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Methods for Utilizing Free Living Blue-Green Algae In Rice Cultivation*

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*Paper presented at the Technology Transfer Workshop, IRRI, Los Banos, 26-27 Oct. 1984; and in the Graduate Course of Botany 291 "Biology of Blue-green Algae and their Symbiotic Forms", 2nd semester 1984-1985, UPLB, Philippines.

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SUMMARY

N₂-fixing free living blue-green algae (BGA), is a possible additional source of N for rice. However, biomass and N₂-fixation measurements as well as inoculation experiments indicate that BGA have a lower potentiality for increasing rice yield than legume green manure or Azolla:

- o A BGA bloom contains 10-20 kg N.
- o Average estimate of N₂ fixation by BGA in rice fields is 27 k N/ha per crop.
- o Field experiments on inoculation with free living BGA shows an average increase in yield of 14%. Comparison with N fertilizers indicates an effect equivalent to the application of 30 kg N.

This moderate potentiality is balanced by the fact that algal inoculation should be a very low input technology. But in the current state of the knowledge, BGA inoculation is a "blind" technology. When successful, mechanisms involved in the reported increase in yield (nitrogen source, "auxinic" effect, antagonism with pathogens, P solubilization....etc...?) and their relative importance are largely a mystery. Reasons for success or failure of inoculation are also very poorly understood.

Reports on the adoption of algal inoculation are controversial, but even considering the most optimistic evaluations, use of algal inoculation seems to be restricted to very limited hectarge in a few Indian states and in Burma. Currently the major limiting factor for utilization of BGA is the lack of reliable technology. Questionable quality of the inoculum and its sporadic establishment are the two stumbling blocks. Therefore it seems appropriate to consider that algal inoculation is more at an experimental level of large field testing than at popularization stage.

In addition, recent ecological studies shows that N₂-fixing BGA are present in rice fields at a much higher extend than it was previously thought. This indicates that inoculation may not be needed in many rice soils and that agricultural practices favoring the growth of indigenous strains may be sufficient to make use of the potentiality of BGA. Practices known to favor growth and N₂-fixation by BGA are: liming of acidic soils, P application, straw application, deep placement of N fertilizers, and control of grazers populations.

More research is needed before BGA technology can be recommended with confidence to farmers.

1) INTRODUCTION

Rice is the staple food of approximately one half of the world's people. About 75% of the 143 million hectares of ricelands are lowlands where rice grows in flooded fields during all or part of the cropping period.

Lowland rice can be grown on the same land year after year without N fertilizer and produce moderate but constant yields. In contrast, upland rice yields decline over time if no N fertilizer is applied. The continuing N fertility in lowland rice fields has been attributed to higher N₂-fixing activity coupled with slower decomposition of organic N compounds under poorly aerated conditions (Buresh et al., 1980). Biological N₂ fixation (BNF) in flooded soil systems was reviewed by Buresh et al. (1980). Processes and ecology of BNF in rice soils were reviewed by Watanabe (1978) and Watanabe and Brotonegoro 1981. An extensive survey of quantitative data was presented in Lowendorf's review (1982). New knowledge of BNF in flooded rice fields was summarized by Watanabe and Roger (1984). Nitrogen is usually the limiting factor to produce high rice yields.

The green revolution in rice production is based on fertilizer-responsive rice varieties. In Asia, one of the constraints to high yields is the limited availability and high prices of N and P fertilizers. In 1978 fertilizer use in tropical Asian countries averaged 30-55 kg NPK/ha arable land (Palacpac, 1982). The idea of utilizing BNF as an alternative or supplementary source of N for rice is not new. N₂-fixing green manures have been used for centuries in some rice growing areas and research on biofertilizers, including algal and bacterial inoculants, began in the early 1930s. One result of the fertilizer price increase during the last decade is a renewed interest in BNF as a mean of reducing the use of chemical fertilizers. Technologies for utilizing BNF in lowland rice were analyzed by Roger and Watanabe (1985) in a review which emphasizes their potentialities, their current usage and the limiting factors. Biological N₂ fixation requires energy generally obtained by the catabolism of photosynthetically fixed carbon (photosynthate). Among the N₂-fixing microorganisms, only blue-green algae (BGA) are able to generate their own photosynthate from CO₂ and water. This tropic independence makes BGA especially attractive as a biofertilizer. The agronomic potential of BGA was recognized in 1939 by De, who attributed the natural fertility of tropical paddy fields to N₂-fixing BGA. Since, many trials have been conducted to increase rice yield by algal inoculation or by cultural practices favoring the growth of indigenous BGA. Literature on BGA and rice was reviewed by Roger and Kulasooriya (1980) and BGA in tropical soils was reviewed by Roger and Reynaud (1982).

2) EVALUATION OF THE POTENTIALITIES

Potential of BGA as a N source for rice can be described in three different ways:

- o evaluation of BGA biomass and N content;
- o measurement of N₂-fixing activity; and
- o field inoculation experiments.

