

BLUE-GREEN ALGAE IN INDIA: A TRIP REPORT

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1) GENERAL INFORMATION ON THE TRIP

11) PURPOSE OF THE TRIP:

- to meet Indian scientists involved in BGA research and discuss possible cooperative work with them.
- to visit sites in Uttar Pradesh, Karnataka and Tamil Nadu where inoculation experiments are being conducted.
- to make a survey of BGA and grazers of BGA in the visited areas.

12) DATE:

7th to 22nd of March, 1985

13) IRRI SCIENTISTS INVOLVED:

P. A. Roger (PAR), Ian F. Grant (IFG), and P.M. Reddy (PMR)

14) MAJOR VISITED PLACES:

- New Delhi, Varanasi, Bangalore, Coimbatore, Trichy, Aduthurai, Chidambaram, Pondicherry, Madras and Tirur.

The itinerary of the tour was prepared 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 interests. We had a feedback of 17 questionnaires, a copy of the questionnaire and the accompanying letter are annexed (Annex 1 and 2).

15) BASIC STATISTICS ABOUT RICE IN INDIA

40 x 10⁶ ha of rice 22 x 10⁶ ha planted with HYV 25 x 10⁶ ha of rainfed rice (upland) 87% of the farmers have less than 2 ha land holdings and use little or no fertilizer 1 kg N = 5 Rupees 1 kg paddy = 1.35 Rupees 1 kg single superphosphate = 5.5 Rupees 1 kg BGA inoculum in Tamil Nadu = 1 Rupee

2) ITINERARY AND SCHEDULE

Thursday March 7th:

Left Manila 15:00 (TG 621) arrived Bangkok 17:10. Left Bangkok 18:55 (AF 175) arrived New Delhi 21:35. Welcomed by Mr. A.P. Haran, IRRI representative in India Checked in at Hotel Kanishka.

Friday March 8th:

09:45 - Picked up at the hotel by Mr. Haran. The whole day was spent at the Indian Agricultural Research Institute in the Division of Microbiology.

10:00 to 11:30 - Meeting with Dr. Goyal, Scientist in charge of algalization experiments.
11:30 to 13:30 - Meeting with Dr. Venkataraman, Coordinator of BGA programme. Dr. Khalil Ghani in charge of BGA inoculum production in Egypt was also visiting IARI and attended the meeting.
13:30 to 14:30 - Lunch at Hotel Karnishka.
14:30 to 15:00 - Meeting with Prof. Subba Rao, Head of the Division.
15:00 to 15:30 - Meeting with Dr. Kaushik and Dr. Roychoudhury, scientists working on the reclamation of saline soils with BGA.
15:30 to 16:30 - Lecture by PAR (Research on BGA at IRRI).
16:30 to 17:00 - Lecture by IFG (Grazing of algae by invertebrates in wetland rice fields).
17:00 to 17:30 - General meeting with Dr. Venkataraman and his colleagues.
17:30 - left for hotel

Saturday March 9th:

Visited the School of Life Sciences, Jawaharlal Nehru University. Meeting with Prof. Mohanty, Dr. Chintamani and some of their students.

10:00 to 11:15 - Discussions
11:15 to 12:00 - Lecture by PAR (Research on BGA at IRRI).
12:00 to 12:30 - Lecture by IFG (Grazing of algae in wetland rice fields)
12:30 to 12:45 - Lecture by PMR (BGA greenhouse experiments at IRRI).
Afternoon - Free

Sunday March 10th:

Left New Delhi 07:30 (IC 407) arrived Varanasi. 10:20 - Welcomed at the airport by Dr. Ashok Kumar and Dr. A. Tripathy. Checked in at Diamond Hotel.

Afternoon - Visited the Center For Advanced Study in Botany, Banaras Hindu University (BHU).

13:30 to 14:00 - Meeting with Dr. H.D. Kumar and Dr. A. Kumar. Finalization of the program.

14:00 to 17:00 - Meeting with the research staff of Prof. Kumar and presentation of the different programs conducted in his laboratory by Ms. Banerji, Ms. Rao, Dr. D.V. Singh, Dr. Misra, Dr. Dube, Dr. Tripathy and Dr. A. Kumar.

Monday March 11th:

08:00 to 13:00 - Field tour in rice and wheat fields in Saranath area, about 10 km west of Varanasi. Collection of soil algal crust, and grazer samples. Observation of perennating Aulosira in the fields. Collection of samples.

13:00 to 14:00 - Lunch at Diamond Hotel.

14:00 to 15:30 - Meeting at BHU with Dr. Kashyap, Dr. S.P. Singh, Dr. Tiwari and presentation of their respective research programmes.

15:30 to 16:30 - Meeting with the research staff of the laboratory of Prof. Talpasayi: Mr. Rao, Mr. Basu, Mr. Parameswaran, Mrs. Verma, Mrs. Bajaj, Mr. Prasad, Mrs. A. Singh and Dr. Bahal.

16:30 to 17:00 - Meeting with Prof. H.D. Kumar and Dr. D.V. Rai. Presentation by Dr. Rai of his research program.

Tuesday March 12th:

9:30 to 10:00 - Meeting with Prof. H.D. Kumar, Dr. A. Kumar and Dr. A. K. Rai. Presentation by Dr. Kumar and Dr. Rai of their research programmes.

10:00 to 11:00 - Lecture by PAR

11:00 to 11:30 - Lecture by PMR

11:30 to 12:15 - Lecture by IFG

12:15 to 13:00 - General meeting with BGA scientists, Prof. Talpasayi and Prof. Sarma joined the meeting.

14:30 - Left to the airport. Plane delayed (IC 498).

Arrived New Delhi 20:00 - Checked in at Hotel Kanishka.

Wednesday March 13th:

Left New Delhi 13:30 (IC 518) Arrived Bangalore

17:30. Checked in at East-West Hotel. Dinner with Dr. Gowda to finalize the programme.

Thursday March 14th:

Welcomed by Dr. Rai. Meeting at the Agricultural University, Bangalore with scientists from the Universities of Bangalore, Mysore and Dharwad.

09:00 to 09:45 - Presentation by Dr. Shetty, Dr. Shivaprakash and Dr. Krishnappa of their research programmes.

09:45 to 10:00 - Presentation by Dr. Hosmani (Mysore University) of his research program.

10:00 to 10:30 - Presentation by Dr. Bongale (Dharwad University) of his research program.

10:30 to 11:30 - Lecture by PAR

11:30 to 12:15 - Lecture by IFG Lunch at the University with Dr. Rai, Dr. Shetty and Dr. Bongale.

13:00 to 14:00 - Visit of the laboratories and other facilities (greenhouses and BGA incubation chamber).

14:00 to 15:00 - General meeting.

15:00 to 18:30 - Field tour in MRS/UAS experimental field (Hebbal) and in farmers fields on the Mysore road (24 km from Bangalore). Collection of samples.

Friday March 15th:

Left Bangalore 08:30 (IC 533). Arrived Coimbatore 09:10 - Welcomed by Dr. Kannaiyan.

11:00 - 13:00 - Discussion with Dr. G. Oblisami and Dr. S. Kannaiyan on BNF programmes and visit of the laboratory.

13:30 PM - Lunch, North house, TNAU.

14:15 to 16:30 - Lectures by PAR, IFG, and PMR.

16:30 to 20:00 - Visit to Paddy Breeding Station, TNAU. Discussion on the on going field trials. Collection of soil and grazers samples.

20:00 - Dinner at TNAU cafeteria.

Saturday March 16th:

06:30 to 11:45 - Field tour in Telugu Palayam area, 20 km west of Coimbatore.

11:45 to 13:00 - Meeting with Dr. Kannaiyan at the Department of Agricultural Microbiology, Coimbatore.

13:00 - Lunch at TNAU Canteen

14:00 to 19:30 PM - Coimbatore - Trichy by Road 20:30 - Dinner at Guest House, Trichy. Halt at Trichy.

Sunday March 17th:

06:00 to 09:00 - Visit to Kumaraperumal Farm Science Centre, Navalur, Kuttapattu. Discussion with Thiru A.K. Kathirvelu, Principal and Thiru Ramanujam, JDA, Trichy. Collection of samples.

09:15 - Breakfast

09:30 to 10:30 - Meeting with Dr. Kathirvelu, Dr. Ramanujam, Dr. Kannaiyan, and Mr. Namanarash (Journalist of the Hindu).

10:30 to 11:30 - Field visit in Trichy and Kumbakonam areas. Collection of samples.

11:30 to 14:00 - Journey to Aduthurai.

14:00 to 14:30 - Lunch at Aduthurai.

14:30 to 16:30 - Field visit in TRRI, Aduthurai. Collection of samples.

16:30 to 17:00 - Visit of farmers fields around Mayavaram.

18:00 to 19:00 - Visit to Poompuhar museum

20:30 - Reached Chidambaram. Dinner at Annamalai University Guest House with Prof. Prasad. Halt at Annamalai University.

Monday March 18th:

06:45 to 08:00 - Meeting with Prof. Prasad and his research staff. Visit to the experimental farm. Collection of samples.

08:00 to 09:00 - Breakfast and discussions at Annamalai University Guesthouse.

09:00 - Visit of Sri Nataraja temple. Left to Pondicherry.

11:00 - Reached Pondicherry. 11:00 - Field tour in Pondicherry area with Mr. K. Natarajan, Deputy Director of Agriculture. Three sites around Mangalam were visited. Collection of samples.

13:30 to 15:00 - Lunch in Pondicherry with agricultural officers.
15:15 to 16:45 - Visit of Agricultural Education Center (KVK) of TNAU Pondicherry with Dr. A. Subrahmanian. Collection of samples.
16:45 to 20:00 - Journey to Madras

Tuesday March 19th:

06:00 to 07:15 - Trip to Tirurkuppam 07:15 to 08:30 - Presentation of the Paddy Experimental Station and discussion with Prof. Thiru Aran. 08:30 to 09:30 - Visit to the Experiment Station and BGA multiplication plots. Collection of samples. 09:30 to 11:00 - Lectures by PAR, PMR and IFG 11:00 to 12:30 - Field trip to Sriperum Budur and in Chengalpattu district. Afternoon - Packing and relabelling of samples collected during the trip.

