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The ORSTOM/IRRI collaborative program on blue-green algae: achievements, contribution to national programs and future plans

(Le programme de recherches collaboratif IRRI /ORSTOM sur les cyanophycées : resultats, contribution à la recherche dans les pays rizicoles et perspectives d'avenir.)

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EXTENDED SUMMARY

Free-living blue-green algae (BGA) that utilize (fix) atmospheric N_2 have a potential as a cheap source of organic N in rice cultivation. For about 30 years, research on agronomical utilization of BGA in the rice-growing countries has focused mostly on inoculation conducted on a trial-and-error basis with strains selected in the laboratory. Progress has been hindered by a limited knowledge of BGA ecology due to inadequate field methods and agronomical utilization of BGA in rice cultivation is still negligible.

The collaborative program on BGA between ORSTOM (Institut Francais de Recherche Scientifique pour le Developpement en Cooperation) and IRRI was initiated in 1979 and formalized in 1981 by the assignment of an ORSTOM scientist to IRRI. The aim of the program is to understand the ecology of BGA in ricefields, and then to develop cultural practices that will consistently maximize the N contribution of BGA to the nutrition of rice.

Permanent personnel assigned to the program is one visiting scientist (P.A. Roger), two research assistants (S. Santiago, R. Jimenez), and one laboratory aide (M. Alumaga). Two postdoctoral fellows (S.A. Kulasooriya in 1980, and P.M. Reddy in 1985/86) and a Ph D student (F. Ghazal, 6 months in 1987) were or are temporarily assigned to the program.

Experiments are mostly conducted on the experimental farm of IRRI. Numerous ecological observations and samplings have also been performed during monitoring tours in Southeast Asian rice-growing countries.

Major results obtained

Standardized field methods for sampling and evaluating BGA populations and for estimating their N_2-fixing activity have been developed.

Different approaches showed that BGA have a N potential of about 30 kgN/ha somewhat lower than that of green manures. However, BGA utilization requires much less work and mony than green manuring because BGA do not compete with the rice crop for soil and water, and they require no incorporation.

Extensive surveys have shown that, contrary to earlier belief, N_2-fixing BGA are ubiquitous in rice soils where they occur at densities frequently higher than that brought about by the quantity of inoculum recommended for application. Major factors limiting the establishment of N_2-fixing blooms are : competition with non N_2-fixing algae when N fertilizer is applied, grazing by invertebrates, and low levels of available P.

Microplot and field experiments showed that nonindigenous BGA do not easily establish themselves when inoculated. These results, together with the

observation that BGA are ubiquitous in ricefields, show that agricultural practices favoring the development of indigenous strains should be emphasized.

Because brodcast chemical N inhibits BGA, they have an advantage in fields where no fertilizer N is applied. Under such conditions their growth can be enhanced by P application, grazer control, and inoculation if needed. Because of their moderate potential, however, BGA cannot be used as the only source of N for producing high rice yields.

A promising approach to utilize BGA is as a component in integrated fertilizer management. Deep-placement of N fertilizers does not inhibit N_2-fixation by BGA. Coupled with the control of BGA grazers by cheap pesticides of plant origin, N fertilizer deep-placement permits the early establishment of a N_2-fixing BGA bloom and simultaneously decreases significantly N losses by volatilization. Replacing P basal application by split application helps maintaining the photodependent N_2-fixing activity during the crop cycle.

Contribution to national programs

Lectures have been given annually during the INSFFER training courses and during INSFFER monitoring tours in India, Vietnam, China, and the Philippines reaching about 600 scientists and trainees. More than 2500 copies of the book " Blue-green algae and rice" have been released. Monitoring tours in Egypt and India permitted BGA surveys, estimation of the current extent of BGA utilization in those countries, meetings with numerous national scientists involved in BGA research and establishment of formal and informal collaboration. Four scientists from Sri Lanka, India, and Egypt were or are being trained at IRRI in the field of BGA.

About 200 strains of N_2-fixing BGA isolated from ricefields are maintained at IRRI. In 1985-86 more than 300 cultures were provided free to laboratories in Southeast Asia and India.

Orientation of the program

In one or two years, the research program will reach a stage at which studies the low range of agroecological conditions available at the IRRI research center or in its vicinity will limit studies. At that time, most field research will have to be conducted by national programs. A 1984 survey of scientists from rice-growing countries showed a strong interest in applied aspects of BGA in rice cultivation (almost 200 scientists indicated interest). The goal now is to encourage national programs to utilize methods developed at IRRI for the study of agronomic practices favoring BGA growth, instead of simply testing inoculation on a trial and error basis. A collaborative program with Egypt is underway. A manual of field methods for studying BGA and a short-term training course on BGA methods are being developed.

While transferring field research to national programs, IRRI will maintain a training component and some basic research on ricefield BGA, to support those programs. These studies will take advantage of IRRI's unique BGA collection, wich is the largest collection of N_2-fixing BGA isolated from ricefields and the fifth largest BGA collection in the world.