21) Biomass evaluations

Records of BGA biomasses were summarized by Roger and Kulasooriya (1980) (Table 1). Fresh weight estimates range from a few kg to 16 t/ha and dry weight estimates from a few kg to 480 kg/ha. However, because of the variable dry matter, 0.5-5%; ash, 5-70%; and N, 2-13% contents of BGA field samples (IRRI, 1983) such data are of little significance.

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Recent evaluations of artificially produced BGA blooms indicate standing biomass of N₂-fixing strains culminating at 150-250 kg dry weight/ha on an ash free basis, equalling 10-20 kg N/ha (IRRI, 1984). Those values may be considered to be the maximum standing biomass that can be expected in a rice field at blooming time. However, they underestimate the value for BNF, which is the result of the activity of a standing biomass and its turnover. No data are available on nutrient turnover rate from field-growing BGA.

Another way to roughly estimate BGA potential is to assume that all C input in the floodwater and surface soil is through BGA (which is an obvious overestimation). Saito and Watanabe (1978), estimated an input of 0.6 t C in phytoplankton/crop per ha. Using that estimate and assuming a BGA C:N ratio ranging from 4 to 16 (IRRI, 1983), the potential contribution of N₂-fixing BGA could be 37 to 150 kg N/ha per crop.

22) Nitrogen fixation measurements

N₂-fixation by BGA has been most frequently studied using the acetylene reducing activity (ARA) method which may provide erroneous results (Lowendorf, 1982). ARA variations during the day and the growing cycle can be rapid and important; moreover ARA has a log-normal distribution (Roger et al., 1977). Therefore many replicates and very frequent measurements are needed to satisfactorily measure total ARA. However this tedious work will lead to an imprecise evaluation of the N₂-fixing activity (NFA) because the conversion factor of acetylene- nitrogen is not constant and needs to be determined (Peterson and Burris, 1976). But ARA is a very convenient and reliable method for qualitative studies when the measurements are brief (David and Fay, 1977), when the problems of gas diffusion and greenhouse effects are minimized and when statistically valid methods are adopted (Roger and Kulasooriya, 1980). Few reliable estimations of ARA have been hitherto published. The number of measurements and replicates have been generally too low. Moreover, the importance of anaerobic nonheterocystous N₂-fixing BGA was not appreciated until recently. Field measurements of nitrogenase activity were carried out under an aerobic gas phase only, therefore it is difficult to evaluate the N₂-input due to N₂-fixation by nonheterocystous BGA (Stewart, 1978). Reported data on BNF related to BGA varied from a few to 80 kg N/ha and averaged 27 kg/ha per crop (Roger and Kulasooriya, 1980).

23) Inoculation experiments

Field experiments on algal inoculation provide indirect information on the overall potential of BGA. Algal inoculation, also called algalization, a terminology introduced by Venkataraman (1972), has been reported to have a beneficial effect on grain yield in different agroclimatic conditions. However reports also indicate failure of algalization. The conclusions of the review of Roger and Kulasooriya (1980) on algalization are summarized in this section. Description of the technology is presented in Section 41.

231) Methodology of the experiments.

Most experiments on algalization were on a 'black box' basis, where only the last indirect effect (grain yield) of algalization was observed and the intermediate effects were not studied. There is little information on the qualitative and quantitative variations of the N₂-fixing algal flora and the N balance in inoculated paddy soils. Pot and field experiments have been conducted, usually on a single crop. The relative increase in grain yield over the control was, on average, 28% in pot experiments and

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15% in field experiments. The better growth of BGA in pot experiments is probably attributable to the reduction of climatic disturbance and to the mechanical effect of the pot wall, where BGA seem to grow preferentially and profusely. Pot experiments may therefore only be suitable for qualitative studies, since they overestimate the effects of BGA inoculation. On the other hand, most of the field experiments were conducted for one growing season only. This may underestimate the effects of algalization since the advantages of a slow N release from dead BGA may not be apparent in the first algalized crop.

232) Effect of algalization on rice yield

Algalization may affect plant size, its nitrogen content, and the number of tillers, ears, spikelets, and filled grains per panicle. The most frequent criterion for assessing the effects of algalization has been better grain yield. Field experiments where algal inoculation was effective (Table 2) show an average yield increase of about 14% over the control, corresponding to about 450 kg grain ha⁻¹ per crop. Comparison with N fertilizers indicate that algalization may be equivalent to the application of 25-30 kg N/ha. A higher grain yield increase was observed when algalization was in combination with lime, P and sometimes molybdenum application. Unfortunately it is not possible to separate the direct effect of PK fertilizers on rice from its indirect effect upon the growth of indigenous or introduced algae.

The effects of algalization used with N fertilizers are controversial. Since BNF is known to be inhibited by inorganic N, the beneficial effect of algalization in the presence of N fertilizers was most frequently interpreted as resulting from growth-promoting substances produced by algae or also by a temporarily immobilization of added N followed by a slow release through subsequent algal decomposition, permitting a more efficient utilization of N by the crop. Such interpretation have yet to be demonstrated. Thus it appears that very little is known about the relative importance of fixed N and other possible effects (auxinic effect, P solubilization, effects on soil properties and microflora, etc.) in the reported yield increase.

