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Improving nitrogen-fixing systems and integrating them into sustainable rice farming

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Key words: aquatic legumes, Azolla, Cyanobacteria, flooded rice soils, nitrogen, N_2 fixation, tropics

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Abstract

This paper summarizes recent achievements in exploiting new biological nitrogen fixation (BNF) systems in rice fields, improving their management, and integrating them into rice farming systems. The inoculation of cyanobacteria has been long recommended, but its effect is erratic and unpredictable. Azolla has a long history of use as a green manure, but a number of biological constraints limited its use in tropical Asia. To overcome these constraints, the Azolla-Anabaena system as well as the growing methods were improved. Hybrids between *A. microphylla* and *A. filiculoides* (male) produced higher annual biomass than either parent. When Anabaena from high temperature-tolerant *A. microphylla* was transferred to Anabaena-free *A. filiculoides*, *A. filiculoides* became tolerant of high temperature. Azolla can have multiple purposes in addition to being a N source. An integrated Azolla-fish-rice system developed in Fujian, China, could increase farmers' income, reduce expenses, and increase ecological stability. A study using Azolla labeled with ^{15}N showed the reduction of N losses by fish uptake of N. The Azolla mat could also reduce losses of urea N by lowering floodwater-pH and storing a part of applied N in Azolla. Agronomically useful aquatic legumes have been explored within

Sesbania and Aeschynomene. *S. rostrata* can accumulate more than 100 kg N ha^{-1} in 45 d. Its N_2 fixation by stem nodules is more tolerant of mineral N than that by root nodules, but the flowering of *S. rostrata* is sensitive to photoperiod. Aquatic legumes can be used in rainfed rice fields as N scavengers and N_2 fixers. The general principle of integrated uses of BNF in rice-farming systems is shown.

Introduction

Rice is the most suitable crop in wet monsoon Asian areas. Heavy rain floods the land during most or part of the rice-growing period. Flooded culture is effective in reducing soil erosion and avoiding acidification, which often occur under dryland conditions. The nitrogen fertility of soil is sustained better under flooded conditions than under dryland conditions (Watanabe and Roger, 1984; Watanabe, 1986). Favorable conditions for biological nitrogen fixation (BNF) is one of the reasons for the relatively stable yield of rice under flooded condition. After soil flooding, differentiation of an oxidized layer and a reduced layer and development of a photodependent biomass in floodwater and on the soil surface occur. The reduced soil conditions favor heterotrophic nitrogen fixation. The floodwater is the site for photodependent nitrogen fixation by free-living cyanobacteria and photosynthetic bacteria, and symbiotic cyanobacteria with Azolla. The rhizosphere of rice also provides favorable conditions for microaerophilic bacterial N_2 fixation.

The centuries-long practice of using green manures was overtaken by the use of chemical fertilizers in the 1960s. Increasing petroleum prices and environmental issues rekindled interest in and attention to the importance of BNF and its use in rice production. The potentialities and practical values differ among BNF systems. Four nitrogen-fixing systems can function in the wetland rice-farming systems. The approximate amount of N gains, the technological availability of these systems, and possible constraints to technological dissemination are shown in Table 1 (modified from Roger and Watanabe, 1986). The quantity of nitrogen fixed by indigenous nitrogen-fixing organisms (bacteria and cyanobacteria) is not high, but this type of nitrogen fixation operates everywhere. Because of competition with native flora, replacement of indigenous strains with better strains is difficult. Cultural techniques to stimulate the growth and activity of indigenous organisms are required. On the other hand, the amount of nitrogen fixed by inoculated organisms (Azolla and legumes) is high, but high cash-and labor inputs are required to make the best use of their nitrogen-fixing potentials.

The management and manipulation of BNF systems in flooded rice systems were reviewed earlier (Roger and Watanabe, 1986; Watanabe, 1986). Since these reviews, progress has been made in understanding cyanobacteria inoculation, genetic enhancement of Azolla-Anabaena symbiosis, integrated uses of Azolla, exploration of fast-growing stem-nodulating legumes and their use in wetland rice-farming systems.