3) TRAVEL NOTES

31) VISIT TO THE INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI 110 012, INDIA (FRIDAY MARCH 8th)

Staff conducting research on BGA: Division of Microbiology: Dr. G.S. Venkataraman, Dr. S.K. Goyal, Dr. B.D. Kaushik, and Dr. (Mrs.) P. Roychoudhury. Division of Agricultural Physics: Dr. G.S. R. Krishna Murti. The BGA scientists of the Division of Microbiology of IARI conduct research on physiology of N₂-fixing BGA.

The program is headed by Dr. G.S. Venkataraman. After survey and isolation from rice soils, BGA strains are screened for growth, nitrogen fixation, tolerance to pesticides and chemical N. Research on the amelioration of salt affected soils, with BGA is also going on. In addition, the microbiology division functions as a coordinating center for algal multiplication and inoculation trials being conducted by different agencies throughout the country. In the Division of Agricultural Physics, Prof. G. S. R. Krishna Murti works on the effect of algalization on physical properties of the soil.

311) Research programmes

3111) Survey of BGA in soils of Maharashtra, and Jammu and Kashmir State (Dr. Goyal). About 350 rice soil samples were collected and studied using the liquid culture enrichment method with Fogg's medium. About 85% of the samples were very rich in N₂-fixing BGA while the remaining 15% were less rich. Results indicate that N₂-fixing BGA are ubiquitous in rice fields of the studied area. The soil screening permitted to isolate 175 unialgal cultures which were tested for N₂-fixation in Fogg's liquid medium without mineral nitrogen, using the measurement of nitrogen accumulated in the culture after 28 days of growth as index of N₂-fixation. This screening yielded 37 efficient strains which were screened again for resistance to pesticides. Finally 8 efficient N₂-fixing strains resistant to pesticides were obtained. The soil survey indicated that Anabaena is an ubiquitous genus present in all the studied soils. In these soils, there was a predominance of saprophytes (species growing on soil). Occurrence of saprophytes in rice field soils seems to be of ecological importance for upland paddies as a considerable area under rice in India falls in the rainfed zone and in these regions, maintenance of waterlogged conditions is generally not possible. Genera of these saprophytes comprise of Tolypothrix, Aulosira, Calothrix, Scytonema, etc. They are generally good N₂-fixers and may make N easily available to rice (less losses) because they are growing at the surface of the soil. When such strains are inoculated, a low level of water is recommended. In flooded rice fields of Jammu and Kashmir state, green algae like Hydrodictyon and Spirogyra usually come up first. After 15 days they die off and it is replaced by N₂-fixing BGA such as Rivularia, Nostoc and Anabaena which may develop into blooms. In the Ratnagari district of the Maharashtra, acid soils (pH - 5.4) have a high incidence of BGA. This may be due to high organic matter content of the soils.

3112) Reclamation of saline soils with BGA (Dr. Kaushik and Dr. Roychoudhury).

There are about 7 x 10⁶ ha of saline soils in India. In the soils where the level of Na is not too high, algal inoculation with tolerant strains decreases pH (9 to 8), electrical conductivity and exchangeable Na, and increases N content. Efficient strains which are able to grow at high levels of NaCl were isolated from such soils. They comprise of Calotrix, Tolypothrix, Hapalosiphon and Anabaena spp. These strains produce large quantities of mucilage that may help in binding Na in

soil aggregates. Present status of saline soils reclamation with BGA is similar to that of algal inoculation. It works but mechanism is still poorly understood.

3113) Algal inoculation in presence of N fertilizers.

Inoculation at high level of N fertilized plots (120 kg/ha) have shown increases in grain yield up to 15%.

312) Current status of algal inoculation in India. (Dr. Venkataraman and Dr. Goyal).

Research on algalization technology started in 1960. It was a component of the All-India Coordinated Project on Algae, headed by Dr. Venkataraman. The project started in 1976 and is now terminated but algalization studies are going on.

Currently algalization has been adopted in two states: Tamil Nadu and Uttar Pradesh.

Trials are currently conducted in experimental farms of Jammu and Kashmir State to spread algalization technology in this state.

In a recent letter (August 85), Dr. Goyal indicated that a preliminary survey by Dr. Venkataraman's group has shown that presently algalization is adopted in about 2×10^6 ha of the 40×10^6 ha of rice fields of India. In Tamil Nadu, only about 5% of the trials were unsuccessful. This is most probably because of a high level of indigenous BGA and a spontaneous high fertility of the soils. According to Dr. Venkataraman algal inoculation is widely adopted in Tamil Nadu. In Uttar Pradesh, adoption of this technology is less. Since 1982 about 50000-70000 ha have been inoculated yearly in U.P. during the Kharif season.

Reasons for successful adoption in Tamil Nadu are:

- Holdings are small and low levels of fertilizers are applied. The recommended level of fertilizers is 60-80-80 (NPK); but farmers rarely apply more than 40 kg N. About 80% of the farmers do not apply any N fertilizer.

- In Tamil Nadu, three crops of rice can be grown per year. Therefore, demonstration of the efficiency of BGA in 6 successive crops could be made in two years. After two years of inoculation there is no need to inoculate anymore. In Aduthurai Station where algal inoculation trials were conducted for several years it is now impossible to obtain a control plot free of N₂-fixing BGA.

- There is an efficient collaboration with the State Agricultural Department and the extension officers. Currently BGA inoculum is produced by the State. Commercial production is not attractive because of the low cost of the final product (about 1 Rupee/kg). In Tamil Nadu, rice soils are submerged most of the year but there is a dry fallow of about 1 month that can be used by farmers for inoculum production.

- Possibly there is a low incidence of grazers such as ostracods. However, detrimental effects of molluscus were recognized in the fields. "Red annelids" (most probably chironomid larvae) were observed to be very detrimental in multiplication ponds. They are controlled by malathion or butachlor at a rate of 1 kg ai/ha.

Reasons for non-adoption in other areas. - The major problem is to convince farmers.

Adoption is slower in the areas where demonstration of BGA inoculation can be done only once a year because only one crop of rice is grown per year. - In Haryana and Punjab, where land holdings are larger and farmers are utilizing N fertilizers, there

was no adoption by farmers despite successful demonstration trials. - Too many

technology transfer programmes (2-3 dozens) are proposed to agricultural officers.

Hence, they are unable to propagate the technologies effectively because they are overburdened with many programs.

32) VISIT TO THE SCHOOL OF LIFE SCIENCES, JAWAHARLAL NEHRU UNIVERSITY, NEW DELHI

(Saturday March 9th) Scientists working on BGA: Prof. P. Mohanty, Dr.(Mrs.) Chintamani, Dr. A.K. Verma.

Several research programs on BGA are being conducted under the guidance of Prof. Mohanty: Photosynthesis (Photosystem II), Energy transfer by phycobilins, electron transport, adaptation to high temperature, effect of Al and Zn on membrane activity.

Comments by Mr. Haran about natural pesticides: neem seeds, neem leaves or mango leaves are traditionally mixed with pulses when stored as dry seed to protect them from insects.

33) VISIT TO THE LABORATORY OF PROF. H. D. KUMAR, CENTER FOR ADVANCED STUDIES IN BOTANY, BHU, VARANASI (Sunday March 10th)

Scientists working on BGA, and algae. Prof. H.D. Kumar, Ms. M. Banerji, Ms. R. Rao, Mr.

D.V. Singh, Dr. Misra, Mr. S. Dube and Dr. A. Tripathy. Research topics can be classified into

three groups: 1. Physiology, genetics and mutagenesis in BGA. 2. Pollution, toxicity of and tolerance to heavy metals in green algae. 3. Inoculation experiments in pot with BGA.

331) Mutagenesis in BGA (Mr. D.V. Singh). *Anabaena* mutants resistant to chloramphenicol, tetracyclin and phenylalanine were produced, using nitrosoguanidin(NTG). The effect of tyrosine and tryptophan on growth and selected enzymatic activities (GS and nitrate reductase) was studied. 332) Mutagenesis in BGA (Dr. Mishra). Beginning a program on the effect of anaerobiosis on mutagenic action of U.V. and N.T.G. on *Oscillatoria limnetica*. 333) Mutagenesis and recombination (Dr. Tripathy). Five mutagenic agents were tested on *Nostoc* to isolate mutants which are able to release amino acids into the medium. The trial was unsuccessful. A mutant releasing hydroxyproline into the medium was isolated. Experiments on recombination and plasmids of BGA were also performed. 334) Ecology of algae in polluted ponds and Ganges River (Ms. Rao). Monthly analysis of phytoplankton, zooplankton and physicochemical parameters are conducted in 3 polluted ponds and in the Ganges river. In the ponds, dominant BGA are *Microcystis* in winter and *Anabaena*, *Nostoc*, *Rivularia* and *Fischerella* in summer. The zooplankton comprises mainly of *Daphnids* and *Ostracods*. Characters of the environment are: a low dissolved oxygen content, a high BOD, a high nitrate content and a high phosphorus content (40 ppm). 335) Pollution by heavy metals (Mr. S. Dube). Study of the toxicity of chromium and tin on *Anabaena doliolum* and protection offered by different carbon sources against the toxicity. Fructose and natural chelators (fulvic and humic acids) have a protective action. Half lethal dose of CrO₃ is 40 ppm for *A. doliolum*. 336) Effect of BGA inoculation on maize in pot (Ms. Banerji). The effect of cultures, culture extracts and supernatant of BGA cultures was tested on maize grown in autoclaved sand in pots. The five treatments were: 1) treated with *Aulosira*, 2) treated with *Oscillatoria*, 3) treated with *Nostoc*, 4) treated with nitrate and 5) water. Algal materials was 15 days old axenic cultures grown in Allen and Arnon's medium without mineral N. Fresh weight, weight of roots, weight of shoots, protein content and chlorophyll content were measured in 15 days old seedlings. Results showed better growth in the BGA treated seedlings than the seedlings in water and in the presence of nitrate. In these studies, *Aulosira* was chosen because it is dominant in the rice fields of the area. The most frequent algal succession in the rice fields after the first rain is in the following order: 1) non N₂-fixing algae; 2) *Anabaena*, *Nostoc*, *Scytonema*, 3) *Cylindrospermum*, and *Aulosira* which become dominant before harvesting when there is a decrease of water level in the fields. However some early growth of *Aulosira* has also been observed.

