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## **BLUE-GREEN ALGAE IN RICE FIELDS**

*Their ecology and their use as inoculant*

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### **Abstract**

This paper is a short review on blue-green algae in rice fields, their ecology and their use as inoculants. Some emphasis has been given to the recent studies of the relations between blue-green algae and rice which include the availability of algal nitrogen to the rice plant and epiphytic relationships.

### **1 INTRODUCTION**

$N_2$ -fixing BGA were recorded in 86 out of 89 paddy soils (Reynaud, 1980). However, Venkataraman (1975) pointed out that "contrary to general belief,  $N_2$ -fixing BGA are not invariably present in tropical rice soils, and that an all India survey showed that out of 2213 soil samples from rice fields, only about 33% harboured  $N_2$ -fixing forms". The heterogenous and sometimes limited distribution of  $N_2$ -fixing BGA is still not well understood because no systematic analysis has correlated the presence or absence of BGA with environmental factors (Lowendorf, 1980).

## 2 METHODOLOGICAL LIMITATIONS FOR THE STUDY OF BGA IN RICE FIELDS

### 2.1 Quantitative evaluations

The lack of completely satisfactory methods for estimating algal abundance and biomasses of the different algal groups (Fogg et al., 1973) is certainly a limiting factor for ecological studies with BGA.

Plating techniques, which are the most frequently used methods, are advantageous in providing qualitative and quantitative results simultaneously. However, the accuracy of the counts depend on the reliability of the particular dilution method. Filamentous forms are difficult to separate into individual cells. Plating techniques can be improved by determining the mean volume of each "count unit" (cell, filament, akinete or colony, according to the species) by directly, microscopically examining the first dilution and multiplying the results of enumerations by the corresponding "volume unit". This permits the expression of the results of enumerations in term of biomasses (Roger and Reynaud, 1976).

Algal enumerations are often limited by an inadequate methodology in sampling. Most of the results are expressed as number of algae per gram of dry soil which does not take into account algae in the floodwater of submerged soils and does not permit any extrapolation at the field level (What is the dry weight of soil colonized by algae in one hectare of a paddy field?). A better way is to express enumerations as number of algae per  $cm^2$  by using core samples with a well defined diameter, each core sample including the first centimetre of soil and the corresponding floodwater, if the soil is submerged (Roger and Reynaud, 1976). This will permit comparisons and extrapolations of the data at the field level if a correct density of sampling has been used.

The problem of the choice of a density of sampling in relation to the distributional ecology of algae has been studied by Roger and Reynaud (1978). Results indicate that soil algae have log-normal distribution (logarithms of number are normally distributed) and that a very high density of sampling is required to obtain a significant evaluation. For example, the mean value of Anabaena sp biomasses based upon 40 samples of 10 cores each, taken in a 0.25 ha paddy field, had a confidence interval of +32% and -27% of the mean. Such an evaluation clearly demonstrates that a composite sample obtained by mixing a large number of core subsamples is at least required to enumerate algae; of course replicate measurements on composite samples will give better results.

Currently, results of BGA enumerations in soils are too fragmentary and the methodology used too frequently open to criticism, to allow the development of general concepts.

## 2.2 Nitrogen fixing activity.

$N_2$ -fixation by BGA has been most frequently studied using the acetylene reducing activity method which may lead to erroneous results (Lowendorf, 1980). ARA variations during both the day and the growing cycle can be rapid and important; moreover ARA also has a log-normal distribution (Roger et al., 1977). Therefore, large number of replicates and very frequent measurements are needed to ensure a satisfactory measure of total ARA. However, this tedious work will still lead to an imprecise evaluation of the nitrogen fixing activity (NFA) as the conversion factor acetylene-nitrogen is not constant and needs to be determined experimentally for each set of experimental conditions (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 sampling 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 non-heterocystous  $N_2$ -fixing BGA was not appreciated until recently and field measurements of nitrogenase activity were carried out under an aerobic gas phase only, therefore it is difficult to evaluate the N-input due to  $N_2$ -fixation by BGA (Stewart, 1978).