Table 1. N gains, technological availability, and constraints in use of various BNF systems in wetland rice farming system

System	N gains[a] (kg N ha^{-1} per crop)	Technological availability	Constraints
Non-symbiotic			
Cyanobacteria	10–80	High but questionable	P deficiency, grazer pressure
Associative bacteria	<10	Not available	
Symbiotic			
Azolla-Anabaena	20–100	High	Poor water control, insect damage, P deficiency, etc.
Aquatic legumes	30–230	High	Seed production, photoperiod sensitivity

[a] N gains are per crop in a symbiotic system, and per crop of rice in a non-symbiotic system.

In this paper, recent developments in the modification of nitrogen-fixing systems and their use in rice-farming systems are presented.

Nitrogen fixation by cyanobacteria and its possible use

Since the discovery of the importance of cyanobacteria in N gains under flooded conditions, many inoculation trials of cultured cyanobacteria have been made. From intensive surveys of the results of inoculation and from his own experiments, Roger (1990) presented a review of the inoculation of cyanobacteria. The inoculation increased rice grain yields by an average of 350 kg ha^{-1}. When successful, the inoculation is low-cost technology, but its effect is erratic and unpredictable. No reliable method is available to assure the success of inoculation with 'efficient' cyanobacterial strains. Recent studies showed (Reddy and Roger, 1988; Roger et al., 1987; Roger, 1990) that N_2-fixing cyanobacteria are present in rice fields at a much higher rate than was previously thought and that non-indigenous strains rarely establish themselves. A shortage of P, the presence of combined N in floodwater, and grazer populations often limit the growth and activities of cyanobacteria. Alleviation of those conditions could stimulate the growth and N_2 fixation of indigenous cyanobacteria, but the economic viability and efficiency of applying P and pesticides to kill grazers have to be determined. Efficiency of P application to cyanobacterial nitrogen fixation in floodwater is lower than that to Azolla (Watanabe and Cholitkul, 1990). The applications of inorganic N fertilizers, and probably herbicides, inhibit cyanobacterial nitrogen fixation. Although NH_4-resistant mutants were reported (Latorre et al., 1986), competitiveness of the strains against the indigenous strain was weak (Roger, ORSTOM, personal communication). A herbicide-resistant strain was isolated (Vaishampayan, 1984). Because the level of herbicide for selection of resistant strains was much higher than the recommended dose of herbicides, the ecological significance of resistant strains is questionable.

Nitrogen fixation by Azolla-Anabaena symbiosis

For many centuries, the aquatic fern Azolla has been used in southern China and northern Vietnam as green manure for rice (Nierzwicki-Bauer, 1990; Shi and Hall, 1988), but in Southeast Asia Azolla was not popular. In the 1980s, Azolla was first used in rice fields in southern Mindanao in the Philippines. Because soils in this area were rich in available P, the growth of Azolla was abundant (Watanabe and Ramirez, 1990). Economical analysis showed that in this area, the use of Azolla before rice transplanting could save inputs of at least US$10 at 1981 prices (Kikuchi et al., 1984). *Azolla pinnata* var. *imbricata* is indigenous in Asia, but recently species belonging to the Euazolla section (*A. filiculoides*, *A. microphylla*, *A. caroliniana*, and *A. mexicana*) have been introduced for agricultural use, because their potential biomass production is higher than that of *A. pinnata*. One crop of Azolla at full cover can accumulate 20–100 kg N ha^{-1} (Watanabe and Roger, 1984). Two crops of Azolla (about 30 days each) can supply N for a crop of rice. At least 70% of N accumulated by Azolla comes from atmospheric N (see Roger and Ladha, 1992).