Field experiments on the effect of fertilizer management on BGA have shown that more emphasis should be placed on the management of the photosynthetic aquatic biomass (algae and aquatic plants) as a whole and the study of its role on the N cycle in wetland rice in replenishing available N in the soil and in causing N fertilizer losses. In the next years the program is going to study this aspect more and reduce the part devoted to BNF by blue-green algae, which will be considered only as a component of the system.

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Résumé détaillé

Les Cyanophycées capables d'utiliser (fixer) l'azote atmosphérique constituent une source potentielle d'engrais azoté organique bon marché pour la riziculture. Depuis prés de 30 ans, la recherche sur l'utilisation agronomique des Cyanophycées dans les pays rizicoles ne s'est intéressée presqu'exclusivement qu'à l'inoculation des rizières avec des souches sélectionnées en laboratoire et l'utilisation pratique des Cyanophycées en riziculture est pratiquement inexistante. Les progrès des études agronomiques ont été entravés par un manque de connaissances sur l'écologie des Cyanophycées qui résulte de l'absence de méthodes de terrain adéquates.

Le programme de recherches collaboratif IRRI/ORSTOM sur les Cyanophycées a été initié en 1979 et a été concrétisé en 1981 par l'affectation d'un chercheur ORSTOM à l'IRRI. L'objectif majeur de ce programme est l'étude de l'écologie des Cyanophycées dans les rizières inondées et l'utilisation des résultats obtenus pour la mise au point de méthodes agronomiques permettant d'optimiser l'apport d'azote par les Cyanophycées fixatrices de N_2 et son utilisation par le riz.

Le personnel permanent affecté à ce programme comprend un chercheur ORSTOM (P.A. Roger), deux assistants de l'IRRI (S. Santiago-Ardales et R. Jimenez) et un aide de laboratoire. Deux "postdoctoral fellows" (S.A. Kulasooriya en 1980 et P. M. Reddy en 1985/86) et un étudiant de thèse (F, Ghazal, 6 mois en 1987) ont été affectés temporairement à ce programme.

Les expériences *in situ* sont conduites principalement dans la ferme expérimentale de l'IRRI. De nombreuses observations écologiques et des prélèvements ont été effectués au cours de tournées dans les pays rizicoles de l'Asie.

Les travaux réalisés ont permis d'accroître de façon notable les connaissances sur l'écologie des Cyanophycées dans les rizières inondées et ont montré que la recherche sur leur utilisation pratique devait s'orienter vers des voies totalement différentes de celles qui ont été suivies depuis de nombreuses années.

Principaux résultats obtenus

Des méthodes de terrain standardisées ont été mises au point pour l'échantillonage, l'évaluation qualitative et quantitative des population de Cyanophycées et la mesure de leur activité fixatice de N_2 (réduction de l'acétylène).

Différentes méthodes indirectes ont montré que les Cyanophycées ont un potentiel agronomique de l'ordre de 30 kg N/ha. Ce potentiel est inférieur à celui des engrais verts traditionnels : Azolla et Légumineuses. Par contre son utilisation devrait nécessiter largement moins de travail et d'intrants monétaires que celle des engrais verts. Les Cyanophycées n'entrent pas en compétition avec le riz pour l'utilisation du sol et de l'eau d'irrigation et ne demandent pas un enfouissement consommateur d'énergie.

Un échantillonnage extensif dans les principaux pays rizicoles à montré que, contrairement à ce qui était précédemment admis, les Cyanophycées fixatrices de N_2 sont ubiquistes dans les sols de rizière. Elles y ont généralement des densités largement supérieures à celles des propagules qui peuvent être apportées par la quantité d'inoculum habituellement recommandée pour l'inoculation des Cyanophycées dans les rizières.

Les principaux facteurs limitant le développement de fleurs d'eau à Cyanophycées fixatrices de N_2 sont la compétition avec les algues eucaryotes lorsque de l'engrais azoté est épandu dans l'eau de la rizière, le broutage par les populations d'invertébrés et le manque de phosphore assimilable dans les sols.

Des expériences en microparcelles et *in situ* ont montré que les souches de Cyanophycées non autochtones inoculées dans les sols ne s'y établissent que trés rarement. Ce résultat et l'observation de l'ubiquité des Cyanophycées indiquent que la recherche sur leur utilisation agronomique doit mettre l'accent sur les pratiques culturales qui favorisent le développement des souches autochtones.

Etant donné que l'épandage d'engrais azoté dans l'eau de submersion inhibe le développement des Cyanophycées fixatrices de N_2, les rizières dans lesquelles l'engrais azoté n'est pas employé constituent un environnement favorable pour leur utilisation. Dans ces conditions, le développement de fleurs d'eau fixatrices de N_2 peut être favorisé par l'épandage de phosphore, le contrôle des populations de prédateurs et , si nécessaire, l'inoculation algaire. Toutefois, en raison du potentiel modéré des Cyanophycées en termes de kg d'azote par hectare, on ne peut espérer produire des rendements trés élevés en riz en les utilisant comme seule source d'azote.

