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The abundance of heterocystous blue-green algae in rice soils and inocula used for application in rice fields

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Summary. Algal populations were quantified (as colony-forming units [CFU] per square centimetre) in 102 samples of rice soils from the Philippines, India, Malaysia and Portugal, and in 22 samples of soil-based inocula from four countries. Heterocystous blue-green algae (BGA) were present in all samples. *Nostoc* was the dominant genus in most samples, followed by *Anabaena* and *Calothrix*. In soils, heterocystous BGA occurred at densities ranging from 1.0×10^2 to 8.0×10^6 CFU/cm^2 (median 6.4×10^4) and comprised, on average, 9% of the total CFU of algae. Their abundance was positively correlated with the pH and the available P content of the soils. In soil-based inocula, heterocystous BGA occurred at densities ranging from 4.6×10^4 to 2.8×10^7 CFU/g dw (dry weight), comprising only a moderate fraction (average 13%) of the total algae. In most soils, the density of indigenous N_2-fixing BGA was usually higher than that attained by applying recommended rates of soil-based inoculum. Whereas research on the practical utilization of BGA has been mostly directed towards inoculation with foreign strains, our results suggest that attention should also be given to agricultural practices that enhance the growth of indigenous strains already adapted to local environmental conditions.

Key words: Blue-green algae – Cyanobacteria – Rice field – Inoculation – *Nostoc* – *Anabaena* – *Calothrix* – N_2-fixation

Free-living blue-green algae (BGA) constitute a major group of N_2-fixing microorganisms in rice fields and have a potential as biofertilizer in rice cultivation (Venkataraman 1972, 1981; Roger and Kulasooriya 1980). Until recently, research on the practical utilization of BGA in rice fields has focused mainly on inoculation with BGA inocula produced on soil (Venkataraman 1981). Justification for algal inoculation comes from early qualitative surveys, which reported a limited occurrence of N_2-fixing BGA in rice soils (Watanabe 1959; Watanabe and Yamamoto 1971; Venkataraman 1975). However, quantitative studies during the last decade showed the consistent presence of N_2-fixing BGA, frequently at high densities, in soils under rice cultivation (Table 1). This prompted a study of the relative abundance of indigenous and inoculated BGA in inoculated rice soils. Algal populations were analyzed in 102 rice soils and the abundance of N_2-fixing BGA was studied in relation to the major chemical properties of the soils. Counts of N_2-fixing BGA in soils were related to similar counts in 22 samples of soil-based inocula of BGA, and the implications for practical utilization of BGA in rice cultivation were analyzed.

Materials and methods

Soil sampling

Composite samples of wet surface soil from the top 0.5 cm layers of 10 core subsamples were collected with plastic tubes, 10 cm long and 3 cm in diameter. Sampling points were located at 0.5-m intervals along a transect through the field. Dry soil samples, for which core sampling was usually not possible, were collected by delineating areas with the tube and removing the upper 0.5-cm layer of the soil with a knife blade. In some cases, the sampling was done on a dry weight basis by collecting a composite sample of ten subsamples of a few grams each. These were kept in plastic containers and processed within 10 days.

Of the 102 soil samples studied, 64 were collected from five major islands of the Philippines (north, central and south Luzon, Palawan, Samar, Bohol, and Mindanao), 31 came from four states in India (Uttar Pradesh, Karnataka, Tamil Nadu, and Andhra Pradesh), 6 from four states of Malaysia, and 1 from Portugal.

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Table 1. Density of N_2-fixing BGA in rice soils (CFU/g dw)

Country	Samples		Minimum	Maximum	Average	Median	Method	Reference
	No.	% with BGA						
Thailand	100	n.i.	n.i.	n.i.	8.6×10^3	n.i.	MPN	Araragi and Tangcham (1979)
Bangladesh	6	100	2.0×10^3	3.0×10^4	1.6×10^4	1.0×10^4	MPN	Bhuiya et al. (1981)
Senegal	15	100	7.9×10^1	1.6×10^6	5.4×10^5	7.9×10^3	MPN	Garcia et al. (1973)
Iraq	7	100	n.i.	n.i.	9.8×10^1	n.i.	MPN	Hamdi et al. (1978)
Philippines	61	100	3.0×10^2	3.0×10^6	2.7×10^5	1.5×10^5	Plating	Irri (1985)
SE Asia	25	100	1.0×10^3	1.0×10^7	1.0×10^6	1.0×10^5	MPN	Kobayashi et al. (1967)
Thailand	40	100	1.0×10^1	1.0×10^5	n.i.	8.0×10^3	MPN	Matsuguchi et al. (1975)
India	16	100	5.7×10^4	4.4×10^6	9.4×10^5	5.0×10^5	Plating	Roger et al., this paper
India	10	100	2.2×10^3	2.2×10^5	7.8×10^4	7.2×10^4	MPN	Saha and Mandal (1979)
Cambodia	n.i.	n.i.	1.0×10^5	1.0×10^6	n.i.	n.i.	MPN	Suzuki and Kawai (1971)
Pooled data	280		1.0×10^1	1.0×10^7	2.5×10^5	10^4		