### 3 ECOLOGY OF BLUE-GREEN ALGAE

In paddy fields growth of BGA and algal successions are governed by climatic, physiochemical and biotic factors.

#### 3.1 Climatic factors

Among climatic factors, light intensity is certainly the most important. Light availability for soil algae depends upon the season and latitude, the plant canopy, the vertical location of the algae in the photic zone and the turbidity of the water. Light intensity reaching the soil may vary from too low to excessive levels (10 to 110,000 lux). In cultivated soils the screening effect of a growing crop canopy can cause a rapid decrease in the light reaching the algae. Thus the canopy of transplanted rice decreased light by 50% when plants were 15 days old, 85% after one month and 95% after two months (Kurasawa, 1956).

BGA are generally sensitive to high light intensities. They develop various protective mechanisms like vertical migrations in the water of submerged soils; preferential growth in more shaded zones like embankments, under or inside decaying plant material, or a few millimeters below the soil surface; photophobotaxis; photokinesis; and stratification of the strains in algal mats, where  $N_2$ -fixing strains grow under a layer of eukaryotic algae, more resistant to high light intensities (Roger and Reynaud, 1982).

In areas with high incident light intensities, BGA develop later in the crop cycle when the plant cover is dense enough to protect them from excessive light (Roger and Reynaud, 1977). On the other hand, light deficiency may also be a limiting factor. In Japan, available light under the canopy was lower than the compensation point of the phytoplankton during the second part of the growth cycle (Ichimura, 1954). In the Philippines, during the wet season, when light was moderate, ARA was higher in bare soil than in planted soil (Watanabe et al., 1977).

The optimal temperature for BGA is about 30-35°C, Temperature is rarely a limiting factor for BGA in paddy fields because the range of temperature permitting their growth is larger than that required by rice; however, temperature influences the composition of the algal biomass and the productivity. Low temperatures decrease productivity

and favour eukaryotic algae. High temperatures favour BGA and increase the algal productivity (Roger and Kulasooriya, 1980).

### 3.2 Soil properties

Among the soil properties, pH is the most important factor determining the algal flora composition. Under natural conditions BGA grow preferentially in environments that are neutral to alkaline. This explains why, positive correlations occur in the rice fields between: water pH and BGA number - soil pH and BGA spores - soil pH and the  $N_2$ -fixing algal biomass in samples homogenous for stage of rice development, fertilization and plant cover density (Roger and Reynaud, 1982). The beneficial influence of high pH on BGA growth is demonstrated by the fact that the addition of lime increases BGA growth and  $N_2$ -fixation (Roger and Kulasooriya, 1980). However, the presence of certain strains of BGA in soils with pH values between 5 and 6 has been reported (Durrel, 1964; Aiyer, 1965).

Besides pH, phosphorus availability is an important factor determining growth of BGA. Okuda and Yamaguchi (1952) incubated 117 submerged soils and noted that BGA growth was closely related to the available P content of the soil.

The growth of  $N_2$ -fixing BGA in rice fields is most commonly limited by low pH and P deficient, and application of P together with lime has frequently produced positive results (Roger and Kulasooriya, 1980).

### 3.3 Biotic factors

Organisms that limit BGA growth are: pathogens, antagonistic organisms and grazers. Of these, only grazers have been documented. The development of zooplankton populations, especially cladocerans, copepods, ostracods, and mosquito larvae prevented the establishment of algal blooms within one or two weeks (Venkataraman, 1961). Grazing rates and algal diet preferences of ostracods were studied by Grant and Alexander (1981) who estimated the potential consumption of BGA at an average field density of 10,000 ostracods  $m^{-2}$  to be about 120 kg (fw)  $ha^{-1} day^{-1}$ . An economical alternative for controlling ostracod populations is the application of crushed seeds of the neem tree (*Azadirachta indica*) (Grant et al., 1982). Snails form another

group of algal grazers in submerged paddy fields, the biomass of snails can be as high as 1.6 t/ha in certain rice fields in the Philippines (Roger and Kulasooriya, 1980). Commercial pesticides capable of controlling grazers are expensive and thus uneconomical to use (Grant et al., 1982).