Une méthode nettement plus prometteuse consite à utiliser les Cyanophycées comme élément d'une fertilisation azotée mixte. Nous avons montré que le placement en profondeur (-5, -7 cm) de l'engrais azoté permet de ne pas inhiber l'activité fixatrice de N_2 photodépendante. Couplé avec l'application de pesticides d'origine végétale (bon marché) pour contrôler les populations de prédateurs, le placement en profondeur de l'engrais azoté permet le développement précoce d'une fleur d'eau fixatrice de N_2 et une diminution importante des pertes d'azote par volatilisation. En remplaçant l'application unique d'engrais phosphaté au repiquage par l'application fractionnée dans le temps de la même quantité d'engrais, on permet à l'activité fixatrice de N_2 photodépendante de se maintenir plus longtemps au cours du cycle cultural.

Contribution à la recherche des pays rizicoles

Des cours sont donnés annuellement aux stagiaires du réseau d'étude de la fertilité des sols et de l'utilisation des engrais azoté(INSFFER). De nombreuses conférences ont été données lors de voyages d'études organisés par ce même réseau en Inde, au Vietnam, en Chine et dans les Philippines. Prés de 2500 copies du livre " Blue-green algae and rice" ont été distribuées. Des tournées de prospection en Egypte et en Inde ont permis 1) la collecte de nombreux échantillons, 2) l'estimation du niveau d'utilisation pratique des Cyanophycées dans ces pays, 3) des rencontres avec de nombreux chercheurs impliqués dans l'étude de l'utilisation des Cyanophycées en riziculture et 4) l'établissement de collaborations formelles où informelles.

Une collection d'environ 200 souches de Cyanophycées isolées à partir de sols de rizière a été constituée. En 1985-86 plus de 300 cultures ont été fournies gracieusement à des centres de recherche du Sud Est Asiatique et de l'Inde.

Orientation du programme de recherches

Dans un à deux ans, le programme arrivera à un stade où la faible variabilité de l'environnement où sont conduites les expériences *in situ* (ferme expérimentale de l'IRRI et région environnante) constituera un facteur limitant majeur et où ces expériences gagneront à être conduites par les programmes de recherches nationaux dans un large éventail de sols et de conditions climatiques. Une enquète effectuée en 1984 auprés des chercheurs des pays rizicoles a montré que prés de 200 d'entre eux travaillaient sur des sujets en relation directe ou indirecte avec l'utilisation agronomique des Cyanophycées. Maintenant, notre principal objectif est d'inciter les organismes de recherches nationaux à utiliser les méthodes mises au point à l'IRRI pour étudier, en termes de biomasse algaire, d'activité fixatrice de N_2 et de rendement, les pratiques culturales favorisant le développement de fleurs d'eau fixatrices de N_2 au lieu de

tester uniquement les effets de l'inoculation sur le rendement en riz.
Quatre chercheurs du Sri Lanka, de l'Inde et de l'Egypte ont reçu une formation sur les Cyanophycées à l'IRRI. Un programme de recherche collaboratif a été mis en place avec l'Egypte. Un manuel sur les méthodes d'études des Cyanophycées dans les rizières et un cours pratique sont en *cours d'élaboration.*

Tandis que la majeure partie des recherches appliquées sera transferrée dans les programmes nationaux, le programme ORSTOM/IRRI maintiendra quelques thèmes de recherches fondamentaux pour pouvoir aider ces programmes, à leur demande, ainsi qu'un programme d'enseignement sur les méthodes d'étude. Les études fondamentales tireront un avantage certain de la collection de Cyanophycées qui est la plus grande collection mondiale de souches isolées de sols de rizière et la cinquième collection mondiale de Cyanophycées fixatrices de N_2.

Les expériences *in situ* concernant l'effet du mode d'application des engrais azotés sur l'activité fixatrice de N_2 photodépendante ont clairement montré que l'étude du rôle de la biomasse photosynthetique submergée (algues et plantes aquatiques) constituait un champ de recherches trés prometteur. D'une part cette biomasse joue un rôle majeur dans les pertes d'engrais azoté par volatilisation (qui peuvent atteindre 50% de l'azote épandu) ; d'autre part elle est en grande partie responsable de la production de l'azote assimilable par le riz dans la couche supérieure des sols submergés. Au cours des prochaines annés l'accent sera mis sur l'étude de pratiques culturales prenant en compte l'utilisation de la biomasse photosynthétique submergée pour diminuer les pertes d'engrais et maximiser la fixation biologique de l'azote. Dans ce programme, les Cyanophycées seront considérées comme un des composants du système et non plus comme le thème majeur de l'étude.