34) FIELD TOUR IN SARANATH AREA: (Monday March 11th) The visited area is about 10 km west of Varanasi. Most of the soils were dried and unplanted. Few fields had a wheat crop. In the vicinity of a small pond, colonies of *Nostoc* were observed on wet soil. On dry soils, large dark patches of perennating *Aulosira* were observed. We noticed the presence of high number of shells of snails in the fields. Three composite samples of 10 cores of surface soil were collected for BGA enumeration (samples 1, 2, 3). One quantitative sampling of algal crusts was made for N and C analysis (sample 4). Samples for quantitative analysis of *Aulosira* crusts (sample 5) and unidentified algae close to the pond (sample 6) were also collected.

35) OTHER MEETINGS AT CENTRE FOR ADVANCED STUDIES IN BOTANY, BHU (Monday March 11th, afternoon and Tuesday March 12th, morning).

351) Dr. S.P. Singh: Study of the effects of heavy metals (cadmium) and factory effluents (bisulphite) on BNF by BGA.

352) Dr. A.K. Kashyap - Viruses of BGA - Viruses could be responsible for the non-establishment of inoculated BGA. Strains of *Aphanothece* not producing mucilage are susceptible to virus attack whereas mucilaginous strains are resistant. Mucilage may offer protection from virus. - Mutagenic effects and transport of manganese in BGA. - Regulation of ammonium transport in *Nostoc muscorum* and in some saline and alkaline strains. - Search for compatible N₂-fixers. (mutants fixing in the presence of combined nitrogen).

353) Dr. D. N. Tiwari - Study of N₂-fixation by *Gloeocapsa*. A *Gloeocapsa* strain with less mucilage is an efficient N₂-fixer with higher specific ARA (30 nm acetylene per mg chlorophyll per min) than filamentous strains. This strain is also 100 times more resistant to 2-4-D than filamentous strains. Its doubling time is 21-24 hours. - Acquisition of resistance to MSX by *Gloeocapsa*. MSX initially inhibits the growth of *Gloeocapsa*. After 6 to 8 days, growth resumes and becomes normal because the alga acquires resistance. This is inferred from the studies where it was found that filtrate was still toxic to fresh inoculum. - Dominant strains of BGA in Varanasi area are:

Aulosira, Gloeotrichia, Wollea bharadwaje, Anabaena unisporea, Anabaena doliolum, Cylindrospermum (2 spp.)

354) Team of Prof. Talpasayi

3541) B.P.R. Narasimha Rao. Comparison of C fixation in natural populations and laboratory cultures of Microcystis. Laboratory cultures are saturated at 400 lux whereas natural populations are not saturated up to 25000 lux. Temperature between 15 to 40°C showed little effect on C fixation in laboratory cultures. Carbon fixation decreases after 40°C. In natural populations optimal temperature is around 30°C. Effects of chemicals such as DCMU on C fixation were studied on natural and cultured materials.

3542) P. S. Basu. Similar studies on C fixation are being performed with thermophilic strains of Synechococcus.

3543) Mr. Parameswaran. Study on the effect of wastes (containing about 25% titanium dioxide) from aluminum factories on BGA growth (Synechococcus).

3544) Mrs. A. Verma and Mrs. N. Bajaj. Studies on Chlorella and Synechococcus strains immobilized in alginate. Effects of inhibitors (MSO, EMSO, DCMU) on nitrate and nitrite reductases of immobilized colony under light and dark are being studied.

3545) Mr. S. M. Prasad. Carbon dioxide fixation by a thermal filamentous BGA (Mastigocladus).

3546) Mrs. Asha Singh. Aerial dispersion of BGA and allergenic BGA. The study is being conducted in the city and at the University campus at different elevations.

355) Dr. L. C. Rai - Control of eutrophication by precipitating P with zirconium oxychloride. About 98% of P at 10 mg/l is precipitated by 100 ppm of Zirconium oxychloride. This is not toxic to algae. - Effects of heavy metals to Chlorella. Ca and Mg are very efficient in reducing toxicity of heavy metals. Toxicity is also reduced by alkaline conditions and by chelating agents such as fulvic acids and humic acids.

356) Dr. Ashok Kumar - Study of the clonal variability in Anabaena doliolum. (It was found that it is a genetical property rather than a physiological one). - Physiological studies on regulation of the heterocyst formation in Anabaena.

357) Dr. A. Kumar Rai - Study of the relations between BNF and photosynthesis in different strains of Anabaena including A. doliolum and A. azollae.

358) General discussion

A short informal meeting with most of the BGA scientists from BHU followed the lectures by IIRI scientists. Prof. Talpasayi emphasized the fact that most of the ecological studies of BGA are only descriptive. He also commented about strain selection, indicating that assessment of strain properties should be made with regard to BNF and ability to withstand stress conditions. PAR presented a brief summary of the results of the survey for the establishment of a BGA network. There was a general consensus: 1) for the development of collaborative research; and 2) to give priority to the standardization of methodologies.

36) MEETING AT THE UNIVERSITY OF AGRICULTURAL SCIENCES, DEPARTMENT OF AGRICULTURAL MICROBIOLOGY, BANGALORE (Thursday March 14th).

The meeting was headed by Prof. P. V. Rai, Head of the Department of Agricultural Microbiology. BGA scientists in the department are Dr. Shetty (Associate Professor) and Mr. M. K. Shivaprakash (Assistant Professor). Dr. A. M. Krishnappa (Associate Professor) from the Fisheries College, Bangalore, Dr. U. Bongale from Karnatak University, Dharwad and Dr. Hosmany of Mysore University were kind enough to come to the department of Agricultural Microbiology to join the meeting. Prof. G. S. Bharati from Karnatak University could not join the meeting because of previous engagements and sent a letter with information about some of his research topics.

361) Dr. Shetty

A program aiming at the popularization of BGA inoculation is being conducted during the 3 last years. This program comprises: a) Research: Isolation, and screening of cultures prior to supplying them production centers. b) Inoculum production: Strains selected at the university are forwarded to five centers selected by the State Agriculture Department of Karnataka. The centers are located in each of the five agroclimatic zones of the state, i.e.: coastal, transitional belt, northern dry zone, eastern dry zone, and central zone. c) Training: Training on BGA is part of orientation courses on biofertilizers which are given to Senior Research Officers (2 days), Extension Officers (4 days), and farmers (7 days). Field demonstration of the use of biofertilizers (2 days) are also organized for farmers. The programme of the orientation course for Senior Officers and a report of a training course for extension workers are annexed to his report

(Annexes 3 and 4). Currently the selection of strains for the coastal zone, the transitional belt and the eastern dry zone has been achieved. Twenty to thirty soil samples are collected from each zone and algal strains are isolated using the enrichment culture method on Fogg's medium without nitrogen. Unialgal strains are tested in liquid medium for growth rate, N₂-fixation, response to P, inorganic N and pH, and pesticides. Selected strains are indigenous strains dominant in the respective zones: *Anabaena variabilis* and *Nostoc commune* are adapted to a wide range of soils; *Calothrix* sp. is adapted to acidic soils; *Hapalosiphon* is adapted to acidic to neutral soils of the eastern dry zone. *Cylindrospermum musicola* is adapted to the soils of the transitional zone. In the production centers soil based inoculum is produced using single strain. Different soil types as well as the effect of the addition of inorganic fertilizers, organic amendments, neem cake, and biogas wastes are tested for the production of the inoculum. Quality of inocula is tested by plating method. Inocula containing 10² to 10⁵ colony forming units per gram dry weight are used for inoculation. Inoculation in pot experiments always gave positive results. In field experiments, there is some increase in yield (6-10%) but not statistically significant. The technology is not yet adopted by farmers. Field trials have shown that algalization is not efficient during Kharif crop probably because of lack of light. During the monsoon, *Aphanothece* usually covers the rice fields. Basal application of 90 kg P/ha as P₂O₅ favor BGA growth. 362) Dr. S.P. Hosmani (Mysore). - Algal flora of 320 soils collected from rice and non-rice grown areas has been studied using liquid enrichment technique in Benedict's medium and soil-water medium. 315 isolates have been obtained. N₂-fixing BGA were present in 50-60% of the total soils and in 70-80% of the rice soils. - Presoaking the seeds of vegetables, mustard, and solanum in filtrate or extracts of BGA (*Hapalosiphon*) increased the rate of germination of the seeds. - Experiments on the effect of fumigants on BGA were also conducted.

363) Dr. Krishnappa (Fisheries College, Bangalore) Study of algal inoculation in acidic (pH 4-5.2) soils of the coastal zone. *Tolypothrix*, *Anabaena*, *Calothrix* and *Nostoc* are present in all the soils but *Tolypothrix* is dominant. As the soils are porous and have a high rate of percolation, inoculum production is made using polyethylene sheaths. Mosquito larvae are a limiting factor for the production of inoculum. Inoculum production was unsuccessful during the rainy season. An inoculation experiment conducted on rice in four plots (10 x 10 m each; no replicates) has given the following yield: control: 3.8 t/ha; 10 kg BGA: 4.5 t/ha; 50 kg N: 4.5 t/ha; 100 kg N: 5.6 t/ha.

