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COOPERATIVE ASPECTS OF THE RESEARCH PROGRAMS ON BIOLOGICAL NITROGEN FIXATION AT IRRI

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COOPERATIVE ASPECTS OF THE RESEARCH PROGRAMS ON BIOLOGICAL NITROGEN FIXATION AT IRRI[a]

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Contents

1) INTRODUCTION

In many areas of rice growing countries, commercial nitrogen fertilizers is not used to a large extent because either it is not available or farmers cannot afford for it. Furthermore, N fertilizers may become more expensive in the future. Thus, alternative or additional cheap sources of nitrogen are urgently required, especially for improved high yielding varieties that have been developed. Biological nitrogen fixation (BNF) is one of the major alternative sources of nitrogen for rice.

BNF has been the major research topic of the Soil Microbiology Department at IRRI. We have taken the opportunity of the 1985 International Rice Research Conference, which would focus on cooperative research, to make a survey of the cooperative aspects of the research programmes on BNF at IRRI. The aim of this paper is, after presenting very shortly the different N_2-fixing systems, to summarize cooperative BNF programs at IRRI in order to analyze what has been their impact on the progress of knowledge on BNF in rice fields and what are the possibilities for future cooperations.

Some emphasis has been placed on a survey for the establishment of a network on blue-green algae (BGA). Analysis of this survey shows some of the problems encountered in developing cooperative research.

2) BIOLOGICAL N_2-FIXATION IN RICE FIELDS AND ITS STUDY AT IRRI

Some free-living and symbiotic bacteria and BGA have the ability to convert nitrogen from the air to ammonia. In free-living microorganisms, ammonia is converted to proteins which then decompose in the soil while in symbiotic systems ammonia is made directly available to the plant. This microbial activity, called biological nitrogen fixation, is a crucial step in the global nitrogen cycle in that it returns to the biosphere, in a combined form, nitrogen that has been lost in the atmosphere mainly through denitrification.

In modern farming systems the importance of BNF is often overshadowed by fertilizer N. However, BNF is the major factor determining the productivity of all but most advanced agricultural areas of the earth. It is vital to subsistence farming , which does not use fertilizer N, and comprises a large part of rice cultivation in Asia. BNF is especially active in submerged soils. Long term fertility experiments have shown that as much as 40-50 kg N can be fixed per rice crop and per hectare by N_2-fixing bacteria and BGA in fields receiving no chemical fertilizer, which explains why wetland rice can be grown on the same land year after year without N fertilizer and produce low but constant yield.

Results of recent research on BNF have helped understanding the role of N_2-fixing microorganisms in rice soils and have shown that leguminous green manure crops, Azolla in symbiosis with BGA, free-living BGA and heterotrophic N_2-fixing bacteria associated with living rice plant and dead plant residues are major N_2-fixing agents active in wetland rice fields. Potentiality of N_2-fixation of the different agents decreases in the above mentioned order.

N_2 fixation in flooded soil systems was reviewed by Buresh et al. (1980). Processes and ecology of BNF in rice soils were reviewed by Watanabe (1978) and Watanabe and Brotonegoro (1981). An extensive survey of quantitative data was presented in Lowendorf's review (1982). New knowledge of BNF in flooded rice fields was summarized by Watanabe and Roger (1985). Technologies for utilizing BNF in wetland rice cultivation were summarized by Roger and Watanabe (1985). A short introductory review was presented by Ladha et al. (1985).

The ultimate objective of the BNF program at IRRI is to enable satisfactory yields of rice to be obtained with the least possible use of chemical N fertilizer. To achieve, this short term objectives of the program are:

- to identify N_2-fixing organisms in rice soils and to determine the quantity of nitrogen fixed by these organisms, and the factors that influence their activity;
- to develop methods and cultural practices to enhance BNF in rice fields;
- to establish a cooperative research network on BNF in rice fields;
- to train scientists who will strengthen the national capabilities for conducting research on BNF.

The BNF program in Soil Microbiology Department comprises three major components: 1) heterotrophic N_2-fixation, 2) free-living BGA, and 3) Azolla (symbiotic BGA). Research on utilization of N_2-fixing legume green-manures is conducted in the Multiple Cropping Department at IRRI.

The three components are reviewed thereafter, placing emphasis on 1) the current status of the knowledge of the N_2-fixing system, 2) progress made in the knowledge through cooperative programs and 3) the possible fields for future cooperations.