Abbreviations: CFU, colony-forming units; n.i., not indicated; MPN, most probable number

BGA inocula

Soil-based inocula. Most samples of multistrain soil-based inocula were provided by scientists involved with algalization studies, while a few were collected by the authors themselves from inoculum production plots in India. Monostrain soil-based inocula were produced in a greenhouse, in 1-m^2 wooden trays covered with polyethylene sheets, a modification of the method described by Venkataraman (1981). Two kilograms of Maahas soil (Aquic Tropudalf, pH 6.8), 50 g superphosphate, and 1.25 g sodium molybdate were mixed together in trays. Demineralized water was added to a level of 4 cm. Fresh algal inoculum (250 g) from laboratory mass cultures in liquid medium was added the next day, when the soil has settled. Two millilitres of Perthane was added to each tray to control algal grazers. Soil-algal mats were collected after 2 weeks' growth, dried in the greenhouse and powdered.

Algal cultures. The strains used for producing monospecific soil-based inocula were grown in 20-l carboys in GO medium (Rippka et al. 1979) without $NaNO_3$. The concentration of Na_2CO_3 was increased ten-fold and continuously bubbled with air enriched with CO_2 to maintain a pH of about 7.0–7.5. Algal material produced under similar conditions was dried at 35 °C in the laboratory, powdered, and used for counts to compare with soil-based inocula.

Algal counts. The total algal flora was evaluated by plating soil suspension dilutions on agarized BG II medium (Stanier et al. 1971) containing mineral N. The same medium minus $NaNO_3$ was used for counting N_2-fixing BGA.

To estimate algal populations in composite soil core samples, the volume of the first soil suspension dilution was adjusted with distilled water to 707 cm^3, a value equal to ten times the surface area of ten core samples in square centimeters (70.7 cm^2), thus providing a 10^{-1} dilution on a surface basis. When sampling was done on a dry weight (dw) basis (for some soil samples and all of the soil-based inocula), the 10^{-1} dilution was prepared by suspending 10 g soil in 90 ml distilled water. Dilutions from 10^{-2} to 10^{-6} were plated using three replicates per dilution. The remaining suspension dilutions were sterilized by autoclaving before being discarded. Before the colonies were counted and identified, petri dishes were incubated for 3 weeks at laboratory temperature (22–30 °C) under a continuous light (about 800 lx) provided by cold white fluorescent lamps. Counts in soils were expressed as number of colony-forming units (CFU) per square centimeter of soil. For soils sampled on a dry weight basis, results were transformed on the basis of 1 g dw, equivalent to 3 cm^2 (average of eight soils). After counting, petri dishes were sterilized before disposal.

The plating method does not distinguish actively growing cells or filaments from spores or propagules dormant in the soil. Also, because of competition between too many colonies, strains present at densities lower than 1% of the total CFU are usually not recorded. The method is, therefore, suitable only for recording the major strains in a soil.

Strains were classified into broad taxa according to morphological features observed directly on the material growing in petri

Table 2. Definition of the taxa of N_2-fixing BGA[a]

Unicellular group	Unicellular strains growing on BG II medium without nitrogen (*Aphanothece, Gloeothece,...*)
Anabaena group:	Heterocystous strains with a thin sheath, without branching, do not form mucilaginous colonies of definite shape (*Anabaena, Nodularia, Cylindrospermum, Anabaenopsis* etc.)
Nostoc group:	Heterocystous strains with a thick sheath, without branching, forming mucilaginous colonies of definite shape (*Nostoc*)
Aulosira group:	Heterocystous strains with a thick sheath, usually without branching, do not form diffuse colonies on agar medium (*Aulosira*)
Scytonema group:	Heterocystous strains with false branching, without polarity, forming velvet-like patches on agar medium (*Scytonema*)
Calothrix group:	Heterocystous strains with false branching, with polarity, forming velvet-like patches on agar medium (*Calothrix, Tolypothrix, Hassalia,...*)
Gloeotrichia group:	Heterocystous strains, with polarity, forming mucilaginous colonies of definite shape (*Gloeotrichia, Rivularia,...*)
Fischerella group:	Heterocystous strains with true branching (*Fischerella, Westiellopsis, Stigonema,...*)

