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RECENT STUDIES ON FREE-LIVING BLUE-GREEN ALGAE AND AZOLLA AT THE INTERNATIONAL RICE RESEARCH INSTITUTE

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Summary. Major findings in 1985-86 at the International Rice Research Institute (IRRI) on ecology and practical utilization of free-living and symbiotic blue-green algae (BGA) are summarized. They cover the chemical composition of cultures and natural samples of N_2-fixing BGA from rice fields ; abundance of heterocystous BGA in rice soils and soil-based inocula ; dynamics of algal populations and acetylene reducing activity in soils inoculated with BGA ; soil properties and Azolla growth ; Azolla response to P, K, Zn in various soils in relation to floodwater chemistry ; Azolla insect pests ; and availability of Azolla N to rice.

During the last 10 years, IRRI has initiated a dynamic research program in the field of biofertilizers and biological nitrogen fixation (BNF). The goal is to obtain good rice yields with the lowest possible use of chemical N fertilizer by utilizing N_2-fixing organisms and farm-grown source of nutrients. The objectives are :

- to collect, identify, and conserve N_2-fixing organisms from rice soils ;
- to determine N fixed by these organisms and the factors influencing their activity;
- to develop methods and cultural practices enhancing BNF in rice fields ;
- to establish with scientists from selected countries a cooperative research network on BNF in rice fields ; and
- to train scientists who can strengthen the national capabilities for conducting research on BNF.

Recent reviews by IRRI authors summarize the current status of knowledge and practical utilization of BNF in rice fields (Watanabe and Roger, 1984; Watanabe, 1985; Roger and Watanabe, 1986).

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RESEARCH ON FREE-LIVING BLUE-GREEN ALGAE.

Agronomic interest in BGA started in the 1930s when De attributed the high spontaneous fertility of submerged rice soils to BGA. Since then, BGA has been cited as a "promising potential source of nitrogen for rice", many trials of algal inoculation have been conducted in rice growing countries, and inoculation of rice fields with BGA has been recommended (Venkataraman, 1981). In 1986, BGA is still only "promising", and the only technology proposed to farmers (algalization) is

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conducted on a "trial and error" basis and not utilized to a noticeable extent. Despite 50 years of research on BGA, our knowledge of their practical utilisation has made relatively little progress because of these major reasons :

1. The imbalance between test tube and field studies : Although most research projects on BGA sought the practical utilisation of BGA, ecological and agronomical field studies are rare.

2. The utilization of grain yield as the only criterion in inoculation experiments : Most experiments give no information on the environmental characteristics, the initial level of indigenous N_2-fixing BGA in the soil, the dynamics of the algal flora during the crop cycle, the fate of inoculated BGA, their influence on BNF and other factors involved in the yield increase. Therefore, little is known about the factors that permit the development of a bloom of indigenous or inoculated BGA in a field.

3. The underestimation of the potential of indigenous strains : Till recently, almost all studies have placed emphasis on inoculation with foreign strains selected in the laboratory. Little attention was given to indigenous strains (already adapted to the environmental conditions), agricultural practices that favor their growth, and integrated management.

Therefore, the BGA research program at IRRI has given priority to field studies with emphasis on the occurrence of BGA in rice soils, their composition and biomass, composition of BGA inocula, fate of inoculated BGA, changes in BGA populations and N_2-fixing activity in inoculated soils, and agricultural practices favoring BGA growth. Major results obtained during the last two years are summarized thereafter. Earlier studies had been summarized (Roger and Watanabe,1982)

CHEMICAL COMPOSITION OF CULTURES AND NATURAL SAMPLES OF N_2-FIXING BGA FROM RICE FIELDS (Roger et al 1985, Roger et al 1986)

Seventy samples of N_2-fixing BGA 1) grown in liquid medium in the laboratory, 2) grown on soil in a greenhouse, and 3) collected from rice fields, were studied.

Dry matter content averaged 4 %. A *Fischerella* sp. showed the highest value (13%). The lowest values (< 1 %) were recorded for genera forming mucilaginous colonies (*Gloeotrichia* , *Aphanothece*, *Nostoc*). High variability of dry matter content (CV = 50 to 80%) was due partly to the nature of the strain (mucilaginous BGA have less dry matter than nonmucilaginous ones). However, high variability was also observed between species of the same genus (*Anabaena* : 1.0 to 8.5% ; *Nostoc* : 0.28 to 6.3 %).