3) HETEROTROPHIC N_2-FIXATION

31) Current status of the knowledge

Heterotrophic N_2-fixing bacteria comprised of aerobes, facultative anaerobes and anaerobes. Anaerobic conditions which developes in flooded soils generally favor N_2-fixing organisms because nitrogenase, the enzyme of BNF, is irreversibly inactivated by oxygen. Therefore N_2-fixation by heterotrophs is higher in wetland rice fields than in dryland.

Heterotrophic BNF depends on the supply of organic matter and therefore develops in sites were such a supply occurs: the rice rhizosphere, the basal portion of rice shoots under water , and the organic debris in the soil. The agronomic significance of heterotrophic BNF has been proved by nitrogen balance studies, acetylene-reducing activity measurements, and ^{15}N studies. Based on actual and potential estimates of N_2-fixation, it may be said that the N potential of associative BNF (kgN/ha per crop) is the least among N_2-fixing agents discussed in this paper. However, this low potential may be balanced by a wider applicability.

There are three possible methods for increasing heterotrophic BNF: 1) bacterial inoculation, 2) selection of varieties high in stimulating N_2-fixation, and 3) incorporation of organic matter.

The overall impression from experiments to enhance BNF by bacterial inoculation is not one of a great success. Inoculation sometimes increased rice yield but there was no evidence that this was due to BNF.

Nitrogen balance experiment, acetylene reduction and ^{15}N studies conducted at IRRI have shown rice varietal differences in their capability to stimulate N_2-fixation. Selection of varieties high in stimulating N_2-fixation is a promising field of research. We are developing methodologies for rapid screening of large numbers of rice varieties.

The only agronomic practice known to enhance heterotrophic BNF which is currently utilized, is organic matter incorporation, especially straw. From data collected by Roger and Watanabe (1985) the average efficiency of heterotrophic BNF is about 2.4 mg N fixed per gram of substrate added. Studies at IRRI indicated that stimulation of N_2 fixation by straw incorporation in flooded soil was more pronounced in planted soil than in fallow soil. Surface application and incorporation of straw enhanced N_2 fixation and number of photosynthetic, heterotrophic aerobic and anaerobic bacteria. Such enhancement was observed between 10 and 40 days after straw application. Total dry matter was also significantly increased in a few varieties due to straw incorporation.

32) Achieved and ongoing cooperative projects

321) Cooperative with Cornell University and Boyce Thompson Institute

Since 1976, the United Nations Development Program has supported the study of BNF at IRRI and particularly that of heterotrophic N_2-fixation associated with wetland rice. The program started with the cooperation of Cornell University and Boyce Thompson Institute. ^{15}N studies by Boyce Thompson Institute confirmed the early work at IRRI demonstrating that significant amount of N is fixed in rice rhizosphere. Results provided direct evidence of heterotrophic BNF associated with rice roots and the flooded soil and demonstrated that part of the newly fixed N is available to the plant (Eskew et al, 1981).

A scientist from Boyce Thompson Institute was stationed at IRRI to study nitrogen balances based on Kjeldahl data over several crops of rice grown in continuously flooded soil in pots. Flooded soil planted to rice had a positive balance which was found to be the result of phototrophic and heterotrophic N_2-fixing agents. Heterotrophic N_2-fixation depended upon the presence of rice plants. Results showed rice varietal differences in the capacity to stimulate nitrogen gains (App et al., 1980).

Cooperative program with Cornell University and Boyce Thompson Institute terminated in May 1985 with the end of the first UNDP project.

322) Cooperation with Thailand

Measurement of acetylene-reducing activity in wetland rice fields were made in cooperation with the Department of Agriculture in Thailand (Watanabe and Cholitkul, 1979). Acetylene reduction activities measurements in acid sulfate soils showed a positive effect of phosphorus application in N_2-fixation associated with rice and on free-living N_2-fixing BGA (Watanabe and Cholitkul, 1982).

323) Cooperation with the United Nations University.

In May 1984 a workshop on N_2-fixation in the rhizosphere of rice sponsored by the United Nations University (UNU) was hosted by IRRI. UNU is supporting research and training in the field of BNF associated with rice. Recommendations for future research, including the establishment of a network, were formulated. IRRI agreed to receive some trainees from the UNU project. IRRI scientists are serving as advisors for cooperative research sponsored by UNU.

