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RECONSIDERING THE UTILIZATION OF BLUE-GREEN ALGAE IN WETLAND RICE CULTIVATION

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INTRODUCTION

The existence of an agronomic potential for BGA in rice cultivation was recognized in 1939 by De, who attributed the natural fertility of tropical ricefields to biological nitrogen-fixation (BNF) by those organisms. Since then, long-term fertility experiments and nitrogen-fixation measurements have confirmed the importance of BGA and other nitrogen-fixing organisms in maintaining a moderate but constant rice production in fields receiving no nitrogen fertilizer.

The importance of BGA in rice culture was made clear in the literature review by Roger and Kulasooriya (1980), which included many reports of ricefields being manipulated to maximize blue-green algal nitrogen fixation, mostly by the addition of dried inocula. However, most of these reports lacked detailed documentation of methods and results, and a more recent review (Roger, 1989) takes a cautious view when interpreting the significance of earlier research.

The aim of this paper is to review the possibilities for promoting the growth of nitrogen-fixing BGA in ricefields by inoculation.

POTENTIAL OF BGA TO INCREASE RICE YIELD

The nitrogen-fixing ability of many BGA species is the principal, but probably not the only reason for increased fertility of ricefields. The extent to which BGA contribute to the nitrogen requirement of the crop is determined mostly by the algal standing biomass, rate of nitrogen fixation, turnover of the fixed nitrogen, and the extent to which BGA nitrogen becomes available to the plant.

Nitrogen Contribution Estimated from Biomass Measurements

Reported standing crops of BGA in ricefields range from a few kg ha^{-1} to 0.5 t ha^{-1} dry weight (aw) (Roger et al., 1987a). However, values expressed as fresh weight (fw) or dw ha^{-1} give little information on the agronomic significance of the biomass because of the wide range of dry matter (0.2-14%) and ash (31-71%) contents in field-grown BGA (Roger et al., 1986b). Nitrogen in one ton fw of BGA averages 1.2 kg but may vary from 0.1 kg to 4 kg. Assuming a maximum biomass of 0.5 t dw ha^{-1} and using average ash and N values obtained for field samples (Roger et al., 1986b), the potential average contribution of a BGA bloom is about 15 kg N/ha.

Figure 1 summarizes 400 biomass measurements performed weekly in 65 plots on the IRRI farm when BGA were visible. In most cases, biomass contained a few kg N ha^{-1} (median: 4 kg N/ha). The highest value was 17 kg N ha^{-1}.

Available data indicate that: (1) a visible growth of BGA usually corresponds to less than 10 kg N ha^{-1}(2) a dense bloom may correspond to 10-20 kg N ha^{-1}, and (3) values were higher only in experimental microplots or in soil-based inoculum production plots (Reddy and Roger, 1988; Roger et al., 1985a).

Nitrogen contribution by BGA is the result of nutrient turnover of the standing biomass, for which no data are yet available.

Assuming that all carbon input in the floodwater and surface soil is through nitrogen-fixing BGA (which is an obvious overestimation) and using an input of 0.6 t C ha^{-1} per crop (Saito and Watanabe, 1978) and an average C/N ratio of 8 (Roger et al., 1986b), the maximum contribution of nitrogen-fixing BGA could be 75 kg N ha^{-1} per crop.

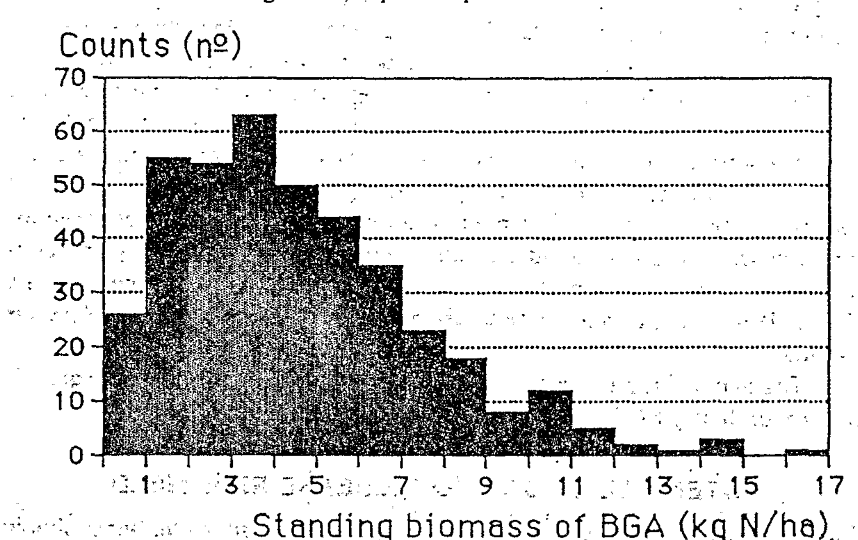

Fig. 1: Distribution of 400 estimates of the standing crop of BGA in 65 plots on the IRRI farm (unpubl. data)

Nitrogen Contribution Estimated by Acetylene Reducing Activity

Nitrogen fixation by BGA has been most frequently estimated by acetylene reducing activity (ARA) measurements. Estimates published before 1980 vary from a few to 80 kg N ha^{-1} and average 27 kg ha^{-1} per crop in plots where no BGA were inoculated (Roger and Kulasooriya, 1980).

Figure 2 presents the distribution of 180 estimates of the average ARA during a crop cycle in experimental plots at IRRI. Sixty plots received no nitrogen fertilizer, 60 received 55 kg N ha^{-1} as broadcast urea, and 60 received 55 kg N ha^{-1} as deep-placed urea. Assuming an acetylene/N ratio of 4, BNF expressed in kg N ha^{-1} per crop is about 1/10 of ARA (μmol C_2H_2 m^{-2} h^{-1}). Values range from 0.2 to 50 kg N ha^{-1} per crop (average 13, median 9.5). Average values per treatment are 20 kg N ha^{-1} in control plots, 8 kg in plots with broadcast urea, and 12 kg in plots where nitrogen was deep-placed (Table 1).