364) Dr. Bongale (Dharwad) The study of 250 dry soil samples from 7 districts out of 20 districts in Karnataka has shown the presence of N₂-fixing BGA in 210 of them. Three areas with acidic and acid lateritic soils were poor in N₂-fixing BGA. Most frequent strains in non-acidic soils are *Nostoc* (*calicicola*, *commune*, *microscopicum*, *puntiforme*), *Calothrix* spp., *Anabaena* (*variabilis*, *spiroides*), *Microchaete* and *Aphanothece*. Two strains of *Hapalosiphon* and *Nostoc microscopicum* have been selected since they grow on different kinds of soils and are utilized for inoculation experiments where indigenous flora do not contain N₂-fixing BGA. The relation between the occurrence of different algal genera with the soil properties and cropping patterns has been worked out. Based on the observations, an assessment of euterrestrial or pseudoterrestrial nature of different algae has been made. Recently, a few strains have been isolated for inoculation in acidic soils. It has also been found that the foliar spray with filtrates of nitrogen fixing algal cultures (60 to 90 days old) improves growth and yield of vegetable crops like beans and onion. Presoaking the seeds in the extracts of *Hapalosiphon welwitschii* and *H. confervaceus* exhibited differential response on growth and spore germination. In a recent letter Dr. Bongale summarized his research interest as follows:

1. Enrichment of Regional Soils: Scope exists for the improvement of such soils that are poor in heterocystous algae, by inoculating a few selected strains from the local areas.
2. Relation of Soil Algal Flora with Soil Properties.
3. Blue Green Algal Spores for Bioassay of Pesticides. Spores of algal strains differ in their response to pesticides. Extensive studies on this aspect would help in evolving a simple bioassay technique for pesticides, applicable even at field level.
4. Recycling of Paddy Field Effluents. Residues of pesticides and their degradation products in field effluents can be utilized for mass cultivation of selected strains of blue-green algal inoculum. This would help in reducing pesticide pollution, and in promoting recycling of water.

37) FIELD TOUR IN MRS/UAS EXPERIMENTAL FIELDS AND IN FARMERS FIELDS ALONG MYSORE ROAD (Thursday March 14th) In the experimental fields of the University of Agricultural Sciences, no experiments on algal inoculation are being conducted. There were blooms of BGA but presence of few *Nostoc* and *Aphanothece* colonies was recorded. There was a high incidence of snails in the irrigated fields. In dry fields, there was a

noticeable coverage of dark algal crusts. One quantitative core sampling was made on dry soil with algal crust (sample 7) and another was performed on slightly wet soil (sample 8). A non-quantitative sampling of algal crusts was made for analysis (sample 9). Presence of a red *Azolla pinnata* was also noted in some of the irrigated fields. Visits to farmers fields along Mysore road permitted us to observe extensive growth of *Oscillatoria limnetica* and other non-nitrogen fixing BGA in fields irrigated with sewage water. A quantitative core sampling was performed (sample 10). In the fields irrigated with freshwater, there was little algal growth. A quantitative core sampling was performed (sample 11).

38) MEETING WITH DR. G. OBLISAMI, HEAD OF MICROBIOLOGY DEPARTMENT, TAMIL NADU AGRICULTURAL UNIVERSITY, COIMBATORE (Friday March 15) There are five thrust areas in the department: 1) BNF: *Rhizobium*, *Azospirillum*, BGA, *Azolla*, *Frankia* 2) Mycorrhizae; endo-and ectomycorrhizae 3) Organic material recycling, biogas, cellulose and lignin degradation 4) Effect of pesticides on soil microflora 5) Industrial pollution Highlights of the research progress are presented in a report of the Center of Advanced Studies in Agricultural Microbiology for post-graduate agricultural education and research (UNDP, FAO, ICAR Project- IND/78/020) (Annex 5). In the programme on biological nitrogen fixation, emphasis is given on legumes (5 scientists): crop legumes, tree legumes and serology of rhizobia. Two scientists are working on *Azospirillum* and three on *Azolla* and BGA. In the presentation of the work conducted in his department, Dr. Oblisami did not include research on BGA which was to be presented by Dr. Kannaiyan on 16th of March.

381) Bacterial and micorrhizal inoculation: The laboratory is producing *Azospirillum* inoculum and releasing in bags for selling to farmers through the Department of Agriculture. About 10000 packets of 200 g each (quantity for inoculating one hectare of rice) are produced every month (Annex 6). The carrier is peat. Seeds are inoculated with inoculum mixed with molasse before seeding. The inoculum has to be used within 6 months after it was produced, and contains about 106 - 108 cells per g.dw. Inoculum is not sold if it contains less than 106 cfu/g. Price is Rs 2/pack. Reinoculation is recommended. Response to bacterization is variable. In about 50% of the cases there was an increase in yield. Increase in yield was statistically significant in about 30% of the cases. A positive effect of bacterization has been observed with sesame, pennisetum, cotton, millet, sorghum and rice. After inoculation, plants are greener, mature earlier (7 days), and N fertilizer application can be reduced by one fourth. Bacterization with *Azotobacter* and *Azospirillum* is recommended for rice. However, it has not yet been adopted by farmers. Other inoculants such as *Beijerinckia* have been tested and abandoned. *Azotobacter* is less efficient than *Azospirillum*. Inoculation of *Casuarina* nurseries with crushed nodules from mature trees proved to be efficient. The microbial symbiont (actinomycetes) has not yet been isolated. Inoculation of cassava, tomato, sweet potato, chilli and citrus with micorrhizae has given good results but it is still at the experimental level. Inoculation of pine trees in the nurseries has been successful. The fungus has been isolated (*Scleroderma*).

382) Other programmes Biogas. Biogas digestors are of the sealed dome type and are widely adopted by landed farmers. The program is aimed at finding out the methods for increasing the methane/CO₂ ratio and for utilizing weeds (water hyacinth), farm residues and poultry litter as substrates. Cellulose and lignin degradation. The program is to study the possibility of utilizing sugarcane bagasse and coconut coir waste as a substrate for the production of single cell protein and soil conditioner. Pesticide effects on soil microflora. Effect of dust pollution on different crops. Dust pollution from cement factories result in a coating as high as 5 mg calcium silicate per cm² of leaf. It reduces significantly the number of root nodules in leguminous crops (50%). Most sensitive non-legume plants are sorghum, maize and cotton. In case of cotton, the quality of the cotton is affected but not the yield.

39) FIELD TOUR TO PADDY BREEDING STATION, TNAU, Friday March 15th afternoon. Paddy Breeding Station of TNAU at Coimbatore is one of the three centers for BGA inoculum production in Tamil Nadu. The inoculum production plots were inoculated one week before our visit. Floating blooms of green algae and LPP blue-green algae were present in all the plots but not covering the whole surface of the water. A secondary bloom of *Anabaena* was coming up at the soil water interface but its color was not very marked though large number of O₂ bubbles were visible. Microscopic examinations in the field and in the laboratory showed the dominance *Spirogyra*, unicellular green algae, and non-N₂-fixing LPP and *Oscillatoria*. *Anabaena* was the dominant N₂-fixing strain. Few filaments of *Calothrix* were also observed. In the experimental plots, blooms of filamentous green algae are common. In dry fields, white patches, probably discolored algae, were observed. A sample was collected (Sample no. 12). One sample of dark crust forming algae was also collected (sample no. 13).

40) FIELD TOUR IN TELUGU PALAYAM AREA (Saturday March 16th morning). We visited farmers fields in Telugu Palayam area, about 20 km west of Coimbatore. The fields were inoculated 2 years back. Water was drained and no bloom of BGA was visible in the field. A quantitative sample of algal crusts was collected in a dry field (sample no. 14).

41) MEETING WITH DR. S. KANNAIYAN, COIMBATORE, March 16th morning. Two other scientists involved with Azolla and BGA research (P. Subrahmanian and A. Thanikachalam) joined the meeting. Tamil Nadu has about 2.8×10^6 ha of rice growing area. About 2 thirds are irrigated. Average yield is 2.2 t/ha. Three crops of rice are grown per year in one third of the area, two in another third and one in the remaining third. Recommended dose of fertilizer is 100-50-50 (NPK) but on average 20 to 30 kg N is used per crop per hectare. About 95% of rice varieties are HYV. The three rice crops in Tamil Nadu are: a. Kharif: short duration crop planted in June, light intensities are high. b. Thaladi: crop planted between October and December, light intensities are low and there is almost no algal growth. c. Navarai: medium duration crop planted in December - January, light intensities are moderate. BGA trials are conducted in collaboration with Dr. Venkataraman. BGA inoculum is produced in 3 research stations (State production units) namely: Aduthurai, Ambasamudram and Tirurkuppam. Each production unit is about 0.30 ha. Production of inoculum in summer months is about 1 kg/m² in 21 day. Average production of the 3 state units together is about 120-150 tons a year since 1981. Besides the state production units, there are three central government production units which together produce about 30 t inoculum per year. Inoculation works under moderate to high light intensities. It does not work under low light intensities. Top dressing of phosphorus, 20 days after transplanting, favors the growth of inoculated or indigenous BGA. Neem stimulates the growth of Anabaena (one kg neem seeds costs 20-25 Paise, one kg of neem cake costs 75-80 Paise). Apparently there is no difference among rice varieties in terms of response to algal inoculation. The percentage of farmers utilizing algal inoculation technology is difficult to evaluate but may be between 10% to 30%. Experiments conducted on algal inoculation by Dr. Kannaiyan and his associates are summarized in a review paper in press.

42) VISIT OF KUMARAPERUMAL FARM SCIENCE CENTER, TRICHY Thiru A.K. Kathirvelu, Principal and Thiru K.M. Ramanujam, Joint Director of Agriculture (Sunday March 17th) accompanied us to the farm. General information on the Center is given in a pamphlet annexed to this report (Annex 8). The Kumaraperumal Farm Science Center comprises the soil salinity research center. The objective of this center is to develop reclamation procedures for different categories of saline and sodic soils. Experiments on reclamation of a saline sodic soils (pH 9.0 - 9.5) are being conducted using gypsum application and BGA inoculation. Recommended gypsum application was 6 tons/ha. Eleven treatments in four replicates were tested. The experimental design and the results are given in Annex 9. The best results was obtained with 50% of the recommended dose of gypsum together with BGA inoculation. Inoculum was applied at a rate of 10 kg per hectare. It was a mixture of soil based inoculum developed with salt resistant strains selected by Dr. B. D. Kaushik (IARI, New Delhi) and indigenous strains isolated from the experimental fields in the center. A bloom was continuously present during the crop. At the time we visited the fields, blooms of algae were present. Microscopic observations in the field showed the dominance of LPP group, filamentous green algae and Aphanothece. In the sandy alkaline soils of the area (about 20% of cultivated soils) spontaneous blooms are very frequent. A quantitative sampling (10 core) of surface soil was made in a dry field of the center where a bloom is usually always present (sample 15). Another sampling was made in the same field where a few millimeter thick dark soil algal crust was present on sandy soil which is otherwise clear in color (sample 16).

