
Stdev	1.64	0.52	14.14	14.88	0.17	0.17	17.1	13.8
Maxi	11.09	2.35	59.63	64.84	0.86	0.88	78.0	54.7
Mini	1.00	-1.50	-25.00	-7.49	0.25	0.25	-20.0	-12.0
Count	121	121	123	51	51	51	60	59
Inoculation with *Azotobacter* (40 field experiments, 18 pot experiments)								
mean	3.9	0.4	16.6	13.3	0.52	0.54	18.5	17.0
stdev	1.6	0.5	20.4	15.5	0.20	0.21	18.9	12.3
nb. of data	40	40	58	28	28	28	26	23
Inoculation with *Azospirillum* (83 field experiments, 11 pot experiments)								
mean	4.1	0.6	15.2	16.0	0.62	0.62	18.8	21.4
stdev	1.7	0.5	18.3	13.3	0.21	0.21	16.0	14.8
nb. of data	81	81	94	45	45	45	34	33
Inoculation with other bacteria (58 pot experiments, no field experiment)								
mean			30.6	17.1	0.75	0.81		
stdev			34.2	18.5	0.15	0.15		
nb. of data	0	0	58	57	57	57	0	0

istogam and statistics of yield differences between inoculated and noninoculated eatments



From published data (Table 3), the average effect of inoculation is a 19.8% increase in grain yield, but the response varies from -33% to +125%.

Average increase is significantly higher in pot experiments (27.6%) than in the field (14.4%). Field experiments show an average increase in yield (+14.4%) that is close to the minimum detectable difference (14.5%) which can be expected from the experimental design most commonly used (16-m^2 plots, with 4 replicates) (Gomez 1972). Thus, experiments with no statistical analysis should be inte preted with caution. In field experiments, the relative differences in grain yield between inoculated and noninoculated plots is also highly variable, ranging from -25 to + 69% (coefficient of variation: 100%). The distribution of the values is very asymetrical, and the median (11%) is a better index of the average effect of inoculation than the mean. The histogram, which exhibits an abrupt raise of the first class of positive values, strongly suggests a bias. At least, it indicates that unsuccessful trials were often not reported.

The strain nature might influence the inoculation effect, but average effects of *Azotobacter* (+16.6%) and *Azospirillum* (+15.2%)(Table 3) do not statistically differ. The higher increase observed with other bacteria is, probably, because these experiments were only conducted in pots.

The beneficial effect of bacterial inoculation can be attributed to a combination of (1) increased associative BNF , (2) production of PGRs that favor rice growth and nutrient utilization, (3) increased nutrient availability through solubilization of immobilized nutrients by inoculated bacteria, and (4) competition of inoculated strains with pathogens or detrimental bacteria in the rhizosphere. The relative importance of these four components has not yet been determined.

Current estimates of BNF in rice rhizosphere are insufficient to explain the average 0.5 t ha^{-1} increase in yield reported in field experiments. Assuming that all N fixed is absorbed by the plant, such a yield increse would at least require an increase in BNF by 10 kg N ha^{-1} $crop^{-1}$. But no data demonstrate a marked and durable increase of BNF in inoculated rice.

The hypothesis that PGR production by inoculated bacteria increases nutrient absorption does not agree with the absence of significant difference in N fertilizer efficiency between control plots (18.7 kg grain per kg N applied) and inoculated plots (19.1 kg) in field experiments (Table 3).

Field inoculation experiments show a relative increase in grain yield (14.3%) similar to that of straw yield (15.1%). Similar harvest index values (grain yield/straw yields) observed in the controls and inoculated plots (0.66 and 0.69) indicates that the effect of inoculation probably takes place early in the crop cycle during the vegetative phase.

Little information is available on the establishment of inoculated strains. In most cases, the range of variations of the number of microorganisms were too low to be significant (Roger *et al.* 1993). Utilizing a marker strain of *Azospirillum lipoferum* resistant to streptomycin and rifampicin, Nayak *et al.* (1986) found survival of the strain for 50 to 70 days but no establishment. Inoculated *Azospirillum* were about 500 times less abundant than putative indigenous populations of *Azospirillum* but inoculation increased the dry weight and total N of the plant.

Trials have been conducted to select the most efficient combination of a N_2-fixing bacterial strain and a specific rice cultivar. Heulin *et al.* (1989) used a two-step process in which bacterial strains were first isolated from the rhizosphere of actively N_2-fixing rice plants. Strains were then tested with rice cultivars using a gnotobiotic system known as the spermosphere model (Thomas-Bauzon *et al.* 1982) in which an axenic rice seedling is grown in darkness in a Pankurst tube on a medium without C and N source. This approach has produced both erratic increases in yield (Charyulu *et al.* 1985) and significant increases (6 to 21%) which were higher at the highest level of N-fertilizer (76-96 kg N/ha) (Omar *et al.* 1989). If the validity of the method is confirmed, its potential for practical utilization will strongly depends upon the degree of specificity required to select an efficient bacteria for given agro-ecological conditions.

322. Utilization of varietal differences in promoting associative BNF

The existence of varietal differences in the ability to support associative BNF was demonstrated by N balance studies (App *et al.* 1986), ARA measurements (Tirol-Padre *et al.* 1988), and N isotope ratios (Watanabe *et al.* 1987). Differences were genetically analyzed by Iyama *et al.* (1983). The plant traits associated ($p < 0.01$) with associative BNF determined by Ladha *et al.* (1988) were, by decreasing importance : dry weight of roots and submerged portions of the plant at heading, dry weight of shoots at heading, N uptake at heading, and N uptake at maturity. Using plant traits and a short-term ARA assay, Ladha *et al.* (1987) established a ranking for BNF and N utilization of 21 rice genotypes, which was fairly reproducible in two consecutive dry season trials. Nothing is known, however, about the physiological basis of the apparent varietal differences. The idea of breeding varieties with higher N_2-fixing potential is attractive because it would enhance BNF without additional cultural practices. However, a prerequisite is the availability of a rapid screening technique. Even short-term ARA assays (Tirol-Padre *et al.*, 1988) are time-consuming and do not allow the screening of a large number of genotypes. ^{15}N dilution could be used for screening and genetic studies, but reference varieties with low BNF stimulation ability must first be identified.

33. Prospects

So far, the results of bacterial inoculation experiments are inconsistent. Reported increases in yield have not been related with an increase in BNF. Usually inoculated strains did not clearly establish. Therefore reasons for yield increases reported in some cases are still unclear. The potential of methods aiming at selecting the most efficient combination of a N_2-fixing bacterial strain and a specific rice cultivar needs (1) further confirmation and (2) the determination of the degree of specificity required, which will determine the feasability for practical use. Current knowledge is insufficient to establish inoculations methods that can be used by rice farmers.

The existence of varietal differences in promoting associative BNF offers a promising way of taking advatage of heterotrophic BNF. This potential can be better utilized if screning takes into account both the ability to stimulate BNF and to utilize soil N (Ladha *et al.* 1988c).

4. FREE-LIVING CYANOBACTERIA

Cyanobacteria are photosynthetic prokaryotic microorganisms, which reproduces vegetatively only. They used to be classified with algae as blue-green algae. Morphologically cyanobacteria can be classified into (1) unicellular and filamentous forms, and (2) scum-, mat- or macrocolony-forming groups. Physiologically they can be classified into N_2-fixing and non-N_2-fixing forms.

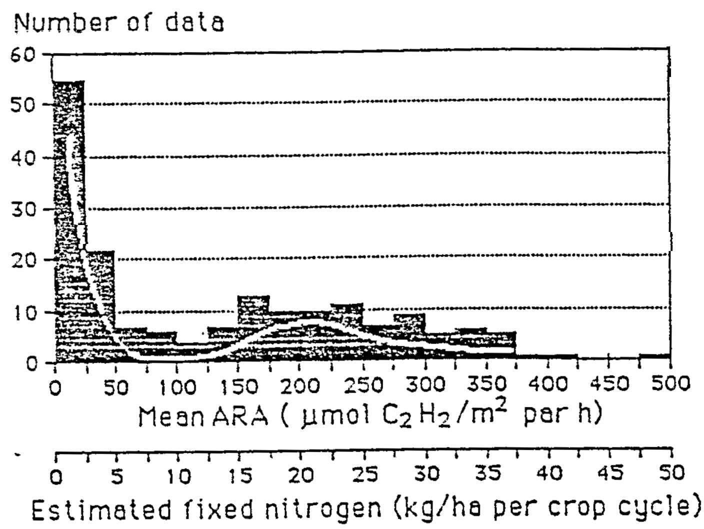
41. Potential of cyanobacteria as a biofertilizer for rice

N_2 fixation by cyanobacteria has been almost exclusively estimated from ARA. Estimates published before 1980 range from a few to 80 kg N ha^{-1} $crop^{-1}$ (mean 27 kg) (Roger and Kulasooriya 1980). About 180 crop cycle measurements in experimental plots at IRRI (Roger *et al.* 1988) show extrapolated values ranging from 0.2 to 50 kg N ha^{-1} $crop^{-1}$ and averaging 20 kg in no-N control plots, 8 kg in plots with broadcast urea, and 12 kg in plots where N was deep-placed (Figure 2). The bimodal histogram shows the combination of a log-normal distribution on the left side, corresponding to plots where BNF was inhibited, mostly by N-fertilizer application, and a bell shaped distribution on the right side, corresponding to plots where a significant BNF developed. BNF was negligible in 75% of the plots where urea was broadcast (Roger *et al.* 1988).