Ash content of laboratory cultures averaged 7.4% of the dry weight, that of field samples averaged 45%, ranging from 27 to 71%.

Nitrogen content, on ash-free basis, ranged from 1.9 to 11.8% (average 6%). Intrageneric variability was large(CV = 20 to 42%). N and pigment contents decreased in aging material.

Phosphorus and N contents were positively correlated. At P contents higher than 1% there was no more increase in N content. The optimal value of 1% was attained in laboratory cultures only. Soil-grown BGA and natural samples had concentrations lower than 0.5%, confirming that P availability limits BGA growth in natural environments.

Potassium content of the ash-free algal material collected from the field (average: 0.5%) was similar to that of laboratory cultures, which indicates that K availability probably does not limit BGA growth.

Multivariate analysis showed that physiological state is at least as important as the nature of the strain in explaining the variability of the composition.

The wide range of dry matter (0.9-7 %) and ash (27-71%) contents in field samples shows that the weight of a bloom of BGA gives little information about its agronomic significance. Average and extreme values of dry matter, ash, and N contents of field samples show that N content in 1 ton of fresh N_2-fixing BGA averages 1.1 kg but may vary from 0.3 to 3.8 kg.

Algal biomass in rice fields range from a few kilograms to 58 tons fresh weight/ha or 500 kg dry weight. N_2-fixing algal biomasses range within the same limits (Roger and Kulasooriya, 1980; Roger, 1986). Assuming a maximum biomass of 500 kg dry weight/ha and using average ash and N values obtained for artificial blooms and field samples, the potential contribution of a BGA bloom is 14 kg N/ha.

C:N values of 4.8-13 show better N availability from BGA than from farmyard and green manures. Tirol et al. (1982) showed that N availability from BGA to two successive rice crops was similar to that of ammonium sulfate.

ABUNDANCE OF HETEROCYSTOUS BGA IN RICE SOILS AND SOIL-BASED INOCULA. (Roger et al., in press a,b)

Heterocystous BGA in rice soils. The quantitative study of the algal population in 102 rice soils from 5 countries showed N_2-fixing strains in all samples. Heterocystous BGA comprised, on the average, 9% of the total algal population. Their density ranged from 1×10^2 to 8×10^6 CFU/cm^2 (median 6×10^4), was higher than 10^3 CFU/cm^2 in 95% of the samples, and was positively correlated with soil pH and available P.

Nostoc was recorded in 99% of the samples and was the dominant N_2-fixing genus in 74 % of them. Genera that form mucilaginous colonies (*Nostoc*, *Aphanothece*) and are less susceptible to grazing (Grant *et al*, 1985) were dominant in more than 90% of the soils; strains that do not form mucilaginous colonies (*Anabaena*, *Calothrix* and *Fischerella*) 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 nonmucilaginous strains active in N_2-fixation.

No significant correlation was observed between the relative abundance of the various groups of heterocystous BGA and soil physicochemical properties.

Analysis of soil-based inocula. Algae were enumerated in 22 samples of soil-based inocula (SBI) collected from Burma, Egypt, and India and produced at IRRI. Densities of heterocystous BGA ranged from 5×10^4 to 3×10^7 CFU/g dry weight. Average values in multistrain and monostrain SBI were similar ($2\text{-}3 \times 10^6$ CFU/g dry weight) and about 100 times lower than in dried laboratory cultures (2×10^8 CFU/g dry weight).

The relative abundance of N_2-fixing BGA in SBI was low, ranging from 3 to 32% (average 15 %). Among N_2-fixing strains, *Nostoc* spp. were clearly dominant, comprising an average 73% of the CFU. The two most abundant heterocystous strains in a given inoculum accounted for an average 95% of the total counts of heterocystous BGA, showing that multistrain SBI were rather unbalanced with regard to the relative abundance of the various strains.

Chemical analysis showed 78-86% ash (average 80.6%), 2.1-4.7% C (average 3.4%), 0.2-0.8 % N (average 0.5%), and 640-1900 ppm P, indicating that SBI contained mostly soil and only about 1% of BGA material.