324) Cooperation with Japan

We have initiated a cooperative project with Dr. Yoneyama, National Institute of Agrobiological Resources, to study rice varietal differences in the capacity to stimulate N_2-fixation, using ^{15}N natural abundance technique. The natural ^{15}N dilution method provides an integrated estimate of the amount of fixed N accumulated by the plant over a growing season. Preliminary results show a good correlation between ^{15}N data and the N_2-fixing trait.

33) Possible fields for future cooperative projects

As suggested during the UNU workshop on heterotrophic BNF on rice, we will develop cooperative relationships with some of the researchers supported by the UNU programme. IRRI provides rice seeds and bacteria strains for common use to scientists working in this field. We also obtained bacteria strains from different parts of the world and maintain it in our culture collection.

Strains of bacteria tagged with genetic and biochemical markers are necessary for inoculation experiments and further studies of associative BNF. Exchange of such strains could be very useful.

Classification of bacteria requires sophisticated techniques especially chemotaxonomical classification which includes analysis of DNA-GC, fatty acids, and quinone by high pressure liquid chromatography. Chemotaxonomical classification of N_2-fixing bacteria isolated from the rice rhizosphere could be developed in cooperation with Japanese scientists.

4) FREE-LIVING BLUE-GREEN ALGAE

41) Current knowledge

Agronomic interest in BGA started in early 1930s when the high spontaneous fertility of submerged rice soils was attributed to BGA by De. Since then, many trials of algal inoculation have been conducted in some rice growing countries and inoculation of rice fields with BGA has been recommended in a few of them. However algal inoculation is a "blind" technology developed on "trial and error" basis. There are many reports of failure. Factors involved in yield increase reported after algal inoculation, factors leading to the establishment of a bloom and the general ecology of BGA in rice fields are still poorly understood. Reports on the extent of area under algal inoculation are controversial, but even considering the most optimistic evaluations, use of algal inoculation seems to be restricted to very limited hectarage in a few Indian states (Tamil

Nadu and Uttar Pradesh), Egypt and Burma (Roger and Watanabe, 1985).

Till recently, almost all studies of agronomical use of BGA have placed emphasis on algal inoculation. Recent ecological studies show that N_2-fixing BGA are widely distributed in rice fields. This indicates that in many rice soils, agricultural practices favoring the growth of indigenous strains may be sufficient to make use of the potentiality of BGA. Practices known to favor growth and N_2-fixation by BGA are phosphorus application, liming, N fertilizer deep-placement, straw application, and grazer control (Roger and Kulasooriya, 1980).

When considering utilization of BGA as a biofertilizer, following points have to be taken into account:

- Potentiality of BGA as an additional source of N for rice is established. Free-living BGA have less potential as N source than legumes or Azolla (around 30 kg N/crop) but their use is promising because little additional labor is required.

- The technology is still at a research level in most of the rice growing countries; and it seems appropriate to consider that algal inoculation is more at an experimental level of large field testing than at a popularization stage. Currently the major limiting factor for utilization of BGA inoculation is the lack of reliable technology. Quality of the inoculum and its establishment in the field are the two stumbling blocks.

- BGA inoculation is not the only possible technology and equal importance should be given to agricultural practices that favor indigenous (and also inoculated) strains.

- Development of reliable technologies for BGA utilization is dependent on: 1) basic research for a better understanding of factors governing the establishment of BGA blooms; 2) field observations and experiments under a large variety of environments with the understanding of the environment, and 3) the effects of BGA on rice besides N_2-fixation.

- In most of the rice countries it would be premature to try to popularize any BGA technology.

42) ACHIEVED AND ON-GOING COOPERATIVE PROJECTS

Since 1980 research on BGA at IRRI is conducted through a cooperative project between IRRI and ORSTOM (Institut Francais de Recherche Scientifique pour le Developpement en Cooperation). A cooperative program on the study of invertebrate fauna and its effect on algae was also conducted with Boyce Thompson Institute.

421) Cooperation with ORSTOM (France)

Major achievements are summarized thereafter.

An extensive review of the literature on BGA in rice fields, of which more than 2000 copies have been distributed, showed that there was adequate evidence to establish that BGA can be utilized as an alternative or supplementary source of N for rice (Roger and Kulasooriya, 1980).