A classical statement in the reports on BGA inoculation is "although the yields obtained in inoculated plots were higher, the difference between the yields of plots using and not using BGA was not significant". This indicates that: the response to algal inoculation varies, the response is small, and the experimental error is larger than the response. The most common design for BGA inoculation experiments has been 4 x 4 m plots with 4 replicates which usually gives a coefficient of variation higher than 10% and a minimum detectable difference of 14.5% (Gomez, 1972). Such a value agrees with the average increase in yield reported after algal inoculation, when successful.

3) EFFECTS OF BGA IN RICE FIELD

31) BGA as a source of N for rice

The uptake of rice of nitrogen fixed by BGA was demonstrated on a qualitative basis by Renaut et al. (1975) and Venkataraman (1977), using ¹⁵N tracer technique. In a quantitative experiment Wilson et al. (1980) recovered from a rice crop 37% of the nitrogen from ¹⁵N-labeled *Aulosira* sp. spread on the soil and 51% of the nitrogen from the same material incorporated into the soil. This study was conducted on a laboratory scale and did not include analysis of ¹⁵N remaining in the soil.

Pot and field experiment conducted at the International Rice Research Institute, using ¹⁵N labeled Nostoc sp, showed that availability of nitrogen from dried BGA incorporated in the soil was between 23 and 28% for the first crop and between 27 and 36% for 2 crops. Surface application of the algal material reduced the availability to

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14-23% for the first crop and 21-27% for 2 crops (Tirol et al., 1982). Availability of nitrogen from fresh algal material was similar to that of dried material when surface applied (14%) but much higher (38%) when incorporated (Roger and Watanabe, 1982). The pot experiment demonstrated that for the first crop algal nitrogen was less available than ammonium sulfate, but for two crops its availability was very similar to that of ammonium sulfate (Tirol et al., 1982). That indicates the slow release nature of BGA nitrogen, which agrees with the cumulative effects of algal inoculation (Roger and Kulasoorya, 1980). The 15N balance in plants and soil after two crops (pot experiment, dried algae) showed that losses from 15N ammonium sulfate were more than twice than from BGA, regardless of the mode of application. From these results the authors concluded that, due to its organic nature, BGA material is less susceptible to nitrogen losses than inorganic fertilizer and that its low C/N ratio (5-7) gives it a better nitrogen availability than those of an organic fertilizer like farmyard manure.

Relative availability of algal nitrogen to rice depends on the susceptibility to decomposition of the algal material which varies with the strains (Gunnison and Alexander, 1975) but also with their physiological state as demonstrated by the discrepancy between the values reported by Wilson et al. (1980) and those reported by Tirol et al. (1982). The former authors used an algal material collected directly from the flask culture and blended after resuspension in distilled water, whereas the latter authors used an algal material dried at room temperature, comprising mainly vegetative cells in dormancy and akinetes, and therefore less susceptible to decomposition.

A study of the tubificid role in mineralization and recovery of algal nitrogen by lowland rice (Grant and Seegers, in press) showed that uptake of BGA nitrogen by rice was affected by tubificids presence. Recovery of algal 15N by the first crop was 24-43% and 4-7% for the second one. Results of this experiment suggested that the presence or absence of tubificid worms offered an alternative explanation of the inconsistent recoveries by Wilson et al. (1980) and Tirol et al. (1982).

Some nitrogen fixed by BGA under field conditions is excreted by living cells but it is clear that only part of it is available to rice, some being either reincorporated by the microflora or volatilized. Release of nutrients through microbial decomposition after the death of the algae appears to be the principal means by which N is made available to the crop. One exception to this rule is the case of nitrogen fixed by BGA that growth addressed on the deep water rice plant (epiphytic BGA).

Epiphytic BGA have been observed on wetland rice (Roger et al., 1981), deepwater rice (Kulasoorya et al., 1981; Martinez and Catling, 1982), and on weeds growing in rice fields (Kulasoorya et al., 1981). ARA measurements indicated that the N contribution by N₂-fixing microorganisms epiphytic on wetland rice is low, whereas epiphytic fixation on deepwater rice makes substantial N contribution to this ecosystem (10-20 kg N/ha) mainly due to the greater biomass available for colonization by epiphytic BGA. The importance of epiphytic N₂ fixation and the availability of epiphytically fixed N was evaluated by Watanabe et al. (1982) and Watanabe and Ventura (1982) using 15N techniques. In a field experiment, rice was grown in pots containing 15N labeled ammonium sulfate, in shallow and deep (110 cm) water in the Philippines and Thailand. Rice plants in deepwater had lower 15N enrichment suggesting that nitrogen in floodwater as molecular nitrogen or combined or both, contributes to nutrition of deepwater rice (Watanabe et al., 1982). Direct evidence of N₂-fixation associated with deepwater rice was obtained by exposing submerged parts of a plant to 15N₂ for 9 days. Higher enrichment of 15N was found in submerged nodal roots and leaf sheaths where BGA grow epiphytically. During a 9 days period 8 mN was fixed by the plant and at maturity, about 40% of the fixed N was found in parts of the plants not directly exposed to 15N₂ (Watanabe and Ventura, 1982).

