

## SENSITIVITY OF BLUE GREEN ALGAE TO SOIL REACTION — A FACTOR AFFECTING ITS EFFICIENT USE AS BIOFERTILIZER

The use of blue green algae (BGA) as biofertilizer for rice cultivation is quite prevalent in many parts of India. It can also be used along with fertilizer nitrogen to improve the existing crop yield. This fact has been well established by the study of many earlier workers like Goyal and Venkataraman (1970), Venkataraman (1977), Singh (1978) and Kannaiyan *et al.*, (1982). Under ideal conditions, BGA can fix about 25 kg N/ha/season. Apart from nitrogen fixation, inoculation with BGA is also reported to reduce considerably the total sulphides and ferrous iron content of the soil (Aiyer, 1965). Many genera of BGA such as *Scytonema*, *Oscillatoria*, *Anabaena* and *Nostoc* are already reported to occur in Kerala (Amma *et al.*, 1966). They also reported that the number of BGA in the acidic soils of Kerala was relatively very low and that the application of lime significantly increased the population of the native flora of BGA in such soils. *Anabaena* and *Nostoc* species are often found to dominate the BGA population.

The large scale use of BGA as a biofertilizer, however, is yet to become a common practice among farmers in Kerala due to its poor response under field conditions. This is because the soils of Kerala are generally acidic in nature and that most species of BGA are sensitive to low pH for normal growth and nitrogen fixation. Under such situations, it is essential to neutralise the soil pH by adequate liming before resorting to BGA application. The present paper is mainly based on pH sensitivity and nitrogen fixing capacity of few indigenous cultures of BGA isolated from three locations in the State.

The initial isolation of BGA was done by enrichment culture technique using Fogg's nitrogen free mineral medium. Soil samples were collected from rice fields of the College of Agriculture, Vellayani, Pazhamchira Ela and Rice Research Station, Moncompu. Fresh soil samples (10 g) from each location were inoculated aseptically into 100 ml of the above medium in 250 ml conical flasks. The flasks were incubated in a growth chamber at  $28 \pm 1^\circ\text{C}$  for 3 weeks under artificial illumination using 40 W fluorescent light. The blue green algal growth from each flask was examined for purity under a microscope and then transferred to fresh Fogg's medium for further studies. The isolates were identified at the Indian Agricultural Research Institute, New Delhi. The nitrogen fixing ability of different isolates of BGA was studied under *in vitro* conditions using the Fogg's medium itself. Sterilized broth of this medium (100 ml) was initially inoculated with 50 mg each of fresh algal mat of various isolates of BGA and allowed to grow for 30 days in a growth chamber under artificial illumination. Three replications were maintained. At the end of the growth period, the amount of nitrogen fixed in the culture filtrate was estimated by microkjeldahl method (Jackson, 1958). The final value is expressed in terms of mg nitrogen fixed per gram of fresh algal mat. The isolate of BGA which fixed maximum nitrogen under above conditions was subsequently used to test its pH sensitivity by culturing aseptically in Fogg's medium adjusted to different levels of pH such as 3.5, 5.5, 7.5, 9.5 and 10.5. These were inoculated with 50 mg of fresh algal mat of the selected species and grown

Table 1. *In vitro* nitrogen fixing ability of BGA isolated from different locations (mg N/g fresh algal mat)

Location	BGA species	N <sub>2</sub> fixed*
Vellayani	<i>Anabaena oryzae</i>	12.7
Vellayani	<i>Anabaena</i> sp.	2.9
Vellayani	<i>Nostoc</i> sp.	1.8
Pazhamchira Ela	<i>Anabaena</i> sp.	1.1
Pazhamchira Ela	<i>Nostoc</i> sp.	0.9
Moncompu	<i>Anabaena ambigua</i>	7.0
Moncompu	<i>Anabaena</i> sp.	2.7
Moncompu	<i>Microchaete</i> sp.	4.1

\*Mean of three replications

for 30 days in a growth chamber under artificial illumination. The extent of growth at different levels of pH was estimated by determining the relative fresh weight of algal mat at the end of the growth period.

In all, eight cultures of BGA were isolated initially from the three locations chosen for that purpose (Table 1). The occurrence of some of these genera in Kuttanad and other areas in Kerala is reported earlier also (Aiyer, 1962; Amma *et al.*, 1986). However, *Anabaena* and *Nostoc* spp. were found to dominate the native flora of the soil. Such predominance of one or two species in a region is observed in other places as well like the presence of *Aulosira* sp. in Uttar Pradesh, *Mastigocladus* sp. in Gujarath, *Westiella* sp. in Maharashtra, *Cylindrospermum* sp. in Karnataka and *Calothrix* sp. in Punjab.

There was much variation in the ability of different isolates of BGA to fix atmospheric nitrogen under *in vitro* conditions (Table 1). The amount of nitrogen fixed, 12.7 mg N<sub>2</sub>/g fresh weight

of algal mat was maximum in *Anabaena oryzae* isolated from Vellayani followed by the two isolates from Moncompu, *Anabaena ambigua* and *Mirochaete* sp. respectively which fixed about 7.0 and 4.1 mg N<sub>2</sub>/g fresh weight of algal mat. The amount of nitrogen fixed by *Anabaena* and *Nostoc* spp. isolated from Pazhamchira Ela was low when compared to other isolates of BGA (Table 2). These cultures were initially isolated from soil samples having relatively a low pH of 4.8. This indicated that eventhough some species of BGA may be present as a part of the normal microflora even in acidic soils, its ability to fix atmospheric nitrogen under such condition is rather very low.

The culture of *Anabaena oryzae* which could fix greater amount of nitrogen under *in vitro* conditions was further selected to test its ability to grow at different levels of pH ranging between 3.5 to 10.5. The growth was maximum at pH 7.5 with a fresh mat yield of 345 mg/100 ml broth (Table 2). The extent of growth at pH 5.5 was also higher when compared to that obtained at pH 3.5, 9.5 and 10.5. It is evident from the study that a near neutral

Table 2. Effect of pH on growth of *A. oryzae*

pH	Fresh weight* (mg)
3.5	303
5.5	330
7.5	345
9.5	280
10.5	228

\* Mean of three replications

pH condition is essential for normal growth and multiplication of BGA. Thus in Kerala, where the soil is generally acidic

at least in some of the major rice growing areas like Kuttanad, there is a **pre-requirement** for adequate liming of the soil to raise the pH if one has to successfully use BGA as a source of biofertilizer. However, very often, it is seen that probably due to economic considerations the farmers are reluctant to adopt this practice and under such conditions the use of BGA will result only in inadequate growth and nitrogen fixation. A solution to this problem will be either to identify new indigenous strains of BGA capable of good growth and nitrogen fixation in acidic soils or to incorporate these traits in existing cultures through modern techniques of biotechnology.

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