#### 4 RELATIONS BETWEEN BLUE-GREEN ALGAE AND THE RICE PLANT

##### 4.1 Availability of algal nitrogen to rice

The uptake by rice of nitrogen fixed by BGA was demonstrated on a qualitative basis by Renaut et al. (1975) and Venkataraman (1977), using  $^{15}\text{N}$  tracer technique. In a quantitative experiment Wilson et al. (1980) recovered from a rice crop 37% of the nitrogen from  $^{15}\text{N}$ -labelled 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  $^{15}\text{N}$  remaining in the soil.

Pot and field experiments conducted at the International Rice Research Institute, using  $^{15}\text{N}$  labelled 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 the 2 crops. Surface application of the algal material reduced the availability to 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 was 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 & Kulasooriya, 1980). The  $^{15}\text{N}$  balance in plants and soil after two crops (pot experiment, dried algae) showed that losses from  $^{15}\text{N}$  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.

#### 4.2 Growth promoting effect

Besides increasing N fertility, BGA have been assumed to benefit higher plants by producing growth-promoting substances. This hypothesis is based on the additive effects of BGA inoculation in the presence of nitrogenous fertilizers. Most of these results have been obtained with rice but similar results were observed also with vegetables such as radishes and tomatoes (Roger et al., 1979).

More direct evidence of hormonal effects has come primarily from treatments of rice seedlings with algal cultures or their extracts. Pre-soaking rice seeds with BGA cultures or extracts enhances germination, promotes the growth of roots and shoots, and increases the weight and protein content of the grain (Roger and Kulasooriya, 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.

#### 4.3 Epiphytism

Epiphytic BGA have been observed on wetland rice (Roger et al., 1981), deepwater rice (Kulasooriya et al., 1981, Martinez and Catling, 1982), and on weeds growing in rice fields (Kulasooriya et al., 1981). Comparing these three different hosts (Kulasooriya et al., 1980) it was found that epiphytism and the associated ARA on wetland rice at seedling, tillering and heading stages and on the submerged weed Chara was predominantly due to colonies of Gloeotrichia sp

visible to the naked eye. The epiphytic algae on wetland rice at maturity, on the submerged weed *Najas*, and on deepwater rice could be observed only under the microscope and the dominant algae were *Nostoc*, *Calothrix*, and *Anabaena*. A unique finding was that BGA also exist inside the cavities of senescent rice leaf sheaths; this "endophytism", however, in addition to being not confined to rice, was not present in living healthy tissues. A frequent observation was that older parts of the hosts and plants with rough surfaces supported more numerous epiphytic BGA. From these facts, it was concluded that epiphytism is possibly related to an abiotic effect, of which a mechanical effect in relation to the roughness of the host surface appears to be of importance.

Rates of ARA on wetland rice gradually diminished from seedling to maturity mainly due to the concomitant decrease of *Gloeotrichia* epiphytism and the reduction of available light. In deepwater rice also there was a decrease in specific ARA (activity per gram of host) from heading to maturity but this was compensated by an increase in the host biomass so that a constant activity per plant was observed at both stages. The results of ARA measurements indicated that the N contribution by  $N_2$ -fixing microorganisms epiphytic on wetland rice is low but epiphytic BGA play an important role in inoculum conservation because floating algae and soil algae are frequently washed out from the field during heavy rains. On the other hand, epiphytic nitrogen fixation on deepwater rice makes a 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  $N_2$ -fixation and the availability of epiphytically fixed N was evaluated by Watanabe et al. (1981) and Watanabe and Ventura (1982) using  $^{15}N$  techniques. In a field experiment, rice was grown in pots containing  $^{15}N$  labelled ammonium sulfate, in shallow and deep (110 cm) water in the Philippines and Thailand. Rice plants in deepwater had lower  $^{15}N$  enrichment suggesting that nitrogen in floodwater as molecular nitrogen or combined or both, contributes to nutrition of deepwater rice (Watanabe et al., 1981). Direct evidence of  $N_2$ -fixation associated with deepwater rice was obtained by exposing submerged parts of a plant to  $^{15}N_2$  for 9 days. The highest enrichments of  $^{15}N$  were found in submerged nodal roots and leaf sheaths where BGA grow epiphytically. During the 9 days period 8 mg-N 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  $^{15}\text{N}_2$  (Watanabe and Ventura, 1982).