Ecology of BGA in rice fields. A set of standardized methods for field studies of BGA has been developed. It comprises of methods for sampling, evaluating BGA populations, and estimating N_2-fixing activity (acetylene-reducing activity). A large scale survey of the occurrence of BGA in soils showed the presence of N_2-fixing strain in all the rice soils tested in the Philippines and indicated that N_2-fixing strains are present in rice fields at a larger extent than it was previously thought. An extensive study of BGA which grows attached to the rice plant (Kulasooriya *et al.*, 1980) showed that epiphytic BGA made a significant contribution to the nitrogen nutrition of deepwater rice (Watanabe et al., 1982). Study of the effect of chemical N fertilizers and their mode of application has shown that surface application of mineral N inhibit N_2-fixing BGA, whereas deep placement does not disturb the natural algal N_2-fixing system that provides a source of nitrogen and decrease the losses of N by volatilization (Roger et al., 1981).

Composition of BGA, productivity, availability of BGA nitrogen to rice plant: Bibliographic, laboratory, greenhouse and field studies have been conducted to assess the overall composition of BGA. Field samples have a lower N content and a higher ash content than laboratory samples. Estimates of N contained in BGA blooms in submerged soils ranged from 10 to 30 kg N/ha. Using ^{15}N it was found that depending on the nature of the BGA material and the mode of application 15 to 36% of algal N is available to the two crop following application (Tirol et al., 1982).

Inoculation experiments. Different methods for medium to large scale inoculum production have been tested and a method for production of high counts, multistrain, balanced, soil based inocula has been developed.

Field inoculation experiments conducted for three consecutive years and a large scale greenhouse experiment have permitted to assess some of the factors involved in the establishment of BGA blooms of indigenous and inoculated strains.

422) Cooperation with Cornell University and Boyce Thompson Institute

Nitrogen balance experiment in the fields have provided information on the contribution of phototrophic nitrogen fixation (App et al., 1984).

An important contribution has been the study of the role of invertebrate populations in wetland rice fields. Invertebrates that feed on algae (microcrustaceans, snails, chironomid larvae ..) are a major limiting factor for BGA growth and photodependent N_2-fixation. A botanical pesticide from Azadirachta indica (neem tree) was found useful in regulating populations of grazers which limit BGA growth (Grant et al., 1983). A survey of wetland rice fields in the Philippines showed a high incidence of grazers of BGA in all the studied areas.

43) Possible fields for future cooperative projects

BGA has attracted the interest of many researchers, especially in rice growing countries. In 1983 we knew of more than 120 individuals working or using to work with BGA in Bangladesh, Burma, China, Egypt, India, Malaysia, Philippines, Sir Lanka, and Thailand.

A major component of the mandate of International Agricultural Research Centers is to support national research organization and agricultural development activities. Therefore research activities that benefit from an international approach, including running and sustaining research networks, are part of IRRI's madate. Because so many scientists and research centers are interested in BGA, it was decided to study through a survey the possibility of establishing a network of BGA researchers that will facilitate sharing information, knowldge, methodologies, and strains, and encourage cooperative research. Also monitoring tours in Egypt and India by IRRI scientists of the BGA group were organized in order to exchange information with their counterparts and to study possible collaborations.

431) Survey for studying the possiblity of establishing a BGA network.

Collection of information and general results. A list of scientists working on BGA was compiled from books and papers published between 1978 and 1983. A letter explaining our goal and a questionnaire (Annex 1) were send to each of them. At IRRI major interest is on agronomic use but it was decided not to restrict the survey to agronomical aspects and to include all aspects of BGA research. Analysis of 177 questionnaires that we received before December 1984 is summarized thereafter. (Since we have received more answers and collected other names. Our file comprises almost 400 names in June 1985). Geographical distribution of the scientists currently recorded in our file is presented in Table 1.

Interests in BGA. Answers show balanced interest in ecological, physiological and biochemical, and applied studies. In ecological studies emphasis is on freshwater and soils. In physiological and biochemical studies emphasis is on BNF. In applied research emphasis is on algal inoculation, *Azolla*, and pollution.

Reprints policy. A large majority prefers to provide other members of the network with their reprints on request (132). Only 31 were interested in a permanent exchange through a mailing list. A personal reprint exchange involving a limited number of colleagues was preferred by some who said that providing reprints upon request tends to become a "one-way-street" system compared with exchange through a limited mailing list.