Factors restricting wide use by farmers

Despite the popularity of Azolla among scientists in Asia, actual use by farmers in Asia is still limited. In China and Vietnam, its use has diminished because of the availability of chemical N fertilizer and the cultivation of more economical crops. Many technical constraints inhibit wide use by farmers in South and Southeast Asia. These are a) the difficulties of maintaining Azolla inoculum throughout the year, particularly in the dry season, b) P deficiency, c) low tolerance for high temperature, d) damage by insect and fungi, and e) poor water control. To overcome these constraints, technological improvement and selection of Azolla strains suitable for various conditions have been sought. To overcome the maintenance of Azolla in winter or summer, the technologies of harvesting and seeding Azolla sporocarps – which have tolerance for adverse conditions were developed in China (Lu, 1987). Scientists in Zhejiang, Guandong, and Hunan

Provinces have studied this technique, but the slow growth in seedbeds and sporulation in only a limited number of strains limit the wide use of this technology. Some strains of Azolla like *A. caroliniana* (IRRI ac. No 3001) are tolerant of adverse conditions and recover quickly after a dry or cool period, despite its inability to sporulate. This *A. caroliniana* strain grows abundantly in South China (Liu and Zheng, 1989).

Surveys of Azolla plants grown in ponds and rice fields in the Philippines showed that at least half of the samples were deficient in P (Watanabe and Ramirez, 1990). The average available P content of soils where Azolla plants grew was 25 ppm – higher than the average for Philippine soils (probably below 10 ppm) – suggesting that Azolla could grow in soils with higher available P content. Averages of plant P content in *A. microphylla* and available P content of soils where this species grew, were higher than those in *A. pinnata*, suggesting that the requirement for available P was higher in *A. microphylla* than in *A. pinnata*. Water-culture experiments also showed that *A. pinnata* grows better under low-P condition than *A. microphylla* and *A. mexicana* (Kushari and Watanabe, 1991). Split application of P to nursery beds of Azolla can greatly increase the efficiency of P in N production by Azolla (Watanabe et al., 1988). After inoculation to the field, Azolla enriched with P can multiply 7–10 times until it becomes P-deficient.

A. filiculoides is least tolerant of high temperature. Introduction of *A. filiculoides* in China in the early 1970s expanded the use of Azolla in the North of China (Liu and Zheng, 1989). Although *A. microphylla* is tolerant of high temperature, growth is greatly reduced at water temperature exceeding 40°C. In the fields, damage by heat is confounded with insect damage because, as temperature increases, insect growth increases and growth of Azolla decreases. Major insect pests are Pyrlalidae (*Ephestiopsis* and *Elophila*) (Mochida et al., 1987) in the humid tropics, and Chironomidae and snails in addition to Pyrlalidae in China (Liu and Zheng, 1989). Use of chemical pesticides in the fields is not economically feasible (Kikuchi et al., 1984). Biological control should be sought. Moist soil culture prevents damage by insect and high temperature, because the absence of floodwater inhibits the movement of aquatic insect larvae (Liu and Zheng, 1989). No strong tolerance for pyralid pests was found among Azolla strains (Liu and Zheng, 1989).

Recent development in genetic enhancement

Although selection of Azolla species or strains and improvement of cultural practices were partly successful in overcoming constraints and in increasing N_2-fixing activity, genetic enhancement of the Azolla-Anabaena system is needed. This has recently been accomplished by sexual hybridization and algal inoculation.

An easily sporulating strain of *A. filiculoides*, introduced in China in the 1970s, gave material for studying the sexual life cycle of Azolla. In 1979, scientists in Jiangsu and Fubei Provinces reported the hybridization of Azolla. Wei et al. (1986) reported the hybridization between *A. filiculoides* and *A. microphylla*. When *A. filiculoides* was a female, a higher number of albinos appeared in the F_1. Hybridization was confirmed by isozyme pattern of esterase. Wei et al. (1986) also reported crosses between *A. mexicana* and *A. filiculoides*. In all of the F_1, no megasporocarps (female organ) were formed, and microspores were often aborted and became non-circular during their development. Improved tolerance for heat and snail attack was observed in hybrids. The annual biomass production of a hybrid (*A. microphylla* × *A. filiculoides*) was better than that of the parents in Fuzhou (Table 2) (Liu and Zheng, 1989). In spring, low tem-

Table 2. Biomass production of hybrid Azolla and its parents in Fuzhou, China (FAO, 1988)