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The ORSTOM/IRRI collaborative program on blue-green algae: achievements, contribution to national programs and future plans

The collaborative research program on blue-green algae between ORSTOM (Institut Francais de Recherche Scientifique pour le Developpement en Cooperation) and IRRI (International Rice Research Institute) was initiated in 1979 during a sabbatical leave at IRRI of P. A. Roger who has then been assigned at IRRI since June 1981.

1. Justification of the program

In the 1930s, P. K. DE ascribed the high nitrogen fertility of wetland rice soils to nitrogen fixation by blue-green algae (BGA). Since then, there have been many attempts to encourage BGA growth in ricefields and to utilize them as an alternative source of nitrogen in rice cultivation. The results and major features of the research conducted during the last 30 years on BGA in rice fields and their agronomical utilization were summarized in 1980 (7) and 1986 (51) as follows:

1. Average estimate of N fixed by free-living BGA is 27 kgN/ha per crop.
2. Till recently, research on practical utilization of BGA in rice cultivation has focused on algal inoculation only.
3. Successful field experiments on BGA inoculation show yield increases averaging 14%. Unfortunately, in most of these experiments, no quantitative analysis of BGA biomass, N_2-fixing rate, or inoculum establishment was performed; therefore reasons for the observed yield increase are unclear.
4. There are many reports of failure, and probably many failures which are not reported.
5. Currently, algal inoculation is a technology developed on a trial and error basis. The extent of its adoption by farmers is negligible.
6. The poor understanding of 1) the general ecology of BGA, 2) the factors contributing to yield increase after inoculation and 3) the ways of establishing an algal bloom, limits the development of technologies that take advantage of the potential of BGA as a biofertilizer in rice cultivation.

The aim of the ORSTOM/IRRI collaborative program is to understand the ecology of BGA in wetland ricefields in order to develop cultural practices that permit to consistently maximize the contribution of photodependent nitrogen fixation to the nitrogen nutrition of rice.

2. Research achievements

The following is a list of the research topics that have been or are being studied. Major results are summarized. Numbers in parentheses refer to the list of publications (section 5).

21. Bibliographic studies

211. Analysis of the literature on BGA and rice

A thorough review of the literature in the book " BGA and rice ", of which more than 3,000 copies have been distributed, showed adequate evidence to establish that BGA significantly contributed to the nitrogen required by the rice crop; however results varied considerably (7).

212. Other reviews

- Free-living BGA in tropical soils (1, 16)
- Ecology of ricefields (44)
- Ecology of nitrogen fixation and nitrogen microorganisms in ricefields (24, 33, 36)
- Algae and aquatic weeds as a source of nutrients for rice (32)
- Technologies for utilizing N_2 fixation in rice cultivation (23, 38, 51, 54)
- Effect of the photosynthetic aquatic biomass on N dynamics in rice fields (48, 58)
- Work conducted at IRRI on BGA and nitrogen fixation (9, 18, 25, 60)
- N cycle in wetland soils (61).

22. METHODOLOGICAL STUDIES

221. Methods for estimating algal abundance and N_2-fixing activity

A set of standardized methods for field studies of BGA has been developed. It comprises methods for sampling, evaluating BGA populations, and estimating their N_2-fixing activity (2, 22, 37, 43). The release of a booklet on methods for field study of BGA in wetland soils is planned for 1987.

222. Isozymes for strains identification

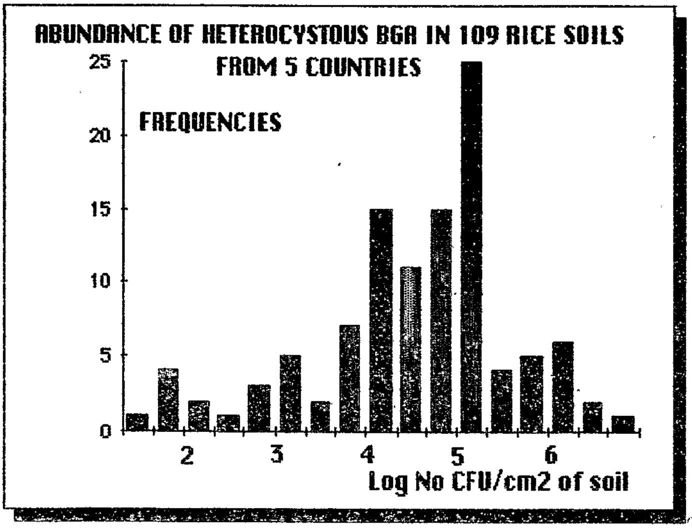
Isozymes of 18 enzymes have been studied on 26 strains of N_2-fixing BGA to evaluate the possible use of isozymes in identifying BGA strains in soil inoculation experiments. Four enzymes were selected. The method is highly discriminatory at genus and species levels and has a satisfactory replicability which allows its use for strain characterization. More data are needed before conclusions on taxonomical use can be drawn (34).