[a] All features refer to strains grown from soil or water sample dilutions plated on agarized BGo II medium without nitrogen

Table 3. Chemical properties and algal populations of the soils

	C (%)	N (%)	C/N	P (ppm)	CEC (meq/100 g)	pH	Tot. (log CFU/cm^2)	Hcys (log CFU/cm^2)
No. of samples	77	78	77	72	59	98	60	102
Minimum	0.2	0.03	6.3	0.0	6.0	3.8	4.00	2.00
Maximum	28.8	2.61	20.0	267	105	8.8	7.73	6.90
Average W	3.19	0.26	10.5	11.8	36.4	6.0	6.01	4.48
D	2.44	0.27	10.8	31.6	37.0	6.8	5.77	5.10
T	2.92	0.26	10.6	19.5	36.6	6.3	5.59	4.75
Median W	1.9	0.19	10.0	8.7	35.3	5.8	6.18	4.63
D	1.5	0.15	10.5	12.0	36.0	7.0	5.73	5.11
T	1.8	0.18	10.1	10.0	36.0	6.4	5.97	4.80
C.V. (%)	162	137	23	195	48	17	14	19
Skewness	+5	+5	+2	+5	+1.3	−0.1	−0.1	−0.5
Kurtosis	+23	+27	+4	+28	+4	−0.7	−0.1	+0.9
Normality	**	**	ns	**	ns	ns	ns	ns

Abbreviations: CEC, cation exchange capacity; Tot., total algal population; Hcys, heterocystous BGA; W, wet soils; D, dry soils; T, all soils; ns, not significant; C.V., coefficient of variation
** Significantly different from normal distribution at $P < 0.01$

dishes (Table 2). In addition to the characters used by Rippka et al. (1979), the ability to form mucilaginous colonies with defined shape, which is associated with resistance to grazing (Grant et al. 1985), was taken as a major character. Taxa with this ability are the unicellular (*Aphanothece, Gloeothece*), *Nostoc,* and *Gloeotrichia* groups (Table 2).

Chemical analysis. Organic carbon, total nitrogen, pH, cation exchange capacity (CEC), and available P (Olsen method) of soils and soil-based inocula were determined by the methods currently used at the IRRI analytical service laboratory (Benckiser et al. 1982).

Statistical analysis. Populations of soil BGA are known to follow distribution patterns that approximate a log normal distribution (Roger and Reynaud 1978). Statistical analysis of the chemical characteristics of the soils showed that C, N, and available P content lacked a normal distribution pattern (Table 3). Therefore Spearman's nonparametric test of rank correlation was used to study the correlation between soil properties and algal populations.

Results and discussion

Representativeness of soil sampling

The 102 soil samples studied correspond to a wide range of chemical properties (Table 3). When compared with 410 soils of tropical Asia (Kawaguchi and Kyuma 1977), their range of pH and average pH value was observed to be very similar but their C and N contents were higher, mainly because a few peat soils were included. After excluding the peat soils, average values for C (1.82%) and N (0.18%) were still slightly higher than those reported by Kawaguchi and Kyuma (1.4% C; 0.13% N).

Data for wet and dry soils (Table 3) have inherent biases. Dry soils, which comprise a large percentage of the samples from India, have a higher average pH (6.8) and available P content (31.6 ppm) than wet soils, which were mostly collected in the Philippines (pH: 6.0; available P: 11.8 ppm). Kawaguchi and Kyuma (1977) also reported higher pH and higher available P content in Indian than in Philippine soils. The very high average available P value observed in dry soils is partly due to the inclusion of three samples from BGA multiplication plots which received a very high dose of P fertilizer. Nevertheless, even when these three values are disregarded, the average available P content still remains higher in dry soils (21.6 ppm) than in wet soils (11.8 ppm), and the values agree with those reported by Kawaguchi and Kyuma for India (21.9 ppm) and the Philippines (13.4 ppm). This indicates that the higher average available P content observed in dry soils is mainly due to their geographical origin.