Culture collections. Ninety eight scientists have a culture collection and 49 have axenic strains. Collections range from 1 to 400 strains. Regarding policies for providing strains to members of the network, about 40% of scientists having a culture collection are ready to do it for free, about 35% would prefer to do it on an exchange basis. A few ask for reimbursement of mailing expenses. About 10% would like to do it: 1) with charge, or 2) either with charge or as an exchange.

It was pointed out that the questions of commercial and scientific value of unialgal and pure culture strains, and accordingly of patents and other protections of this value, have not been resolved to date. It was said one should view an algal culture not only as a potential experimental material and future source of information but also as a storage of all information already collected in the process of obtaining, isolating and cultivating the organisms. Therefore considering the investment of time, effort, and know-how required to obtain a pure culture, and particularly one with certain taxonomic identity and ecological relevance, questions related to policies for strains protection, exchange or distribution should not be taken lightly.

Information exchange. Fourty percent of our colleagues were in favor of publishing a newsletter when enough material has been collected for filling an issue. However one of our colleagues pointed out that if there is no regular publication date no issue will come out. The second score was for a biannual publication (34%). A majority was in favor of a cost lower than 5 US $ a year (40%) followed by those who want it free (36%). It was said that the idea of a newsletter is excellent provided it is restricted to BGA and rice. The author of this statement commented: "A newsletter with ill-defined aims, tending to cover all BGA topics but with emphasis on rice would stand the risk of doing neither job properly. There should be more than enough material on BGA and rice and the newsletter might encourage real dialogue and cooperation."

Activities of the newtwork. Standardization of methods was quoted in 72% of the questionnaires. There were comments about a need for standardizing methods for 1) identifying and classifying BGA, 2) designing inoculation experiments, and 3) testing inocula. (One colleague pointed out that there are a few bogus commercial products claimed to be "miracle inocula" containing N_2 fixing BGA which did not produce any growth when cultured on N free BGA medium). Sixty two percent of the people interested in ecology of BGA quoted ecological survey as a possible activity of the network. Laboratory or field experiments designed to test methodologies or agricultural practices were quoted in 60% of the questionnaire. There were many comments emphasizing the importance of cooperative projects for research on agricultural utilization of BGA. Workshop and seminars organization was quoted in 76% of the questionnaires and five comments were about organizing conference and workshop on BGA at IRRI. Organizing training courses was quoted in 58% of the questionnaire.

Among other activities, the set up of a center that contains all BGA cultures now carried in the various collections is a major concern. There was a suggestion regarding the establishment of a computerized repository of published information dealing with BGA and one about the establishment of a journal devoted to BGA.

332) Cooperation with Egypt

The Department of Agricultural Microbiology of the Soils and Water Research Institute has conducted extensive trials showing a positive effect of algal inoculation. The Ministry of Agriculture is currently developing a locally financed project called "Production of biofertilizers". One of the objectives of the project is to produce yearly the algal material required for inoculating about 1.3×10^6 acres.

Possible cooperation between Egypt and IRRI was discussed during a visit of P.A.Roger to the Department of Agricultural Microbiology in Giza, in August. Cooperation could be initiated by a stay at IRRI of one Egyptian scientist who will study problems regarding the quality control of algal inoculants.

433) Cooperation with India

In March 1985, three IRRI scientists went to India in order to: 1) meet Indian scientists involved in BGA research and discuss possible cooperation with them, 2) visit sites in Uttar Pradesh, Karnataka and Tamil Nadu where inoculation experiments are conducted, and 3) make a survey of BGA and grazers of BGA in the visited areas. The itinerary of the tour was established from a survey made through a questionnaire sent to the 40 Indian scientists working on BGA who answered the questionnaire related to the establishment of a BGA network and who indicated that algalization is one of their research interest. We had a feed back of 17 questionnaires. We visited 11 laboratories in universities or research centers, had 15 field tours during which we visited 10 sites in farmers fields and 6 sites in experimental stations and had discussions with 50 scientists among which 10 are currently involved in ecological or applied field studies on BGA in rice fields.

There was a general agreement for initiating cooperative work in the field of methodologies and more specifically in standardizing field methods used by the BGA scientists working in rice fields.