23. ECOLOGICAL STUDIES

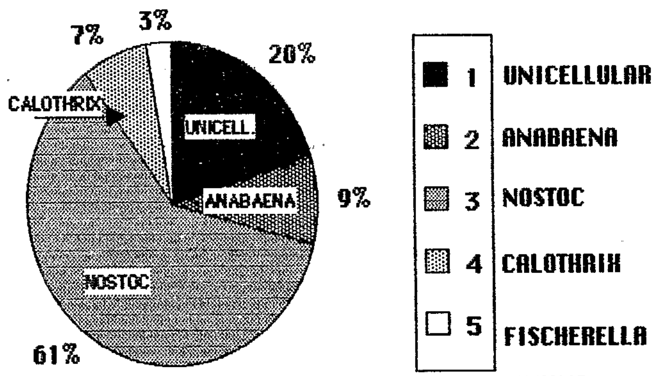
231. Occurrence of N_2-fixing BGA in ricefields

An extensive survey of the algal flora in 120 ricefield soils from six countries (40, 55, 56, 57, 59) has contradicted the earlier belief that N_2-fixing BGA are absent in many rice soils. N_2-fixing strains were found in all studied soils. Densities ranged from a few dozen to more than 10^6 colony-forming units (CFU)/cm^2 of soil; the median was 8×10^4 CFU/cm^2 (Fig 1).

Figure 1 :



Field surveys and bibliographic study showed that *Nostoc* spp., *Anabaena* spp., *and Calothrix* spp. are present in 80% or more of the studied soils. *Nostoc* is most frequently the dominant genus (Fig. 2).



RELATIVE ABUNDANCE OF MAJOR TAXA OF N2-FIXING BGA IN 109 RICE SOILS FROM 5 COUNTRIES.

Positive correlations were found between the abundance of heterocystous BGA on one hand and pH (Fig. 3), available P, and CEC of the soils on the other hand (Table 1).

ABUNDANCE OF HETEROCYSTOUS BGA IN RELATION WITH SOIL pH (R = 0.23 *)

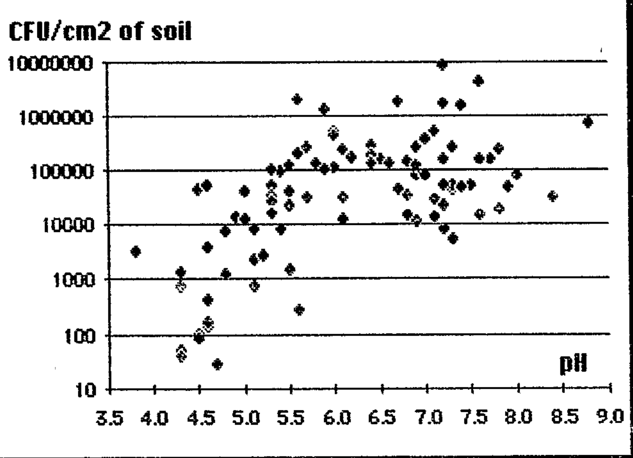


Table 1 : Correlation between soil properties and algae counts in 102 rice soils (CFU/g dw) (Pearson's correlation coefficient)[a].

	C	N	C:N	P	CEC	pH	Tot.[b]	Hcy.[c]	Tot./Hcy.
Carbon		++	ns	ns	ns	ns	++	ns	++
Nitrogen			ns	ns	ns	ns	++	ns	+
C:N				--	ns	ns	ns	-	+
Available P(Olsen)					ns	ns	ns	++	ns
CEC						++	ns	++	ns
pH							ns	++	--

[a] : + / ++ / - / -- : positive or negative correlations significant at the 5 and 1% levels respectively ; [b] : Total algal population. [c] : Heterocystous BGA.

232. Epiphytism

Epiphytic BGA (5) are present on wetland rice plants (12), deepwater rice (10), and weeds (11). Epiphytic BGA makes a low contribution to shallow water rice. The contribution becomes of agronomic value with deepwater rice (21). The transfer of fixed N from algae to the deepwater rice plant was demonstrated.

233. Grazing

In wetland ricefields, grazing by populations of invertebrates is a major limiting factor for BGA growth. Grazers are responsible for the development of blooms of mucilaginous BGA, which are resistant to grazing but frequently less efficient N_2 fixers than fast growing nonmucilaginous strains susceptible to grazing (3, 35, 40, 46)

24. BGA AS A SOURCE OF NUTRIENTS FOR RICE

241. Composition of BGA

The study of laboratory-, greenhouse-, and field-grown BGA has shown substantial variation in BGA chemical composition (42, 49, 50). Dry matter content ranged from 0.9 to 14%. Ash content ranged from 17 to 71%. Nitrogen content ranged from 1 to 12% on ash-free basis. Variations were larger within genera than between genera which indicates that chemical composition may not be very useful for taxonomic purposes. Environmental factors and the physiological status of the BGA influence their composition more than their taxonomic position.

Very significant differences were observed between material grown in artificial medium and field samples. The latter were characterized by very high ash contents, lower N contents, and P deficiency (Table 2).

Table 2 : Comparison of the composition of laboratory cultures and field samples of N_2-fixing blue-green algae.