Biomass measurements provide a rough estimate of the N_2-fixing potential of cyanobacteria because they bloom only when the photic zone is depleted of N and most of their N can be assumed to originate from BNF. Inubushi and Watanabe (1986) estimated that cyanobacteria in ^{15}N-labeled plots had about 90% Ndfa. However, biomass measurement may underestimate N_2 fixed because the turnover of the algal biomass is not taken into account.

Fig. 2. Study of 180 estimates of the average **photodependent ARA** during a crop cycle in experimental plots at IRRI (adapted from Roger *et al.* 1988, 1990 a, and unpublished data).

1. Histogram of pooled data[a]



2. Average ARA during the crop cycle and rice yield according to N-fertilizer management[b].

Treatment	Average acetylene reducing activity (μmol C_2H_2 m^{-2} h^{-1})	Grain yield (t ha^{-1})
• Control (No N applied)	195 ± 14	4.08 ± 0.10
• 38 kg N ha^{-1} broadcast at transplanting +17 kg N ha^{-1} at panicle initiation	**80 ± 13**	4.82 ± 0.12
• 55 kg N ha^{-1} deep-placed at transplanting	116 ± 16	5.78 ± 0.09

a: Each of the 180 values is the average of 9-13 daily measurements performed at intervals during a crop cycle. Each daily measurement was performed on a composite sample of 13 core samples comprised of the fist cm of soil and floodwater.
The left part of the histogram corresponds mostly to plots where N-fertilizer was broadcast in the floodwater. The right part of the histogram corresponds mostly to control plots where no N-fertilizer was applied and plots whereN-fertilizer was deep-placed.
b Each value is the average of 60 data.

A visible growth of cyanobacteria usually corresponds to less than 10 kg N ha^{-1}, a dense bloom may correspond to 10-20 kg N ha^{-1} ; larger biomasses (20-45 kgN ha^{-1}) are recorded only in experimental microplots or in inoculum production plots (IRRI 1986, Roger *et al.* 1985ab, 1987 ab). More than two blooms of N_2-fixing cyanobacteria is a rare occurrence during a crop cycle. Therefore 20-30 kg N ha^{-1} per crop seems a reasonable estimate of photodependent BNF when a dense cyanobacterial growth is visible.

The theoretical maximum BNF by cyanobacteria can be calculated by assuming that the photosynthetic aquatic biomass is composed exclusively of N_2-fixing cyanobacteria (C/N = 7) and primary production is 0.5 t C ha^{-1} $crop^{-1}$. This would be equivalent to 70 kg N ha^{-1} $crop^{-1}$ (Table 2).

Table 3. Compilation of estimates of nitrogen recovered from biofertilizers by rice (adapted from Roger *et al.* 1977a, Biswas 1988, et Diekmann *et al.* 1991).

Studied material: Nature	Studied material: State	Fauna[a]	Experiment design	% N recovered: Surface applied	% N recovered: Soil incorporated	Reference
Cyanobacteria						
Anabaena	fresh	?	pot	37	52	Wilson *et al.* 1980
Nostoc	dry	-	pot	14	28	Tirol *et al.* 1982
" "	dry	+	*in situ*	23	23	" "
" "	frais	-	pot	-	38	" "
Anabaena	fresh	-	pot	24	44	Grant and Seegers
" "	fresh	+	pot	25	30	1985
Anabaena	dry	-	pot	-	35-40	Mian & Stewart 1985
Mean				25	36	
Aquatic macrophytes						
Eichornia sp.	fresh	+	*in situ*	-	25	Shi *et al.* 1980
Azolla pinnata	fresh	+	*in situ*	-	26	Watanabe *et al.* 1981
A. caroliniana	fresh	+	*in situ*	12/14	26	Ito et Watanabe 1985
A. caroliniana	dry	?	pot	-	34	Mian & Stewart 1985
A. caroliniana	fresh	+	*in situ*	-	32	Kumarasinghe *et al.* 1986
Mean				13	29	
Legumes						
Sesbania rostrata	fresh	+	*in situ*	---	32	Biswas 1988
" "	fresh	+	*in situ*	---	49	Biswas 1988
" "	fresh	+	*in situ*	---	42	Diekmann *et al.* 1991
Aeschynomene	fresh	+	*in situ*	---	47	Diekmann *et al.* 1991
afraspera	fresh	+	*in situ*	---	40	Diekmann *et al.* 1991
Mean					42	

a : + : present in the soil ; - : absent

Recovery of cyanobacteria N by rice, estimated from studies with ^{15}N labelled strains, averages 30% and varies from 13 to 50%, depending on the nature of the material, the method of application, and the presence or absence of soil fauna (Table 4). Recovery was highest with fresh cyanobacteria incorporated into a soil depleted of fauna (Grant and Seegers 1985).

Possible beneficial effects of cyanobacteria other than provision of N include (1) competition with weeds, (2) increased soil organic matter content and aggregation, (3) excretion of organic acids that increase P availability to rice, (4) decrease of sulphide injury in sulfate reduction-prone soils by increased O_2 content and plant resistance to sulfide, and (5) production of plant growth regulators (PGR) that enhance rice growth. But this last aspect still needs to be demontrated because (1) cyanobacteria extracts may also negatively affect rice germination (Pedurand and Reynaud 1987), and (2) as Metting and Pyne (1986) pointed out, despite the numerous reports on algal PGR effects, none shows the isolation and characterization of a microalgal PGR.

4.2. Algal inoculation technology and its current status

Experimental cyanobacteria inoculation of ricefields initiated in Japan by Watanabe et al.(1951) was subsequently abandoned there. Applied research on cyanobacteria inoculation has been conducted mostly in India where the All-India Coordinated Project on Algae was initiated in 1977 and, to a lesser extent, in Burma, Egypt, and China. A similar technique of inoculum production in shallow open-air ponds is used in India, Egypt, and Burma

(Venkataraman 1981). A multistrain starter inoculum produced from laboratory cultures is propagated, on the spot, in trays or microplots with 5-15 cm water, about 4 kg soil m^{-2}, 100 g superphosphate m^{-2}, and insecticide. When necessary, lime is added to adjust soil pH to 7.0-7.5. In 1-3 weeks, an algal mat develops which is then allowed to dry. Algal flakes are scraped off and stored for further use at 10 kg ha^{-1}.