Ratio between inoculated and indigenous BGA. The average density of heterocystous BGA in SBI was 2×10^6 CFU /g dry weight. Applying the recommended dose of 10 kg/ha (Venkataraman ,1981) of this inoculum brings 2×10^{11} CFU/ha or 2×10^3 CFU/cm^2. This is 150 times less than the average density of indigenous N_2-fixing BGA in the soils examined (3×10^5 CFU/cm^2) . The study of the ratio of indigenous heterocystous BGA from 102 soils to inoculated heterocystous BGA from 22 SBI showed that in 90% of the cases, indigenous BGA in the first centimeter of soil of a one-hectare rice field were more abundant than BGA brought by 10 kg of algal inoculum. In about 50% of the cases, the ratio of indigenous heterocystous BGA to inoculated BGA was higher than 100.

Discussion. The study showed that multispecies SBI are unbalanced : one or two strains, mostly the *Nostoc* spp. dominate. A suitable method for producing a balanced multistrain inoculum of known quality would be to produce monospecific inocula of various strains, evaluate their concentration in CFU after drying and mix them accordingly.

All studied rice soils had heterocystous BGA. In most cases, the heterocystous BGA in the quantity of SBI recommended for application were less numerous than indigenous ones on a unit-area basis. Despite that, inoculation was reportedly successful in soils which, according to our results, contained a fairly high level of indigenous heterocystous BGA. Among other possible explanations, such a success might be attributed to the accumulation of P by propagules of BGA inoculum (produced with high levels of P), which gives them an initial advantage for growth over the propagules of most often P-deficient indigenous BGA (Roger *et al*, 1986). Since spore germination is photodependent (Reddy, 1984), inoculated propagules applied on soil surface might have better germination than the indigenous propagules mixed with the soil. However, such hypothesis need to be demonstrated as well as other possible ones not related to BNF.

DYNAMICS OF ALGAL POPULATIONS AND ACETYLENE REDUCING ACTIVITY IN SOILS INOCULATED WITH BGA (Reddy and Roger, in press)

The dynamics of inoculated strains of heterocystous BGA and indigenous algae were studied for one month in 1-m^2 microplots of five soils previously air-dried or oven-dried at 110 ºC. Oven drying was performed to assess whether a reduction of the indigenous algal population favors the establishment of inoculated BGA. Inoculum was a mixture of dry SBI containing nonindigenous *Anabaena variabilis, Tolypothrix tenuis, Aulosira fertilissima, Fischerella* sp., and *Nostoc* sp., spread at 20 kg /ha. The same soils were then dried and resubmerged for two months to study the effect of controlling algal grazers (a major limiting factor for BGA) with neem (*Azadirachta indica*) seeds on the revival and dynamics of indigenous and inoculated algae. ARA was measured and algal populations were enumerated for four weeks as in the first experiment. Then soils were kept flooded for another month and the nature and biomass of algae were then determined.

Heat treatment markedly reduced algal populations. Survival in the soils, ranged from 4 to 17%. Eukaryotic algae and unicellular BGA were the most affected. Heterocystous BGA were more resistant.

Dynamics of algal populations were similar in air-dried and oven-dried treatments of a given soil during the month following inoculation . Heterocystous BGA populations exhibited poor growth in soils richer in organic matter. In other soils, they comprised a significant percentage of the total algal population and became dominant by the 14th day of submersion in two soils. In most cases, indigenous heterocystous BGA were more numerous than inoculated ones. The ratio of CFU of indigenous to inoculated heterocystous BGA ranged from 0.1 to 840 (average 104). Only on 7 out of 40 occasions were inoculated BGA more numerous than indigenous heterocystous BGA. This was observed with no growth or late growth of indigenous heterocystous BGA , or after the decline of a bloom of indigenous heterocystous BGA.

Once the soils were dried, two inoculated strains disappeared. During the first month following rewetting, heterocystous BGA growth was poor in all soils but better in neem-treated plots than in the controls. Indigenous strains were usually more abundant than inoculated ones. Only on 4 out of 40 occasions did inoculated heterocystous BGA become more abundant than the indigenous ones. This was observed only after the decline of the indigenous populations of heterocystous BGA.

Establishment of inoculated algae. Counts of inoculated BGA showed no clear effect of oven-drying of soil or neem application on the establishment of inoculated BGA. But strains markedly differed in ability to persist in the soils. *T. tenuis* was the most abundant inoculated strain in 65% of the cases. Despite a low level of inoculation, *A. fertilissima* developed and persisted during the second experiment. *A. variabilis* and *Nostoc* SL significantly multiplied during the first experiment but disappeared during the second.