32) Other effects on rice

Besides increasing N fertility, BGA have been assumed to benefit higher plants by producing growth-promoting substances (see Roger and Kulasoorya, 1980). This hypothesis is based on the additive effects of BGA inoculation in the presence of N fertilizers. More direct evidence of hormonal effects has come primarily from treatments of rice seedlings with algal cultures or their extracts. Presoaking rice seeds with BGA cultures or extracts enhanced germination, promoted the growth of roots and shoots, and increased the weight and protein content of the grain (see Roger and Kulasoorya, 1980). It has also been established that algal growth-promoting substances are beneficial to other crops besides rice and that the production of such substances is not confined to BGA. Whether these substances are hormones, vitamins, amino-acids or any other components is still unknown. Possible effects as P solubilizers or as antagonists of rice pathogens have not yet been demonstrated.

33) Effect on soil properties and microflora

Grain-yield measurements suggest that algalization produces both a cumulative and residual effect. This was attributed to a build up of both the organic N content and the number of BGA propagules in the soil, facilitating the reestablishment of the BGA biomass. Several reports indicate an increase in organic matter and organic N; algalization was also reported to increase soil aggregation (Shield and Durrel, 1964), water-holding capacity (Singh, 1961), and available P, total microflora, Azotobacte(end-und)r, Clostridium, and nitrifiers populations (Ibrahim et al., 1971).

4) TECHNOLOGIES FOR UTILIZING BGA

Research on methods for using BGA in rice cultivation, emphasizes 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. It appears now that results concerning the occurrence of N₂-fixing BGA in rice fields are controversial. Watanabe and Yamamoto (1971) found that only 5% of 911 soil samples from Asia and Africa harbored N₂-fixing species. Venkataraman (1975) reported that 33% of 2213 soil samples from rice fields in India contained N₂-fixing strains. Okuda and Yamaguchi (1952) reported the presence of N₂-fixing strains in 71% of the samples they collected in Japan. Reynaud and Roger (1978) found N₂-fixing strains in 95% of the samples they collected in Senegal. In a survey of 40 rice fields in Thailand, Matsuguchi et al. (1974) found BGA in all soils. In a survey of Philippine rice soils, we found N₂-fixing strains in all of the 79 samples collected (Roger et al., unpublished). N₂-fixing strains, most probably, are more common in rice fields than it was previously thought. Unsuitable survey methodology, especially sampling method, probably caused the low values recorded. Therefore, research should equally emphasize inoculation and indigenous strain enhancement.

41) Algal inoculation

The methodology of BGA inoculum production was reviewed by Watanabe and Yamamoto (1971) and Venkataraman (1972). Methods of field application were reviewed by Venkataraman (1981).

411) Inoculum production

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Inoculum production in artificially controlled conditions was developed mainly in Japan (where algalization is not used). Inoculum production under artificially controlled conditions is efficient but expensive.

Open air soil culture, developed in India, is more simple, less expensive, and easily adoptable by the farmers. It is based on the use of a multistrain starter inoculum of *Aulosira*, *Tolypothrix*, *Scytonema*, *Nostoc*, *Anabaena*, and *Plectonema* provided by the "All India Coordinated Project on Algae" (1979). The inoculum is multiplied by the farmer in shallow trays or tanks 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 and sometimes 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 fields.

Using that method, the final proportion of individual strains in the algal flakes is unpredictable, but it is assumed that, because the inoculum is produced in soil climatic conditions similar to those in the field, dominant strains will be the best adapted to the local conditions. The recorded rates of production of algal flakes in the open air soil culture range from 0.4 to 1.0 kg/m² in 15 days, indicating that in 2-3 months a 2 m² tray can produce algal material to inoculate a 1 ha rice field.

412) Inoculation.

The algal inoculum is generally applied 1 week after transplanting. When rice is direct-seeded, seeds can be coated by mixing with the algal suspension and 2-3 kg calcium carbonate per 10-20 kg seed and air-dried in the shade. Recommendations for field application of dried algal inoculum (algal flakes) given by the India Coordinated Project on Algae (1979) indicate that:

- o 8-10 kg of dry algal flakes applied 1 week after transplanting is sufficient to inoculate 1 ha, a larger inoculation will accelerate multiplication and establishment in the field;
- o algalization can be used with high levels of commercial N fertilizer, but N application should be reduced by 30%;
- o to benefit from the cumulative effect of algalization the algae should be applied for at least three consecutive seasons; and
- o recommended pest-control measures and other management practices do not interfere with BGA establishment and activity in the fields.

42) Cultural practices to encourage BGA growth

421) Non N fertilizers application.

Soil properties that limit the growth of N₂-fixing BGA in rice fields are most commonly low pH and P deficiency. Application of P and lime has frequently increase growth, particularly in acidic soils (Watanabe and Cholitkul, 1982). Laboratory experiments (IRRI, 1976) indicated that application of phosphorus (50 kg P₂O₅/ha) enhanced NFA during the initial stages of a rice crop. Response to phosphorus varied with the soil type (Fig. 1). In the most responsive soils the increase in nitrogen fixation was estimated to be 0.7-1.2 g N/g P₂O₅ applied.

422) N fertilizer deep placement.