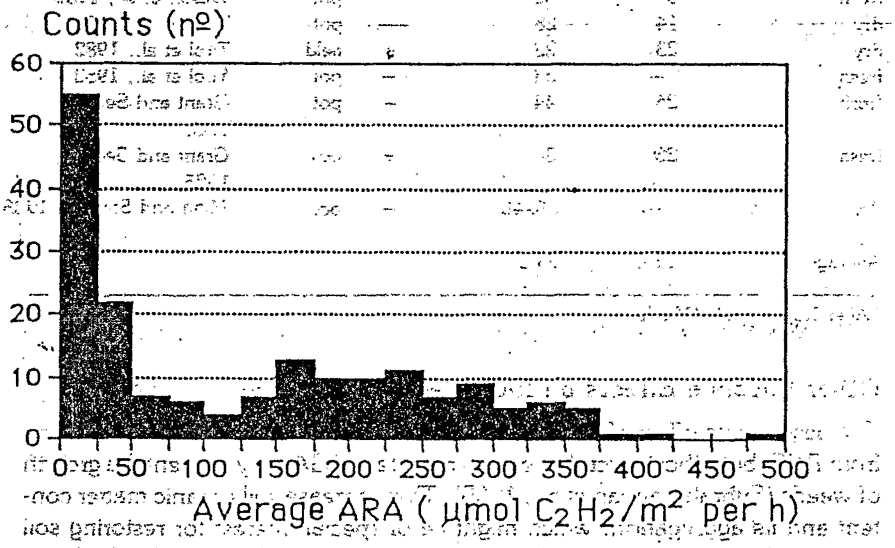

Fig. 2: Distribution of 180 estimates of the average ARA during a crop cycle in experimental plots at IRRI (unpubl. data).

Table 1. Average ARA during the crop cycle and rice yield under different nitrogen fertilizer (urea) management*

Treatment	Average ARA (μmol C_2H_2/m^2 per h)	Grain yield (t/ha)
Control (No N applied)	195±14	4.08±0.10
38 kg N/ha broadcast at transplanting + 17 kg N/ha at panicle initiation	80±13	4.82±0.12
55 kg N/ha deep-placed at transplanting	116±16	5.78±0.09

*Unpubl. data. Each value is the average of estimates in 60 plots. Each estimate is the average of 9 to 13 measurements during the crop cycle using composite samples of 8 soil cores.

Utilization of BGA Nitrogen by the Rice Plant

Recovery of BGA N by rice (Table 2) varies from 13 to 50%, depending on the nature of the material (fresh versus dried), the method of application, and the presence or absence of soil fauna (Grant, 1985a; Tirol et al., 1982; Wilson et al., 1980a). Rice use of nitrogen of the BGA biomass is more efficient when the biomass is incorporated into the soil. Recovery was highest with fresh BGA incorporated into a soil depleted of fauna (Wilson et al., 1980a). Recovery was lowest with dried BGA applied on the surface of a soil rich in tubificid worms (Tirol et al., 1982), which reduce the recovery of algal nitrogen by rice by making more soil nitrogen available through mineralization (Grant, 1985a).

Table 2: Availability of nitrogen from BGA to rice*

Material	N recovery (%)		Fauna	Experimental	Reference state
	Surface applied	Incorporated			
fresh	37	52	?	pot	Wilson et al., 1980
dry	14	28	—	pot	Tirol et al., 1982
dry	23	23	+	field	Tirol et al., 1982
fresh	—	38	—	pot	Tirol et al., 1982
fresh	24	44	—	pot	Grant and Seegers, 1985
fresh	25	30	+	pot	Grant and Seeger, 1985
dry	—	35–40	—	pot	Mian and Stewart, 1985
Average	24.6	36.2			

*After Roger et al. (1987a).

Other Possible Effects on Rice Yield

The best known effect of BGA on rice is increased nitrogen availability resulting from BNF, but other effects have been reported. BGA may prevent the growth of weeds (Subrahmanyan et al., 1965). They increase soil organic matter content and its aggregation, which might be of special interest for restoring soil structure for an upland crop after rice (Roychoudhury et al., 1983). Excretion of organic acids by BGA might increase phosphorus availability to rice (Arora, 1969). Presoaking rice seeds in BGA cultures (Jacq and Roger, 1977) or field inoculation (Aiyer et al., 1971) was reported to decrease sulfide injury in soils subject to sulfate reduction processes detrimental to rice.

There have been many claims that soaking seeds or growing seedlings with BGA cultures can benefit rice plants by producing plant growth regulators (PGR). Roger and Kulasooriya (1980) cite 11 references where the additive effects of BGA inoculation in the presence of nitrogen fertilizers were interpreted as an effect of a PGR produced by the algae. Such interpretations are obviously highly hypothetical. More direct evidence of PGR effects has come primarily from the treatment of rice seedlings with algal cultures or their extracts. Roger and Kulasooriya (1980) cite 12 references reporting that presoaking of seeds or seedlings in BGA cultures or extracts had some of the following effects: enhanced germination; faster growth; early seedling recovery; rhizogenous effect; prolonged tillering; stimulated vegetative growth; and increase in length and number of ears, in number of grains per ear, and in weight and protein content of the grains. However, when 133 unialgal strains isolated from sites in Africa (not all ricefields) were tested (Pedurand and Reynaud, 1987) for their effects on rice germination and growth, 70% had a negative effect on germination and only 21% a stimulatory effect; many *Nostoc* strains had a negative effect.

As pointed out by Metting and Pyne (Metting and Pyne, 1986), the numerous reports of algal PGR showed no definitive study in which a microalgal PGR was isolated and characterized.

BGA Potential Estimated from Inoculation Experiments

The effects on rice yield of soil inoculation by BGA were first reported by Watanabe et al. (1951): a 25% increase in a poorly drained ricefield inoculated with *Tolypothrix tenuis*. Several authors reported increases well over 200% from pot trials (Singh, 1961; Sundara Rao et al., 1963). Subsequent studies have indicated much lower increases in field than in pot trials, even where comparative studies have been made (Huang, 1978). Possible causes for overestimated effects of BGA inoculation in pot experiments include the strong reduction of grazer populations (dry soil, depleted of fauna, is usually used), less climatic disturbances, and a mechanical effect of the pot wall, where BGA may grow profusely.

Studies on BGA inoculation have been discontinued in Japan, but subsequently have been pursued in India and a few other countries. Inocula have mostly been derived from laboratory-grown strains, following the early studies with *T. tenuis* in Japan (Watanabe, 1961).

The interest generated in BGA in India led in 1977 to the All-India Coordinated Project on Algae, which involved the production, distribution, and testing of soil-based inocula of BGA. Books based on the results give details of practical methods (Venkataraman, 1972 and 1981a). Inocula are prepared from a mix of strains isolated originally from ricefields and grown in shallow trays with soil, phosphate, and insecticide. If necessary, lime is added to adjust soil pH to 7.0–7.5. The algal mats that develop are allowed to dry and the dried flakes are stored in bags for use at 10 kg ha^{-1} in farmers' fields. Ricefield inoculation with BGA has received considerable publicity and some reviews have accepted the success of the method in raising grain yields as a well-established fact (Agarwal, 1979). Many studies have reported increased grain yield, grain nitrogen content, or straw nitrogen content (Venkataraman, 1981b; Singh and Singh, 1985), the effects of BGA being equivalent to the addition of 20-30 kg ha^{-1} N, provided phosphorus fertilizer is added (Sharma and Gupta, 1983). Reports on field experiments available to Roger and Kulasooriya (1980) showed that on the average, algal inoculation causes 14.5% relative increase in grain yield, corresponding to 475 kg grain ha^{-1} per crop.