	Cultures		Field samples	
	$\bar{X}$	range	$\bar{X}$	range
Dry matter *	4	0.3-14.0	4	0.9-7.0
Ash**	7	6-12	45	27-71
Nitrogen***	6	4-12	5	3.8-7.4
Carbon***	42	34-72	40	37-45
C/N	7	5-13	8	5-12
Phosphorus ***	0.7	0.2-2.0	0.2	0.05-0.39

*: %fw **: %dw ***: % dw ash free

The low P content of field samples of BGA (Fig. 4) and the beneficial effect of P application on photodependent BNF indicate that low P availability is a major limiting factor for N_2-fixing BGA in rice soils.

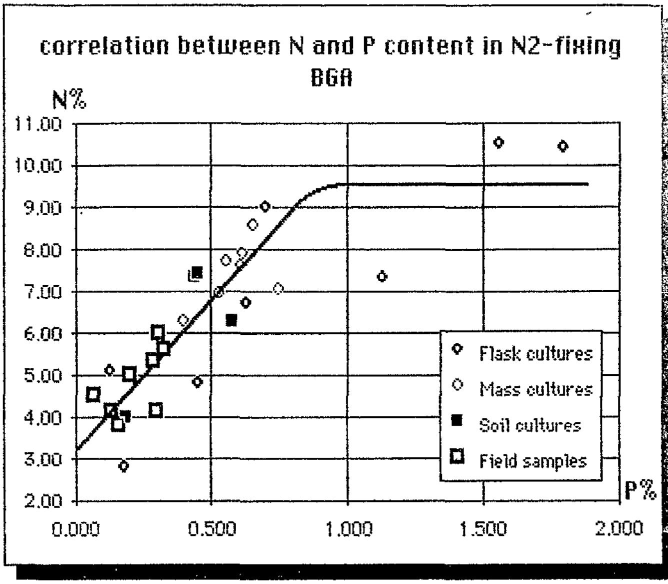
243. Availability of BGA nitrogen to rice plant

Use of ^{15}N showed that, depending on the nature of the algal material and the method of application, 15 to 36% of BGA nitrogen is available to the two crops following BGA application (13, 19, 20)

244. "Auxinic" effect of BGA

Experiments in vitro, in pot, and in situ have failed to demonstrate or disprove the so-called "auxinic effect" of BGA. In all experimental designs, experimental artifacts had much more effect on the plants than the treatment with BGA.

Figure 4 :



25. AGRONOMICAL PRACTICES TO ENHANCE BGA GROWTH

251. Algal inoculation

2511. *Algal inoculants.* A method for the production of high-count, balanced, multistrain inocula has been developed.

The composition of soil-based inocula produced in India, Eygpt, Burma, and at IRRI was surveyeded. Results combined with those of the study of the occurrence of heterocystous BGA in rice soils show that in most soils, indigenous N_2-fixing BGA occur at densities largely higher than that brought by the quantity of soil-based inoculum recommended for application (40, 59) (Table 3).

This indicates that inoculation is not the only possible way to utilize BGA and that emphasis should also be placed on agricultural practices that enhance indigenous BGA growth.

Table 3 . Relative and cumulative frequency of the ratio of indigenous to inoculated heterocystous BGA[a].

Range of values	Frequencies	Cumulative frequencies
Higher than 10000	4.8 %	4.8 %
from 1000 to 10000	15.7 %	20.5 %
from 100 to 1000	26.1 %	46.6 %
from 10 to 100	22.3 %	68.9 %
from 1 to 10	20.6 %	89.5 %
lower than 1	10.5 %	100.0 %

[a] Calculated from enumerations in 102 rice soils and 22 soil-based inocula.

2512. *Establishment of inoculated BGA.* Microplot and field experiments have shown that nonindigenous strains do not easily establish in a field. When practices ensuring the formation of a BGA bloom were applied in various soils inoculated with nonindigenous strains, the bloom was most frequently composed of indigenous strains. Persistence of inoculated strains was more related to the nature of the strains than to that of the soil (35, 47, 53).

2513. *Inoculation experiments.* Various field experiments were conducted (35, 42, 47 and unpublished results). A significant but moderate effect on BGA biomass or/and N_2 fixation and/or grain yield was observed only when the inoculum was produced from the soil of the field to be inoculated. This suggests that inoculation with indigenous strains is a possible method for increasing BGA growth.

252. Other agronomic practices

2521. *N fertilizer deep placement.* Surface appplication of mineral nitrogen inhibits BGA whereas fertilizer deep placement permits BGA growth and a N_2-fixing activity equal to 30% to 100% of the control where no mineral N was applied (8, 31, 47).