Acetylene-reducing activity (ARA) was, on the average, higher in air-dried and in neem-treated plots than in oven-dried and non neem-treated plots. ARA was correlated with the counts of total heterocystous BGA and indigenous heterocystous BGA but not with the counts of inoculated BGA. This indicated that ARA was principally due to indigenous BGA. However, correlation coefficients were generally higher with total heterocystous BGA than with indigenous heterocystous BGA, which may indicate some contribution by inoculated BGA.

BGA biomass after two months of submersion was higher in neem-treated plots. In 35 of 40 cases, indigenous strains of algae became dominant. However, inoculated *A.fertilissima* developed in two neem-treated soils and became dominant in one where it produced a bloom corresponding to 450 kg dry weight/ha (35 kg N/ha), the highest biomass recorded in a plot during this experiment.

Discussion. The longer persistence of some of the strains and the late establishment of blooms of inoculated *A. fertilissima* in plots of two soils treated with neem, where this strain developed the highest BGA biomass in the experiment, might indicate some potential for foreign strain inoculation in rice soils. However establishment of inoculated nonindigenous strains was infrequent in the studied soils. This agrees with the results of Grant et al. (1985), showing the failure of nonindigenous inoculated BGA to establish in the field, even when grazers were controlled. The results also confirmed the beneficial effect of neem application on photodependent BNF reported by the same authors.

RESEARCH ON AZOLLA

Whereas BGA technology is more at an experimental level of large field testing, *Azolla* technology has been used for several centuries in China and Vietnam. In these countries, however, environmental and economical constraints limited *Azolla* use to about 2 x 10^6 ha in 1983 (Roger and Watanabe, 1986). Use of *Azolla* as green manure in China and Vietnam is decreasing, but interest in its use as fish and animal feed, mineral scavenger, and depollutant, has increased (Liuchungchu, 1984). In other parts of Asia, *Azolla* technology is not beyond small-scale trials except in South Cotabato (Philippines). In this area, high available P, 9-11 months of rainy season, well-irrigated rice fields, and the presence of many small ponds allowed wide adoption of *A. pinnata* which was then replaced by a more efficient strain of *A. microphylla* (Watanabe,1984).

Under optimum conditions (22 °C), maximum biomass is 100 kg N /ha for *A. pinnata*, 140 kg N/ha for *A. filiculoides* (Watanabe and Berja, 1983), and 190 kg N/ha for *A. microphylla* (Watanabe, 1986). In INSFFER trials average productivity per *Azolla* crop was 1.5 kg/m^2 (about 30 kg N/ha) before transplanting and 1.1 kg /m^2 after transplanting (Watanabe in press). At IRRI, the maximum biomass was 80 kg N /ha for *A. microphylla* in 28 days (Watanabe, in press). Productivity discrepancy under optimum and field conditions is due to many constraints in the field. Technical constraints in the tropics are as follows :

<u>1) Low temperature requirement.</u> Optimum temperature for most species is below the average temperature in the tropics. Cool weather is a key to successful *Azolla* cultivation in Vietnam and China. High temperature and humidity result in high pests incidence.

<u>2) Need for P fertilizer.</u> P is the most important nutrient (Watanabe et al, 1986)

<u>3) Insect damage.</u> Insect control is an important economic limitation. If more than 200 g carbofuran ai/ha is needed to control insects, benefits are eliminated (Kikuchi et al ,1984).

<u>4) Year-round maintenance of inoculum.</u> Because *Azolla* is multiplied vegetatively, inoculum must be maintained in nurseries all year and multiplied for distribution before field cultivation.

<u>5) Need for good water control.</u>

Research at IRRI has placed emphasis on resistance to adverse temperatures, soil and nutritional requirements, insect pests, and availability of *Azolla* N to rice :

SCREENING FOR TEMPERATURE

Temperature limitations can be reduced by selecting cold or heat-tolerant strains (Watanabe and Berja, 1983). Among strains tested at IRRI, *A. microphylla* #418 was most tolerant of high temperature : 37°C day/29° C night (unpublished).