However a recent review, considers 260 experiments reported between 1980 and 1987, and shows a lower average yield increase of about 300 kg/ha (8.5%) in inoculated plots. The *t*-test of Student-Fischer for paired samples (ddf = 258; $t = -1.734$) shows no significant difference in average yield between inoculated and noninoculated plots at p = 0.05 (The level of significance is 0.084.)

Figures 3 and 4 and Table 3 present the analysis of a bibliographic compilation of field data (634 experiments) reporting yield values in both a noninoculated control and the corresponding inoculated treatment at the same fertilizer level. The intercept of the regression curve with the y-axis has a value

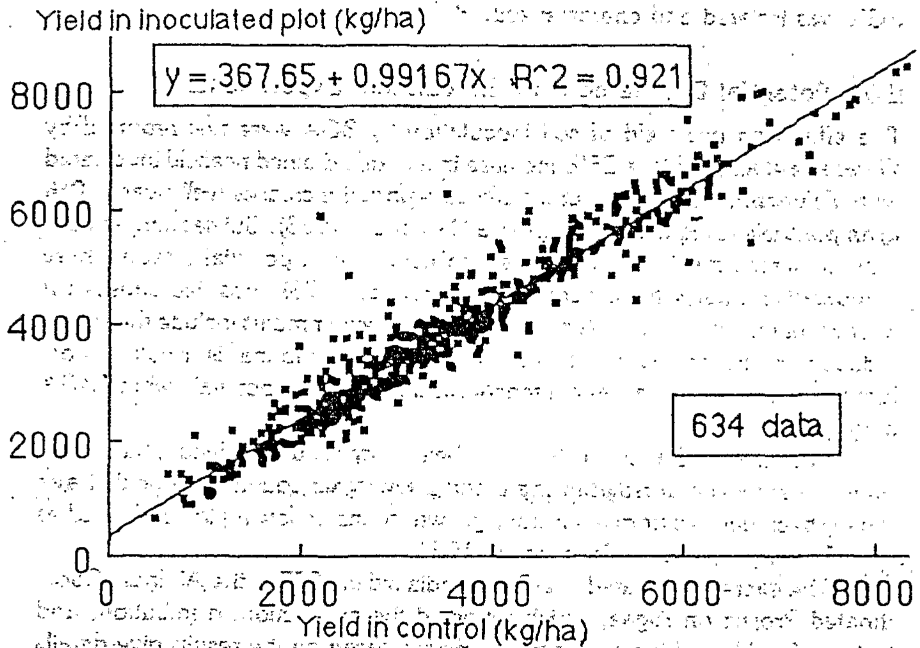

Fig. 3: Correlation between yield in inoculated plots and controls. Bibliographic compilation.*

* Data from Aiyer et al., 1972; Alimagno and Yoshida, 1975; Beri and Meelu, 1983; Bhuiya, 1984; Bhuiya et al., 1984; Chandrakar et al., 1983; Gaur and Singh, 1983; Grant et al., 1985; Hamissa et al., 1979; Indian Agricultural Research Insititute, 1978 and 1980; Jagannathan and Kannaiyan, 1977; Jalapathi Rao et al., 1977; Jha et al., 1965; Kannaiyan, 1981a, 1981b, 1985 and 1986; Kannaiyan and Govindarajan, 1982; Kannaiyan et al., 1980; Kannaiyan et al., 1982; Konishi and Seino, 1961; Mudholkar et al., 1973; Pantastico and Gonzales, 1976; Patel et al., 1984; Pillai, 1980; Ram and Rawat, 1984; Ram et al., 1985; Reddy et al., 1986; Relwani, 1963 and 1965; Roychoudhury et al., 1983; Sankaram et al., 1967; Sarkar and Islam, 1984; Singh, 1981 and 1982; Srinivasan and Ponnayya, 1978; Subramani et al., 1980; Venkataraman, 1975, 1977 and 1980.

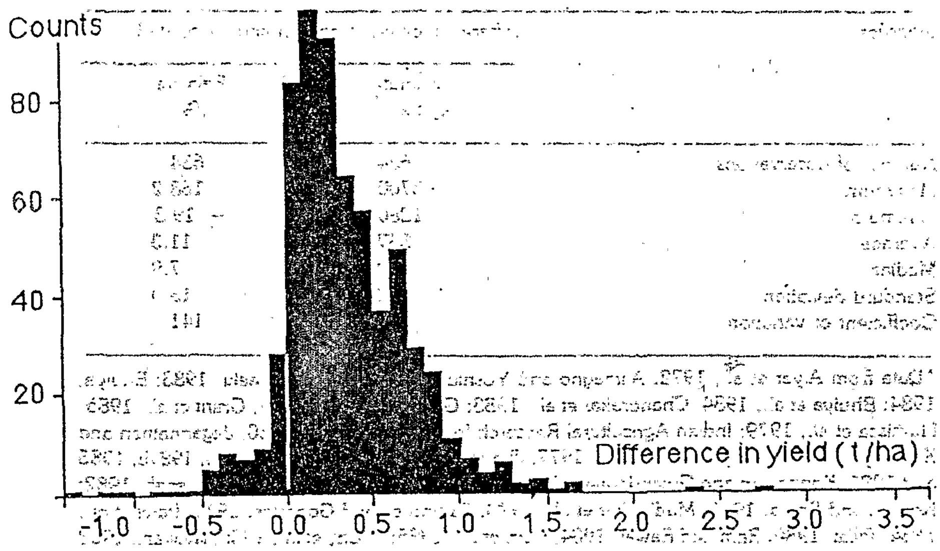

Fig. 4: Distribution of absolute yield differences between inoculated plots and controls. Bibliographic compilation.*

* Data from Aiyer et al., 1972; Alimagno and Yoshida, 1975; Beri and Meelu, 1983; Bhuiya, 1984; Bhuiya et al., 1984; Chandrakar et al., 1983; Gaur and Singh, 1983; Grant et al., 1985; Hamissa et al., 1979; Indian Agricultural Research Institute, 1978 and 1980; Janannathan and Kannaiyan, 1977; Jalapathi Rao et al., 1977; Jha et al., 1965; Kannaiyan, 1981a, 1981b, 1985 and 1986; Kannaiyan and Govindrajan, 1982; Kannaiyan et al., 1980; Kannaiyan et al., 1982; Konishi and Seino, 1961; Mudholkar et al., 1973; Pantastico and Gonzales, 1976; Patel et al., 1984; Pillai, 1980; Ram and Rawat, 1984; Ram et al., 1985; Reddy et al., 1986; Relwani, 1963 and 1965; Roychoudhury et al., 1983; Sankaram et al., 1967; Sarkar and Islam, 1984; Singh, 1981 and 1982; Srinivasan and Ponnayya, 1978; Subramani et al., 1980; Venkataraman, 1975, 1977 and 1980.

of 360 kg grain ha^{-1}, and the difference in yield between inoculated and control plots is almost independent of the yield of the control (Fig. 3). Statistical analysis (Table 3) shows a very large variability of the difference in yield between inoculated and noninoculated plots, with some surprising extreme values and a coefficient of variation of more than 100%. Average difference is 337 kg grain ha^{-1}. However, a very dissymetrical histogram (Fig. 4) and a standard deviation close to the mean (Table 3) indicate a log-normal distribution of the data. Therefore, the median (257 kg ha^{-1}) is a better index of the average effect of inoculation that the mean.