2522. *Grazer control.* Control of ostracods and microcrustaceans that graze on BGA by cheap pesticides of plant origin , increases N_2 fixation and N gains in the soil (35, 46, 47). Treatment with phosphorus and pesticides of plant origin (*Azadirachta indica* and *Phytolacca dodecandra*) resulted in differences between the treated plots and the control ranging from -2 to 10 g N/m^2 after two month of submersion. Control of ostracod populations with *A. indica* significantly increased N balance through the conservation of the photosynthetic biomass. *P. dodecandra* successfully controlled snails populations, however this pesticide, which acts as a detergent, also inhibited algal growth (Table 4).

Table 4 : N balance in 0,5 m^2 rice soil microplots after two months of submersion.

Treatment (kg . ha^{-1})			Nitrogen balance (g . m^{-2})	
Phosphorus (P_2O_5)	*Azadirachta indica*	*Phytolacca dodecandra*		mean
0	0	0	0,6	
0	0	8	-1,5	
0	100	0	1,6	
0	100	8	2,3	0,7
20	0	0	2,1	
20	0	8	1,1	
20	100	0	10,2	
20	100	8	3,9	4.3

2523. *Surface application of straw.* Preliminary trials have shown a beneficial effect of surface application of straw on the formation of N_2-fixing blooms of BGA and/or the photodependant N_2-fixing activity of the soil (17, 26).

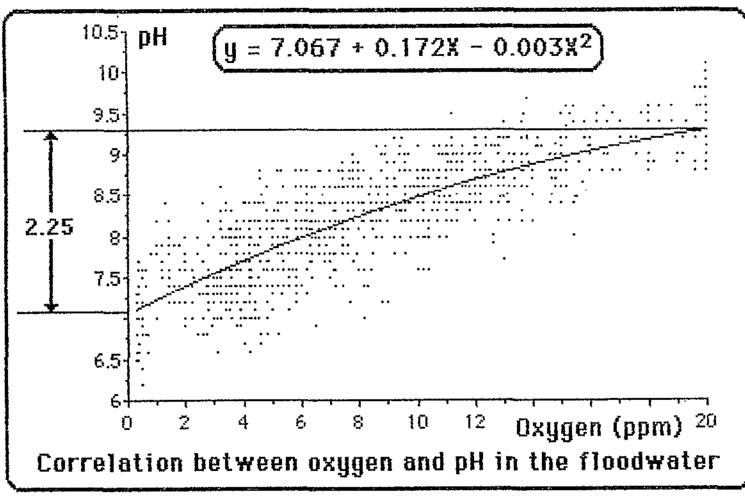
26. EFFECT OF ALGAE ON N CYCLE IN RICE FIELDS

Bibliographic surveys of the different aspects of the topic have been conducted (48, 58, 61). A few studies of the algal flora in re[illegible]on with N cycle were conducted in collaboration with the Agronomy Department.

Results from three independent experiments show that a large algal biomass is not required for the development of daily variations of pH that cause significant losses of N by ammonia volatilization (45).

A study of the correlation of dissolved oxygen and pH of the floodwater of five rice soils showed that, on the average, pH increases by 2.25 units when the photosynthetic activity in the floodwater increases from none (less than 1 ppm O_2 in the floodwater) to the value corresponding to a maximum oxygen oversaturation of 20 ppm (Fig. 5).

Figure 5 : Correlation between dissolved oxygen and pH in the floodwater of rice soils[a].



[a]: 1200 data from five soils.

27. BGA CULTURE COLLECTION

About 200 strains of N_2-fixing BGA (Table 5) isolated from ricefields in Southeast Asia and West Africa are maintained as unialgal material in liquid medium and as dried inoculum on filter paper strips. The last method has proved to be efficient for unialgal material. It is more efficient for genetic conservation than continuous subculturing on artificial media, which frequently affect physiological and morphological properties of the strains. The method is also easier and less expensive than conservation in liquid nitrogen.

Strains are provided free on request. In 1985 more than 200 samples of BGA strains were sent to laboratories in Southeast Asia and India.

Table 5: Number and origin of the collection of blue-green algae.

Genera	Africa		Asia		Europe	Other regions	TOTAL
	Senegal	Others	Philipp.	Others			
Anabaena	20	2	5	11	9	3	**50**
Aphanothece	0	1	1	0	0	0	**2**
Aulosira	1	1	0	1	0	1	**4**
Calothrix	14	2	5	3	0	1	**25**
Cylindrospermum	4	1	1	0	0	0	**6**
Gloeotrichia	1	0	3	0	0	0	**4**
Fischerella	0	3	7	0	0	0	**10**
Nodularia	2	0	1	1	0	0	**4**
Nostoc	20	6	13	13	2	5	**59**
Scytonema	6	0	1	1	0	0	**8**
Tolypothrix	0	0	0	4	0	0	**4**
Wollea	0	0	1	0	0	0	**1**
Westiellopsis	0	1	0	0	0	0	**1**
N_2 Fixing	68	16	38	34	11	10	**177**
Non-fixing	16	0	0	1	1	0	**18**
TOTAL	**84**	**16**	**38**	**35**	**12**	**10**	**195**

3. OTHER ACHIEVEMENTS

31. Monitoring tours

Trips in Egypt (29) and India (40) enabled us :
- to meet with national scientists involved in BGA research and discuss possible cooperative work with them,
- to visit sites of BGA inoculum production and sites where inoculation experiments are conducted,
- to estimate the current extent of algal inoculation in these countries, and
- to make a survery of BGA and grazers in the visited areas.