Occurrence of N_2-fixing BGA in rice soils

Total algal populations ranged from 1.0×10^4 to 5.3×10^7 CFU/cm^2 and averaged 3.5×10^6 CFU/cm^2. N_2-fixing strains were present in all samples studied. Heterocystous BGA comprised, on average, 9% of the total algal population, ranging from 1.0×10^2 to 8.0×10^6 CFU/cm^2 (average 3.2×10^5; median 6.4×10^4) (Fig. 1). Heterocystous BGA occurred at densities higher than 10^3, 10^4, and 10^5 CFU/cm^2 in 95%, 85%, and 45% of the samples, respectively.

Quantitative surveys during the last decade in rice fields in several countries recorded heterocystous BGA at densities ranging from 10 to 10^7 CFU/g dw (Table 1). The average value that we observed was 3.2×10^5 CFU/cm^2, about four times higher than those already published (2.5×10^5 CFU/g dw or 8.3×10^4 CFU/cm^2) (Table 1). This can be explained

Table 4. Occurrence and dominance of major groups of N_2-fixing BGA in the samples

Groups	Average relative occurrence (%)			Percentage of samples where a group was			
	Dry soils	Wet soils	All soils	Dominant	Second dominant	Recorded but not dominant	Recorded (total)
Unicellular	27.4	6.7	18.5	18	13	22	53
Anabaena	10.0	6.6	8.5	5	29	44	78
Nostoc	47.5	80.3	61.6	74	22	3	99
Scytonema	0.5	0.1	0.4	0	1	13	14
Calothrix	8.1	4.9	6.8	3	22	35	60
Gloeotrichia	0.3	0.1	0.3	0	1	21	22
Fischerella	4.3	1.1	2.9	0	11	30	41

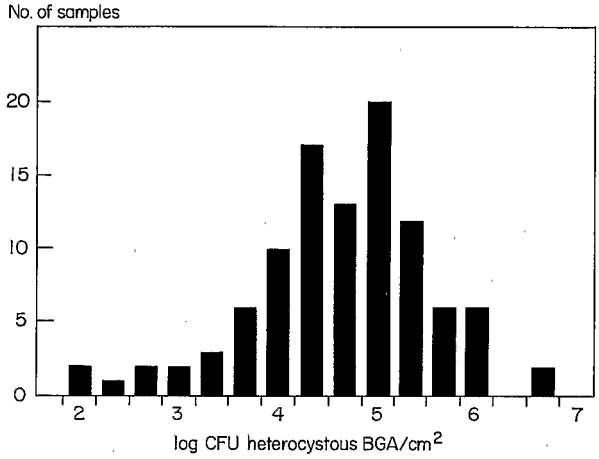

Fig. 1. Histogram of the counts of heterocystous BGA (log CFU/cm^2) in 102 soil samples from rice fields

by the fact that most data recorded in the literature were obtained by the most probable number (MPN) method, which gives lower counts than the plating method utilized in our study (unpublished data). Moreover, those data were for samples from a thicker layer of soil (top 1 – 15 cm) than the one we used (0.5 cm), leading to a dilution of the more abundant algae in the uppermost portion of the soil. The average value of these pooled data (observed and collected) is 1.5×10^5 CFU/g dw, and the median is about 2.0×10^4 CFU/g dw. Present quantitative data show that N_2-fixing BGA are more frequent in rice soils than was estimated in earlier qualitative studies (Watanabe 1959; Watanabe and Yamamoto 1971; Venkataraman 1975).

Dominant N_2-fixing BGA in rice soils

Among the N_2-fixing BGA, the *Nostoc* group was the most frequently recorded (Table 4), comprising, on average, 62% of the CFU, followed by unicellular BGA (18%), *Anabaena* (8%), and *Calothrix* (7%). Other groups accounted for less than 5% of the CFU. The high incidence of *Nostoc* may be partly the result of half the samples being dry soils. As reported by Roger and Reynaud (1976) in Senegal rice fields, desiccation results in some selection of BGA that have the ability to form spores, such as *Nostoc*. In our survey, the incidence of *Nostoc* was also higher in dry soils (80.3%) than in wet soils (47.5%).