5) AZOLLA

51) Current status of the knowledge

Azolla is a symbiosis between an aquatic fern of the *Azolla* genus and a N_2-fixing BGA, *Anabaena azollae*. Because of its rapid growth and high

N content, Azolla has been used as a green manure in rice culture for centuries in northern Vietnam and southern China (Lumpkin and Plucknett, 1982; Watanabe, 1982). In 1979-1980, Azolla was used on 1.3 millions hectares in China and 500,000 hectares in North Vietnam. In the Philippines, it was recently adopted by farmers on about 26,000 hectares. In other countries, Azolla use is incidental and limited.

Azolla grows well with rice in flooded conditions. It is easier to incorporate than other organic manures and has a N potential similar to that of legumes green manures. When grown dual cropped with rice, Azolla can accumulate from 25 to 170 kg N/ha in 60 days (Kikuchi et al, 1984), which may exceed the N requirement of rice. As shown by the low hectarage were Azolla is currently being utilized, there are limiting factors to its adoption. Because Azolla cannot withstand desiccation, a first requirement for its use is water availability and control. Additionally, because Azolla grows from vegetative multiplication, inoculum must be maintained in nurseries all year and multiplied for distribution before field cultivation. High temperature retard Azolla growth. The major detrimental effect in relation of high temperature and humidity is the resulting high incidence of insect and fungus pests. Among nutrients, P is most important. Optimal available P concentrations for Azolla growth are quite rare in rice soils and P application is usually needed. Economics are important. Kikuchi et al., (1984) calculated that labor cost of Azolla use becomes critical where agricultural wage rates approach $2/dollar. Insect control was also an important economic limitation. If more than 200 g carbofuran ai/ha is needed to control insects, benefits are eliminated.

Problems arising from inoculum conservations, multiplication, and transport could be solved to a large extend if Azolla could be propagated from spores. Temperature limitations and P requirements can be reduced by selecting cold or heat resistant strains with low P requirements and by using P fertilizer. Basic studies for making cross among *Azolla* species are needed to improve agronomic characters of *Azolla*. Cheap ways of controlling pests by biological control or plant origin pesticide should be exploited. Labor costs not apply in many rice growing countries. Among green manures, *Azolla* is still less utilized than legumes but, contrary to legume use, *Azolla* use is reported to be increasing, and many countries are evaluating it for popularization.

52) Achieved and ongoing cooperative projects

521) INSFFER trials on *Azolla*

INSFFER initiated a cooperative program to examine the effect of *Azolla* incorporation on rice yield in 1979. Trials in 1979 and 1980 had 9 common treatments at 33 sites in 7 countries. Results showed that one crop of *Azolla* incorporated before or after transplanting rice caused an increase in grain yield equivalent to that obtained with 30 kg N/ha urea or ammonium sulfate. In 1981 and 1982 trials, 8 common treatments were tested in 32 sites in 8 countries. Results showed that 30 kg N/ha urea + 2.0 kg/m^2 *Azolla* fresh weight incorporated before transplanting gave the same yield as that obtained by 60 kg N/ha urea. Incorporating two crops of *Azolla*, one before and the other after transplanting gave a yield slightly lower than that obtained with 60 kg N/ha urea. A third set of trials with 8 common treatments began in 1983. Because it was difficult to incorporate 2.0 kg/m^2 *Azolla* fresh weight without bringing in *Azolla* produced outside the test plots, only 1.5 kg/m^2 *Azolla* was incorporated along with 30 kg N/ha urea. *Azolla* biomass was recorded in 58% of the trials. Rice yield increase per unit fresh weight Azolla was proportional to the effect of N fertilizer at each site. The average fresh weight of one Azolla crop was 1.5 kg/m^2 before transplanting and 1.1 kg/m^2 after transplanting. Plans for 1985 were discussed by collaborating scientists who joined the Azolla workshop in Fuzhou in April 1985. Because the soil disturbance resulting from Azolla incorporation may affect rice yield, a treatment simulating incorporation process was included. Otherwise, choosen treatments were similar to those in 1983-1984 trials.

The number of cooperators on Azolla trials has decreased to some extent in 1985. This may reflect the loss of enthusiasm on Azolla in certain countries. Nevertheless, interest increases in other countries like the Philippines and India.