43) FIELD TOUR IN TRICHY AREA (Sunday March 17th) Near Trichy (Somarasampettai) we visited a BGA demonstration field where algal inoculum was applied at a rate of 10 kg/ha. NPK fertilizer was applied at a rate of 30-15-15. N was applied as 10 kg basal, 10 kg 3 DAT and 10 kg at P.I. No BGA growth was visible with the naked eye. A quantitative surface soil sample was collected. In Trichy area, we observed 2 fields with dense algal blooms spontaneously growing (not inoculated). In the first one, a simultaneous growth of Azolla and mucilaginous colonies of Nostoc were observed. The field was adjacent to the housing colony and irrigation water was contaminated with waste waters. In the next field, the nature of the bloom was very different. It could be Anabaena, or a mixture of unicellular BGA/green algae with diatoms. Time was too short for microscopic observations.

44) VISIT TO TAMIL NADU RICE RESEARCH INSTITUTE, ADUTHURAI (Sunday March 17th) First, we visited the BGA multiplication plots with Dr. Kannaiyan and Dr. Dawood. pH of the soil is 7.2. Superphosphate is added at a rate of 2 kg/40 m² (500 kg/ha). Insecticide

Ekalau is applied at a rate of 200 ml per acre, which is half of the dose recommended for rice. Dr. Kannaiyan said algal crusts may contain as much as 1.5% N. When BGA are growing well as much as 5 t algal flakes can be harvested per hectare. At the time we visited the station, BGA multiplication plots were drained a week ago and algal flakes were covering the plots. Some heterogeneity was observed in the density of the flakes. Also one plot had better productivity than the other one. White patches of undissolved superphosphate were present at the surface of the soil. In the experimental plots of the station, we observed algal blooms comprised mainly of Oscillatoria and LPP strains. Mr. Srinivasan reported later that Aphanothece and Wollea are almost always presents in the field while other N-fixing BGA are seasonal. He said that he could collect N₂-fixing BGA bloom ranging from 5.5 to 18 t fresh weight per hectare. A quantitative sample (sample no. 18) and a nonquantitative sample (sample no. 17) of algal flakes from BGA multiplication plots and a composite sample of surface soil in experimental plots (sample no. 19) were collected.

45) FIELD VISIT TO MYLADUTHURAI AREA. There was no visible growth of N₂-fixing BGA.

46) MEETING WITH PROF. N. N. PRASAD AND HIS ASSOCIATES, ANNAMALAI UNIVERSITY, ANNAMALAINAGAR. (18-3-85) Research in Prof. Prasad's department is mostly on Rhizobium. Some work is also being conducted in other areas such as Azotobacter inoculation, bacterial diseases of rice, root exudates etc. Experiments with Azotobacter inoculation have shown that the application of 37.5 kg N/ha + Azotobacter inoculum was as effective as the application of 50 kg N/ha. Research on BGA is also conducted in Prof. Prasad's department and three M.Sc. thesis are available on BGA. It was shown that increasing P application from 20 to 80 kg/ha increased grain yield and BGA growth in fields where no nitrogen was applied. The presence of gibberellic acid was detected in one strain of BGA (Au. 3). One quantitative soil sample was collected in the experimental farm (sample 20).

47) FIELD TOUR IN PONDICHERRY AREA (AROUND MANGALAM) (Monday March 18th) We visited three farmers field where dense Anabaena blooms were seen. The first site had an alkaline soil (pH 8.1 - 8.5). It was inoculated the last year with 10 kg/ha of the inoculum produced on a neutral soil by TRRI, Aduthurai. We were told that indigenous blooms of Anabaena were observed in this field even without inoculation. NPK fertilization was 100-50-50: 50 kg N basal, 25 kg N one month after transplanting and 25 kg N at booting stage. Twenty three kg Zn/ha, and insecticides (Phosphomedan) were also applied. A soil sample was collected (sample 21). In the second site, soil was sandy and alkaline. Fertilizer (100-50-50) as well as gamma BHC (20 kg a.i./ha) were applied. Irrigation water was from a well. It was not inoculated but an Anabaena bloom was present. The third site was similar to the two other plots visited before. It was a sandy alkaline (pH 8.5 - 9.0) soil. Fertilizer had been applied. The field was not inoculated but an Anabaena bloom was seen.

48) VISIT OF THE AGRICULTURAL EDUCATION CENTER (KRISHI VIGYAN KENDRA) OF TNAU, PONDICHERRY (Monday March 18th Afternoon). We were received by Dr. A Subramanian, principal, who presented the activities of the Center. There are currently eleven major programs: Rice breeding, Biogas, Transfer of innovations, Training in improved agrotechnologies, Village adoption of improvements, Supply of seeds, Social forestry, Breeding of fish, Mixed farming, and Identification of pulse and oil crops. The center has released three rice varieties P.Y.-1 (130 days) P.Y.-2 (115 days) P.Y.-3 (120 days). P.Y.-3, released under the name Bharathidasan, is totally resistant to BPH and can yield 5 to 5.5 t/ha. The station is beginning a program on biofertilizers in cooperation with the Agriculture department of Pondicherry and has a small multiplication plot for BGA. During the visit of the experimental farm a composite soil sample (red lateritic soil, pH 6.8 to 7.5) was collected (sample no. 21). Agriculture department of Pondicherry produces about 10 tons of inoculum per year. We did not visit this plot located in Aiyankuttupalayam. 49) VISIT OF TIRURKUPPAM PADDY EXPERIMENTAL STATION (Tuesday March 19th). We were welcomed by Prof. Thiru Aran. Major goal of the station is to cater to the needs of three districts (Chingleput, South Arcot and North Arcot). There are three cropping seasons in this area where most of the rice is rainfed. Rainfall (700-1200 mm) is between July and December. - Sornavari crop: Planted in May/June. It is rainfed only, receives maximum sunlight and is usually high yielding. - Samba crop: Planted in September/October during the monsoon. This crop receives water differently in different localities: a) rainfed with one month drought at the beginning (wet, dry, wet) b) starts as dry and is then irrigated (about 44000 ha) (dry and irrigated) c) wet (irrigated) - Navarai: crop planted in December/January. The research station has released several varieties including TKM6 resistant to stemborer. TKM9, a HYV, susceptible to blast and TKM-80-89

resistant to blast were also released. The station operates a BGA program and has BGA multiplication plots. In this area, recommended dose of fertilizer is 100-50-50. Soils are light loam with pH ranging from 6.5 to 7.5. The soils are rich in indigenous BGA. Inoculation is recommended to reinforce the indigenous flora and to reduce N application by 25 kg (100-50-50 is equivalent to 75-50-50 + BGA). Application of insecticide (carbofuran) is recommended along with algal inoculation. A summary of the BGA scheme (Annex 10) and summarization of the going on experiments (Annex 11) are annexed. We visited the experimental farm and the BGA multiplication plots. Good growth of BGA was observed in multiplication plots. A sample of the BGA inoculum was collected (Sample 22).

50) FIELD TOUR TO SRIPERUM BUDUR DIVISION, CHINGLEPUT DISTRICT.

During the field tour a handout on the BGA scheme in Sriperumbudur division was provided to us (Annex 12). BGA inoculation is being adopted by some farmers of this area.

4) BGA AND GRAZERS SAMPLING

41) BLUE GREEN ALGAE 411) Methods 4111) Sampling Composite samples of surface soil were collected from each visited site. Samples comprised of the top 0.5 cm of ten core subsamples collected with plastic tubes 10 cm long and 3 cm in diameter. Sampling points were, at least, at 0.5 m intervals along a transect through the field. Twelve composite samples were collected from dry soils and three from wet soils. During the trip samples were kept in tight plastic bags, they were processed immediately after the trip. We also collected quantitative samples of algal crust (including soil-based inocula). A sample of the soil under the crust was also taken for comparison.

4112) Algal counts Evaluation of the total algal flora was made by plating soil suspension-dilutions on agarized BG II medium (Stanier et al. 1971) containing mineral nitrogen. The same medium depleted of mineral nitrogen was used for enumerating N₂-fixing BGA. For core samples, the volume of the first soil suspension-dilution was adjusted with distilled water to a value in cm³ equal to ten times the value in cm² of the surface corresponding to 10 core samples, thus providing a 10⁻¹ dilution on a surface basis. Dilutions from 10⁻² to 10⁻⁶ were plated using three replicates per dilution.

Remaining suspension-dilutions, were sterilized by autoclaving before discarding. Petri dishes were incubated for three weeks at laboratory temperature (22-30°C) under continuous light provided by cold white fluorescent lamps before counting and identification of colonies.

Depending on the method of sampling, counts were expressed as numbers of colony forming units (CFU) per cm² of soil or as CFU/g soil d.w. After counting, Petri dishes were sterilized before discarding the culture medium and the algal colonies. The plating method does not permit to distinguish between living organisms and spores or propagules dormant in the soil. Also, because of reading and competition problems on dishes having too many colonies, strains present at a density lower than 1% of the total CFU are most frequently not recorded. The method is therefore suitable for making an inventory of the major strains present in a soil.

Strains were classified into broad taxa according to criteria directly observable on the colonies growing on Petri dishes (Table 1). The ability to form mucilaginous colonies of defined shape, which is associated with resistance to grazing, is taken as a major character. Taxons having this ability are: unicellular, Nostoc and Gloeotrichia groups.

4113) Chemical analysis We determined pH, organic C, total N, exchangeable K, Mg, Ca, C.E.C., total P and available P (Olsen) of 1

) the algal crust samples and the contiguous subsoil, 2) the soil-based BGA inocula, and 3) some selected soils. Methods are those currently used by the analytical laboratory of IRRI.