The *t*-test of Student-Fischer for paired samples (ddf = 63; t_c = 3.03) shows a statistically significant difference in average yield between inoculated (3937 kg/ha) and noninoculated (3599 kg/ha) plots at $p \leq 0.01$. However, only 17% of the experiments report a statistically significant difference bet-

Table 3: Statistics of absolute and relative yield differences between inoculated plots and controls. Bibliographic compilation*

Statistics	Difference between control and inoculated	
	Absolute ($kg\ ha^{-1}$)	Relative (%)
Number of observations	634	634
Maximum	3700	168.2
Minimum	−1280	− 19.3
Average	337	11.3
Median	257	7.9
Standard deviation	393	16.0
Coefficient of variation	118	141

*Data from Aiyer et al., 1972; Alimagno and Yoshida, 1975; Eeri and Meelu, 1983; Bhuiya, 1984; Bhuiya et al., 1984; Chandrakar et al., 1983; Gaur and Singh, 1983; Grant et al., 1985; Hamissa et al., 1979; Indian Agricultural Research Institute, 1978 and 1980; Jagannathan and Kannaiyan, 1977; Jalapathi Rao et al., 1977; Jha et al., 1965; Kannaiyan, 1981a, 1981b, 1985 and 1986; Kannaiyan and Govindarajan, 1982; Kannaiyan et al., 1980; Kannaiyan et al., 1982; Konishi and Seino, 1961; Mudholkar et al., 1973; Pantastico and Gonzales, 1976; Patel et al., 1984; Pillai, 1980; Ram and Rawat, 1984; Ram et al., 1985; Reddy et al., 1986; Relwani, 1963 and 1965; Roychoudhury et al., 1983; Sankaram et al., 1967; Sarkar and Islam, 1984; Singh, 1981 and 1982; Srinivasan and Ponnayya, 1978; Subramani et al., 1980; Venkataraman, 1975, 1977 and 1980.

ween inoculated plots and controls. This indicates that (1) the response to algal inoculation varies, (2) the response is small, and (3) the experimental error is larger than the response. The most common design for BGA inoculation experiments has been 4 × 4 m plots with four replications, which usually gives a coefficient of variation higher than 10% and a minimum detectable difference of 14.5% (Gomez, 1972) larger than the average yield increase reported after algal inoculation.

Another important aspect of the statistical analysis of BGA inoculation experiments refers to the experimental design to compare an inoculated treatment receiving nitrogen fertilizer with a noninoculated treatment receiving an additional 25 or 30 kg N ha^{-1}. If the yields in both treatments do not significantly differ, the authors conclude that the effect of BGA is equivalent to the application of 25 or 30 kg N ha^{-1}. This design should not be used because it does not permit the testing of yield response at the additional level of nitrogen. In addition, with such a design, concluding that two values are not significantly different requires $p > 0.95$ whereas most authors use $p > 0.05$, which is erroneous.

When interpreting data from the literature, it should also be kept in mind that, probably, unsuccessful trials were often not reported. When they were mentioned, it was usually without quantitative data that could explain the possible reason for failure. For example, a report of a multilocation trial in India (Pillai, 1980) indicates that notwithstanding the 22 sets of data presented, "the results from many other locations ... were not received because of the failure of multiplying BGA at these locations."

Available data show that algal inoculation can increase rice yield but its effects often seem to be erratic and limited, which may explain the farmers limited adoption of algal inoculation. A study of the economics of BGA use by 40 farmers in Tamil Nadu (Univ. of Madras, 1982) found no significant difference in the average per hectare cost of cultivation between crops using ($247) and not using ($246) BGA, but the average return of BGA utilization was only $4/ha. When asked whether BGA had increased yield, only five out of 40 farmers responded positively. Among the 10 farmers not using BGA, four were unaware of the technology, two were aware of it but could not get any inoculum, and four said they were not convinced of the yield-increasing quality of the algae.

LIMITING FACTORS FOR BGA GROWTH

Grazing

Invertebrates like cladocerans, copepods, ostracods, mosquito larvae, and snails are common grazers of algae in ricefields. Their development reportedly prevented the establishment of inocula (Hirano et al., 1955; Watanabe et al., 1955) and caused algal blooms to disappear (Venkataraman, 1961). Recent studies confirm that grazing is a major limiting factor for BGA growth (Grant et al., 1983; Grant et al., 1985; Wilson et al., 1980 b). Ingestion rates of *Heterocypris* determined *in vitro* by Grant and converted to BGA consumed by a field population ($8700.m^{-2}$) totalled 187 g $N \cdot ha^{-1} \cdot day^{-1}$[4] or 73 kg fw algae $ha^{-1} \cdot day^{-1}$ or 19 kg N ha^{-1} . $crop^{-1}$. In microplot (0.5 m^2) experiments, nitrogen accumulation at the soil surface increased by one to three-and-a-half times when grazers were controlled (IRRI, 1986).

A trend among nitrogen-fixing BGA is that strains forming mucilaginous colonies (*Aphanothece, Nostoc, Gloeotrichia*) are less susceptible to grazing than strains that do not form such colonies (Grant et al., 1985). Therefore, grazing has a selective effect on BGA. Plate counts (Roger et al., 1987) showed that genera forming mucilaginous colonies were dominant in more than 90% of 102 studied soils, while other genera were present in many soils but were rarely dominant. This may indicate that grazing leads to the dominance of mucilaginous BGA, which are usually less active in BNF (Grant et al., 1985; Antarikanonda et al., 1982).

Phosphorus

Phosphorus is often a limiting factor for BGA growth as shown by a positive correlation between available phosphorus and the abundance of BGA in rice soils and experiments demonstrating that phosphorus application stimulates BGA growth and photodependent BNF (Roger and Kulasooriya, 1980; Cholitkul et al., 1980; Wilson and Alexander, 1979).