SOIL PROPERTIES AND AZOLLA GROWTH

Azolla adaptability in soils of the Philippines (Callo et al,1985). A growth test in 972 Philippine sites used local soil in pots without fertilizer addition and 6 *A. pinnata* fronds previously starved by 7 days of growth on tapwater. Doubling time was estimated after 14 days. Soils were classified as highly suitable when

Azolla doubling time was less than 3.5 days ; moderately suitable with a doubling time of 3.5 to 5.0 days; and nonsuitable with a doubling time longer than 5 days. Doubling time was less than 5 days in 40% of the samples growing under full sunlight and 34% of those growing in the shade. It was less than 3.5 days in 13% of the samples in full sunlight and 8% of those growing in the shade. Without P fertilisation, only limited areas have soils suitable for *Azolla* growth.

Soil available P and *Azolla* growth (Watanabe and Ramirez, 1984). The productivity, N content, and P content of *Azolla* grown on 29 soils from 4 Philippine sites were studied with regard to the soil available P and P sorption capacity. Using simultaneously data on available P and P sorption capacity, it was possible to calculate a discriminate function separating soils according to their *Azolla* productivity. Results showed that *A. pinnata* grows satisfactorily without P application in soils with Olsen P values higher than 30 mg/kg and P sorption capacity lower than 1500 mg P_2O_5/100 g. Such conditions are rare in rice soils and P fertilization for *Azolla* growth usually is needed. None of the other analyzed soil properties (total C, total N, and exchangeable K, Ca, and Mg) correlated with *Azolla* productivity.

AZOLLA RESPONSE TO P, K, AND Zn IN VARIOUS SOILS, IN RELATION TO FLOODWATER CHEMISTRY (Ali and Watanabe, 1986)

The supply of P, K, and Zn to *Azolla* from flooded soils and the kinetics of P, K, and Zn in floodwater were studied in pots, using 11 Philippine rice soils, ranging from loamy sand to clay (pH range : 4.7 - 7.7). Treatments were a control (no fertilization), PK, KZn, PZn, and PKZn applied just before *Azolla* inoculation. *Azolla* was harvested 3 weeks after inoculation.

Phosphorus concentration in the floodwater of P fertilized soils decreased rapidly and became similar to that in the control within a week. Phosphate in floodwater 1 week after *Azolla* inoculation was correlated with soil Olsen P (r = 0.83), except for one soil. Phosphate application did not increase P content in *Azolla* at harvest. The K and Zn contents of *Azolla* at harvest increased with K and Zn application. In two soils, *Azolla* biomass was lower in the treatments without K or Zn than in the PKZn treatment, but *Azolla* analysis showed no K or Zn deficiency.

Azolla biomass ranged from 9 to 53 g dry weight/m^2 and N content from 2.1 to 5.2%. *Azolla* N was highly correlated with dry weight (r = 0.953). Dry weight and N were highly correlated with P content (r = 0.63-0.55), but not with K or Zn, indicating that P supply limited *Azolla* growth. The critical level of P in *Azolla* was about 0.15%. Floodwater P was better correlated with *Azolla* P (r = 0.739) one week after *Azolla* inoculation than at 2 or 3 weeks. When floodwater P at 1 week was less than 0.1 ppm, *Azolla* was P deficient. When floodwater P was more than 0.4 ppm, P supply to *Azolla* was sufficient.

PHOSPHATE APPLICATION

Surveys of N and P contents in field-grown *Azolla* showed a threshold value of P deficiency of 0.4% P in *Azolla* (dry weight basis). When *Azolla* P was higher than 0.4%, N content was about 4% dry weight (Watanabe and Ramirez, 1984; Ali and Watanabe, 1986). About 80% of *Azolla* samples collected from fields and ponds in the Philippines had less than 0.4% P and 4% N (unpublished). Application of P can increase *Azolla* growth under economically feasible conditions, especially split application of superphosphate which can increase N gains of *Azolla* by 4.6 g N/g P (Watanabe et al., 1980). Recent data from *A. microphylla* showed 10 g N/g P (unpublished).

Application of P to the inoculum permits its multiplication without P application in the main field until it becomes deficient ($P<0.2\%$). P-enriched inoculum may be used for rapidly producing a large *Azolla* biomass (unpublished).