Species	Fresh weight (t ha^{-1})		
	Apr.–Jul.	Aug.–Nov.	Total/year
Hybrid[a]	118	43	161
A. microphylla (female)	79	50	129
A. filiculoides (male)	101	25	126
A. caroliniana	93	44	137

[a] *A. microphylla* × *A. filiculoides*, Strain name–Rong-Pin 1–4.

perature-tolerant *A. filiculoides* grew better than high temperature-tolerant *A. microphylla*. In summer and autumn, the opposite was found. In spring, the hybrid produced biomass comparable to that of *A. filiculoides*; in summer and autumn it was comparable to *A. microphylla*. Consequently, annual production of the hybrid in Fuzhou was higher than that of the parents (Table 2) (Liu and Zheng, 1989). Do van Cat et al. (1989) also reported hybrids between *A. microphylla* and *A. filiculoides* (IRRI ac. No. 4028, and 4030). Hybrids were confirmed by zymograms of three enzymes. Megasporocarps were not formed and microspores were also abnormal. The N content of hybrids grown in IRRI's field or laboratory was higher than that of the parent *A. microphylla*. Biomass production of hybrids in IRRI fields was higher than that of *A. microphylla* except in May, the hottest month. These results indicate that sexual hybridization could change Azolla's reactions to environments. Scientists in University of Hanoi (Do van Cat, person. comm.) and the University of Philippines at Los Baños (P.C. Payawal, personal comm.) reported the hybridization between *A. microphylla* and *A. mexicana*. Although cyanobacteria were isolated from the fern, their immuno-chemical and DNA properties were not identical with those of the endosymbiont (Zimmerman et al., 1989). Lin et al. (1989) showed that the inoculation of one of such isolated Anabaena could not establish complete symbiosis. It is unlikely that genuine cyanobionts have been isolated. Anabaena cells are present beneath the indusium of the megasporocarp and transfer to a new generation of sporophytes. By transferring the indusium to *Anabaena*-free megasporocarps of another species, Lin et al. (1989) succeeded in transferring *Anabaena* of *A. microphylla* to *A. filiculoides* and vice versa (Fig. 1). *A. filiculoides* having a cyanobiont from *A. microphylla* became more tolerant of heat (37°C-day/29°C-night) than *A. filiculoides*, but less tolerant than *A. microphylla*, suggesting that heat tolerance is partly controlled by *Anabaena* (Watanabe et al., 1989).

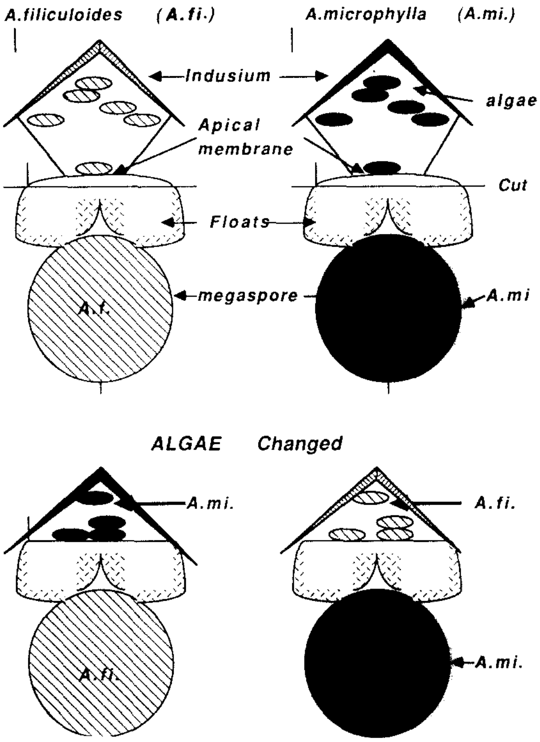

Fig. 1. Diagram for exchanging symbiotic Anabaena in megasporocarps.

Although these achievements in sexual hybridization and the exchange of cyanobionts were preliminary, the methods opened a way to genetic enhancement and study of the mechanisms of Azolla-Anabaena symbiosis. We expect that some of the unfavorable characteristics of various Azolla species – high P requirement, low nutrient content and low digestibility to animals, susceptibility to high temperature, and sensitivity to insect attack – may be improved by hybridization or by changing the symbiotic cyanobacteria or even by introducing foreign DNA-like insect resistance genes to either the fern or the cyanobiot.