Nitrogen Fertilizer

Roger and Kulasooriya (1980) list seven references reporting that nitrogen fertilizer inhibited BGA growth in ricefields, but also suggest that inhibition *in situ* might not be as marked as that *in vitro*. Studies at IRRI (IRRI, 1987 and 1988) show that broadcasting urea strongly inhibited photodependent acetylene-reducing activity (ARA) in about 75% of 60 cases; while in others, a significant ARA was recorded (Fig. 5). The negative correlation between photodependent BNF and rice yield (Fig. 5) indicates that a significant ARA developed after nitrogen application when nitrogen efficiency (kg rice produced/kg nitrogen applied) was low.

Inoculation experiments at various levels of nitrogen fertilizer also show inconsistent trends. Some authors observed a more or less marked decrease of the effects of BGA inoculation when nitrogen fertilizer was applied (Indian Agricultural Research Institute, 1978; Gaur and Singh, 1983; Kannaiyan and Govindarajan, 1982) while others report a similar effect of BGA inoculation at all levels of nitrogen applied (Indian Agricultural Research Institute, 1978; Jalapathi et al., 1977).

A trial at 22 locations in India (Pillai, 1980) showed: (1) a better average effect of BGA inoculation without nitrogen fertilizer (310 kg grain ha^{-1}) that when 25 kg N ha^{-1} was applied (190 kg/ha) (Table 4) but at both levels the

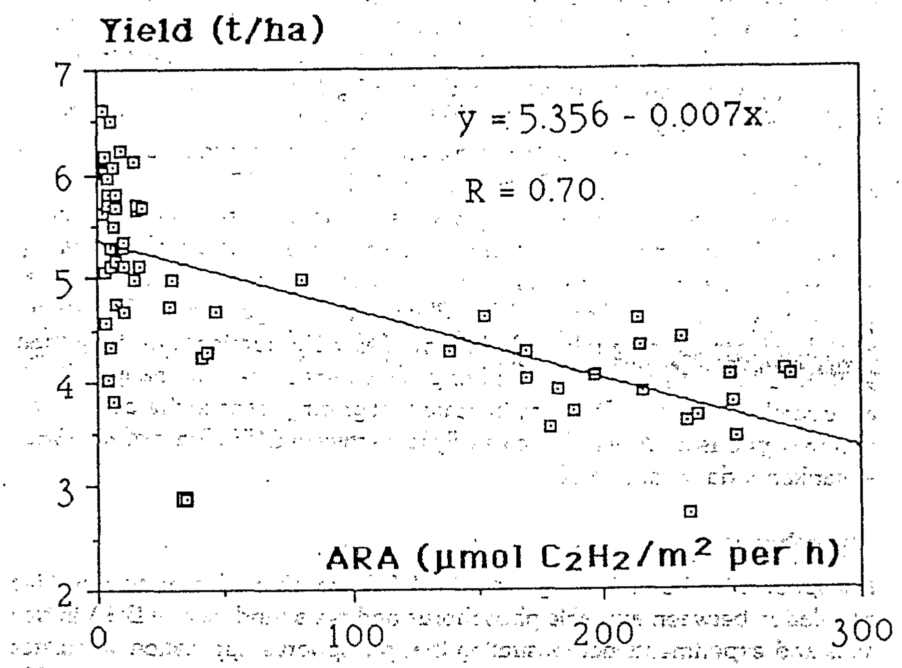

Fig. 5: Rice yield and average photodependent N_2-fixing activity during the crop cycle in plots with urea broadcast at 80 kgN/ha (unpubl. data)

Table 4. Average effect of nitrogen fertilizer and BGA inoculation on yield in 22 sites from a multilocation trial, calculated from Pillai (1980)

Treatment	Grain yield (t/ha)	N efficiency kg grain/kg N*	Yield increase due to BGA (t/ha)	N equivalent to inoculation (kg/ha)
Control	2.87			
25 N	3.52	26.02		
50 N	3.90	15.40		
75 N	4.26	14.31		
0 N + BGA	3.18		0.31	11.9
25 N + BGA	3.71		0.19	12.3

*for each 25 kg/ha increment in nitrogen fertilizer applied

effect of BGA was equivalent to the application of 12 kg of nitrogen fertilizer. The same data also show a negative correlation between nitrogen efficiency and the effect of inoculation in fertilized plots (Fig. 6).

The effect of nitrogen fertilizer on BGA seems to be inconsistent and is not yet fully understood. After broadcast nitrogen fertilization, nitrogen in the floodwater returns to its original concentration within a few days; therefore BGA inhibition by broadcast nitrogen fertilizer might be mostly indirect. Nitrogen fertilizer favors the growth of unicellular green algae early in the crop cycle. That permits the establishment of grazers that first cause the disappearance of green algae and might further inhibit BGA growth, even when the nitrogen concentration in the floodwater has decreased to a level insufficient to inhibit BGA.

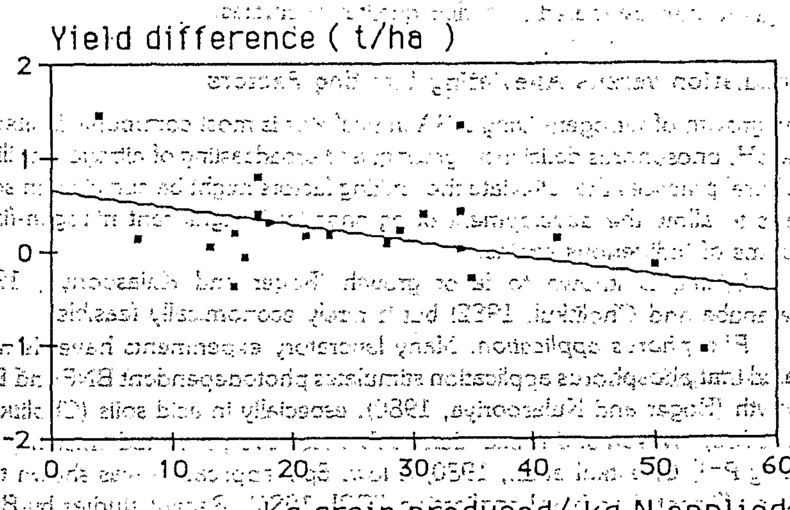

Fig. 6. Correlation between yield change due to inoculation and fertilizer efficiency (25 kg N/ha) in 22 locations. Calculated and drawn from data by Pillai (1980)

RECONSIDERING BGA INOCULATION

Till recently, almost all studies on the agronomic use of BGA have emphasized algal inoculation with foreign strains selected in the laboratory. This arose from the earlier belief that nitrogen-fixing BGA were not normally present in many ricefields. Recent results show that nitrogen-fixing BGA are probably ubiquitous in rice soils and foreign strains inoculated in a soil rarely establish. Therefore, research on BGA inoculation should pay attention to agricultural practices to alleviate factors limiting BGA growth and inoculum quality.