412) List of soil-algae samples 1) Composite sample of 10 cores collect in Sarnath area in dry fallow plots close to the road (11-3-85). 2) Composite sample of 10 cores collected in Sarnath area in dry fallow plots in the vicinity of a pond (11-3-85). 3) Composite sample of 10 cores collected in Sarnath area in dry fallow plots near a railway track (11-3-85). 4) 3 square samples of surface soil crusts and deep soil collected in Sarnath area, dry fallow soil (11-3-85). 5) Aulosira crusts (non-quantitative sampling) collected in Sarnath area (11-3-85). 6) Unidentified algal colonies close to a pond collected in Sanath area (11-3-85). 7) Composite sample of 10 cores collected in MRS, UAS, Hebbal in fallow dry plots after harvest (14-3-85). 8) Composite sample of 10 cores collected in MRS, UAS, Hebbal in fallow wet plots after harvest (14-3-85). 9) Algal crust nonquantitative sample collected in MRS, UAS, Hebbal in fallow dry plots after harvest (14-3-85). 10) Composite sample of 10 cores collected along Mysore road in a farmer field irrigated with sewage water (14-3-85). 11) Composite sample of 10 cores taken along Mysore road in a farmer field irrigated with fresh water at tillering stage (14-3-85). 12) Unknown material, possibly discolored algae, collected non-quantitatively from TNAU paddy breeding station, in fallow plots after rice harvest (15-3-85).

13) Algal crusts collected non-quantitatively from TNAU Paddy Breeding station in fallow plots after rice harvest 15-3-85. 14) Two square sample of surface soil crusts and deeper soil collected in Telugu Palayam area in a farmer field inoculated 2 years back (16-3-85). 15) Composite sample of 10 cores collected in Kumaraperumal Farm Science Center. Saline soil pH 9.2, dry fallow plots after rice harvest (16-3-85). 16) One square sample of surface soil crusts and deeper soil collected in Kumaraperumal Farm Science Center. Sol saline soil pH 9.2 dry fallow plot after rice harvest (16-3-85). 17) Algal flakes from Aduthurai BGA production plots. Non quantitative sampling (17-3-85). 18) Two squares samples of surface crusts and deeper soil collected in the BGA production plots at Aduthurai (17-3-85). 19) Composite nonquantitative sample of surface soil collected in Aduthurai Research Station (17-3-85). 20) Composite sample of 10 cores collected in Annamalai Nagar (18-3-85). 21) Surface soil from rice field embankment in Mangalam (near Pandicherry) where an Anabaena bloom was observed. 22) Composite sample of 10 cores collected in the Agricultural Education Center (18-3-85). 23) BGA inoculum from Tirukuppam (19-3-85). 24) 10 core samples from lowland stream irrigated rice fallow in Ellamada, Piler (sandy soil) 25) 10 core samples from lowland well irrigated rice fallow in Ellamada, Piler (clay soil) 26) 10 core samples from lowland rice fallow irrigated with freshwater (on Mysore road. 3 km from Bangalore) 27) 10 core samples from lowland rice fallow irrigated with freshwater (on Mylore road. 3 km. from Bangalore).

413) Results

4131) Algal enumerations Results of algal enumerations are presented in a computerized table (Table 2) of 48 rows and 52 columns. We refer to the data according to their coordinates expressed as rows (R) and/or columns (C) in the table. 41311) Composite samples of 10 cores. Results of the enumeration of algae in composite soil samples, expressed on cm² basis, are presented in columns 1 to 17; average, maximal and minimal values are presented in column 44 to 46. Total algal populations (R-46) ranged from 4.4×10^5 to 1.0×10^7 CFU/cm² and averaged 2.1×10^6 . Heterocystous BGA (R. 33) ranged from 1.3×10^5 to 4.2×10^6 (average 8.7×10^5) and comprised 7.7 to 70% of the total algal populations (average 36%) (R.48). N₂-fixing strains were present in all studied samples. Among N₂-fixing BGA (R 37 to 44) Nostoc group was the most frequently dominant, followed by unicellular forms and Calothrix and Anabaena groups. Other groups were never dominant. 41312) Enumerations on dry weight basis Algal populations were enumerated on dry weight basis in 1) algal crusts, 2) soil under the algal crusts, 3) soil based inocula (S.B.1), and 4) two other soils. Results are presented in columns 18 to 36 of Table 2; average, maximal, and minimal values are presented in columns 50 to 52. Total algal populations ranged from 1.8×10^5 to 1.6×10^8 CFU/g and averaged 4.1×10^7 CFU/g. Heterocystous BGA ranged from 4.3×10^4 to 2.4×10^7 and averaged 2.5×10^6 CFU/g; they comprised 2 to 35% of the total algal populations (average 15%). N₂-fixing strains were present in all studied samples. Among N₂-fixing BGA, Nostoc group was most dominant in all samples. 41313) Pooled data Average, maximal and minimal values are presented in columns 38 to 40 of Table 2. Total populations ranged from 1.8×10^5 to 1.6×10^8 CFU/counting unit. N₂-fixing BGA ranged from 4.3×10^4 to 2.8×10^7 . Heterocystous BGA ranged from 4.3×10^4 to 2.4×10^7 . The relative importance of the different taxa of N₂-fixing BGA is presented in rows 37 to 44. Table 3 summarizes these data and shows that Nostoc group was dominant in 75% of the samples. Unicellular BGA were dominant in 10% of the samples. Anabaena and Calothrix groups were incidentally dominant (5% of the samples) whereas other groups were never dominant. 4132) Chemical analysis of algal crusts and soil based inocula. The comparison between the composition of the algal crust and that of the soil just under the crust (Table 4) shows an accumulation of organic material in the algal crust equivalent to 150-850 kg C/ha, 14-110 kg N/ha, and 1 to 35 kg P/ha. Soil based inocula (Table 5) had C contents ranging from 2.36% to 4.73%, N contents ranging from 0.299 to 0.755%, and P contents ranging from 640 to 1260 ppm 414)

Discussion

4141) Occurrence of N₂-fixing BGA in rice soils Dr. Goyal in his survey of soils of Maharashtra, and Jammu and Kashmir State found that N₂-fixing strains are ubiquitous in the studied area. About 85% of the 350 rice soil samples he collected were very rich in N₂-fixing while the remaining 15% were less rich (Section 3111). In a survey of 320 rice and non-rice soils, Dr. Hosmani found that N₂-fixing BGA were present in 70-80% of the rice soils (Section 362). Dr. Krishnappa found N₂-fixing strains in all the samples he collected from acidic soils of the coastal zone of Karnataka state (Section 363). Dr. Bongale found N₂-fixing strains in 210 of the 250 soils samples he collected in Karnataka state (Section 364). Algal enumeration in soil samples we

collected during the trip shows the occurrence of heterocystous BGA at a density higher than 7×10^4 CFU/cm² in all the samples. The average value is 8.7×10^5 . These values are slightly higher than those we recorded in 67 rice soils in the Philippines (minimum 1.5×10^2 CFU/cm², average 1.1×10^5 CFU/cm²). Research on methods for using BGA in rice cultivation, emphasizes algal inoculation (algalization) alone or together with agricultural practices favoring the growth of inoculated strains. This arose from the earlier belief that N₂-fixing strains were not normally present in many rice fields. Results of BGA surveys conducted by Indian scientists, results of the sampling we made during the trip and large surveys we conducted in West Africa and the Philippines show the wide occurrence of N₂ fixing BGA in rice soils. N₂-fixing strains most probably are more common in rice fields than was previously thought. Unsuitable survey methodology, especially sampling and culture methods, probably resulted in the low values recorded in earlier studies. Therefore research on the practical use of BGA in rice cultivation should equally emphasize both on inoculation and enhancement of indigenous flora.

4142) Dominant strains in rice soils According to the results of the enumerations of BGA in the soil samples we collected, Nostoc seems to be the dominant genus in most of the soils, even in the places where Aulosira fertilissima was thought to be dominant. However, the dominance of Nostoc in the counts may be due to the fact that most of the samples were taken from dry soils and that desiccation might have resulted in the survival of only spore forming BGA.

In wet soils unicellular BGA and Anabaena were dominant. This aspect has to be taken into account when making soil surveys (dry soil versus wet soil). It may also indicate that a suitable methodology for BGA surveys could be quantitative measurements by the plating method complemented by a qualitative study using the enrichment culture method.

4143) Enumeration in algal crusts Enumeration of algae in the algal crusts and in the soil beneath the crusts shown that algal density was between 2 and 170 times higher in the crusts than in the soil beneath the crusts. Lowest ratio between algal density in the crust and in the soil (Table 3, R8, C6) was 2.5 in Kumaraperumal soil. In this, soil being sandy, algal growth extended over several millimeters of the upper layer. Because of the texture of the soil, algal spores or propagules might have been washed down very easily, and this may be a reason for higher density of BGA in the deeper soil. The ratio between algae in the crust and algae in the soil was usually much higher for total algae than for heterocystous BGA. This may be related with the ability of heterocystous BGA to form spores which accumulate in the upper horizon.