Study of different methods of nitrogen fertilizer application on the algal flora and photodependent BNF by Roger et al. (1980) (Table 3) have shown that surface broadcast application of N fertilizer which is widely practiced by farmers, not only inhibits photodependent BNF but also encourages the growth of green algae. These deleterious algae immobilize N fertilizer, making it temporarily unavailable to the

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plant. A profuse growth of green algae also increases the pH of the floodwater, encouraging fertilizer losses by ammonia volatilization. Deep placement of N fertilizer, in contrast, decreases the losses of N fertilizer by volatilization and does not disturb the natural algal N₂-fixing system (Table 4) that provides a bonus of N to the ecosystem.

423) Straw application.

Straw incorporation is a traditional agricultural practice which has been primarily used to add nutrients and organic matter to the soil. Beneficial effects on BNF were first reported for heterotrophic anaerobic microorganisms. More recently it was shown that straw application also favors aerobic heterotrophs and phototrophs (see Roger and Watanabe, 1985). Matsuguchi and Yoo (1981) measured ARA of straw fraction, root fraction and soil 7 weeks after transplanting in a soil with 8 t incorporated straw/ha. In the 0-1 cm surface layer, photodependent and photoindependent ARA of straw were roughly 1000-fold and 100-fold higher than those in the soil fraction. In deeper layer the trend was similar. The same authors compared the effect on BNF of deep placement and topdressing of 8 t rice straw/ha in two soils. In all cases straw stimulated BNF. Stimulation was more marked for photodependent ARA in the 0-1 cm surface layer than for photoindependent ARA in the whole profile. Topdressing rice straw induced higher photodependent ARA and better rice growth than deep placement. Photodependent ARA in the topdressed straw was 103-fold more than in the soil fraction and was enhanced by N and herbicide application. Beneficial effects of surface straw application on BGA growth and photodependent ARA were also reported by Roger et al., (1982) and IRRI (1982) (Fig. 2). This may be due to an increase of CO₂ in the photic zone, a decrease of mineral N and O₂ concentration in the floodwater, and the provision of microaerobic microsites by the straw. Increased CO₂ availability and low N concentration favor the growth of N₂-fixing BGA. Low O₂ concentration in the photic zone may increase specific N₂-fixing activity.

424) Grazers control.

Invertebrates like cladocerans, copepods, ostracods, mosquito larvae, snails, etc. are common grazers of algae in rice fields. The development of such populations may prevent the establishment of algal inocula and cause the disappearance of algal blooms within one or two weeks (see Roger and Kulasooriya, 1980). Insecticides have been shown to enhance algal growth and sometimes to favor BGA over green algae and diatoms. Development of grazers populations can be controlled by cheap pesticides of plant origin (Grant et al., 1983) and by seasonal drying. Detailed information on grazers control is available from the chapter by Grant in this volume.

43) Current utilization of BGA technology

Most of applied research on algal inoculation is conducted in India where a national program has been developed, the All-India Coordinated Project on Algae. To a lesser extent, applied research is also conducted in Burma, China, and Egypt. Reports on the adoption of algal technology are controversial, but even considering the most optimistic evaluations, use of algal inoculation seems to be restricted to very limited hectareage in a few Indian states and in Burma.

In a review on adoption of biofertilizers in India, Pillai (1980) 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 a review on non-symbiotic N₂-fixation, Venkataraman (1982) wrote: "A conservative estimate suggests

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that about two million hectare under rice are currently covered with algal biofertilizer technology". In a review on biofertilizers, Subba Rao (1982) wrote that the production capacity of BGA flakes in India was around 40 t/yr, which was approximately 0.01% of the total inoculum requirement for the country (40 t will inoculate 4000 ha).

From the recent extensive report on BGA field trials published by the Agricultural Economics Research Center of the University of Madras (1982), it seems that, despite an official radio and print publicity campaign, BGA use remains at the trial level and that in many cases inoculated algae did not multiply. Therefore, it seems appropriate to consider that this technology is more at an experimental level of large scale field testing than at a popularization stage.

44) Limiting factors

The major limiting factor to popularize algal inoculation is the lack of reliable technology for recommendation to farmers. Inoculum establishment is sporadic and the reasons for failure are frequently not known. In reviewing BGA literature, it is surprising to observe the unbalance between the different topics. Taxonomy, morphology, micromorphology, physiology, and enzymology are highly documented and test tube BGA growth has been studied extensively. However, field studies are rare, most probably limited by lack of suitable methodology. Therefore, BGA ecology is still poorly undertaken. The physiological characteristics of N₂-fixation desirable for strains suitable for field inoculation are known (Stewart, et al., 1979), but the selection of "Super N₂-Fixing Strains" is meaningless unless they survive, develop, and fix N₂, as programmed, in rice fields. As indicated by Gibson (1981), virtually nothing is known of the attributes permitting introduced strains to colonize the various hostile environments to which they will be exposed.

Similarly, the factors permitting the establishment of an N₂-fixing bloom of inoculated or indigenous strains still are unknown. Low pH, low temperatures, and P deficiency limit BGA growth. However, because in some soils algalization is inefficient despite the addition of lime and phosphate (Okuda and Yamaguchi, 1952) pH and available P are not the only limiting factors. Texture, organic matter content, CEC of saturated extracts, and total N are probably not important (Subrahmanian et al., 1965).