522) Azolla germplasm

One hundred thirty two strains in six species from --- countries are maintained at IRRI. Part of the collection was obtained through Dr. T. Lumpkin, Washington State University and Dr. Rains from California University. We are now collecting strains from Latin America which are suitable for growth under tropical conditions. For several years IRRI acted as an informal germplasm center for Azolla conservation and distribution. In 1983-84 more than 250 samples ranging from a few gram to 1/2 kilo have been given to laboratories or individuals from 15 countries. A. filiculoides and A. caroliniana from IRRI's collections are now successfully utilized in Vietnam and China. In the Azolla workshop held in Fuzhou (China) in April 1985, it was agreed that IRRI should 1) act as the Azolla germplasm world center, 2) collect Azolla strains but also herbariums, and 3) disseminate information on Azolla through a newsletter.

523) Cooperation with the Philippines.

In 1980, IRRI started Azolla on-farm trials in cooperation with the Philippine Council for Agriculture and Resources Research (PCARRD) and the Ministry of Agriculture. It was through these trials that Azolla was introduced to South Cotabato (Mindanao), where it has subsequently thrived. Following recognition of its successful adaptation in South Cotabato, the Ministry of Agriculture took the lead in evaluating its adaptation throughout the Philippines using a test developed at IRRI. Since then, IRRI has been cooperating with the Ministry of Agriculture of the Philippines in its efforts of dessiminating Azolla technology launched as Unified Azolla Action Program. Aspect of dessimination of Azolla technology will be discussed in another section of this meeting.

We have also initiated cooperation with UPLB scientists in the field of sexual reproduction of Azolla from sporocarps. UPLB scientists found that sporocarp are formed abundantly in cooler Mountain Province. The Mountain Province State University provides good facilities for cooperatives studies of Azolla sporulation, identification of the species through spores, sexual recombination, and mass culture from sporocarps.

524) Cooperation with Portsmouth Polytechnique.

The classification of Azolla is still open to criticism. New criteria and and a better knowledge of spore formation are needed for developing a better taxonomic treatment. Dr. Fowler of Portsmouth Polytechnique gathers herbarium and sporocarps specimens from IRRI collection to try to identify the species. Original identification of most of the species was confirm (A. pinnata, A. filiculoides). The validity of A. microphylla and A. mexicana was confirmed but results indicated that the validity of A. caroliniana is questionable. We are now providing Prof. Fowler with fertile herbariums for further studies.

53) Possible fields for future cooperative projects

The first international workshop on Azolla use, cosponsored by the Fujian Academy of Agricultural Sciences (FAAS) and IRRI, was organized in April 1985 in Fuzhou, coinciding with the inauguration of the FAAS National Azolla Research Center. Thirty-two participants from 10 countries and 44 Chinese scientists discussed: 1) the use of Azolla as biofertilizer and feed, 2) methods for determining N_2-fixed by Azolla, 3) Azolla classification, and 4) possible ways of Azolla research in international cooperation.

IRRI was obviously regarded as an important channel through which cooperative research, distribution of strain and information should be done. The possibility to send scientists and trainees to the National Azolla Research Center of Fuzhou provides another opportunity for cooperative work with China.

6) CONCLUSION

IRRI's policy regarding cooperative research is defined in the 'Plan for IRRI's Third Decade (1982). It reads: "Cooperation with researchers in rice-growing countries is critical to IRRI's success because technology that is tailored to local conditions can most efficiently be developed in the environment for which it is intended" and "The research and training strategies for IRRI's third decade will call for ... relevant extension of cooperative and network programs with national research systems in rice growing countries. Basic research will also be strengthened through cooperation with appropriate scientists...". In 1984, IRRI explicitly recognized the importance of cooperation by adding program area 1100, cooperative research.

IRRI research and dissemination programs utilize seven major pathways of cooperation: global research services, networks, country programs, cooperative research, cooperation with advanced institutions, training and technology transfer, and knowledge sharing. All of them have been used by the research program on BNF (Table 2). Relatively slightly more importance has been given to cooperation with advanced institutions. This reflect the fact that among three research topics on BNF only Azolla is available for popularization, whereas heterotrophic bacteria and free-living BGA are at a stage where more basic research is needed.

Cooperation on basic research on BNF has been very successful. More than one fourth of the scientific papers published by the Soil Microbiology Department during the past five years were the result of cooperative work (Table 3)

A major success in the field of applied research has been spreading Azolla knowledge in many countries where Azolla is now being adopted or tested for adoption. Five reports summarizing the results of cooperative INSFFER trials have been published and distributed to cooperators and to individuals or organizations that requested for them. Azolla program utilizes almost all pathways of cooperation.