Nostoc was recorded in 99% of the samples and was the dominant (highest in relative abundance) N_2-fixing genus in 74% of them. *Anabaena* was recorded in 78% of the samples but was dominant in only 5%. Relatively high levels of occurrence associated with low frequencies of dominance were also observed for *Calothrix* and *Fischerella*. A general trend observed among N_2-fixing BGA is that strains forming mucilaginous colonies (unicellular, *Nostoc*, and *Gloeotrichia* groups) are less susceptible to grazing by invertebrate predators than strains that do not form such colonies (Grant et al. 1985). Mucilaginous strains were dominant in more than 90% of the soils; strains that do not form mucilaginous colonies were present in most soils but were rarely dominant. This may indicate that grazing is a major limiting factor in the development of blooms of non-mucilaginous strains active in N_2-fixation in rice fields. However, more information regarding the selectivity of the plating method is needed before definite conclusions can be drawn.

Among correlations between the relative abundance of the individual groups of heterocystous BGA (as defined in Table 2) and the soil physicochemical properties, only the correlation between pH and the relative abundance of *Nostoc* was statistically significant. However, this same correlation became non-significant when tested separately in wet and dry soils, indicating that a bias in soil sampling associated the higher pH with dry soils where *Nostoc* was more abundant.

Table 5. Correlation between soil properties and algal counts (CFU/g dw) (Spearman's correlation coefficient)

	C	N	C:N	P	CEC	pH	Tot.	Hcy.	Tot./Hcy.
Carbon		++	ns	ns	ns	ns	++	ns	++
Nitrogen			ns	ns	ns	ns	++	ns	+
C:N				−−	ns	ns	ns	−	+
Available P (Olsen)					ns	ns	ns	++	ns
CEC						++	ns	++	ns
pH							ns	++	−−

Abbreviations: Tot., total algal population; Hcy., heterocystous BGA; + and ++, positive correlations significant at the 5% and 1% levels, respectively; − and −−, negative correlations significant at the 5% and 1% levels, respectively; ns, not statistically significant

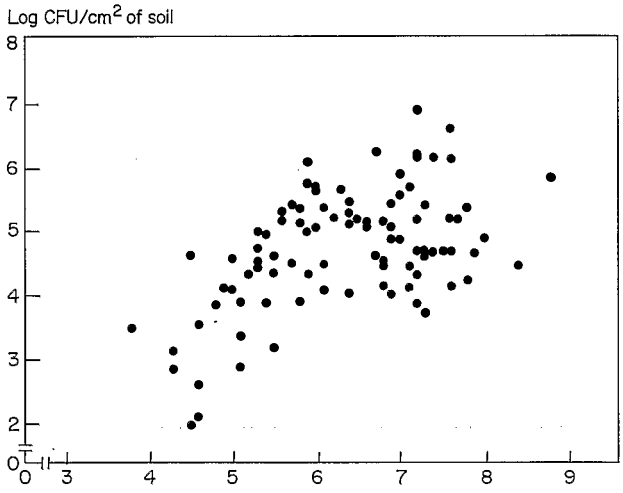

Fig. 2. Abundance of heterocystous BGA (log CFU/cm^2) in soils as a function of soil pH ($r = 0.5$; $P < 0.01$)

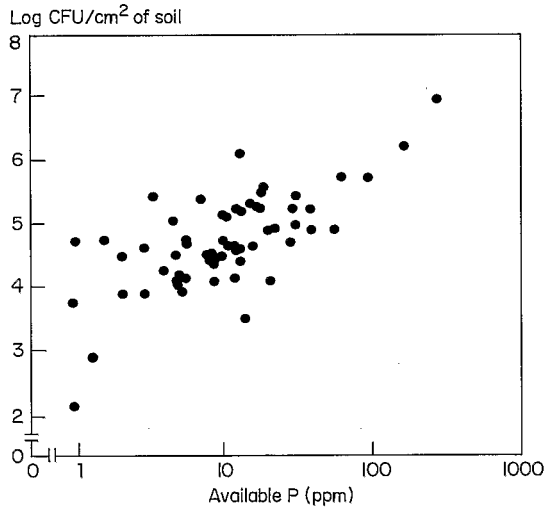

Fig. 3. Abundance of heterocystous BGA (log CFU/cm^2) in soils as a function of soil available P ($r = 0.5$; $P < 0.01$)

Correlation between total abundance of heterocystous BGA and soil properties

Highly significant positive correlations were observed between the abundance of heterocystous BGA and soil pH, as well as available P (Table 5). The correlation with pH was significant in soils having a pH lower than 6.5 but not in those in which pH was higher (Fig. 2). Correlation with available P (Fig. 3) took into account some unusually high values from highly P-fertilized plots, but still remained significant when only values lower than 50 ppm were considered. These two correlations agree with earlier reports (Matsuguchi et al. 1975; Roger and Reynaud 1977) and the general observation that N_2-fixing BGA are usually more abundant in neutral to alkaline soils rich in P (Roger and Kulasooriya 1980).