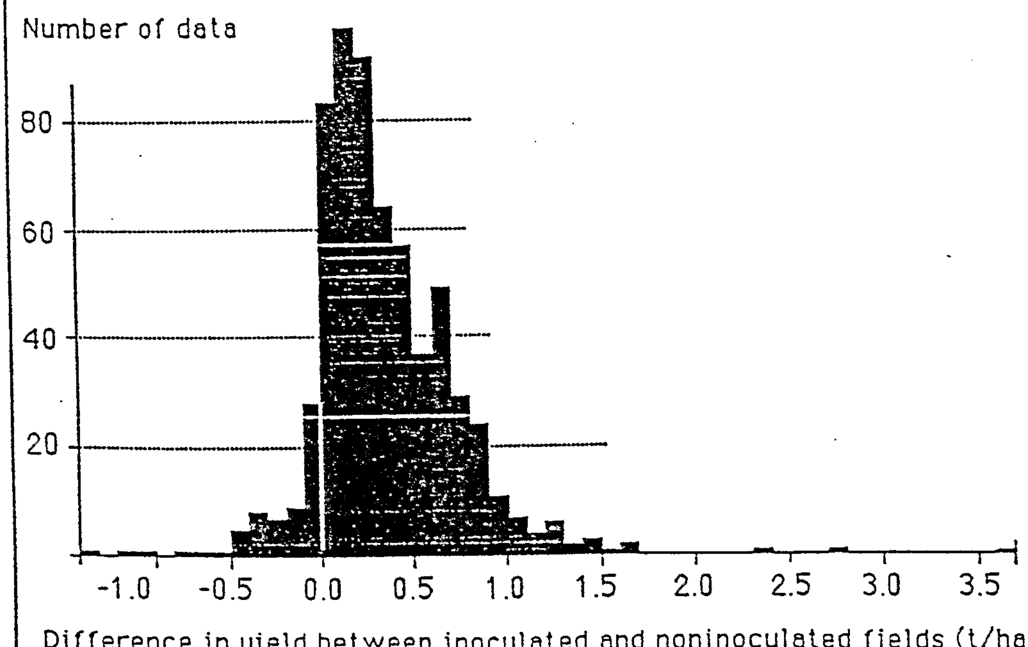
Table 5 presents the analysis of 634 field experiments. The difference in yield between inoculated and noninoculated plots is very variable (C.V. > 100%). Because of the asymmetrical data distribution, the median grain yield (257 kg ha^{-1}) was considered a better index of the average effect of inoculation than the mean (337 kg ha^{-1}). While the difference in average yield between inoculated and noninoculated plots was significant at $p < 0.01$, only 17 % of the 634 individual observed differences were statistically significant.

Table 5. Bibliographic study of the effect of cyanobacterial inoculation on rice yield[a]. (from Roger 1991)

1. Major statistics of the data

	Difference between control and inoculated plots: Absolute (kg ha^{-1})	Relative (%)
Number of observations	634	634
Maximum	3700	168.2
Minimum	-1280	-19.3
Average	337	11.3
Median	257	7.9
Standard deviation	398	16.0
Coefficient of variation	118	141

2. Histogram of the data



[a] Data compiled from 41 references listed in Roger 1991.

This indicates a small and variable response of yield to algal inoculation and also an experimental error frequently larger than the response. When interpreting data from the literature, it should also be kept in mind that unsuccessful trials have often not been reported. When they were mentioned, it was usually without quantitative data that could explain the possible reason for failure. For example, a report of a multilocation trial (Pillai 1980) indicates that notwithstanding the 22 sets of data presented, "the results from many other locations in South India, Deccan, and the Konkan region were not received because of the failure of multiplying cyanobacteria at these locations."

Cyanobacteria inoculation is currently used on a trial-and-error basis. Methods to estimate the chance of success of inoculation in a given agroecosystem are unavailable because the factors underlying yield increases associated with successful algal inoculation are not clearly understood or quantified. No published study reporting a significant increase in yield after cyanobacterial inoculation includes estimation of inoculum quality, BNF measurement, or biomass estimates.

Reports on the adoption of algal inoculation are somewhat controversial, but even with the most optimistic evaluations, adoption seems to be restricted to a limited area in a few Indian states, in Egypt, and possibly in Burma. In 1985, Roger *et al.* reported that algalization was adopted in only two states of India (Tamil Nadu and Uttar Pradesh) where inoculated fields constituted a small percentage of the total area planted to rice. Farmers' limited acceptance of algalization probably reflects the low and erratic increases in yield obtained.

43. Prospects

Methods for utilizing cyanobacteria in rice cultivation need to be reconsidered in view of the results of the agroecological studies of the last decade:

- Because of the earlier belief that N_2-fixing cyanobacteria were not common in many rice soils, research has focused on inoculation. However, surveys have shown that they are ubiquitous in rice soils at densities averaging $5\ 10^4$ cm^{-2} (Roger *et al.* 1987b).
- The study of the ratio of indigenous heterocystous cyanobacteria in 102 soils (1st cm of soil over one ha) to heterocystous cyanobacteria contained in the recommended dose of 22 soil-based inocula (10 kg ha^{-1}) showed that in 90% of the cases, indigenous cyanobacteria were more abundant than cyanobacteria in the inoculum (Roger *et al.* 1987b).
- Results also show the infrequent establishment of nonindigenous strains inoculated in various soils, even when grazers were controlled (Grant *et al.* 1985, Reddy and Roger 1988, Reynaud and Metting 1988). While cyanobacteria inoculated in five soils persisted for at least 1 month, their growth was rare (one out of 10 cases). Blooms developed on all soils when grazers were controlled, but were mostly of indigenous strains (Reddy and Roger 1988).

This suggests that attention should be paid to practices that enhance the growth of indigenous strains already adapted to the environment. Their growth is most commonly limited by low pH, P deficiency, grazing, and broadcasting of N-fertilizer. Cultural practices that alleviate these limiting factors often favor cyanobacteria growth.

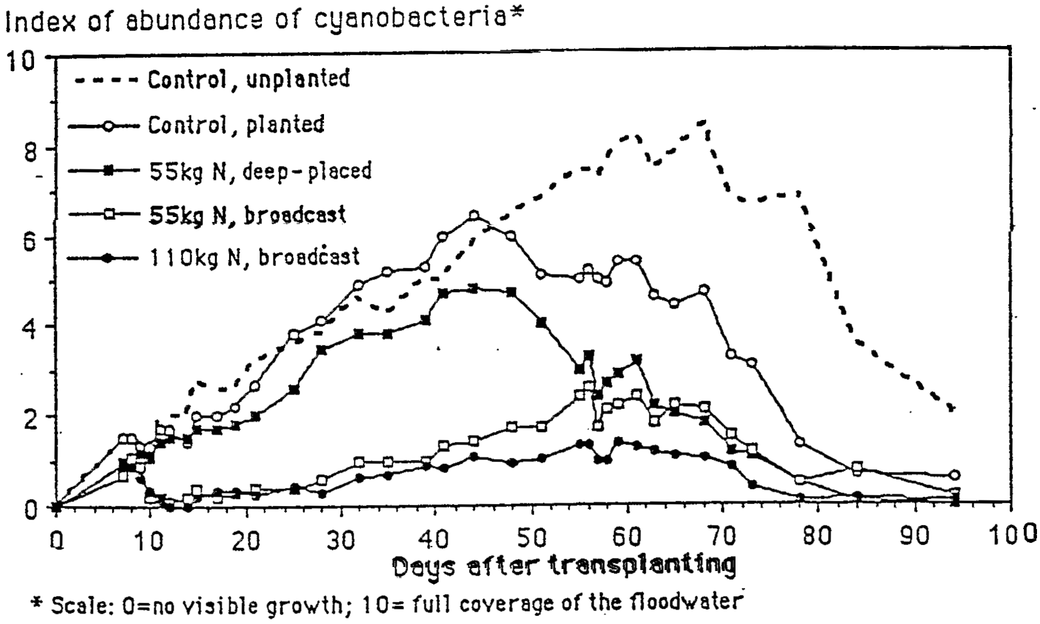
Liming is known to favor cyanobacteria growth (Roger and Kulasooriya 1980) but is rarely economically feasible.

Many reports show that P application stimulates photodependent BNF and cyanobacteria growth (Roger and Kulasooriya 1980). But efficiency is low (2.3 g N g^{-1} P) (Cholitkul *et al.* 1980). Split application is more efficient than basal application (IRRI 1986).

Controlling grazers especially ostracods, enhances cyanobacteria growth (Grant *et al.* 1985). It can be achieved with conventional pesticides but their cost is prohibitive for the result achieved. Pesticides of plant origin might be more economical. Wetting and drying at select times is an alternative way of controlling grazers in wetland soils.

The study of different methods of N-fertilizer application has shown that surface broadcast application of N-fertilizer, which is widely practiced by farmers, inhibits photodependent BNF (Table 2) and causes N-losses by ammonia volatilization (Roger *et al.* 1980, Fillery *et al.* 1986). In contrast, the deep-placement of N-fertilizer decreases its inhibitory effect on cyanobacteria and reduces N-losses by volatilization (Fig. 3). Delaying N-fertilizer application could also possibly allow the growth of a N_2-fixing algal bloom at the early stages of the crop, but the resulting effects on N- losses from fertilizer applied in an algal-rich water are unknown.

Fig. 3. Dynamics of N_2-fixing cyanob cteria during a crop cycle (IRRI 1991).