Occurrence of Nitrogen-fixing BGA in Rice Soils

Early qualitative surveys reported a limited occurrence of nitrogen-fixing BGA in rice soils (Venkataraman, 1975; Watanabe, 1959; Watanabe and Yamamoto, 1971). However, quantitative studies during the last decade showed the consistent presence of nitrogen-fixing BGA, frequently at high densities, in soils under rice cultivation (Table 5). In published data (Table 1), the average value is 1.5×10^5 CFU g^{-1} dry soil (median 2.0×10^4). A quantitative study (Roger et al., 1987) of 102 samples of rice soils from the Philippines, India, Malaysia, and Portugal showed that heterocystous BGA occurred at densities ranging from 10^2 to 8×10^6 colony-forming units (CFU) cm^{-2}. The abundance of heterocystous forms showed a positive correlation with pH and available P content of soils.

BGA seem to be either ubiquitous in rice soils or, at least, much more frequent than estimated in earlier qualitative studies.

Inoculation versus Alleviating Limiting Factors

The growth of nitrogen-fixing BGA in ricefields is most commonly limited by low pH, phosphorus deficiency, grazing, and broadcasting of nitrogen fertilizer. Cultural practices that alleviate the limiting factors might be sufficient in some cases to allow the development of agronomically significant nitrogen-fixing blooms of indigenous strains.

Liming is known to favor growth (Roger and Kulasooriya, 1980; Watanabe and Cholitkul, 1982) but is rarely economically feasible.

Phosphorus application. Many laboratory experiments have demonstrated that phosphorus application stimulates photodependent BNF and BGA growth (Roger and Kulasooriya, 1980), especially in acid soils (Cholitkul et al., 1980); Wilson and Alexander, 1979). However, reported efficiency (2-3 g N g P^{-1}) Cholitkul et al., 1980) is low. Split application was shown to be more efficient than basal application (IRRI, 1986). Recent studies by Bisoyi and Singh (1988) provide detailed information on the effect of phosphorus on BGA production. Phosphorus increased both BGA biomass and its nitrogen content. Split application was more efficient than basal application. 17.4 kg P ha^{-1} increased yield (dw after two weeks) by 2.5, 3.5, and 5 times with *Aulosira, Aphanothece,* and *Gloeotrichia,* respectively. Biomass production was maximum at 17.4 kg P/ha and decreased at higher phosphorus

Table 5: Density of N_2-fixing BGA in rice soils (CFU/g dw)[a]

COUNTRY	Samples n ×	Samples % with BGA	Mini	Maxi	Average	Median	Reference
Thailand	100	n.i.[b]	n.i.	n.i.	$8.6\ 10^3$	n.i	Araragi & Tangcham, 1979
Bangladesh	6	100	2×10^3	3×10^4	2×10^4	1×10^4	Bhuiya et al., 1981
Senegal	15	100	8×10^1	2×10^6	5×10^5	8×10^3	Garcia et al., 1973
Iraq	7	100	n.i.	n.i.	1×10^2	n.i.	Hamdi et al., 1978
Philippines	61	100	3×10^2	3×10^6	3×10^5	2×10^5	IRRI, 1985
S.E. Asia	25	100	1×10^3	1×10^7	1×10^6	1×10^5	Kobayashi et al., 1967
Thailand	40	100	1×10^1	1×10^5	n.i.	8×10^3	Matsuguchi and Tangcham, 1974
India	16	100	6×10^3	4×10^6	9×10^5	5×10^5	Roger et al., 1985a,
Philippines	14	100	8×10^3	1×10^6	2×10^5	5×10^4	Roger et al., 1986
Asia	102	100	1×10^2	8×10^6	3×10^5	6×10^4	Roger et al., 1987
India	10	100	2×10^3	2×10^5	8×10^4	7×10^4	Saha and Mandal, 1979
Cambodia	n.i.	n.i	1×10^5	1×10^6	n.i.	n.i.	Suzuki and Kaway, 1971
All data	396		1×10^1	1×10^7	2×10^5	2×10^4	

a : adapted from (Roger, 1989); b: ni = not indicated.

values. Highest yield (900 kg dw/ha or 30 kgN ha^{-1} in 15 days) was obtained with 17.4 kgN ha^{-1} applied in three splits (0, 5, 10 d) using an indigenous inoculum of fresh *Aulosira* (60 kg dw ha^{-1}) containing 2 kg N.

Grazer control. Field measurements of ARA, and BGA and grazer abundance showed that suppressing ostracods with insecticides stimulated BGA growth and BNF (Grant et al., 1983; Grant et al., 1985) even when insecticidal action was limited to a few weeks (Grant et al., 1985). When snails were present, insecticide application was not sufficient to ensure BGA growth. Grazers can be economically controlled by pesticides of plant origin (Grant et al., 1983) and by seasonal drying.

Deep placement of nitrogen fertilizer. The study of different methods of nitrogen fertilizer application on the algal flora and photodependent BNF has shown that surface broadcast application of nitrogen fertilizer, which is widely practiced by farmers, not only inhibits photodependent BNF (Table 1) but also favors the growth of green algae, which increases the pH of the floodwater and causes fertilizer losses by ammonia volatilization (Fillery et al., 1986). Fertilizer deep placement, in contrast, decreases N losses by volatilization and reduces the inhibitory effect of N fertilizer on BGA (Table 1) (Roger et al., 1980).

While the effectiveness of those practices in increasing BGA growth and/or ARA has been established, no experiment has yet quantified the relative contribution of the increased BGA activity and the direct effect of the practice to yield increase, when some was observed.

Why Inoculate

The ubiquity of heterocystous BGA in rice soils does not imply that inoculation is not needed. There seem to be no data to support the claim by Agarwal (Agarwal, 1979) that the introduced BGA can establish themselves almost permanently if inoculation is repeated for three to four cropping seasons.

Inoculation might be useful because the accumulation of phosphorus by the propagules of the BGA inoculum (produced with high levels of phosphorus) gives them an initial advantage over the propagules of the most often phosphorus-deficient indigenous BGA (Roger et al., 1986b). This is likely to be more important in the case of those species that survive dessication as whole filaments, as akinetes (spores) are apparently characterized by a high phosphorus content (Whitton, 1987) and so are unlikely to differ much whatever their source.

Since spore germination is photodependent (Reddy, 1983), inoculated propagules applied on the soil surface might germinate better than indigenous propagules mixed with the soil.

The effect of inoculation is likely to be much more important where there are marked seasonal changes in the use of land, such as when an upland crop is grown before rice or after a long dry fallow. Under such circumstances, the natural BGA population density may be low at the beginning of the subsequent rice season, leading to a lag of several weeks before it makes a significant contribution to nitrogen fixation.