Grazing by invertebrate populations is an important biotic limiting factor (Grant and Alexander, 1981). Other possible limiting mechanisms such as antagonism, competition, etc. have been suggested, but their role is unclear. Low temperatures, heavy rains, and cloudy weather also have been reported to limit the inoculum establishment (Roger and Kulasoorya, 1980).

Inoculum quality also may be a limiting factor. In published methods of inoculum production, no tests of composition and viability have been included. We have shown that the density of colony-forming unit in BGA inocula may vary from 10³ to 10 per gram of dry inoculum and that in many cases N₂-fixing strains are not dominant (IRRI, 1984). Some commercial inoculants also have been reported to have limited potential for BGA population enhancement (Tiedman et al., 1980). Therefore, special attention must be paid to inocula quantity.

Economics apparently do not limit BGA utilization. In a study of the economics of BGA use of 40 farmers in Tamil Nadu, (University of Madras, 1982) no significant difference was found in the average per hectare cost of cultivation between crops using (\$247) and not using (\$246) BGA. The average return of BGA utilization was \$4/ha. When needed, grazer population can be controlled with inexpensive natural insecticides.

5) CONCLUSION

Blue-green algae are a possible N source in slightly acidic to alkaline soils with moderate to high P availability and low grazer incidence. Field experiments on algal inoculation (algalization) provide indirect information on the overall potential of BGA. They report an average 14% yield increase over the control corresponding to about 450 kg grain/ha per crop, where algal inoculation was effective. Comparison with N fertilizers indicated that algal inoculation may be equivalent to the application of 25-30 kg N/ha. A similar increase was observed with and without N fertilizers (Table 2). Because BNF is known to be inhibited by inorganic N, the beneficial effect of algalization in the presence of N fertilizers was most frequently interpreted as resulting from growth-promoting substances produced by algae or by a temporary immobilization of added N, followed by a slow release through subsequent algal decomposition that permitted more efficient crop N utilization. Such interpretations have yet to be demonstrated.

BGA have less potential in terms of N₂-fixed than legumes green manures or azolla, (around 30 kg N/ha per crop) but their use is promising because little additional labor is required. However, algal inoculation is still at a research level in most of the rice growing countries. Factors involved in yield increase reported after algal inoculation, factors leading to the establishment of a bloom, and the general ecology of BGA in rice fields are still poorly understood. BGA utilization is limited by technological problems concerning inoculum quality and establishment. Therefore, algal inoculation cannot be confidently recommended yet in many rice growing countries.

A common characteristic of the BNF technologies currently adopted by farmers (legume green manures and azolla) is intensive labor use. They are most often used under socioeconomic conditions where labor intensive practices are economically feasible or where economics is not a major factor. In the future it is unlikely that BNF could be an exclusive N source for producing high yields under economically feasible conditions. Most probably the future of BNF in rice cultivation is in integrated management. A better knowledge of the microbiology and the ecology of rice fields will encourage high rice yields through a more efficient usage of chemical fertilizers and the simultaneous utilization of BNF. N fertilizer deep placement (De Datta et al., 1983), which significantly decreases losses of N by volatilization and does not inhibit photodependent BNF by BGA coupled with BGA inoculation is a good example of the kind of technology that must be developed for integrated management of BNF and chemical fertilizers.

REFERENCES CITED

- All India coordinated project on algae. 1979. Algal biofertilizer for rice. India Agric. Res. Inst. New Delhi, 61 p.
- Buresh, R. J., M. E. Casselman, and W. H. Patrick, Jr. 1980. Nitrogen fixation in flooded soil systems, a review. *Adv. Agron.* 23: 149-192.
- David, K. A. V. and P. Fay. 1977. Effects of long-term treatment with acetylene on nitrogen fixing micro-organisms. *Appl. Environ. Microbiol.* 34:640-646.
- De, P. K. 1939. The role of blue-green algae in nitrogen fixation in rice fields. *Proc. R. Soc. Lond* 127 B, 121-139.

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De Datta, S. K., I. R. P. Fillery, and E. T. Craswell. 1983. Results from recent studies on nitrogen fertilizer efficiency in wetland rice. *Outlook in Agriculture* 12(3):125-134.

Gibson, A. H. 1981. Some required inputs from basic studies to applied nitrogen fixation research, Pages 6-7. In *Current Perspective in Nitrogen Fixation*. Gibson, A. and Newton, W. E. eds., Australian Academy of Science.

Gomez, K. A. 1972. Techniques for field experiment with rice. International Rice Research Institute, Los Banos, Philippines.

Gunnison, D. and M. Alexander. 1975. Resistance and susceptibility of algae to decomposition by natural microbial communities. *Limnol. Oceanogr.* 20:64-70.

Grant, I. and M. Alexander. 1981. Grazing of blue-green algae (Cyanobacteria) in flooded soils by Cypris (Ostracoda), *Soil Sci. Soc. Amer. J.* 45:773-777.