In shallow water-rice, epiphytic BGA make only a small contribution to the nitrogen input, whereas, in deepwater rice they produce a substantial nitrogen input which is especially important because in this cropping system nitrogen fertilizer is seldom applied.

## 5 ALGAL INCOULATION IN RICE FIELDS

BGA were among the first  $\text{N}_2$ -fixing agents recognized to be active in flooded rice soils. Many trials have been conducted to increase rice yield by inoculating the soil with BGA. This practice, also called algalization, a terminology introduced by Venkataraman (1966), has been reported to have a beneficial effect on grain yield in different agroclimatic conditions. However, some reports also indicate failure of algalization. The conclusions of the review of Roger and Kulasooriya (1980) on algalization are summarized hereafter.

### 5.1. Methodology of the experiments

Most experiments on algalization have been 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  $\text{N}_2$ -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 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 walls, where BGA frequently seem to grow preferentially and profusely. Pot experiments may therefore only be suitable for qualitative studies, since they overestimate the effects of BGA inoculation. Most of the field experiments on the other hand, were conducted in only one growing season and 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.

## 5.2. Effect of algalization on the rice plant

Algalization may affect plant size, nitrogen content, and the number of tillers, ears, spikelets, and filled grains per panicle. The most frequently used criterion for assessing the effects of algalization has been better grain yield. Results of field experiments conducted mainly in India report an average yield increase of about 14% over the control, corresponding to about 450 kg grain  $\text{ha}^{-1}$  per crop, where algal inoculation was effective. 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 biological  $\text{N}_2$  fixation 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 temporary immobilization of added N followed by a slow release through subsequent algal decomposition permitting a more efficient utilization of N by the crop.

## 5.3 Effect of algalization 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: aggregation status of the soil (Shield and Durrel, 1964), water-holding capacity (Singh, 1961), available P, and total microflora, Azotobacter, Clostridia, and nitrifiers (Ibrahim et al., 1971).

## 5.4. Limiting factors for algalization

Among the possible limiting factors responsible for the failure of algalization only pH and available P content of the soil have been studied. Since in some soils algalization is inefficient in spite of

available P content is probably not the only factor limiting the effect of algalization. On the other hand, texture, organic matter content, CEC of saturated extracts, and total N are probably not important limiting factors (Subrahmanyam et al., 1965). Among the biotic factors that can possibly limit BGA inoculum growth, grazing by the zooplankton has been already mentioned. Other possible mechanisms involved such as antagonism, competition, etc. have been cited, but their role is not clear. Low temperatures, heavy rains, and cloudy weather have also been reported to limit the establishment of the inoculum.

#### 5.5. Algalization technology

The methodology of BGA production has been reviewed by Watanabe and Yamamoto (1970) and Venkataraman (1972). The methods of field application have been reviewed by Venkataraman (1981). Methods of inoculum production in artificially controlled conditions have been developed mainly in Japan where algalization is not used. Producing the inoculum in artificially controlled conditions is well defined but relatively expensive. On the contrary, the open air soil culture, used in India, is simpler, less expensive and easily adoptable by the farmers. It is based on the use of a starter culture that is a multistrain inoculum of Aulosira, Tolypothrix, Scytonema, Nostoc, Anabaena and Flectonema, 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 m<sup>-2</sup>, 100 g triple superphosphate m<sup>-2</sup> 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 dry up in the sun. Algal flakes are then scraped off and stored in bags for use in the fields. With such a method, the ultimate proportion of individual strains in the algal flakes is unpredictable. It is assumed that, because the inoculum is produced in soil and climatic conditions similar to those in the field, dominant strains will be the most 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 m<sup>-2</sup> in 15 days, indicating that a 2 m<sup>2</sup> tray can produce in 2-3 months enough algal material to inoculate 1 ha of rice field. For