A highly significant positive correlation was also observed between C content of the soil and (1) total number of algae and (2) the ratio between total algae and heterocystous BGA (Table 5). However, there was no significant correlation between C content and the abundance of heterocystous BGA. A similar trend was observed for N content. These results indicate that soils rich in organic matter have higher total algal populations and lower relative populations of heterocystous BGA.

The negative correlation between C:N and the abundance of heterocystous BGA (Table 5) is in agreement with a positive correlation between C:N and the ratio between total algae and heterocystous BGA. These correlations may be partially explained by the negative correlation between C:N and available P which was observed among the soils studied. Similarly, the positive correlation between CEC and the abundance of heterocystous BGA may be partly due to the highly significant correlation observed between CEC and soil pH.

Analysis of soil-based inocula

Soil-based inocula contained heterocystous BGA at densities ranging from 4.6×10^4 to 2.8×10^7 CFU/g dw (Table 6). Similar average values were observed

Table 6. Analysis of BGA inocula

	Total algae (CFU/g)	Heterocystous BGA		Taxa of heterocystous BGA in multistrain soil-based inocula (% of heterocystous BGA)			
		(CFU/g)	(% of total)	*Anabaena*	*Nostoc*	*Scytonema*	*Calothrix*
Multistrain soil-based inocula[a]							
Max.	8.7×10^7	2.8×10^7	32	45	100	43	26
Min.	2.0×10^6	4.6×10^4	2	0	36	0	0
Mean	2.4×10^7	3.1×10^6	13	16	73	5	6
Monostrain soil-based inocula[b]							
Max.	2.2×10^7	3.5×10^6	16				
Min.	4.0×10^6	2.8×10^5	7				
Mean	1.1×10^7	2.0×10^6	18				
Dried laboratory cultures[c]							
Max.	6.1×10^8	6.1×10^8	100				
Min.	1.0×10^5	1.0×10^5	100				
Mean	1.7×10^8	1.7×10^8	100				

[a] 15 samples produced in outdoor plots (Burma: 2; Egypt: 6; India: 7)
[b] 7 samples produced in a greenhouse at IRRI in microplots inoculated with *Anabaena, Aulosira, Fischerella, Nostoc* (2 strains), *Tolypothrix* (2 strains)
[c] 7 strains (*Anabaena, Aphanothece, Aulosira, Fischerella, Nostoc, Scytonema, Tolypothrix*) grown in BGo 11 liquid medium

for multistrain (3.1×10^6) and monostrain inocula (2.0×10^6). Powdered dried laboratory cultures had an average density more than 100 times as high (1.7×10^8 CFU/g dw).

The relative abundance of N_2-fixing BGA in soil-based inocula ranged from 2 to 32% and averaged 13–18%. It was highest (32%) in a sample composed of algal flakes selectively collected from an inoculum production plot at Aduthurai (Roger et al. 1985). The present results show a low relative abundance of heterocystous BGA in soil-based inocula, which agrees with observations in BGA multiplication plots at Coimbatore that green and non N_2-fixing BGA initially dominated the flora colonizing the plots (Roger et al. 1985). Among N_2-fixing BGA recorded in the multistrain soil-based inocula, the *Nostoc* group was clearly dominant, comprising on average 73% of the CFU. The incidence of *Anabaena*, *Scytonema*, and *Calothrix* groups ranged from 5% to 16%. Other groups were not recorded, which means they were either absent or comprised less than 1% of the total. The two most abundant heterocystous strains in a given inoculum accounted, on average, for 95% of the total counts of heterocystous BGA. These results indicate that multistrain soil-based inocula were rather unbalanced with regard to the relative abundance of the various strains.