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

The ubiquity of N_2-fixing cyanobacteria in rice soils does mean that inoculation is unnecessary. Inoculating indigenous strains--able to establish-- might be useful because P accumulation by the propagules of the inoculum (produced with high levels of P) gives them an initial advantage over the indigenous propagules which are usually P-deficient (Roger *et al.* 1986). Since spore germination is photodependent (Reddy 1983), inoculated propagules applied on the soil surface might germinate better than the indigenous ones mixed with the soil. Inoculation with indigenous strains is likely to be useful after an upland crop grown before rice or after a long dry fallow because the low density of natural population density may lead to a lag of several weeks before N_2-fixation becomes significant. In fact, the positive effects of cyanobacteria inoculation observed with the method recommended in India could be due to indigenous strains, because when inoculum is multiplied on the spot in shallow trays or plots, it is probable that strains present in the local soil may outgrow the original isolates even before inoculum is added to the field.

In the absence of knowledge on factors that allows foreign strains to establish in a field, the agronomic potential of cyanobacteria inoculation is probably limited to indigenous strains used in agroecosystems favorable to cyanobacteria growth when inoculation allows to speed up the formation of an N_2-fixing bloom early in the crop cycle. Soil properties, climatic conditions and cultural practices needed for such conditions to occur probably limit the usefulness of cyanobacteria inoculation to a small percentage of the world's ricefields.

5. SYMBIOTIC CYANOBACTERIA: *AZOLLA*

Azolla is an aquatic fern which harbors the symbiotic N_2-fixing cyanobacteria *Anabaena azollae*. It has been used as agreen manure in rice cultivation in Vietnam and China for centuries. Spontaneous development of *Azolla* in ricefields is much less frequent than that of free-living cyanobacteria, which are ubiquitous. *Azolla* usually needs to be inoculated and grown when used as green manure.

51. Potential as a biofertilizer for rice

BNF by *Azolla* has usually been estimated from biomass measurement and the assumption that most of *Azolla* N originates from BNF. Recent measurements show an average Ndfa of 75% in *Azolla* (Table 6).

The N potential of *Azolla* was summarized by Roger and Watanabe (1986) from data obtained mostly in experimental plots. The N content in maximum standing crops ranged from 20 to 146 kg ha^{-1} and averaged 70 kg ha^{-1} (n = 17; c.v. = 58%). N_2-fixing rate ranged from 0.4 to 3.6 kg N ha^{-1} d^{-1} and averaged 2 kg N ha^{-1} d^{-1} (n = 15, c.v. = 47%). The maximum N potential of *Azolla*, calculated from maximum bimass recorded in experimental plots and assuming that two crops of *Azolla* with a Ndfa of 80% are grown per rice crop is 224 kg N^{-1} crop $cycle^{-1}$ (Table 2). But, in a field trial at 37 sites in 10 countries, productivity was lower than in experimental plots (Watanabe 1987). Biomass was 5-25 t fresh weight ha^{-1} (10-50 kg N ha^{-1}) for*Azolla* grown before or after transplanting (average 15 t ha^{-1} or 30 kg N). Comparisons with inorganic fertilizers showed that one crop of *Azolla* incorporated before or after transplanting was equivalent to the application of 30 kg N ha^{-1} as urea (Table 7).

N recovered by rice from ^{15}N-labeled *Azolla* incorporated into the soil was 20-34%. As with cyanobacteria, availability decreased when *Azolla* was surface applied (Table 3).

Besides providing N to the rice crop, *Azolla* has several other advantages. Because of its lower K absorption threshold in floodwater than rice, *Azolla* becomes a source of K for rice when incorporated (Liu 1987). *Azolla* also enhances the utilization of P fertilizer (Sampaio *et al.* 1984), decreases weed incidence (Diara *et al.* 1987, Lumpkin and Plucknett 1982), reduces water evaporation (Diara and Van Hoove 1984), and improves soil structure, which is important where rice is grown sequentially with an upland crop (Roychoudhury *et al.* 1983).

Table 6. *Azolla* N derived from biological N_2-fixation (after Watanabe *et al.* 1991)

Reference	Non-fixing control	Ndfa %	Notes
Kumarasinghe *et al.*	*Salvinia*	92	+ 15 mg N/microplot
(1985)	"	79	+ 75 mg N/microplot
	"	76	+ 150 mg N/microplot
	Lemna /Salvinia	81-83	planted
	"	82-79	unplanted
You C.B. *et al.*	*Lemna*	40-59	
1987)	"	63-71	labelled *Azolla*
ulasooriya *et al.*	*Salvinia /Lemna*	52-55	unplanted, + 40ppm N
988)	"	58-64	planted, + 40ppm N
atanabe *et al.*	*Lemna /Spirogyra*	86-93	3 *Azolla* crops
990)	*Lemna*	80-81	after one crop of rice
verage		74	

Table 7. INSFFER *Azolla* trials at 37 sites in 10 countries (after Watanabe 1987).

Treatment	Yield (t/ha)	Index
Control, no N, no *Azolla*	3.00	100 c
30 kg N/ha, 3 split applications	3.65	121 b
60 kg N/ha, 3 split applications	4.24	141 a
Azolla incorporated before transplanting	3.73	124 b
Azolla incorporated after transplanting	3.67	122 b
Azolla inoculated but not incorporated	3.61	120 b
Combination of treatments 2 and 4	4.15	138 a
Combination of treatments 2 and 5	4.07	135 a
Azolla incorporated before and after transplanting	4.09	136 a
ndard error between and within sites	0.05	

Table 8. Evolution of *Azolla* utilization in rice cultivation.

• Countries where *Azolla* has been used by rice farmers:		
China:		
before 1978:	> 6.5 million ha	(FAO 1978)
before 1979:	1.34 million ha	(Liu Chung Chu 1979)
before 1980:	0.7 million ha	(Lumpkin *and* Plucknett 1982)
1987:	decrease of use as green manure, reseach on *Azolla* as animal feed	(Liu Chung Chu 1987)
1989:	reseach on Rice-fish-*Azolla* and beginning of adoption	(FAO 1988) (Liu Chung Chu 1988)
Vietnam:		
in 1980:	about 500 000 ha	(Roger *and* Watanabe 1986)
since 1980:	use has been continuously decreasing	
Philippines:		
in 1981:	adoption on 5,000 ha	(Kikuchi *et al.* 1984)
in 1986:	84,000 ha	(*Azolla* Workshop, IRRI 1987)
since 1986:	use has been continuously decreasing	
• Countries where *Azolla* has been tested for adoption:		
Brasil, India, Italy, Pakistan, Senegal, Sri Lanka, Thailand.		
•In 1992 *Azolla* is probably used in less than 1% of the rice growing area.		

5.2. Current usage

Estimates of the extent of *Azolla* use (Table 8) show a marked decrease during the 1980s in Vietnam and China, where it has been a traditional technology. During the same period, *Azolla* has been tested in Brazil, India, Pakistan, Philippines, Senegal, Sri Lanka, and Thailand. Philippine is the only country were adoption was sufficient to be quantified during the 1986 *Azolla* Workshop (IRRI 1987). In this country, farmers adopted *Azolla* on 5,000 ha in South Cotabato in 1981 (Kikuchi *et al.* 1984); success was due mainly to a high level of available P in the soils and a short dry season. *Azolla* utilization extended to 26,000 ha in 1983, and 84,000 ha in 1985 (Mabbayad 1987). Since then, *Azolla* use has not progressed in the Philippines and has probably decreased.

Decrease in *Azolla* use in China has been attributed to the advent of available, cheap sources of urea and potash, and the changing governmental economic policy which has led to the disbanding of many agricultural communes and the reallocation of labor (Roger *et al.* 1993). This has also been observed for legume green manures (Stone 1990). However, interest in *Azolla* use as fish and animal feed, primary producer in rice-*Azolla*-fish culture, mineral scavenger, and depollutant has increased in China (Liu ChungChu 1987). World use is now a fraction of the estimated 2 million hectares of rice that were fertilized with *Azolla* in China and Vietnam in the late 1970s (Roger and Watanabe 1986).

5.3. Limiting factors for *Azolla* use and possible remedies

The optimum temperature for most *Azolla* species (20-30 ºC) is below the average temperature in the tropics. Cool weather is a key to successful *Azolla* cultivation in Vietnam and China. High temperature associated with humid conditions favors *Azolla* pests and diseases that limit *Azolla* growth in humid tropics. P application is required in most soils for growing *Azolla*. *Azolla* technology is labor intensive and therefore has economic limitations.