For genetic enhancement, knowledge of the genetic or strain or species variabilities is required. Table 3 summarizes known variabilities in various traits. The gene pool for these widely variable characteristics is, however, still virtually unanalyzed.

Multiple uses

Recently, multiple benefits from Azolla besides being an organic N fertilizer have been recognized. These are as (a) weed suppressor (Janiya and Moody, 1981), (b) K scavenger from floodwater (Liu, 1987), (c) animal feed, (d) fish feed,

Table 3. Genetic variabilities of Azolla in various traits

Traits	Species	Among strains	Mutants or hybrids	Details[a]	References
Sporulation	Yes	Yes	Yes	In hybrids, no female spores	Wei et al. (1986) Payawal and Paderson (1987)
Heat tolerance	Yes	Yes	Yes	Amic, Amex > Api > Af Latin America Af > Europe af	Watanabe, unpublished Watanabe, unpublished
Cold tolerance	Yes	?	?	Af > others	Li et al. (1982)
P deficiency	Yes	Yes	Yes	Ap > Af, Amic, Amex A mutant 4138	Kushari and Watanabe (1991) Liu and Watanabe, unpublished Desmadyl et al. (1988)
NaCl tolerance	Yes	Yes	Yes	Amic, Amex > Ap A mutant 8028	Liu and Zheng (1989) H. Brunner, IAEA, pers. comm.
Al tolerance	Yes	Yes	Yes	Ap > other A mutant 413	Wauthelet et al. (1988) Liu, unpublished
Appetency to fish	Yes	Yes	?	Amic > Api	Antonie et al. (1986)
Inhibition of N_2 fixation by NH_4	Yes	Yes	?	App > others	Okoronko et al. (1989)
Insect tolerance	Yes	Yes	?	App > others	Mochida, IRRI, pers. comm.

[a] Ap: *A. pinnata*, Api: *A. pinnata* var. *imbricata*, App: *A. pinnata* var. *pinnata*, Af: *A. filiculoides*, Amic: *A. microphylla*, Amex: *A. mexicana*. Numbers are accession number of IRRI Azolla Collection.

(e) P scavenger in sewage treatment (Shiomi and Kitoh, 1987), and (f) suppressor of ammonium volatilization (Villegas and San Valentin, 1989). The use as fish feed and effect on losses of inorganic N fertilizer are described here.

Rice-Azolla-fish culture

Asian farmers have been rearing fish with flooded rice culture. Fish production is limited, however, by aquatic biomass production in flooded rice fields. The Fujian Academy of Agriculture Science (FAAS) developed the rice-Azolla-fish system to increase farmers' income by the higher production of fish and reduced consumption of fertilizers and pesticides (FAO, 1988; Liu and Zheng, 1989).

The essence of this technique lies in (a) the mixture and proper ratio of fish with different eating and rearing habits, (b) proper ratio of the area for pits and ditches to rice-planted area in one plot of the field, (c) double narrow row planting of rice, (d) mixture of Azolla species, and (e) supplement of fish feed, if necessary. Three or more kinds of fish – grass carp (herbivorous), *Tilapia nilotica* (omnivorous), and other omnivorous fish – are mixed. Rice spacing is such as to allow sufficient space to fish without reducing rice yield. Pits and ditches are dug in the rice field, and rice is often grown on the ridges.

Several kinds of Azolla are mixed to give stable biomass production in different seasons. The mixture of fish having different eating habits may accelerate N transformation among Azolla-fish-fish faeces-rice. Experiments with ^{15}N-labelled Azolla showed Azolla N to be incorporated into fish protein and the rice plant. The recoveries of Azolla N labelled with ^{15}N were 26% to rice and 35% in soil and floodwater (39% loss) in the rice-Azolla system; in the rice-Azolla-fish system, 27% was recovered in fish, 23% in rice, and 35% in soil and floodwater (15% loss). These data show a more efficient use of biologically fixed N in the rice-Azolla-fish system. Potassium is also recycled in the Azolla-fish-fish faeces system. Consequently, this system could reduce substantially the use of inorganic fertilizers. The soil surface in rice fields and fish pits was enriched with N, P, and K. The incidence of rice disease and insect pest also decreased. This system, therefore, not only protects the environment, but also increases farmers' income. The experiments to reduce the use of chemical fertilizer and pesticides confirmed that this system increases farmers' income by producing fish without loss of rice yield and by reducing inputs (Table 4). This system could give an additional income of about $2000/ha per year, compared to the conventional two rice crops (FAO, 1988; Liu and Zheng, 1989).