Grant, I. F. and R. Seegers. Tubificid role in soil mineralization and recovery of algal nitrogen by lowland rice. *Soil Biol. Biochem.* (in press)

Grant, I. F., A. C. Tirol, T. Aziz, and I. Watanabe. 1983. Regulation of invertebrate grazers as a means to enhance biomass and nitrogen fixation of Cyanophyceae in wetland rice fields. *Soil Sci. Soc. Amer. J.* 47:669-675.

Ibrahim, A. N., M. Kamel, and M. El-Sherbeny. 1971. Effect of inoculation with alga *Tolypothrix tenuis* on the yield of rice and soil nitrogen balance. *Agrokem. Talagtan* 20:389-400.

International Rice Research Institute. 1982. Annual Report for 1981.

International Rice Research Institute. 1983. Annual Report for 1982.

International Rice Research Institute. 1984. Annual Report for 1983.

International Rice Research Institute. 1976. Annual Report for 1975.

Kulasooriya, S. A., P. A. Roger, W. L. Barraquio, and I. Watanabe. 1981. Epiphytic nitrogen fixation on deepwater rice. *Soil Sci. Plant Nutr.* 27(1):19-27.

Kulasooriya, S. A., P. A. Roger, W. L. Barraquio, and I. Watanabe. 1981. Epiphytic N₂ fixation on weeds in a rice field ecosystem. Pages 56-61. In R. Wetselaar et al. ed., *Nitrogen Cycling in South Asian Wet Monsoon Ecosystems*. Austral. Acad. Sci. Canberra.

Lowendorf, H. S. 1982. Biological nitrogen fixation in flooded rice fields. *Cornell Int. Agric. Mimeogr.* 96, Nov. 1982, 75 pp.

Martinez, M. R. and M. D. Catling. 1982. Contribution of algae to the nutrition of deepwater rice. pp. 201-214 in *Proc. 1981 Int. Deepwater rice workshop*, the International Rice Research Institute, Los Banos, Philippines.

Matsuguchi, T., B. Tangcham, and S. Patiyuth. 1974. Free-living nitrogen fixers and acetylene reduction in tropical rice fields. *Jpn Agric. Res. Q.* 8(4):253-256.

Matsuguchi, T. and I. D. Yoo. 1981. Stimulation of phototrophic N₂ fixation in paddy fields through rice straw application. pp. 18-25. In *Nitrogen Cycling in South-East*

13

Asian WEt Monsoonal Ecosystems. Wetselaar, R., Simpson, J. R. and Rosswall, T. eds. Canberra, Austral. Acad. Sci.

Okuda, A. and M. Yamaguchi. 1952. Algae and atmospheric nitrogen fixation in paddy soils. II. Relation between the growth of blue-green algae and physical or chemical properties of soil and effect of soil treatments and inoculation on the nitrogen fixation. Mem. Res. Inst. Food Sci. 4:1-11.

Palacpac, A. C. 1982. World rice statistics. The International Rice Research Institute, Los Banos, Philippines 152 p.

Peterson, R. B. and R. H. Burris. 1976. Conversion of acetylene reduction rates to nitrogen fixation rates in natural populations of blue-green algae. Anal. Biochem. 73:404-410.

Pillai, K. G. 1980. Biofertilizers in rice culture. Problems and prospects for large scale adoption. AICRIP Publ. No. 196.

Renaut, J., A. Sasson, H. W. Pearson, and W. D. P. Stewart. 1975. Nitrogen-fixing algae in Morocco. In Nitrogen Fixation by Free-living Microorganisms", pp. 229-246. Stewart, W. D. P. (ed.), Cambridge University Press, Cambridge.

Reynaud, P. A. and P. A. Roger. 1978. N₂-fixing algal biomass in Senegal rice fields. Ecol. Bull., Stockholm 26:148-157.

Roger, P. A. and S. A. Kulasooriya. 1980. Blue-green algae and rice. The International Rice Research Institute, Los Banos, Philippines. 112 pp.

Roger, P. A., S. A. Kulasooriya, W. L. Barraquio, and I. Watanabe. 1981. Epiphytic nitrogen fixation in wetland rice. Pages 62-66. In Nitrogen Cycling in Southeast Asian Wet Monsoonal Ecosystem, Australian Acad. Sci. Canberra.

Roger, P. A., S. A. Kulasooriya, and E. T. Craswell. 1980. Deep placement: a method of nitrogen fertilizer application compatible with algal nitrogen fixation in wetland rice soils. Plant and Soil 57:137-142.

Roger, P. A. and P. A. Reynaud. 1982. Free living blue-green algae in tropical soils. pp. 147-168. In Microbiology of Tropical Soils and Plant Productivity, Dommergues, Y. and Diem H. eds. Martinus, Nijhoff, La Hague.

Roger, P. A., P. A. Reynaud, G. E. Rinaudo, P. E. Ducerf, and T. M. Traore. 1977. Log-normal distribution of acetylene-reducing activity in situ. Cah. ORSTOM Ser. Biol. 12:133-140 (in French, English summary).

Roger, P.A., A. Tirol, I. Grant, and I. Watanabe. 1982. Effect on surface application of straw on phototrophic nitrogen fixation. Int. Rice Res. Newsl. 7(3):16-17.