4144) Enumeration in soil-based inocula Enumeration of heterocystous BGA in the soil based inocula (Table 5) showed values of the same order of magnitude as that in algal crusts (106/g d.w.). Material from Aduthurai and Tirur were characterized by the dominance of Nostoc group which comprised about 87% of the C.F.U. of N₂-fixing BGA. Relative density of N₂-fixing BGA ranged from 3 to 30%. It was low (3-6%) in material collected by scraping the soil surface (samples 18A and 23) and higher in algal flakes selectively collected from areas exhibiting a good growth (sample No. 17). When considering that recommended level for algal inoculation is 10 kg/ha, the application of the best inoculum (Adulthurai No. 17 containing 2.8×10^7 CFU/g) correspond to 2.8×10^{11} C.F.U. of N₂-fixing BGA per hectare. This corresponds to 2.8×10^3 CFU per cm², which is about 50 times less than the lowest density of indigenous N₂-fixing BGA and about 400 times less than the average density of indigenous N₂-fixing BGA in the soils we collected. Though more results are needed before drawing conclusions, the present observations indicate that: inoculation may not be needed in the soils of the areas we visited. Further multispecies inocula which we collected were rather unbalanced and dominated by Nostoc strains. We may add here that "multispecies inocula" we collected or obtained from various countries as well as and those we produced in the Philippines were almost always unbalanced and largely dominated by one strain. The soil-based BGA inoculum production method developed in India is simple, inexpensive and easily adoptable by farmers. It is based on the use of a multistrain starter inoculum of Aulosira, Tolypothrix, Sytonema, Nostoc, Anabaena, and Plectonema provided to the farmers by inoculum production units. Starter inoculum is multiplied in shallow trays or plots with 5-15 cm water, about 4 kg soil/m², 100 g triple superphosphate/m², and insecticide. If necessary, lime is added to correct the soil pH to about 7.0-7.5. In 1 to 3 weeks a thick mat develops on the soil surface which subsequently floats. Watering is stopped and water in the trays is allowed to evaporate in the sun. Algal flakes are scraped off and stored in bags for use in the fields. Using this method, the final proportion of individual strains in the algal flakes is unpredictable, but it is assumed that the strains best adapted for local conditions will dominate in the inoculum because it is produced in soil and climatic conditions similar to those in the field. However, this method is valid only if the starter

inoculum provided to the farmer is a balanced one comprising a wide range of strains. Results of BGA enumerations in collected inocula, together with those we produced at IRRI indicate that it would be safer to 1) produce monospecific inocula of various strains, 2) dry them, 3) check their quality, and 4) mix them according to their CFU contents. This method would permit to obtain a multistrain, balanced starter inoculum of known quality.

4145) Chemical analysis Results of chemical analysis of algal crusts (Table 4) soil based inocula (Table 5) and gave some estimates of the potentiality of algae in accumulating N in their biomass. The exceptionally high values in the samples obtained from Kumaraperumal center were due to a profuse growth of filamentous green algae and the sandy nature of the soil that permits the algae to develop a crust up to 1 cm depth. This may be partly related with a deeper penetration of light in sandy soils. The contribution of N₂-fixing BGA to organic matter accumulation in the crust was less important than in the three other soils (Table 4) as indicated by the algal enumerations. Results of analysis of algal crusts in inoculum production plots at Aduthurai gives an estimate of the potentiality of BGA which is about 45 kg/ha. However it has to be kept in mind that supersphosphate is added at a rate of 500 kg/ha to obtain such a profuse growth of BGA. Recent experiments conducted in micro plots showed that at IRRI nitrogen content in BGA blooms ranged from 10 to 20 kg/ha and exceptionally attained a value of 35 kg N/ha. These values may be considered to be a reasonable estimate of the nitrogen content in the maximum standing biomass that can be expected in a rice field at blooming time. However, they underestimate the value of BNF, which is the result of the activity of a standing biomass and its turnover. No data are available on nutrient turnover rate of field-grown BGA.

42) Grazers

421) Sampling Samples were collected with net when depth of water was sufficient. When the floodwater was too shallow samples (500 ml x 6) were collected with a syringes and the contents of the syringe were emptied into the net, and the organisms retained in the latter were transferred to a bottle containing 70% C₂H₅OH. Molluscs were hand picked from the soil for identification. A semi-quantitative ranking of abundance was achieved by counting animals retained by the net, or by visual observation in the case of larger molluscs.

422) Sampling sites At the time of the survey, only sites at Bangalore and Pondicherry, were irrigated and planted to rice. Three sites with crops at tillering stages were located at experimental farms in Tamil Nadu. These were all irrigated with well water. One site, at Sarnath (Varanasi), was dry except for water in drainage ditches and marsh areas, and was not planted to rice. Ditches and marsh areas (previously planted) were sampled at this site.

423) Results Taxonomic report of the grazers collected during the survey is presented in Table 6. A total of forty one species of macroinvertebrates were recorded. The greatest number of taxa at any one site was 25 (Bangalore). Pondicherry, had 17 spp. and the marshy site in Sarnath, had 11 spp. The lowest number of taxa were at the 3 university experimental stations: Coimbatore (2), Madras-Tirurkuppam (7) and Trichy (10). It was evident that in all these stations the plots were infrequently irrigated with well water and consequently the soils remained cracked despite of the overlying water. At the most soils had been flooded 2-3 days before our visit. At Coimbatore, irrigation canals contained abundant Lymnaea. Molluscs, particularly Lymnaea, Vivipara, and Gyraulus spp., were common at most sites. Also, the Cladocerans and Copepods contributed a large number of species common amongst sites. Ostracods were quite restricted and appeared in quantity only at the Bangalore sites. The hemipterans, Anisops and Micronecta, were widely distributed and mosquito larvae, when present were often abundant. Chironomid larvae were quite dense in Pondicherry where they were associated with large BGA growths. The percentage of grazers, expressed as a % of the total number of taxa, was between 68 and 82% for farmers' fields and 50-57% for experimental stations. These former percentages are in agreement with our findings in the Philippines. Primary production and recruitment of animals in experimental stations using well water for short periods of irrigation must be low and probably explains the reduced % of grazers. The community structure of invertebrates is also very close to that observed in Philippines rice fields. The 12 taxa designated characteristic of rainfed and irrigated rice fields in the Philippines and suffice to describe the Indian rice fields visited. Community domination by Ostracods in Indian fields was less pronounced than in the Philippines but mosquito larvae (Culicidae) and Copepoda and Cladocera were more widely distributed and more abundant.

Casual observations Most of the fauna were collected from sites of, or near, government BGA inoculation experiments or the BGA adoption sites of farmers (Sarnath excepted). Regional officers of the BGA adoption programme informed us that at least 100 kg urea N ha⁻¹,

50 kg P and 50 kg K were applied to all their fields. Local extension workers explained that this recommended level of fertilizer use was often doubled by the farmers. Furthermore, the recommended application rates of pesticide (invariably 20 kg ai dry powder, B.H.C.) were also doubled as an insurance policy against pests. Frequently the applications were made many times during the crop. At these sites (not all sampled) mosquito larvae and molluscs (*Lymnaea* and *Vivipara*) were either dominant or at least abundant. Many sites visited smelled strongly of insecticide, and the beige dusting powder was frequently seen. Under these conditions, *Oscillatoria*, LPP and at Pondicherry, *Anabaena* were growing well. Only molluscs, and mosquitoes, it seemed, could withstand the concentrations of BHC. Grazers were not a problem in these areas of non limiting N & P concentrations and heavy pesticide application rates.

5) CONCLUSIONS

51) Summarizing the trip with numbers and lists We visited eleven laboratories or research stations. We had discussions with about 50 scientists among which 38 are working at least partly on BGA. Eleven of them are involved at least partly in ecological or applied field studies on BGA in rice fields, whereas 27 are involved in basic research. We gave seminars on IRRI's work on BGA in IARI, Jawaharal Nehru University, Banaras Hindu University, Agricultural University of Bangalore, Tamil Nadu Agricultural University and Tirurkuppam Paddy Experimental Station. We had nine field tours where we visited eleven sites in farmers fields and six experimental sites. We collected a total of 27 samples for algal flora and chemical analysis. Grazers populations were sampled at six sites. During the trip we provided our hosts with a list of IRRI and our personal publications on BGA and grazers to permit them to request materials they were interested in. Thirty four sets of reprints have been sent to India after we returned to IRRI. During the trip we collected 130 reprints: - 6 are bibliographic reviews - 100 deal with fundamental research using laboratory grown BGA. Some emphasis is given to the effects of pollutants and pesticides on laboratory cultures (18 papers). - 10 are ecological and taxonomical studies of algae including BGA. - 3 are surveys of BGA in rice fields - 6 are on reclamation of saline soils with BGA - 5 are on algal inoculation. References of the articles dealing with BGA in rice fields and their agronomical utilization is given in the annexes.

52) Conclusions from the trip in India

521) Increasing professional relationships A very beneficial aspect of the trip has been the development or enhancement of professional, and, in many cases, friendly relationships, with colleagues whom we otherwise knew only through their names on scientific papers. We learnt a great deal from them and hope that we also provided them with some useful information.

522) The status of applied research on BGA in India Research on the use of BGA as biofertilizers is mainly conducted at the Indian Agricultural Research Institute (New Delhi), University of Agricultural Sciences (Bangalore), Central Rice Research Institute (Cuttack), Tamil Nadu Agricultural University and Tamil Nadu State Agriculture Departmental Farms (Coimbatore, Trichy, Adulthurai, Pondicherry and Tirurkuppam). Some field trials are made in Tamil Nadu, Uttar Pradesh, Andhra Pradesh, Punjab and Haryana to assess the effect of algalization on rice yield. Research has been initiated in Jammu and Kashmir and Maharashtra States. Most of the research is conducted on the following bases: - Isolation of strains from the rice fields. - Culture and testing of the strains in the laboratory for N₂-fixing activity and resistance to adverse conditions. - Testing selected strains in field experiments where effects of algalization are assessed through grain yield. An important part of the activity of many research centres is the production of starter inoculum.