Table 4. Experiments in new farming (rice-Azolla-fish) system at Fujian Acad. Agric. Sci. (1987–1988)

	Fertilizers used (kg ha^{-1} yr^{-1})			Azolla applied (t ha^{-1})	Rice yield (t ha^{-1})			Fish yield (t ha^{-1})
	N	SP[a]	K_2O		Early	Late	Total	
1987								
Rice-rice system	207.8	194.3	127.4	/	6.0	6.4	13.0	/
Rice-Azolla-fish	37	42.25	42.25	30	5.8	4.4	10.2	4.0
1988								
Rice-rice system	274.8	180.4	152.9	/	4.7	4.8	9.5	/
Rice-Azolla-fish	67.3	69.4	50.9	45	4.4	4.5	8.9	4.3

[a] Superphosphate.

Effect of Azolla on the fate of applied inorganic N fertilizer

The rise in floodwater-pH because of the photosynthetic activity of the aquatic community stimulates N volatilization from surface-applied urea. Under the Azolla cover, floodwater-pH does not rise because of light interception by Azolla (Kroeck et al., 1988). This may decrease ammonia volatilization. Furthermore, the applied N is absorbed by Azolla and used by rice. A small-scale experiment in the laboratory showed ammonia volatilization to be 7–40% of applied ammonium, depending on pH, temperature, and wind speed. The presence of an Azolla cover reduced ammonia volatilization by 20–50% of that without Azolla (Villegas and San Velentin, 1989). The experiment in FAAS, using ^{15}N-labeled urea, showed that 50% of urea N was lost when urea was applied to floodwater 2 weeks after transplanting rice. When N fertilizer was applied to the Azolla mat, the loss was 42%. When Azolla was further grown 2 weeks after applying urea and incorporated in the soil, recovery of ^{15}N in rice further increased, and loss was reduced to 25%. The experiments coordinated by IAEA (International Atomic Energy Agency)/FAO (Food Agriculture Organization of the United Nations)/SIDA (Swedish International Aids Agency) project on the use of ^{15}N in N_2 fixation of Azolla and blue-green algae confirmed the beneficial effects of Azolla cover on reducing ammonia volatilization (Table 5).

Nitrogen-fixing aquatic legumes

Exploration of fast-growing aquatic legumes as green manure crops

Leguminous green manure crops have been used as green manures for rice. The major ones are *Astragalus sinicus* in the temperate region and *Sesbania cannabina* (syn. *aculeata*) in the tropics. Although green manure use generally decreased, it has continued in some Asian regions like Bangladesh and some parts of India. The interest in green manure legumes was revived recently (Ladha et al., 1988; Meelu and Morris, 1988). Under the conditions of intensive agriculture, in which land and time are major constraints, green manure crops can be inserted in the cropping calender when the growth interval is short, or

Table 5. Effects of Azolla cover and incorporation on rice grain yields and ^{15}N recoveries (IAEA/FAO/SIDA joint project on the use of ^{15}N in Azolla and blue-green algae in rice fields, 1989)[a]

Treatment	Indonesia		Sri Lanka		Beijing, China		Fuzhou, China		Philip.	Thailand
	Yield (t ha^{-1})	^{15}N % recovery	Yield (t ha^{-1})	^{15}N % recovery	Yield (t ha^{-1})	^{15}N % recovery	Yield (t ha^{-1})	^{15}N % recovery	Yield (t ha^{-1})	Yield % recovery
Urea	4.2	59(76)	2.9	27	10.1	16	4.1	25(50)	4.7	3.5
Azolla + urea	4.2	62(86)	3.2	35	10.4	30	4.2	29(58)	6	4.0
Incorporated	4.4	59(90)	3.8	42			4.3	35(75)	6.7	4.7