Water control and maintenance of inoculum. *Azolla* cannot withstand desiccation and requires water in the field throughout its cultivation cycle. Because *Azolla* is propagated vegetatively, inoculum must be maintained in nurseries year-round and multiplied for distribution before field cultivation. Those requirements imply that an irrigation network and a network for inoculum conservation, production, and distribution are prerequisites for *Azolla* use. This also implies that *Azolla* adoption by farmers first depends on a government policy to establish such networks (Roger and Watanabe 1986). Problems in inoculum conservation, multiplication, and transport could be solved if *Azolla* could be propagated from spores. A method for utilizing sporocarps for inoculum conservation has been developed in China but the growth of sporophytes was too slow to meet the inocum requirement in the field; 160 kg fresh weight ha^{-1} sporocarps yielded 16 to 21 t fresh weight ha^{-1} of *Azolla* in 52 days (Lu ShuYing 1987). Conditions for sporocarp formation and germination are incompletely understood. Low temperatures stimulated sporulation in the tropics (Kannaiyan and Rains 1985). Germination of spores of *Azolla filiculoides* required 1) mixed cultivation of mature megasporocarps and microsporocarps, 2) sunlight, and 3) an average daily temperature of 20-30 ºC. Increase in temperature within this range shortened the period of germination (Xiao QingYuan*et al.* 1987).

Need for P-fertilizer. P application is required in most soils for growing *Azolla*. Reported threshold values of P deficiency are 0.4% dry weight in *Azolla* , 0.15 ppm in floodwater, and 20 ppm available Olsen P in soil (Ali and Watanabe 1986). *A. pinnata* can grow satisfactorily without P application when the available Olsen P in the soil is higher than 30 ppm and P sorption capacity lower than 1500 mg P_2O_5 per 100 g (Watanabe and Ramirez 1984). Such P-rich soils are uncommon. *Azolla* doubling time estimated in a growth test on 972 Philippine soils was less than 5 days (moderately suitable soil) in 40% of the samples and less than 3.5 days (highly suitable) in only 13% of the samples, showing that P fertilization of *Azolla* would be required in many soils (Callo *et al.* 1985). This was confirmed by the observation that 80% of field-grown *Azolla* have a P content below 0.4% and a N content below 4% (Watanabe *et al.* 1990).

To be economically feasible, P fertilization requires a ratio of N fixed to P applied that is greater than the ratio of the prices of the corresponding fertilizers. Basal application of P might have a low efficiency and be economically infeasible, while split P application has an efficiency of 5-10 g N_2 fixed per g P applied (Watanabe *et al.* 1988b). Phosphorus fertilization limited to the inoculum production plot permits the P-enriched *Azolla* to multiply 6 to 7 times without P application in the main field and ensures a high efficiency of P applied (Watanabe *et al.* 1988b).

Pests. Although commercial pesticides effectively control *Azolla* pests, their application is not economically feasible in the field (IRRI 1986) and should be limited to inoculum production (Mochida 1987). Pesticides of plant origin might be economically feasible for field use. Alternate drainage and irrigation, cultivation in wet field or with a thin water layer, and reduced application of organic manures, may help controlling *Azolla* pests (Zhang Zhuang-Ta *et al.* 1987).

Temperature requirement. The optimum temperature for most *Azolla* species (20-30 ºC) is below the average temperature in the tropics (Lumpkin 1987). Cool weather is a key to successful *Azolla* cultivation in Vietnam and China. Temperature limitations can be reduced by selecting cold- or heat-tolerant strains. Among strains tested at IRRI, *A. microphylla* #418 was most tolerant of high temperature (37 ºC day/29 ºC night) (Watanabe *et al.* 1992).

Economics. Technologies used in Vietnam and China are labor intensive and therefore, have economic limitations. Kikuchi *et al.* (1984) studied the economics of *Azolla* use in the Philippines in South Cotabato, where *Azolla* spread spontaneously and no P-fertilizer and little labor were needed. Economic return from *Azolla* adoption, including cost savings in chemical fertilizers and weed control, was more than \$35 ha^{-1} at 1981 prices. However, conditions in the study area were exceptionally favorable and should be viewed realistically. The authors concluded that the economic potential of *Azolla* is greatest where the opportunity cost of labor is low, and that labor cost becomes critical where wage rates approach \$2 day^{-1}. Insect control was also an important economic limitation. If more than 200 g carbofuran ai ha^{-1} was needed to control insects, benefits were eliminated. In areas of the Philippines where conditions for *Azolla* growth were not favored by an exceptionally high level of available P, economics were not in favor of *Azolla* use (Rosegrant *et al.* 1985). It is clear that a case study in the Philippines is not enough to allow definite conclusions regarding *Azolla* economics, which may vary according to socio-agricultural systems. Economic calculations should also consider the long-term benefits of *Azolla* as an organic fertilizer with the concomitant increase in soil organic matter and fertility, instead of only those costs directly comparable to commercial N-fertilizer prices. The economics of integrated rice-fish-*Azolla* culture might be more favorable and should be considered.

5.4. Prospects

Azolla has a N-potential similar to that of legumes but is easier to incorporate and grows well with rice in flooded conditions. Environmental, technological, and economic factors limit its use. Problems in inoculum conservation, multiplication, and transport could be solved if *Azolla* could be propagated from spores. Temperature limitations and requirements for P can be reduced by selecting cold- or heat-resistant strains with low P requirements, and by split application of P-fertilizer, limited or not to inoculum production.

Azolla strains exhibit a wide range of behavior with regard to environmental factors, P requirement, N_2 fixation, productivity, etc. The ability to combine favorable characters such as resistance to high temperature and pests, low P requirement, and erect growth (permitting higher productivity) would allow strains to be designed for specific conditions. For this purpose, recombination of algal and plant symbionts and sexual hybridization between *Azolla* species proved feasible (Table 10). *Anabaena* from *A. filiculoides* was recombined with *A. microphylla* and vice versa. Megasporocarps of each species were freed of their algal symbiont (Lin Chang and Watanabe 1988), then an indusium of the other *Azolla* species, containing the corresponding *Anabaena*, was placed on the alga-free megasporocarps; symbiosis was thus established with the newly forming sporophyte (Lin Chang *et al.* 1988).
The formation of *Azolla* hybrids requires that macro- and micro-sporocarps be obtained. Sporulation of many strains can be observed under natural conditions (Payawal and Paderon 1986) but no method has been yet designed to induce sporulation at will. This is a major limiting factor for *Azolla* hybridization. Hybrids of *A. microphylla* (female parent) and *A. filiculoides* have been obtained (Wei WenXiong *et al.* 1986). The IRRI biofertilizer germplasm contains 23 hybrid strains obtained by algal transfer and 85 obtained by sexual hybridization, some of which exhibit improved characters (Watanabe *et al.* 1992).

The key economic costs in *Azolla* use are those of P application, labor, and pest control. Economic limitations are important and need further evaluation but calculations should also consider the long-term benefits of *Azolla* on soil fertility. *Azolla* has a potential not only as a green manure but as a multipurpose biofertilizer that can be a weed suppressor, a K source through its ability to concentrate this element, an animal feed, and a primary producer in rice-fish-*Azolla* culture (FAO 1988). This potential may renew interest in *Azolla* use.

Table 10. Biomass, N_2 fixing activity and N content of two Azolla species and their hybrid grown for 28 days at two temperature ranges
(Watanabe et Santiago-Ventura, IRRI, personal communication).

Espèce	température	Poids frais	ARA	% N
A. microphylla	37°/29°C	1400	3.1	3,8
microphylla x *filiculoides*	37°/29°C	1100	3.4	5,0
A. filiculoides	37°/29°C	300	0.4	1,5
A. filiculoides	26°/18°C	1800	4.6	5,2

Table 11. N accumulated by legumes used as green manure in rice cultivation

1. Crops grown until maturity (from Roger and Watanabe 1986).

Species	Nitrogen accumulated (kg ha^{-1})	(% fresh weight)
Astragalus sinicus	108-123	0,35-0,47
Canavalia ensiformis	98	0,47
Cassia mimosoides	97	0,44
Crotalaria anagyroides	98	0,33
Crotalaria juncea	105-129	0,30
Crotalaria quinquefolia	88	0,19
Dolichos biflorus	89	0,58
Gycine koidzumii	71	0,42
Phaseolus sp.	-	0,28
Phaseolus lathyroid	90	-
Phaseolus calcaratus	42	0,22
Sesbania aculeata	96-122	0,32-0,36
Sesbania rostrata	267	-
Sesbania sesban	100-202	0,39
Sesbania microcarpa	87	0,50
Sesbania sirececa	146	-
Average	114	0,37