Chemical analysis of the samples (Table 7) gave 78%–86% ash, 2.1%–4.7% C, 0.2%–0.8% N, and 640–1900 ppm P. Average contents of N (0.5%), C (3.4%), and ash (80.6%) showed that soil-based inocula contained a high percentage of soil. A comparison of the average density of heterocystous BGA in soil-based inocula (2.5×10^6 CFU/g dw) with that in powdered dried laboratory cultures (1.7×10^8 CFU/g dw) indicates that soil-based inocula may contain about 1% of BGA material. The high P content is obviously due to the high level of P fertilizer applied for inoculum production (500 kg superphosphate/ha in the inoculum production plots in India!; Roger et al. 1985).

Table 7. Composition of soil-based inocula (22 samples)

	Ash (%)	C (%)	N (%)	P (ppm)
Max.	86.6	4.70	0.8	1897
Min.	78.5	2.1	0.2	640
Mean	80.6	3.4	0.5	1385

Ratio between inoculated and indigenous BGA

The average density of heterocystous BGA in soil-based inocula was 2.5×10^6 CFU/g dw. Applying the recommended dose of 10 kg/ha (Venkataraman 1981) of this inoculum introduces 2.5×10^{11} CFU/ha or 2.5×10^3 CFU/cm^2. This is 1/130th the average density of indigenous N_2-fixing BGA (3.2×10^5 CFU/cm^2) in the soils examined. A study of the ratio of indigenous heterocystous BGA in the 102 soils to inoculated heterocystous BGA from 22 soil-based inocula (Table 8) showed that in 89.5% of cases, indigenous BGA were more abundant than BGA introduced in 10 kg algal inoculum. In 46.6% of cases the ratio of indigenous to inoculated heterocystous BGA was higher than 100.

Table 8. Relative and cumulative frequency of the ratio of indigenous to inoculated heterocystous BGA[a]

Range of values	Frequencies	Cumulative frequencies
>10000	4.8%	4.8%
1000–10000	15.7%	20.5%
100–1000	26.1%	46.6%
10–100	22.3%	68.9%
1–10	20.6%	89.5%
<1	10.5%	100.0%

[a] From 2244 ratios (number of indigenous heterocystous BGA in 1 ha of a rice field to the number of heterocystous BGA contained in 10 kg of soil-based inoculum) calculated from enumerations in 102 rice soils and 22 soil-based inocula

Conclusions

Quality of the inoculum

The BGA inoculation method in India uses a multistrain starter inoculum provided to the farmers from inoculum production units (Venkataraman 1981). The starter inoculum is multiplied by the farmer in shallow trays or plots. Under such conditions, the final proportion of individual strains in the algal flakes is unpredictable. It is assumed that the strains best adapted to local conditions will become dominant in the inoculum because it is produced on soil, and under climatic conditions, similar to those in the field (Venkataraman 1981). The starter inoculum must therefore contain a wide range of strains with similar relative abundance. Our study showed that soil-based inocula that are meant to be multistrain are most frequently unbalanced and generally dominated by one or two strains, mostly a *Nostoc*. It seems that a suitable method for producing a multistrain, balanced inoculum of known quality would be to produce monostrain inocula of various strains, evaluate their concentration after drying, and mix them according to CFU content to obtain a balanced mixture.

Need for inoculation

The results show indigenous heterocystous BGA in all rice soils studied quantitatively. In most instances, heterocystous BGA in the recommended dose of a soil-based inoculum are less numerous than indigenous ones on an unit area basis. Nevertheless, inoculation proved successful in soils containing a fairly high level of indigenous heterocystous BGA. This success might be attributed to the possible accumulation of P by propagules of BGA produced in inoculum multiplication plots with high levels of P, as high P content gives the inoculum an initial growth advantage over the propagules of P-deficient indigenous BGA (Roger et al. 1986). Furthermore, because spore germination is photodependent (Reddy 1984), inoculum propagules applied on the soil surface might benefit from more light and germinate better than the indigenous propagules mixed with the soil.

Whereas research on the practical utilization of BGA has been mostly oriented towards inoculation with foreign strains, our results, showing the presence of indigenous strains in all soils, suggest that attention should also be given to agricultural practices that enhance the growth of indigenous strains. Indigenous strains are already adapted to the local environmental conditions and can also be utilized for producing inocula. Practices such as the application of pesticides of plant origin (e.g. neem, *Azadirachta indica*) favor the growth of indigenous and efficient N_2-fixing strains (Grant et al. 1985) which are otherwise limited by grazer pressure.

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