[a] ^{15}N recovery in plants and in soil and plant in parentheses.

can be grown as an intercrop. In particular, the discovery of the potential as green manure of stem-nodulating and fast-growing aquatic legumes – *Sesbania rostrata* (Dreyfus and Dommergues, 1981) and various Aeschynomene species (Alazard and Becker, 1987) – led to new ways in using green manure legumes in rice-based farming systems. Many species of *Sesbania* and *Aeschynomene* have nodules on the stems (Ladha et al., 1991). There are three patterns of stem nodulation.

Type A: Profuse nodulation all over the aerial stem. *S. rostrata*, *A. afraspera*, *A. nilotica*

Type B: Scarce nodulation on the aerial stem, but good nodulation on the stem portion submerged in water. *A. aspera*, *A. cilita*, *A. denticulata*, *A. evenia*, *A. indica*, *A. pratensis*, *A. rudis*, *A. schimperi*, *A. scabra*, *A. sensitiva*, *A. tambacoundensis*.

Type C: No nodulation on the aerial stem and scarce nodulation on the submerged stem. *A. crassicaulis*, *A. pfundii*, *A. elaphroxylon*, *S. sesban*, *S. punctata*, *S. speciosa*, *S. javanica*, *Neptunia oleracea*

Because of stem nodules, the plant can continue to fix N_2 under submerged conditions and can be grown before wet-season rice, when flooding frequently occurs. N_2 fixation by stem nodules is more tolerant of combined N than is that by root nodule. Field-and pot experiments showed that N_2 fixation by *S. rostrata* was stimulated at 30 kg N ha^{-1}, and was not inhibited at 60 kg N ha^{-1} (Becker et al., 1988; 1990). Among them, *S. rostrata* and *A. afraspera*, which originated from Africa, have been studied most (Becker et al., 1988; 1990; Ladha et al., 1988; Meelu and Morris, 1988). Experiments in the Philippines showed that within 45 days *S. rostrata* could accumulate 40–110 kg N ha^{-1} and *A. afraspera* 80–90 kg N ha^{-1}. N accumulation varied with variation in period to flowering. *S. rostrata* is more sensitive to photoperiodism than is *A. afraspera*. Days to accumulate 100 kg N ha^{-1} in the Philippines ranged from 41 (May planting) to 61 (December planting). In *S. rostrata*, the values ranged between 45 and 50 d in *A. afraspera* (Becker et al., 1988; 1990). The difference in day length is only 1.5 h in a year. *S. rostrata* is, therefore, suitable for the long-day season. *A. afraspera* has lower C/N ratios (13–16 at 7-week growth) than *S. rostrata* (22–24). Because the bulky biomass of these green manure crops requires additional labor for incorporation, a green manure crop with higher N content or less biomass at the same rate of applied N is preferable (Becker et al., 1988; 1990).

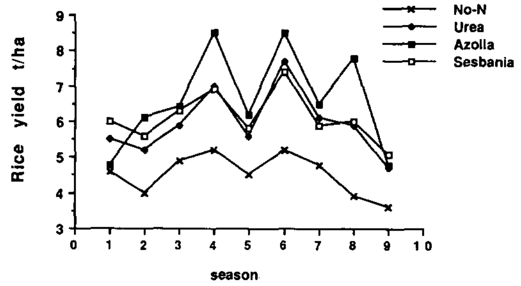

Fig. 2. Rice grain yields, as affected by various sources of N (1985–1989 at IRRI).

Urea, Azolla, *S. rostrata*, and *A. afraspera* have been compared as sources of N in IRRI fields. Azolla was incorporated twice before transplanting rice and once after transplanting. Legumes were grown for 40 to 50 d before incorporation. The average amount of N incorporated per hectare over 4.5 yr (9 crop cycles) was 57 kg urea N, 84 kg N from Azolla, and 73 kg N from legume green manures. Azolla produced more biomass N than legumes during the dry season, but not during the wet season. Average rice yields were 4.5 t ha^{-1} in the control, 6.0 t ha^{-1} with urea, 6.6 t ha^{-1} with Azolla, and 6.1 t ha^{-1} with legume green manures (Fig. 2).