32. Participation in the INSFFER Network

Lectures have been given annually during the INSFFER training courses and also during the INSFFER monitoring tours in India , Vietnam, China, the Philippines (6, 14, 15, 39, 40, 52).

33. Teaching in degree courses (University of the Philippines)

- Advances in Phycology, Botany 202 of the Institute of Biological Sciences (12 hours/year).
- Botany 291, Special topics, The biology of BGA and their symbiotic forms, UPLB (10 hours/year).

Handouts have been prepared for the major topics (27, 28, 30, 39)

4. CONCLUSION AND ORIENTATION OF THE PROGRAM

For about 30 years, research on the agronomical utilization of BGA has mostly focused on inoculation conducted on a trial and error basis with strains selected in the laboratory. Progress in the knowledge of BGA in ricefields has been hindered by the lack of field methods for ecological and agronomical studies and the resulting utilization of grain yield as a unique criterion in experiments with BGA. Research on BGA under the IRRI/ORSTOM collaborative program has permitted significant progress to be made in the field of the ecology of BGA in ricefields and has shown that methods for their practical utilization needs to be reconsidered.

41. Major results obtained

Development of field methods for the study of BGA

We have developed a set of methods for estimating BGA abundance and their N_2-fixing activity. Because of the uneven distribution (log-normal) of BGA in rice soil, special attention has been paid to sampling strategies.

Estimation of the nitrogen potential of BGA in rice cultivation

BGA have a N potential of about 30 kg N/ha, lower than that of legume green manures and *Azolla* However, the agronomical utilization of this potential requires much less work and monetary input than green manuring. BGA do not compete with the rice crop for soil and water.

Ecology of BGA in rice fields

Extensive surveys have shown that, contrary to earlier belief, N_2-fixing BGA are ubiquitous in ricefields. Major factors limiting the establishment of N_2-fixing blooms are competition with non N_2-fixing algae when N fertilizer is applied, grazing by invertebrate populations, and low levels of available P.

Methods for utilizing BGA in rice cultivation

Our results, showing that BGA are ubiquitous in ricefields and that non-indigenous strains rarely established when inoculated, indicate that attention should be paid to agricultural practices favoring the development of a N_2-fixing bloom of indigenous strains.

Because of the inhibition by broadcasted chemical N, BGA have an advantage in fields where no fertilizer N is applied. Under such conditions their growth can be favored by P application, grazer control, and, if needed, inoculation. But because of their moderate potential, they cannot be used as the only source of N for producing high rice yields.

The most promising approach for utilizing BGA is as a component of integrated fertilizer management. We have shown that deep placement of N fertilizers coupled with the control of BGA grazers by cheap pesticides of plant origin permits the early establishment of a N_2-fixing BGA bloom. Simultaneously, deep placement very significantly decreases N losses by volatilization. Replacing basal application of P by its split application helps in maintaining the photodependent N_2-fixing activity during the crop cycle.

42. Orientation of the program

In one or two years, the research program will reach a stage where studies will be limited by the very low range of agroecological conditions available at the IRRI research center or in its vicinity and where most of the field research can be conducted by national programs using methodologies designed at IRRI (and eventually adapted to local conditions).

A survey conducted in 1984 has shown a strong interest of scientists from ricegrowing countries in applied aspects of BGA in rice cultivation (almost 200 scientists showed their interest). Our goal now is to encourage national programs interrested in BGA to utilize methods developed at IRRI for the study of agronomic practices favoring BGA growth instead of only testing inoculation conducted on a trial and error basis.

We have already started a collaborative program with Egypt and an

Egyptian scientist is trained in our department. A short-term training course on methodologies for a limited number of participants is planned.

While transferring field research to national programs, we should, however, maintain some basic research on rice field BGA, to be able to help those programs. A major point is the identification of strains, which is one of the major gaps in BGA research. This study will take advantage of our unique collection which is the largest collection of N_2-fixing strains isolated from ricefields and the fourth largest collection of N_2-fixing BGA in the world.

Field experiments on the effect of fertilizer management on BGA, algae, and aquatic plants show that we should place more emphasis on the management of the photosynthetic aquatic biomass as a whole and study its role on the N cycle in wetland rice especially in replenishing soil-available nitrogen and in N fertilizer losses. BGA are an important component of the photosynthetic biomass, but their contribution is of the same order of magnitude as the N losses from broadcast N fertilizers that are due to the photosynthetic activity of algae and aquatic plants.

We started research on the role of the photosynthetic biomass in 1986. In the next years, the program will give more attention to this aspect and reduce the part devoted to BNF by blue-green algae, which will be considered only as a component of the system.

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