Quality of the Inoculum

Little attention has been paid to inoculum quality. Published methods of inoculum production do not include tests of composition and viability. Recent studies show that the density of colony-forming units of nitrogen-fixing strains in soil-based inocula may vary from 10^3 to 10^7/g dw and comprise an average 13% of the total algae (Roger et al., 1987).

The study of the ratio of indigenous heterocystous BGA in 102 soils to heterocystous BGA contained in 10 kg (recommended dose) of 22 soil-based inocula showed that in 90% of the cases, indigenous BGA were more abundant than BGA in the inoculum. In 59% of the cases, the ratio of indigenous BGA to inoculated BGA was higher than 100 (Roger et al., 1987).

The low content in BGA propagules of the inoculum might be one of the reasons for the failure of inoculation.

Indigenous Strains versus Foreign Strains

Most algalization trials have been carried out using inocula developed from a mixture of laboratory cultures, but almost none of the published inoculation experiments have paid attention to the establishment of inoculated strains.

The first quantitative study to establish the fate of strains subsequent to inoculation is that of Reddy and Roger (1988). In this study, the fate of five laboratory-grown heterocystous strains representing 75% of the inoculum was studied for one month in 1-m^2 plots of five different soils. During the month following inoculation, the inoculated strains multiplied to some extent in all soils, but rarely dominated the indigenous BGA. They did so only when the growth of indigenous nitrogen-fixing species was poor or after the population of indigenous species declined. The soils were dried at the end of the period and then resubmerged together with neem (*Azadirchta indica*) to control grazers. Two of the inoculated strains did not reappear, but one (*Aulosira fertilissima*) developed an agronomically significant bloom on two soils. In all the soils control of grazers by neem combined with phosphorus application permitted the establishment of nitrogen-fixing blooms, but in 23 of the 25 combinations tested, blooms were of the indigenous strains.

In a recent study of the effect of phosphorus on BGA production, Bisoyi and Singh (1988) found no effect of inoculation with foreign strains, whereas inoculation with indigenous strains increased inoculum production by more than 10 times as compared with a noninoculated plot.

A similar observation was made by Reynaud and Metting (1988) who studied the establishment of 23 strains in an irrigated soil and found that the only strain that significantly developed was a local isolate to *Nostoc*.

In fields with a rich natural flora it seems likely that other strains usually will rapidly outgrow populations derived from the original laboratory isolates. Where farmers increase their inocula in shallow trays or ponds, it is probable that strains present in the added local soil may outgrow the original isolates even before inocula are added to the fields.

Available data are not enough for drawing definite conclusions but clearly suggest that the potential of inoculating ricefields with an inoculum produced from the soil to be inoculated should be tested whenever inoculation experiments are conducted.

Dried Inoculum versus Fresh Inoculum

In inoculum production plots, we usually observed easier establishment of the algae when a fresh inoculum was used (unpubl.). Bisoyi and Singh (1988) obtained the highest BGA biomass in an inoculum production plot (900 kg dw ha^{-1} or 30 kgN ha^{-1} in 15 d) with 17.4 kgP ha^{-1} applied in three splits (0, 5, 10 d) using an indigenous inoculum of fresh *Aulosira* (60 kg dw/ha) containing 2 kg nitrogen. Such conditions are quite different from what has been currently recommended for field inoculation. In experiments where inoculum corresponded to 0.126 kg N ha^{-1} (about 4 kg algae dw ha^{-1}), net nitrogen yield ranged from 1.6 to 5.5 kg N ha^{-1} and averaged 3.3 kg N ha^{-1}

Fresh inoculum applied at a high density might be more efficient than a dry soil-based inoculum to promote the growth of an early bloom of nitrogen-fixing BGA which is more efficient to provide nitrogen to the current crop. The feasibility of such a technology has to be studied.

Does the Super-strain Exist?

Since the identification of BGA as Cyanobacteria, significant progress has been made in their genetics, including, from an applied point of view, the transformation of *Anacystis* for herbicide resistance studies (Golden and Haselkorn, 1985). One can speculate on the possibility of selecting or designing more efficient strains for field inoculation.

Efforts have been made to select strains with especially high nitrogen-fixing ability. Screening of a range of strains obtained from enrichment cultures has provided strains that are fast-growing in the laboratory (Antarikanonda and Lorenzen, 1982; Huang, 1983), but there is yet some evidence that such strains will not perform equally well in liquid culture under laboratory conditions and *in situ*. It seems unlikely that an introduced strain will survive long in competition with the natural flora, unless there is a simultaneous change in the environment, which gives it a competitive advantage. In fact, most fast-growing strains, with doubling time of five to 12 hours, belong to the genus *Anabaena*, have short filaments, and do not form mucilaginous colonies (Antarikanonda and Lorenzen, 1982; Huang, 1983). Such strains are very susceptible to grazing. A laboratory screening of 12 strains of six genera and the study of their establishment in the field showed that strains with high specific activity under laboratory conditions did not establish in the field (Huang, 1983).

A further use for selected strains might be the production of inocula resistant to pesticides. It is clear that the growth and activities of BGA are affected adversely by some commonly used pesticides (Padhy, 1985). A strain of *Gloeocapsa* was reported by Singh et al. (1986) to be highly resistant to the herbicides Machete and Basalin, whereas *Nostoc muscorum* was quite sensitive. Repeated laboratory culture with increasing levels of pesticide led to increased resistance of three nitrogen-fixing strains to four fungicides and insecticides (Sharma and Gaur, 1981). Artificially induced mutants resistant to Blitox have been obtained for two *Nostoc* strains and other mutants resistant to Carbaryl, Zineb and Mancozeb have been obtained (Padhy, 1985). Most studies on pesticide tolerance in BGA have been laboratory-based. Data based directly on field observations and assays on strains of particular species taken from sites with a known pesticide history are needed to understand whether or not BGA easily acquire genetic tolerance for pesticides under field conditions.

Biological engineering on BGA is currently limited to unicellular strains that are morphologically, physiologically, and ecologically very different from the nitrogen-fixing strains considered for inoculating ricefields.

Probably super nitrogen-fixing BGA can be selected or designed and grown in test tubes, but the characteristics that will enable them to survive, develop, and fix nitrogen as programmed in ricefields are still largely unknown. The immediate need is for a better understanding to BGA ecology, but on a long term, genetic engineering may also contribute to the agronomic use of BGA.

CONCLUSION

In ricefields, BNF in general and BGA in particular have been the most efficient systems in sustaining production in low-input, nonintensive traditional cultivation. It is possible to influence the amount of nitrogen fixed by BGA in many ricefields. One of the ways is by inoculation, a method which is sometimes effective.