Areas with potential for aquatic leguminous green manures

Because rainfed fields afford few opportunities for using chemical fertilizers and have uncontrolled water regimes, aquatic legume green manure would be more suitable there than in irrigated rice fields. Tolerance of aquatic legumes for flooding allows their continuous growth after flooding, which often occurs at the beginning of the wet season. The usefulness of Sesbania in infertile rainfed rice areas is being studied (Arunin et al., 1988). *S. rostrata* is tolerant of salt, and is grown in salt-affected areas as a source of mineral nutrients and organic matter (Arunin et al., 1988). Addition of organic matter is effective in preventing salt accumulation and in accelerating leaching of salt (H. Wada, Agricultural Development Research Center of Northeast Thailand, personal communication). Legumes planted before the wet season save soil N, which is otherwise lost by leaching or denitrification by heavy rain and flooding. This effect is an additional benefit of aquatic legumes, discussed by George et al. (1992).

Constraints of fast-growing, stem-nodulating leguminous plants are low seed production, labor-requiring incorporation of bulky biomass into the soil, *S. rostrata*'s high sensitivity to photoperiod, and insect incidence.

To overcome these constraints and to find green manure crops suitable for different conditions, exploration of various aquatic legumes, particularly Aeschynomene species, has been started.

Genetic improvement of recently explored aquatic legumes is not yet in progress. As in Azolla, the means of increasing income by growing leguminous plants should be considered.

Concluding remarks

As discussed above, nitrogen-fixing organisms give many benefits additional to accumulating N from the atmosphere. These benefits should be fully used. Nitrogen-fixing agents like Azolla and legumes contain not only N, but also useful substances like carbohydrates, amino acids, and vitamins. When these plants are incorporated into the soil, the organic components are decomposed. Mineralized N accumulated in soil may be lost, or may contaminate the environment before its absorption by the rice plant. Integrated use of BNF aims at multi-purpose uses and recycling of nutrients among animal, plant, and soil. The proteins in the primary producer (BNF agents) are converted into animal-or fish proteins. Excreta of an animal or fish are decomposed to provide mineral nutrients to BNF agents and rice or to provide energy and carbon for biogas production. The effluent of a biogas digester provides mineral nutrients to BNF agents and rice in the rice fields (Fig. 3). A Philippine farmer practices integrated use of Azolla on his small (0.7 ha) farm to support his family (Johnson, 1988). Azolla is used as animal feed and fertilizer to rice and upland crops. Excreta of animals and unconsumed feed are used for biogas and the effluent of the digester is returned to the rice field. No P fertilizer is applied to Azolla, because the effluent from the biogas digester can provide that P. This is an example of sustainable agriculture with reduced dependence on external sources.

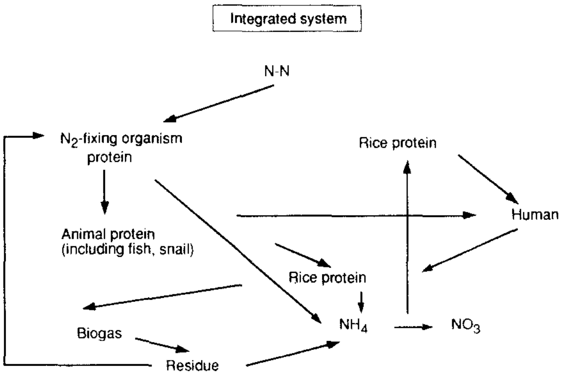

Fig. 3. Integrated use of BNF.

Genetic enhancement of BNF agents used in rice fields is far less developed than is that of grain legumes. The improvement of BNF systems by traditional breeding and modern biotechnology is absolutely necessary. For Azolla, this effort is in progress. For aquatic leguminous plants, the exploration of genetic variabilities among species and population has just begun.

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