2. N accumulated in crops grown for a definite time (calculated from data tabulated by Buresh et DeDatta 1991)

	Dry weight t ha^{-1}	N accumulated kg ha^{-1}	kg ha^{-1} day^{-1}
110 crops of 16 species grown for 30 to 178 days (average 52 days)			
Mean	4.3	99	1.9
Maximum	13.3	267	5.1
Minimum	0.2	7	0.2
32 crops of 11 species grown for 30 to 45 days (average 40 days).			
Mean	2.5	63	1.6
Maximum	6.7	143	3.2
Minimum	0.2	7	0.2

6. LEGUME GREEN MANURES

A range of legumes has been used as green manure in rice cultivation (Table 11) Potentialities of legume green manures (LGM) for rice were early recognized. In 1936 the International Institute of Agriculture reported that: "application of green manure may involve great progress in rice growing by ensuring yields higher than those at present attained". Similar statements were recorded by Pandey and Morris (1983) from the proceedings of symposia in 1952, 1953 and 1954. Then, it seems that less attention was paid to LGM in rice production. They were mentioned in only a few paragraphs of the proceeding of the symposium on "Nitrogen and Rice" held in 1979 at IRRI. The discovery of stem-nodulating legumes (Deyfus and Dommergues 1981, Alazard and Duhoux 1987) able to grow, fix N_2, and develop large biomasses under waterlogged conditions and the concern for agricultural sustainability have renewed the interest of the scientific community in LGM for wetland rice. A symposium held at IRRI in 1987 was devoted to green manure in rice farming (IRRI 1988).

6.1. Potential to increase rice yield

BNF by legume green manure (LGM) used for rice has usually been estimated from total N measurement and the assumption that 50-80% of accumulated N is Ndfa. Values of N accumulated in traditional LGM crop summarized by Roger and Watanabe (1986) average 114 kg ha^{-1} (Table 11). Those values were most often for a crop grown until maturity, which is rarely done with a LGM.

Values listed by Buresh and DeDatta (1991) (Table 11) show that in 30-45 days a LGM accumulate 7 to 143 kg N ha^{-1} (mean 63). Values published after 1985 average 133 kg N ha^{-1} (Ladha *et al.* 1988b). Ranges in kg N ha^{-1} are 40-225 for stem nodulating legumes, 33-115 for grain legumes, and 24-39 for perennial trees. Assuming 50-80% Ndfa, one LGM crop can fix an average 1.0-1.6 kg N ha^{-1} d^{-1} or 60-100 kg N ha^{-1} in 50-60 d.

Several studies have shown the high N_2-fixing potential of some stem nodulating legumes (Table 12). Using ^{15}N dilution, Pareek *et al.* (1990) estimated that Ndfa in 25-d *Sesbania* spp. was 50% in dry season (DS) and 75% in wet season (WS). Lower values (30-50%) were reported by Rinaudo *et al.* (1988), and N'Doye and Dreyfus (1988) for 53- to 63-d-old *S. rostrata*, probably because they used an uninoculated *S. rostrata* as control. A few estimates of BNF by *S. rostrata* as a pre-rice LGM are available from small-scale balance studies. Rinaudo *et al.* (1988) reported a gain of 267 kg N ha^{-1} after incorporating a 52-d crop. In a [45-d Sesbania-rice (WS)/55-d Sesbania-rice (DS)] sequence, Ladha *et al.* (1988b) estimated that *Sesbania* fixed 303 kg N ha^{-1} $year^{-1}$ when uninoculated, and 383 kg N when inoculated with *Azorhizobium*..

Beside N and other nutrient provision to the crop, the beneficial effects of LGM incorporation that have been reported include:

- improvement of soil properties especially (1) total N and organic matter content, (2) available Zn, (3) water holding capacity, and (4) soil aggregation (Becker et al. 1988, Bouldin 1988)
- control of some rice pests, in particular nematodes (Table 13); and
- immobilization of nitrogen nitrified during dry fallows, that would otherwise be lost by denitrification when soils are reflooded.

Table 12. Estimates of N_2 fixed by two stem nodulating legumes grown under flooded conditions.

Day length	Growth duration (days)	Ndfa* %	N fixed (kg/ha)	Method of measurement	Reference
Sesbania rostrata					
long	56	38		^{15}N dil.	Rinaudo *et al.* 1988
long	60	36-51	83-109	^{15}N dil.	Ndoye and Dreyfus 1989
long	56	88	175	ARA/$^{15}N_2$	Becker *et al.* 1990
short	56	83	70	---	---
long	25	76	10	^{15}N dil.	Pareek *et al.* 1990
long	45	88	140	---	---
long	65	94	458	---	---
short	25	53	7	---	---
short	45	71	100	---	---
short	65	86	324	---	---
Aeschynomene afraspera					
long	56	77	145	ARA/$^{15}N_2$	Becker *et al.* 1990
short	56	68	105	---	---
Mean			70		

* Nitrogen derived from the air.

Table 13. Effect of culture and/or incorporation of *Sesbania* on rice nematodes and rice yield (after Prot *et al.* 1992)

	Control	T 1	T 2	T3
1st crop	Rice	Rice	*Sesbania*	*Sesbania*
		incorporation of LGM imported from T2	culture and exportation to T1	culture and incorporation
2nd crop	Rice	Rice	Rice	Rice
Nematodes (nb. dm^{-3} of sol)				
before 1st crop	860 a	1024 a	755 a	750 a
at 1st harvest	766 a	1055 a	9 b	12 b
at 2nd harvest	766 a	621 ab	446 ab	352 b
Yield of the 2nd crop ($t\ ha^{-1}$)				
	2.2 e	3.2 bc	2.9 cd	4.0 a

Estimates of yield increase due to LGM range from 30 to 100 kg grain per ton of LGM incorpotared (Roger and Watanabe 1986). Incorporating one 40-60 day old culture of *Sesbania* or *Aeschynomene* may increase yield by about 1 $t\ ha^{-1}$ (Fig. 4a) and is equivalent to the application of 50 to 100 kg fertilizer N ha^{-1} (Ladha *et al.* 1988b; Becker *et al.* 1988). Nitrogen efficiency (kg grain produced per kg N applied) of LGM decreases with the quantity of N applied and is similar to that of urea (Fig. 4b).

62. Current utilization

Despite their potentiality, a setback of leguminous green manures has been observed during the last years. China is the only country where legumes are still used. The total area of LGM crops and *Azolla* in the major rice producing areas in China regions had increased from 2.45 millions ha in 1952 to 8.35 millions hectare in 1979, representing about 346,000 t N. In 1980 the planting area of green manure in some provinces (Jiangsu, Zhejiang, Jiangxi, Huuan and Husei) was about 20-50% of the total farmland. The total area of green manured land was estimated to 10 millions ha (Roger and Watanabe 1986). Recent data show a clear setback of LGM use in China (Fig. 5). A spectacular setback of green manuring has also been observed in Japan since 1955 (Watanabe 1984).

In other countries, usage of green manure seems to have become incidental. About 100,000 hectares of *Sesbania sesban* were estimated to be grown each year in northern Vietnam in the early 1980's for the summer rice crop, when Azolla cannot be utilized because of the high incidence of pests in relation with high temperatures (Roger and Watanabe 1986).

In India, Singh (1984) reported that in several rice growing areas 20-30% of soils were planted to LGM at mid century but that green manuring received a setback with the increase in cropping intensity and the low cost and ready availability of fertilizer during the last decades. Whereas green manuring is well known to farmers, these practices are no longer extensive in India since the introduction of inorganic fertilizers (Venkataraman 1984).

63. Limiting factors

Reasons for decline and constraints associated with LGM use were reviewed by Roger and Watanabe (1986), Garrity and Flinn (1988) and Becker *et al.* (1988a).