When looking at the possible fields for future cooperation, it is obvious that the nature of the possible cooperation pathways depends on the state of the current knowledge.

In the case of heterotrophic N_2-fixation more basic research is needed before field technologies can be develop. We have only a fragmentary knowledge of the complex association between the rice root and heterotrophic bacteria. We do not know what degree of specificity exists between plant and bacterial genotypes. The two major fields for future cooperation in research on heterotrophic BNF are: 1) isolation, collection and identification of N_2-fixing bacteria associated with rice from a wide range of environments, and 2) development of genetically and biochemically marked strains of bacteria. Therefore most of the cooperation on heterotrophic BNF will be with advanced institutions.

In the program on BGA, increasing cooperative research is definitely needed. We are not yet at a stage where the main purpose is to test technologies but it is clear that observations and studies under a wide range of agroecological conditions are needed to understand the ecology of N_2-fixing BGA and the factors that permits the establishment of a bloom. During the survey conducted in 1984-1985 we sent questionnaires to 384 scientists from 59 countries, who have been studying BGA or may have interest in BGA studies. The high return rate (about 75%) showed that there is a general consensus for establishing a network and a newsletter. We need, however, to examine carefully the scope of the network that could be established by IRRI, its relationship to national programs, implications to rice research, and the linkage with other on-going networks, especially INSFFER. There is a huge gap between basic knowledge of test tube growing BGA and BGA inoculation technology, especially in the field of ecology. Therefore, priority should be given to standardization of methods for field studies with BGA, ecological surveys, and field experiments under a range of agroecological conditions. Such studies would obviously benefit from a collaborative approach. In addition, collaborative work would certainly strengthen national programs. We believe that the future of BNF in rice cultivation lies in integrated management. Therefore, some linkage with INSFFER network would be necessary.

As pointed above *Azolla* program is currently the only one with a technology available for popularization. It utilizes almost all pathways of cooperation. To solve probelms related to the application of *Azolla* technology, cooperation with rice growing countries will be strengthened. Some attention should be given to the economics. Contacts have already been taken with advanced organizations to develop cooperation in the field of taxonomy, genetics, and germplasm collection.

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Table 1. Geographical distribution of scientists working on BGA included in our file.

Austria 3	Italy 6
Australia 5	Japan 12
Belgium 1	Kuwait 1
Bangladesh 10	Malaysia 13
Brazil 8	Nepal 3
Bulgaria 1	Netherlands 2
Burma 4	Norway 1
Canada 4	Pakistan 3
China 11	Philippines 10
China (Taiwan) 3	Poland 1
Czechoslovakia 2	Senegal 3
Egypt 7	Saudi Arabia 2
Finland 1	Singapore 1
France 13	Spain 1
Germany 25	Sri Lanka 2
Greece 5	Sweden 7
Hungary 4	Switzerland 4
India 103	Thailand 1
Indonesia 2	U.K. 18
Iraq 1	U.S.A. 57
Iran 1	U.S.S.R. 6
Israel 7	Vietnam 7
	Total 384

Table 2. Pathways of cooperation between IRRI and other scientists and institutions in the field of BNF.

Pathway	Examples
Research services	Azolla germplasm BGA collection
Networks	INSFFER (Azolla component)
Cooperative country programs	Cooperation with the Philippines on Azolla
Cooperative research	Cooperation with Thailand for field studies of BNF
Cooperation with advanced institutions	Cornell University and Boyce Thompson Institute (Balance experiments, study of zooplankton) ORSTOM, France (BGA) National Institute of Agrobiological Resources, Japan (Heterotrophs) UPLB (Azolla)
Training and technology transfer	Training on Azolla (INSFFER) Lectures on BNF and BGA at IRRI Lectures at UPLB (BGA)
Knowledge sharing	Nitrogen and Rice Symposium Advisory Committee Monitoring tours Workshops Written information exchange Bibliography on Azolla

Table 3. Published papers of the Soil Microbiology Department since its creation (1965).

Year	Published papers No.	Papers resulting from collaborative works
1965 - 1969	16	0
1970 - 1974	27	0
1975 - 1979	59	2
1980 - 1984	97	25