transplanted rice the algal inoculum is generally applied 1 week after the transplanting. When rice is sown, seeds can be coated by mixing 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 "All Coordinated Project on Algae" (1979) indicate that 8-10 kg of dry algal flakes applied 1 week after transplanting is sufficient to inoculate 1 ha; although a larger amount will accelerate multiplication and establishment in the field. Algalization can be used with high levels of commercial nitrogen fertilizer, but reduction of the N dose by one third is recommended. To benefit from the cumulative effect of algalization, the algae should be applied for at least three consecutive seasons. Recommended pest-control measures and other management practices do not interfere with the establishment and activity of these algae in the fields.

#### 6 RESEARCH NEEDS ON BGA IN RICE FIELDS

Looking at the literature on BGA, it is surprising to observe the disequilibrium between the different topics. Taxonomy, morphology, micromorphology, physiology and enzymology are highly documented whereas ecology is still very poorly understood, most probably because of methodological problems. Test tube grown BGA have been extensively studied, and several desirable physiological characteristics of BGA strains for field inoculation have been summarized by Stewart et al. (1979) as follows: "Strains selected for use in the field should be fast-growing and capable of fixing  $N_2$  under aerobic, microaerobic and anaerobic conditions. They should also be able to grow photoautotrophically and chemoheterotrophically, and store endogenous carbohydrate reserves. They should evolve little  $H_2$  and liberate nitrogen in excess of their requirements for optimum growth. Cyanobacteria differ in whether or not they liberate extracellular  $NH_4^+$  on inhibition of glutamine synthetase. The ways in which glutamine synthetase of Cyanobacteria is regulated could be of importance in strain selection". However the selection of "super  $N_2$ -fixing strains" has no meaning unless such strains are able to survive, develop and fix nitrogen as programmed in the rice fields. Information is available on inoculum production under laboratory and outdoor conditions, but the successful field establishment of these

inocula seems to be sporadic. As pointed out 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. Recent studies have shown the importance of grazers as a limiting factor for BGA growth. Neem cake or neem oil application permits cheap control of microcrustaceans, but a similar technology is needed for snails that graze on BGA.

Another important gap, is the mode of action of inoculated BGA. In field experiments average yield increase in absence of nitrogen fertilizers (14.6%) does not significantly differ from that in presence of nitrogen fertilizers (14.3%). Since biological  $N_2$ -fixation is known to be inhibited by inorganic N, the beneficial effect of algalization in the presence of N fertilizers has been most frequently interpreted as resulting from growth-promoting substances produced by BGA. Such a hypothesis still needs to be proved as algalization experiments have been conducted on a "black box" basis, where only the last indirect effect (yield) of an agronomic practice (algalization) was observed. No data are available on nitrogen fixation and algal biomass measurements in an inoculated paddy field. Therefore the relative importance of nitrogen fixation by inoculated BGA in increasing the rice yield, compared with other possible effects like auxinic effects, effects on soil properties, increase of P availability etc. is still unknown. Although it is claimed that BGA are widely used for rice production in certain countries (India, Burma), it appears that algal technology is still at a research level in most of the rice growing countries. This is most probably due to insufficient knowledge, which does not permit algalization to be recommended with confidence to the farmers. Singh (1979) reported that the experiments conducted at CRRRI have shown very clearly that fresh algae inoculation is always better than dried algae inoculation. However, due to the high water content of BGA, the weight of a fresh inoculum is very high: a value of 750 kg/ha was reported in a FAO Soils Bulletin on organic manure in China (1977). Using fresh inocula for algalization reduces one of the main advantages of this technology, that is the utilization of a stable, easy to handle, non-bulky, dry inoculum. It is essential to achieve the aim of reliable, dry inoculum to establish competitively in the field. For such a purpose we need a better knowledge of the factors

affecting i) the formation and the germination of algal spores and propagules, ii) the establishment of algal inocula in situ.

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