Roger, P. A. and I. Watanabe. 1982. Research on algae, blue-green algae, and phototrophic nitrogen fixation at the International Riuce Research Institute (1963-81), summarization, problems and prospects. IRRI Res. Paper Series No. 78, IRRI, Los Banos, Philippines.

14

Roger, P. A. and I. Watanabe. 1985. Technologies for utilizing biological nitrogen fixation in wetland rice: potentialities, current usage, and limiting factors. *Fertilizer Review* (in press).

Saito, M. and I. Watanabe. 1978. Organic matter production in rice field flood water. *Soil Sci. Plant Nutr.* 24(3):427-440.

Shield, L. M. and L. W. Durrel. 1964. Algae in relation to soil fertility. *Bot. Rev.* 30:93-128.

Singh, R. N. 1961. Role of blue-green algae in nitrogen economy of Indian agriculture. *Indian Council of Agric. Res., New Delhi*, 175 pp.

Stewart, W. D. P. 1978. Nitrogen fixing Cyanobacteria and their association with eukaryotic plants. *Endeavour* 2:170-179.

Stewart, W. D. P., P. Rowel, J. K. Ladha, and M. J. A. Sampaio. 1979. Blue-green algae (Cyanobacteria) - some aspects related to their role as sources of fixed nitrogen in paddy soils. pp. 263-285. In *Nitrogen and Rice*, The International Rice Research Institute, Los Banos, Philippines.

Subba Rao, N. S. 1982. Biofertilizer - interdisciplinary science reviews. 7(3):220-229.

Subramanyan, R., G. B. Manna, and S. Patnaik. 1965. Preliminary observations on the interaction of different rice soil types to inoculation of blue-green algae in relation to rice culture. *Proc. Indian Acad. Sci. Sect. B* 62(4):171-175.

Tiedman, A. R., W. Lopushinsky, and H. H. Larsen. 1980. Plant and soil responses to a commercial blue-green algae inoculant. *Soil Biol. Biochem.* 12:471-475.

Tirol, A., P. A. Roger, and I. Watanabe. 1982. Fate of nitrogen from a blue-green alga in a flooded rice soil. *Soil Sci. Plant Nutr.* 28(4):559-569.

University of Madras. 1982. Blue-green algae as a source of bio-fertilizers in Thanjauur district - Tamil Nadu. Res. Study No. 74 of the Agric. Econ. Res. Center, Multigr. 79 pp.

Venkataraman, G. S. 1972. *Algal biofertilizers and rice cultivation. Today and Tomorrow's Printers, Faridabad (Haryana)* 75 pp.

Venkataraman, G. S. 1975. The role of blue-green algae in tropical rice cultivation. pp. 207-218. In *Nitrogen fixation by free-living microorganisms*, Stewart, W. D. P. ed., Cambridge Univ. Press.

Venkataraman, G. S. 1977. Blue-green algae as a biological input in rice cultivation. Pages 132-142. In *Proc. Natl. Symp. Nitrogen assimilation and crop productivity*. Hissar.

Venkataraman, G. S. 1981. Blue-green algae for rice production - A manual for its promotion. *FAO Soils Bull. No. 46*, 102 pp.

Venkataraman, G. S. 1982. Non symbiotic nitrogen fixation. Pages 225-235. In *Indian Society of Soil Sci. ed. Review of Soil Research in India. Vol. 1*, New Delhi.

15

Watanabe, A. and Y. Yamamoto, Y. 1971. Algal nitrogen fixation in the tropics. *Plant and Soil* (special volume), 403-413. Watanabe, I. 1978. Biological nitrogen fixation in rice soils. Pages 465-478. In *Soils and Rice*, The International Rice Research Institute, Los Banos, Philippines.

Watanabe, I. and S. Brotonegoro. 1981. Paddy fields. Pages 241-263. In *Nitrogen Fixation*, Vol. 1, Ecology, Broughton, W. J. ed., Clarendon Press, Oxford.

Watanabe, I. and W. Cholitkul. 1982. Nitrogen fixation in acid sulfate soils. *Trop. Agric. Res. Ser. No. 15*, 219-226.

Watanabe, I. and P. A. Roger. 1984. Nitrogen fixation in wetland rice field. Pages 237-276. In *Current Developments in Biological Nitrogen Fixation*. Edited by N. S. Subba Rao. Oxford & IBH Publishing Co. New Delhi, Bombay, Calcutta.

Watanabe, I. and W. Ventura. 1982. Nitrogen fixation by blue-green algae associated with deepwater rice. *Current Sci.* 51:462-465.

Watanabe, I., W. Ventura, W. Cholitkul, P. A. Roger, and S. A. Kulasooriya. 1982. Potential of biological nitrogen fixation in deepwater rice. Pages 191-200. In *Proc. 1981 Int. Deepwater Rice Workshop*. The International Rice Research Institute, Los Banos, Philippines.

Wilson, J. T., D. L. Eskew, and M. Habte. 1980. Recovery of nitrogen by rice from blue-green algae added in a flooded soil. *Soil Sci. Soc. Am. J.* 44:1330-1331.