In general, BGA have less potential in terms of nitrogen fixed than legumes or *Azolla* (Roger and Watanabe, 1986) but inoculation, when successful, is a low-cost technology with a cost-to-benefit ratio far more favorable than that of green manure (Venkataraman, 1981b). Numerous experiments indicate that, when successful, BGA inoculation of ricefields may increase grain yield by 300-450 kg ha^{-1} per crop, but its effects seem erratic and limited. Cultural practices to enhance the growth of indigenous or inoculated BGA are known, but their efficiency and economic viability have to be determined.

Because knowledge of factors that allow BGA to establish and bloom in ricefields is limited, BGA inoculation is conducted on a trial-and-error basis. No method for estimating the chance of success of inoculation in a given agroecosystem is available. It may explain why algal inoculation is practiced in only a limited hectarage in some Indian states and possibly in Burma. Recent studies show that: [1] N_2-fixing BGA are present in ricefields at a much higher rate than was previously thought, and [2] foreign strains rarely establish. Identifying and alleviating limiting factors in fields where BGA are present but do not bloom might sometimes be enough to make use of their potential. They are also a prerequisite for establishing inoculated strains.

Many of the studies on algalization have been uncritical. They most often appeared to be concerned only in grain yield and to lack any interest in the microbial and ecological processes that might influence this. The dearth of quantitative studies on the fate of inoculated strains has hindered the evaluation of algalization studies. A prerequisite for any progress in BGA technology is the adoption of experimental design and measurement that allow interpretation of yield data. They include: [1] experiments over several crops using experimental designs that permit statistical analysis, [2] qualitiative and quantitative analysis of BGA populations in the soil and the inoculum, [3] chemical analysis of the soil, [4] record of major climatic parameters, [5] estimation of the algal biomass during the crop and, if possible, its nitrogen-fixing activity, and [6] record of visual observations important for understanding the results [grazer populations, rice pests, incidence of weeds, etc.]

The extent to which algalization will eventually prove worthwhile will depend on local circumstances. The response is likely to be most evident in non-acidic soils with moderate to high phosphorus availability where nitrogen fertilizer is not broadcast. Inoculation is less efficient in the presence of broadcast nitrogen fertilizer, but still may increase rice yield. Agrosystems with a long dry fallow or an upland crop before rice might be especially responsive to inoculation because of a low incidence of grazers and a lower indigenous density of viable propagules of BGA after the dry period.

Available information is not sufficient for a definite conclusion but suggests that research priority should be on:

1] identifying the major characteristics of rice ecosystems responsive to BGA inoculation,

2] comparing the relative efficiency of indigenous strains versus foreign strains, and

3] comparing the relative efficiency of dry inoculum and that of fresh inoculum.

BGA have a potential in low input farming systems where fertilizer is not available or affordable; however, they are unlikely to be an exclusive nitrogen source for producing high yields. Therefore an important aspect of their possible use is integrated nutrient management. A concern in recent high-input, intensive rice cultivation is the sustainability of the high yield and the possible environmental impacts of crop intensification, considering that regardless of the quantity of chemical nitrogen fertilizer applied, rice obtains most of its nitrogen from the soil. Knowledge in this aspect is still limited, but the importance of the photosynthetic aquatic biomass and its BGA component in maintaining the fertility of rice soils under intensive cultivation has been recognized (Golden and Haselkorn, 1985). However, the agronomic potential of BGA is currently underutilized, and benefits from the intentional use of BGA are far less than their spontaneous contribution to the nitrogen fertility of natural and cultivated ecosystems. This is mostly due to a lack of basic knowledge on the conditions that allow their establishment in the field. In-depth agroecological research is required before BGA technology can be substantially improved.

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DISCUSSIONS

Q: *G.B. Manna:* A Suggestion. The priority of research on BGA should go for optimum ecological requirement for desired manifestation irrespectively of native or inoculated BGA.

A: I agree for giving priority to the characterization of environments responsive to BGA management/inoculation. However the estimation of the relative potential of foreign and indigenous strains to establish is very important if the farmer does not produce inoculum in his field but is using an inoculum produced somewhere else or a commercial inoculant.

Q: *R.K. Mishra:* What is the prospective of B.G.A. spraying on rhizosphere? Sen and Mandal in Aulosoria acclaimed good results. What is your view?

A: I suppose you refer to the so called "auxinic effect" of BGA. My views and those of other authors are summarized in the paper. Currently no PGR has been isolated from a BGA and characterized.

Q: *Anonymous:* Do you think the literature survey data you presented is based towards positive responses, because experiments with effect or negative response would not be published.

A: Yes. When all results are negative, authors usually do not published their data. To my knowledge there are only 3 papers reporting no effect of BGA inoculation. But in the literature survey presented in my paper there are some negative results obtained from multilocation trials where both positive and negative results were recorded.

Q: *David Eskew:* Does any one know what is the active bio-chemical principle in nim-cake?

A: The main active ingredient in neem is azadirachtin, which act as an insecticide and antifedant for algal grazers. Several studies on this topic have been publish by Grant and coworkers (see refs. in the paper.)

Q: *Anonymous*: What is the type of statistical analysis technique being contemplated for experimental designs in case of field trials on B.G.A.?

A: A booklet on methods for field studies with BGA is going to be published by IRRI and will include a chapter on statistical analysis and experimental designs.

Q: What are the major characteristics of rice ecosystem planned to be studied for responsive to B.G.A. inoculation?

A: The environmental characteristics of ecosystems responsive to not only BGA inoculation but also to BGA management is certainly a key problem and little progress will be made in BGA utilization as long as we have not answered this question. This requires multilocation trials, proper experimental designs and records of environmental data which have been traditionally overlooked in inoculation experiments.
From empirical observations, I expect neutral to alkaline soils, in areas with high incident light, high temperature, and long dry fallow to be more responsive to BGA management.

Q: *G. Oblisami:* The performance of B.G.A. inoculation is varying in different agro-climatic systems in the state or region. Do you agree to take up indepth studies on the survival mechanism of the introduced B.G.A. inoculum (composite culture) and competitiveness over the native (indigenous) B.G.A. strain?

A: Yes, yes, yes!

Q: *S.K. Roy:* To avoid any further confusion on the prospect of B.G.A. inoculation, there should be detail survey of the native B.G.A. flora as well as the agroecological conditions prevailing in different locations. This will obviously guide us to plan the algalisation programme effectively. The detection of efficient strains in some field should simply be followed by support of nutrients particularly phosphorus and to allow the maximum growth and contribution to the rice and the other subsequent crops.

A: I fully agree with your views. Besides P, which is obviously a key factor, attention has to be paid to grazer control by economically feasible means handle with general pest management. There is a scope for pesticides of plant origin for such a purpose.