Some detrimental effects have been reported mainly in temperate condition. Watanabe (1984) attributed the setback of green manures in Japan to:

1) possible growth damage to rice due to anaerobic decomposition of the LGM
2) unmatching between N release and N needs of the plant leading to growth depression at earlier stages and excessive growth, detrimental to yield, at later stages,

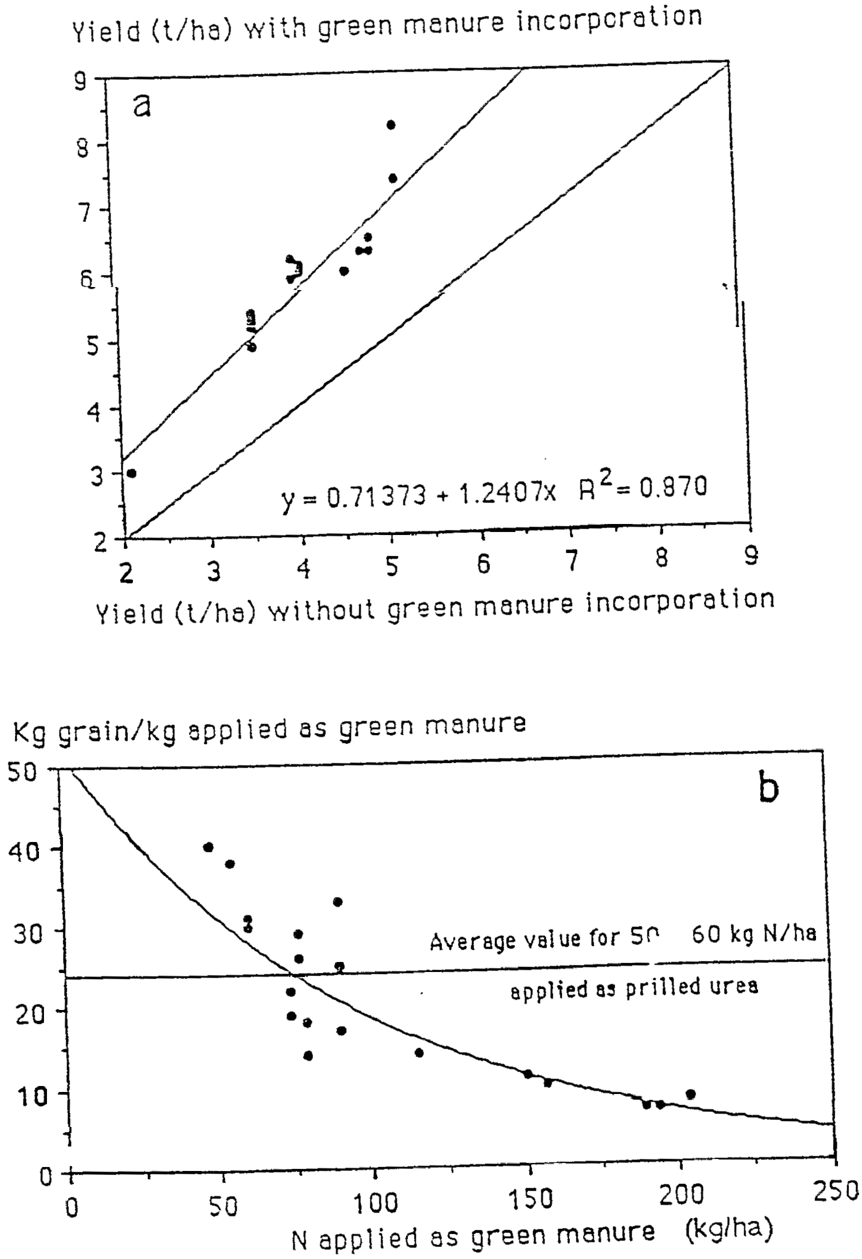


Fig. 4. Effet on rice yield of 42 to 59-day old *Sesbania* and *Aeschynomene* as green manure. a : effect on rice yield; b: nitrogen efficiency
(drawn from data by Ventura *et al.* 1987, Diekmann *et al.* 1991, Becker 1990, Furoc and Morris 1989, and Biswas 1988).

Fig. 5. Utilization

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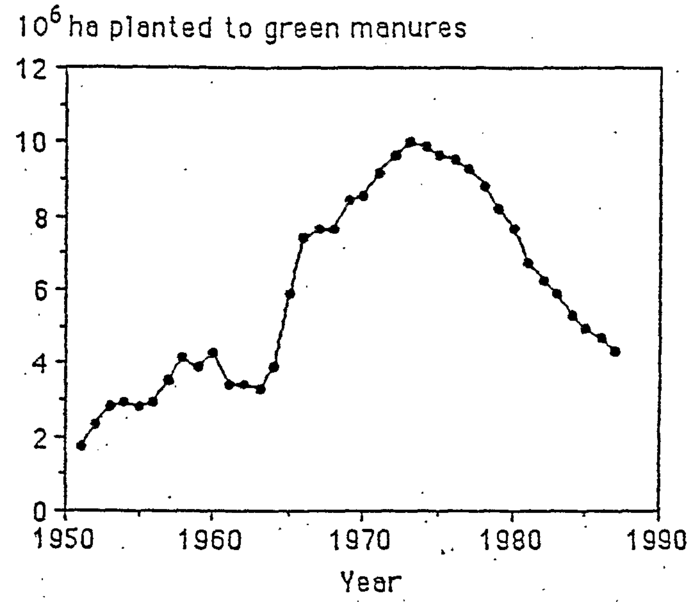

Fig. 5. Utilization of green manures in China between 1950 and 1988 (after Stone 1990)

3) degradation of the soil, and
4) unpredictability of the amount of N applied.

One other factor is the lack of draft or man power. Nitrogen content in legumes vary from 0.2 to 0.6% therefore the fresh weigh corresponding to 50 kg N/ha vary from 10 to 26 tons f.w. Incorporation of such a biomass, given animal draft power and traditional implement is difficult on a large scale. In developing countries the wage rate and the lack of man power may be limiting. One of the reasons for the abandon of organic manure in Japan is the decrease of the amount of labor available for farming because about 90% of rice farmers are part-time farmers.

There are also incidental reasons. For example, in certain areas of India indiscriminate cattle grazing due to inadequate social control is a reason for the reluctance of the farmer to raise a green manure crop (Venkataraman 1984).

However, major limitations are socio-economical. First, it is clear that legume green manure are not appealing because they do not yield food or cash directly. Then there are many economical limitations. In situations where N fertilizer is available, assuming an average yield of 15 kg grain per kg N applied, the cost of inorganic N fertilizer relative to the price of rice is very favorable. Furthermore, many governments have adopted a fertilizer subsidy policy and made cheap credit available for farmers to buy N fertilizer. On the other hand, the costs of green manure seed and land preparation are not favorable. If there is residual moisture in the soil, after the harvest of the rice crop, the economic advantage is very often in favor of a catch crop of legumes, ground nut, maize, millet, onion etc. and to resort to application of inorganic fertilizers for the next rice crop (Venkataraman 1984).

Situations where N fertilizer is not available is most frequently that of subsistence farmers, with small land holdings, who cannot afford to release land used for food or forage crops to green manure cultivation and therefore prefer to grow a catch crop. In some areas where no N fertilizer is available and organic manure was traditionally applied to rice, green manure is now applied preferentially to vegetable cash crops rather than to rice food crop.

Furthermore, the increased availability of inorganic fertilizers and low emphasis on GM by research and extension have contributed to the decline in LGM use.

7.4. Prospects

The sustainability issues together with the discovery of stem-nodulating legumes have revived scientist interest in green manuring. The formation of stem nodules has been reported in 25 species of the genera *Sesbania*, *Aeschynomene*, and, possibly, *Neptunia*. Stem-nodulating legumes exhibit adaptation to waterlogged or water-saturated conditions of growth. Their symbiotic bacteria cannot be accommodated into the existing Rhizobia classification. Two new additional genera have been proposed: *Azorhizobium* and *Photorhizobium*. Some of their strains exhibit unique properties such as the ability to fix N_2 under free-living conditions or a photosynthetic activity of bacterial type. Positive effects of stem inoculation on growth were observed in greenhouse and pot experiments. In the field, response seems limited to dry season and/or upland condition. The application of K and P stimulate nodulation and BNF. N-fertilizer at field level of application does not inhibit BNF in situ and a low dose had generally a stimulatory effect (Ladha et al. 1992).

Data on N accumulated in 40-55 days in a LGM crop show a higher N potential of stem-nodulating LGM than of traditional LGM. Fast-growing, high-nodulating, and erect species producing enough biomass have a potential as LGM in wetland areas. *S. rostrata*, *A. afraspera*, and *A. nilotica* have been reported as most promising. Ranges in kg N ha^{-1} are 40-225 for efficient species. ^{15}N dilution and $\partial\,^{15}N$ studies showed that Ndfa of 45-55 day-old *S. rostrata* was about 70% and increased to 90% at 65 days. Field experiments show that stem-nodulating LGM offer a better N potential for wetland rice than traditional LGM do.

Photoperiod sensitivity limits the utilization of *S. rostrata* while *A. afraspera* seems to be considerably less photoperiod sensitive (Becker 1990). The non availability of seeds of stem-nodulating legumes currently limit their adoption by farmers. Also many of the socioeconomic factors that limit the use of traditional LGM use also will limit that of stem-nodulating LGM.