EFFECT OF N FERTILIZER

In the absence of competing organisms, BNF by *Azolla* is more tolerant of combined N than that by free-living microorganisms. Azolla ARA was reduced by about 50% by 10 mM urea-N or ammonium-N and 25 mM of nitrate-N, whereas a 24-hr exposure to 1 mM ammonium-N completely repressed ARA in free-living BGA (Ito and Watanabe, 1983). *In situ*, however, competing aquatic plants growing at the expense of foodwater N, may hinder *Azolla* growth. In the presence of green algae, 1.4 mM ammonium-N inhibited *A. pinnata* growth by 60% (Watanabe et al., 1977).

The *Azolla* canopy prevents light from penetrating the floodwater, inhibits the growth of other phototrophs, and depresses photodependent CO_2 uptake. Thus, the floodwater pH is lower than in *Azolla*-free conditions. *Azolla* may therefore be expected to reduce N losses by ammonia volatilization.

Using the delta ^{15}N technique and *Lemna minor* as non N_2-fixing control, the contribution of BNF to N of *A. microphylla* grown on two soils was estimated to be 70-100% (Yoneyama et al, in press).

AZOLLA INSECT PESTS (Mochida et al , in press)

Five Diptera (*Chironomus crassiforceps*, *C javanus*, *C kiiensis*, *Polypedilum anticum*, and *P. suturalis)*, one Coleoptera (*Nanophyes* sp.), and four Lepidoptera (*Ephestiopsis vishnu* [webworm] and three species of *Elophila* [caseworms] were recorded as *Azolla* pests in the Philippines. The life cycle of the webworm and three caseworms were studied, and their populations were monitored at IRRI using light traps and counts on *A. microphylla* and *A. pinnata*. At 27 ºC, *Elophila* life cycle is about 31-32 days and *Ephestiopsis*, about 20 days. The standing biomass of *A. microphylla* (IRRI No. 418) averaged 1.0 kg fresh weight/m^2 in insecticide-treated plots and 0.7 kg/m^2 in untreated plots. That of *A. pinnata* (No.

5) averaged 0.7 kg/m^2 in insecticide-treated plots and 0.45 /m^2 in untreated plots. Webworm larval and pupal populations in untreated plots averaged 146/m^2 on *A. microphylla* and 135/m^2 on *A. pinnata*. Caseworm populations on the same strains averaged 57 and 48/m^2, respectively. *Azolla* yield loss due to these insects ranged from 6 to 64 % for *A. microphylla* and from 13 to 57% for *A. pinnata*. Although pesticide application was effective for controlling insect pests, no method is yet economically feasible.

AVAILABILITY OF AZOLLA NITROGEN TO RICE (Ito and Watanabe, 1985)

Decomposition of *Azolla* in water and soil in relation to the availability of *Azolla* N to rice plants was determined using ^{15}N as a tracer. When *Azolla* was placed on the surface of flooded soils, about 66% of its N was lost within 6 weeks. Losses were about 30% when *Azolla* was incorporated. Rice plants grown in a pot absorbed 50% of N from *Azolla* incorporated at transplanting and less than 10% of N from *Azolla* kept on the water surface. In the field, N availability from incorporated *Azolla* was higher than that from *Azolla* placed at the soil surface. However, N availability from incorporated Azolla (12-27%) was much lower than that in a pot. With late application, 78 days after transplanting, *Azolla* contribution to grain N was higher than with application 30 or 53 days after transplanting.

BIOFERTILIZER GERMPLASM

As part of its BNF program, IRRI has collected a large germplasm of N_2-fixing organisms, and currently acts as an international center for *Azolla* germplasm collection and distribution. IRRI maintains the largest collection of *Azolla* (168 strains from 7 species and 4 *Anabaena*-free species) and of blue-green algae (about 200 strains) isolated from rice fields. A large number of N_2-fixing bacteria are also preserved. Hundred of strains of these organisms have been given to laboratories all over the world.

CONCLUSION

Studies on BGA show the presence of indigenous strains in all soils, and the usual poor establishment of inoculated foreign strains. Attention should therefore be given to agricultural practices that enhance the growth of indigenous strains which are already adapted to the environmental conditions and can also be utilized for producing inocula. Studies on Azolla confirm that P is a key factor. Insecticide and P application to an inoculum of a selected strain might be an economical management, permitting Azolla multiplication in the field without further P and insecticide application.

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