523) Extent of adoption of algalization in India In 1980, in a review on adoption of biofertilizers in India (Biofertilizers in rice culture. Problems and prospects for large scale adoption. AICRIP publ no. 196) Pillai wrote: "Apart from the work carried out at Research Stations, very little organized work on development of the material for being adopted by the farmers has been taken up, especially in areas where it could be of potential benefit". In 1982, in a review of biofertilizers (Biofertilizers, Interdisciplinary science reviews, 7(3): 220-229), Subba Rao stated that the production capacity of BGA flakes in India was around 40 t/yr, which was approximately 0.01% of the total inoculum requirement of the country (40 t will inoculate 4000 ha). From an extensive report on BGA field trials published in 1982 by the Agricultural Economics Research Center of the University of Madras (Blue-green algae as a source of bio-fertilizers in Thanjavur district - Tamil Nadu, Res Study No. 74 of the Agric. Econ. Res. Center, 79 pp), it appears that despite an official radio and print publicity campaign, BGA use was mostly at the trial level in 1982 and that in many cases inoculated algae did not multiply. According to the information we received during our trip, algalization is now being adopted by

farmers. Dr. Venkataraman said it is adopted in Tamil Nadu and, to a lesser extent in Uttar Pradesh, where about 50000-70000 ha have been inoculated yearly since 1982 (section 312). Dr. Kannaiyan said that evaluation of the adoption of algalization is difficult. His estimation was about 10 to 30% of the 2.8×10^5 ha of rice growing area in Tamil Nadu are currently inoculated (section 41). Data regarding inoculum production may help in assessing the approximate order of magnitude of the percentage of inoculated fields. Total production of starter inoculum in Tamil Nadu is about 150-180 t/year since 1981. This material will inoculate 15000 - 18000 ha of rice fields (10 kg/ha) but will permit larger areas to be inoculated if multiplied by farmers. If 2 to 5 kg of starter inoculum is required for application per cent (1 cent = 1/100 acre = 1/40 m²) in a BGA inoculum production plot (Annex 7), then 180 t starter inoculum permits to inoculate 144 to 360 ha of BGA inoculum production plots. Assuming that all starter inocula produced in Tamil Nadu is utilized by farmers to produce soil based inocula and its productivity is 2 kg algal flakes per m² (data calculated from Annex 7 which indicates that 9 tons were produced in 1 year on 105 cents by 15 farmers) then it will lead to a final production of 1440 to 3600 t of algal flakes per year, which is enough to inoculate 288000 to 720000 ha (10 to 24% of Tamil Nadu rice fields). Such an extrapolation may sometimes lead to an overestimation. For example, the "notes for BGA inoculum production in Sriperumbudur division" (Annex 7) shows that BGA inoculum produced by farmers or obtained from experimental farms was about 15 t in 1984-1985 which was enough to inoculate 1500 ha in an area comprising 44300 ha of rice fields (3.4%). Therefore it seems reasonable to assume that inoculated fields comprise a moderate percentage of the total area under rice in Tamil Nadu and a very limited percentage of rice fields in India.

524) Inoculation versus enhancement of indigenous strains Results of surveys conducted by Indian scientists in Maharashtra, Jammu and Kashmir, and Karnataka States indicate that N₂-fixing BGA are present in most of the rice soils. Results of enumeration of N₂-fixing BGA in soil samples we collected in Uttar Pradesh and in Tamil Nadu showed that the occurrence of heterocystous BGA is higher than 7×10^4 CFU/cm² in all the samples and density averages $7-8 \times 10^5$ /CFU per cm² (For comparison, the average value of 93 soil samples collected from the Philippines, Malaysia and India is 6.3×10^5 CFU/cm² and the median is 1.1×10^5). The soil based BGA-inoculum having the highest density in CFU of N₂-fixing BGA, among those we obtained from India, Egypt and Burma, was from Aduthurai. It contained 2.8×10^7 CFU/g dry weight. Applying 10 kg of this inoculum per hectare is equivalent to $10 \times 10^3 \times 2.8 \times 10^7 = 2.8 \times 10^{11}$ CFU per hectare or $2.8 \times 10^{11} \times 10^{-8} = 2.8 \times 10^3$ CFU per cm². This is about 50 time less than the lowest density of indigenous N₂-fixing BGA in the soils we collected and 400 time less than the average density. From these data it can be concluded that indigenous N₂-fixing BGA are already present in many soils at a density largely higher than that brought by an algal inoculum. BGA inoculation may not be needed in many rice soils. Research in practical utilization of BGA should emphasize both inoculation and agricultural practices for enhancing indigenous flora.

53) Reconsidering international research on agronomical utilization of BGA

531) Introduction The following paragraph presents the analysis of the current status of applied research on BGA in rice cultivation, conducted in rice and non rice-growing countries. Emphasis is on "bottle necks" and a cooperative research strategy to alleviate these problems. Since 1945 BGA has been a "promising potential source of nitrogen for rice". In 1985 it is still promising and the only technology proposed to farmers (algalization) is not utilized to a noticeable extent in rice growing countries. From a large number of field experiments it has been reported that inoculation with N₂-fixing BGA may increase grain yield by a few percent. However, "a few percent" should not be negligible for many rice growing farmers when considering that producing BGA inoculum and spreading it in the field is a very low input technology (low cost and little additional work). Obviously there are limiting factors for adoption of algalization by farmers. The two major ones are probably the lack of a reliable technology and the low potential of BGA in increasing rice yield. Currently practical utilisation of BGA is focused on inoculation conducted on "trial and error basis". Very little is known about the factors that permit the development of a bloom of indigenous or inoculated algae in a field. Also little is known about the factors involved in the reported increase in yield after algalization. The increased yield in inoculated plots can be due to fixed nitrogen, production of growth promoting substances, oligoelements from the inoculum, phosphorus solubilization by the algae, even in some cases a better care of the inoculated plot by the farmer involved in the experiments, etc.

532) Negative features of the current applied research on BGA In 40 years of ecological and applied research on BGA our knowledge has made relatively little progress. Some of the possible reasons are listed below.

5321) The imbalance between test tube and field studies In reviewing literature on BGA, it is surprising to observe the imbalance between the different topics. Taxonomy, morphology, micromorphology, physiology, enzymology, and genetics are highly documented and test tube growth of BGA has been studied extensively. On the contrary, field studies are rare. Therefore, BGA ecology is still poorly understood and algal inoculation is still conducted on "trial and error" basis. (Literature collected by us during our trip in India shows a similar imbalance: among 130 papers collected, only 15 deal with ecological or applied aspects). The practical utilisation of BGA has been quoted as the reason for many basic studies on BGA and has been given as the major goal of many research projects on BGA to be financed by national or international fundings. However, it is amazing to observe that only very few of the studies deal with field work and truly applied aspects.

5322) The underestimation of the potential of indigenous strains We have already emphasized twice in this report (sections 4141 and 524) that inoculation is not the only method to utilize BGA. Obviously N₂-fixing strains are present in many soils at a density largely higher than that of strains that could be inoculated (in 50% of the soils we studied during the last two years, more than 4 tons/ha of the best soil based inoculum we collected would be necessary to equal the indigenous N₂-fixing populations). Therefore emphasis should be placed also on agricultural practices that favor BGA growth and on integrated management. Deep placement of N fertilizers and control of grazers populations with cheap pesticides of plant origin are currently two possible realistic methods.

5323) Grain yield as the only criterium in field experiments First field inoculation experiments were conducted about thirty years ago. In most of the experiments the only measured variable was grain yield. Currently inoculation experiments are still conducted in the same manner, which give no information on the agro-ecological characteristics of the experimental field, the initial level of indigenous N₂-fixing BGA in the soil and the dynamics of the algal flora during the crop cycle. In addition, only the results of successful experiment are usually published, whereas most of the results of unsuccessful trials, that could provide information on limiting factors, go most frequently into a forgotten file.

324) The scarcity and limited scope of ecological studies There are very few ecological studies about BGA in rice fields. A majority of them are qualitative surveys of algae comprising of a list of species and little or no information on the physico-chemical characteristics of the environment.

533) A major limiting factor: methodological problems The three major deficiencies of available methods for studying BGA in situ are: 1) the absence of standardization, 2) a poor accuracy, and 3) the requirement of a very large number of simultaneous measurements that prevent a single operator from studying several factors at a time.

5331) Absence of standardization As an example, about 30 different media have been described for the culture of BGA. At least 6 of them are currently used as major media in laboratories working on ecology and applied aspects of BGA. In many laboratories, utilization of a given medium is not the result of a screening but the 'heritage' of a Professor or a laboratory where algology was learnt. Similarly, different methods of sampling and different methods of evaluating algal populations in soils prevent algologists to compare their data.

5322) Poor accuracy and need for manpower The uneven distribution of algae (together with the fact that many of the measurements are indirect ones), lead to a very poor accuracy of field measurements of algal populations and their activities. The uneven distribution of algae should be balanced by taking a large number of replicates which needs manpower and facilities for handling them. As an example, grain yield can be measured in 32 plots without major problems whereas it is impossible to evaluate quantitatively in those plots algal populations once every two weeks during a crop cycle without a staff of 3-4 persons and laboratory facilities permitting to incubate under light about 1500 petri dishes simultaneously.

534) A promising solution: cooperative research Progress in the field of ecology and practical use of BGA can not certainly be made by a single individual or a laboratory. To understand factors leading to the development of indigenous or inoculated BGA blooms in rice fields, number of observations conducted with standardized methodologies under a wide range of agroecological conditions are needed. This is obviously a research project that needs a collaborative approach. The first goal should be to select and standardize common methodologies, not necessarily the most accurate ones, but those which are scientifically feasible and can be used in the wider range of environments, permitting samplings in remote areas and studies in laboratories with moderate

facilities. Table 7 lists some of the methodologies that need standardisation and the points to be considered. (During our trip in India, methodologies was the most quoted topic for collaborative work). If cooperation in the establishment of common methodologies is successful, other aspects such as surveys and field experiments on common bases will develop spontaneously. A list of scientists currently involved in BGA research is annexed to this report. Those who indicated their interest in applied aspects are indicated with an asterisks. Their large number shows how great is the potential for collaborative research in agronomical utilisation of BGA.

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Table 1. Definition of the taxons of N₂-fixing

BGAa₁

Unicellular: Unicellular strains growing on BG II medium without nitrogen.

"Anabaena" group: Heterocystous strains with a thin sheath, without branching, not forming mucilaginous colonies of definite shape (Anabaena, Nodularia, Cylandrospermum, Anabaenospis...)

"Nostoc" group: Heterocystous strains, without branching, forming mucilaginous colonies of definite shape.

"Aulosira" group: Heterocystous strains with a thick sheath, usually without branching, forming dilluse colonies on agar medium.

"Scytonema" group: Heterocystous strains, with false branching, without polarity, forming velvet like patches on agar medium.

"Gloeotrichia" group: Heterocystous strains, with false branching, with polarity, forming velvet like patches on agar medium (Calothrix, Tolypothrix, Hassalia, ---)

"Gloeotrichia" group: Heterocystous strains, with polarity forming mucilaginous colonies of definite shape (Gloeotrichia, Rivularia...)

"Fischerella" group: Heterocystous strains with true branching. (Fischerella, Westilopsis, Stygonema)

a₁/All features refer to strains growing from soil or water samples plated on solid BG II medium without nitrogen. Table 2. Enumeration of algae in soil and algal crusts from India.

Table 2. Continued