Conditions for economical use of legume green manures are that (1) there is no alternative for a more profitable crop, (2) legume establishment does not require soil preparation and is inexpensive, (3) LGM productivity is stable in time, (4) man power and adequate implements are available for incorporation, and (5) there is no concurrence with man power needed for rice transplanting (Garrity et Flinn 1988). In particular, N_2 fixation by a LGM (and conservation of NO_3 mineralized during the dry season) may be an economically viable proposition if production costs can be kept low, and if the green manure does not compete with marketable or subsistence crops.

8. SUMMARY AND CONCLUSION

Recent methodological progress in measuring BNF in ricefields includes improved strategies for sampling and a better understanding of the potential of the ^{15}N dilution methods (labeled substrate and natural abundance). ^{15}N dilution, using available soil N as control, is promising for screening rice varieties for their ability to promote associative BNF.

BNF by individual systems can be estimated more or less accurately (Table 2). Estimates for *Azolla* and legumes are based on biomass measurements combined with Ndfa determination and are probably more reliable than estimates for indigenous fixers based mostly on indirect methods (ARA) or balance in small-scale trials. Total BNF in ricefields has been estimated from balance experiments, however, it has not yet been estimated by measuring simultaneously the activities of the various components *in situ*. As a result, the relation between the different N_2-fixing systems, especially indigenous ones, are not fully understood and it is not clear if their activities are independent or related. A method to estimate *in situ* the contribution of N_2 fixed to rice nutrition is still not available. Dynamics of BNF during the crop cycle is known for indigenous agents but the pattern of fixed N availability to rice is known only for a few green manure crops. As a result, BNF in models of N cycling in wetlands, is either not taken into account or taken into account as a non-dynamic input (Roger and Ladha 1992).

Nitrogen is usually the limiting factor to high yields in rice fields. Therefore, the use of BNF as an alternative or supplementary source of N for rice has been the major approach in microbiological management of wetland rice. Whereas N_2-fixing green manures have been used for centuries in some rice-growing areas, research on cyanobacterial and bacterial inoculants for wetland rice is relatively recent, being initiated in the early 1950s for free-living cyanobacteria and in the 1960s for rhizosphere bacteria.

Currently, most bacterial strains tested for inoculation have been N_2-fixing forms, but ARA, ^{15}N, and N balance studies have not provided clear evidence that the promotion of growth and N uptake was due to increased BNF. Therefore, several authors refer to the production of PGRs to explain the beneficial effect of bacterial inoculation. No experiment has yet supported this hypothesis. If it is verified that the ability of inoculated strains to produce PGRs is more important than their N_2-fixing ability, it is clear that screening of bacterial strains for inoculation should not be limited to N_2-fixing strains. The few data available on strain establishment showed that, in most cases, inoculated strains disappeared or established themselves for various periods of time, but did not multiply. That would limit the effect of inoculation to the earlier growth stage of the plant, a hypothesis that agrees with the absence of an inoculation effect on harvest index. Given the current status of knowledge on bacterial inoculation of rice, no definite conclusion regarding the potential of this technology can be drawn.

The selection and breeding of rice according to the variety's ability to stimulate an associative microflora that promotes BNF and soil N utilization is still limited by the absence of an efficient screening method. The relatively low N_2 fixation potential of associative BNF is not a hindrance to this promising approach, whose major advantage is that the N potential is inherent to the plant and thus requires no additional cultural practice by the farmer.

Biomass estimates, ARA measurements, and inoculation experiments indicate that free-living cyanobacteria have a moderate potential of about 30 kg N ha^{-1} crop $cycle^{-1}$ which may translate to a yield increase of 300-450 kg ha^{-1}. However, the technology for cyanobacteria inoculation has not progressed beyond the experimental stage of large-scale field testing. As long as cyanobacteria inoculation is applied on a trial-and-error basis, it will have little chance of success. Recent developments indicate that the whole principle of cyanobacteria inoculation should be reconsidered. Foreign strains usually do not establish and more attention should be paid to promoting indigenous strains. Cultural practices to enhance their growth are known but environments where those practices can be efficient and economically viable are probably limited. In-depth agroecological research is required before cyanobacteria technology can be substantially improved.

With regard to the rapid progress in genetic engineering, one can speculate on the possibilities to select or design efficient N_2-fixing strains of cyanobacteria and bacteria for inoculation. In the long term, genetic engineering may contribute to the microbiological management of wetland rice fields, but it is not yet known if and how engineered strains can establish and/or compete with the indigenous microflora.

Azolla has proved useful as a N biofertilizer in some rice-growing countries. Like legumes it has a high N potential, but is easier to incorporate and grows well with rice under flooded conditions. Environmental, technological, and economic factors limit *Azolla* use. Recent progress in strain hybridization and recombination has opened new ways to alleviate some environmental and nutritional limitations. Socioeconomic limitations are important and are probably increasing, as shown by the setback of *Azolla* in China and Vietnam where it was traditionally used. However, recent studies have shown that *Azolla* has a potential not only as a green manure but as a multipurpose biofertilizer. These limiting factors and the potential of *Azolla* as a multipurpose crop, which may revive interest in its use, will decide the extent of its future utilization.

Leguminous green manures have traditionally been used in many rice-growing countries. Estimates of N accumulated in a traditional prerice LGM crop range from 42 to 202 kg N ha^{-1}. Assuming 50-80% Ndfa, one average crop can fix 50-80 kg N ha^{-1}. Despite this potential, a strong setback of LGM use has been observed during the last decades. In the

1980s, China was the only country where LGM were noticeably used, but this use has been continuously declining. In other countries, the use of LGM seems to have become incidental. Main reasons for the setback or the nonacceptance of LGM by rice farmers include (1) some detrimental effects on rice and soil, reported mainly in temperate areas, (2) the lack of draft or manpower for incorporation and (3) socioeconomic limitations. LGM are not appealing because they do not yield food or cash directly. Where N fertilizer is available, green manuring is usually more expensive than inorganic N. Situations where N fertilizer is not available concern mostly subsistence farmers, with small holdings, who cannot afford to release land used for food or forage crops to LGM and prefer to grow a cash crop.

During the last decade the discovery of the high N_2-fixing potential of some flood-resistant stem-nodulating legumes has revived the interest of rice scientists in LGM. Field experiments show that stem-nodulating LGM offer a better N potential for wetland rice than traditional LGM do. However, many of the socioeconomic factors that limit the use of traditional LGM use also will limit that of stem-nodulating LGM. N_2 fixation by a LGM (and conservation of NO_3 mineralized during the dry season) may be an economically viable proposition if production costs can be kept low, and if the green manure does not compete with marketable or subsistence crops.

BNF in rice fields has been the most effective system for sustaining production in low-input traditional cultivation. The general impression when considering the management of N_2-fixing organisms in ricefields is that 40 years after the first inoculation experiments, the agronomic potential of BNF is underutilized and its intentional use is decreasing.

Considering that rice obtains most of its N from the soil, regardless of the amount of chemical N fertilizer applied, concerns in recent high-input, intensive rice cultivation are sustainability of high yields and the possible environmental impacts of intensive management on soil fertility. Knowledge on this aspect is still limited, but the key roles of the rhizosphere, the photosynthetic aquatic biomass, and their N_2-fixing components in maintaining the fertility of rice soils under intensive cultivation have been recognized and need further study (Watanabe *et al.* 1988).

An additional 300 million tonnes of rice will be needed in 2020 to meet the need of a fast-growing human population. This requires a 65% production increase within 30 years without much expansion of actual cultivated area (IRRI 1989). But increased rice production should not be at the expense of future generations and should fulfill the concept of sustainability. A major challenge is managing pests and nutrients in ways that reduce agrochemical use. Increased use of inorganic fertilizer is inescapable, but, as pointed out by Postgate (1989), a parallel return to greater exploitation of BNF, still responsible for providing 60-70% of the new N in the biosphere, seems common sense. Currently, intentional use of BNF in wetland ricefields concerns only a few percent of the global rice-growing area. Designing economically viable methods for utilizing N_2-fixing organisms in rice cultivation still remains a major challenge for scientists.

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IFS Workshop
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Nanjing, China

April 18-24, 1993

Sponsored by
International Foundation for Science

Supported by
The National Natural Science Foundation of China
The Chinese Academy of Science

Organized by
Institute of Soil Science, Academia Sinica (ISSAS)

Nanjing, IFS, 1993