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Use of blue-green algae and *Azolla* in rice culture

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Introduction

Blue-green algae (cyanobacteria) are distributed world-wide and contribute to the fertility of many agricultural ecosytems, either as free-living organisms or in symbiotic association with the water-fern *Azolla* (Fay, 1983). The nitrogen-fixing ability of many species is the principal, but by no means the only, reason for this increased fertility. The particular importance of these organisms in rice culture was made clear in the review by Roger and Kulasooriya (1980). This included many reports of the manipulation of rice field ecosystems to maximize blue-green algal nitrogen fixation, especially by the deliberate addition of dried inocula. However, most of these reports lacked detailed documentation of methods and results, and a most recent review (Roger, 1989) takes a cautious view when interpreting the significance of earlier research. The literature on the use of *Azolla* is, however, much more detailed (Lumpkin and Plucknett, 1982; Shi and Hall, 1988).

This chapter examines the possibilities for deliberate modifications of blue-green algal populations in rice fields, with the ultimate aim of increasing rice yield. Topics dealt with in detail by Roger (1989) are treated here only briefly, especially those concerning *Azolla*.

Free-living blue-green algae

Occurrence and agronomic significance

The abundance of blue-green algae in rice fields has been reported in numerous papers since Fritsch's accounts (Fritsch, 1907a,b). Culture studies were introduced by Bannerji (1935) and the importance of blue-green algal nitrogen fixation in helping to maintain fertility of the rice fields was first recognized by De (1939). Many rice fields show visually obvious growths of blue-green algae, although eukaryotic green algae may be more abundant where high quantities of nitrogenous fertilizer have been added. Reports from many countries indicate that the blue-green algal flora is often rich in species (Gupta, 1966; Ali *et al.*, 1978; Saha and Mandal, 1979; Anagnostidis *et al.*, 1981; Al-Mousawi and Whitton, 1983; Kulasooriya and de Silva, 1981). Typically, about half the blue-green algal genera represented are heterocystous (*Anabaena*, *Aulosira*, *Calothrix, Cylindrospermum, Fischerella, Gloeotrichia, Nostoc, Scytonema, Tolypothrix, Wollea*) and thus nitrogen-fixers. A quantitative study (Roger *et al.*, 1987) of 102 samples of rice soils from the Philippines, India, Malaysia and Portugal showed that heterocystous blue-green algae occurred at densities ranging from 10^2 to 8×10^6 colony-forming units (CFU) per cm^2. The abundance of heterocystous forms shows a positive correlation with pH and available phosphorus content of soils. Some communities dominated by non-heterocystous forms also fix nitrogen, though laboratory studies show that associated bacteria are sometimes responsible for the fixation (R.Islam and B.A.Whitton, unpublished).

In contrast to most subsequent studies, Watanabe *et al.* (1951) found only a low number (13) of species of nitrogen-fixing blue-green algae in 643 rice field samples from various parts of Asia. In a subsequent study, Watanabe (1959) reported that there was a complete absence of blue-green algae in many rice field soils, such as the Kanto loams of Japan, which have pH values of 5–6. However, more recent studies have shown the consistent presence of nitrogen-fixing blue-green algae, frequently at high densities, in soils under rice cultivation (Roger *et al.*, 1987). It remains uncertain whether the soils tested by Watanabe (1959) really were atypical, but the culture medium given by Watanabe *et al.* (1951) appears to be unsuitable for the isolation of nitrogen-fixing blue-green algae.

The best known effect of blue-green algal growth on rice increased nitrogen availability resulting from nitrogen fixation but other effects have been reported. They may prevent the growth of weeds (Subrahmanyan *et al.*, 1965) and they add to the soil organic content, aiding particle aggregation (Roychoudhury *et al.*, 1980). Increased availability of phosphorus to rice found by Arora (1969) was explained by the excretion of organic acids by blue-green algae. The presence of blue-green algae in the immediate vicinity of rice seeds can decrease sulphide injury; this can be achieved either by presoaking the seeds in blue-green algal cultures (Jacq and Roger, 1977) or by field inoculation (Aiyer *et al.*; 1971). There have been many claims that soaking seeds or joint growth of seedlings with blue-green algal cultures can benefit rice plants by producing plant growth regulators. Roger and Kulasooriya (1980) listed many physiological and morphological responses of rice plants which have been interpreted by the original authors in this way. However, when 133 cyanobacterial isolates from sites in Africa (not all rice fields) 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. They concluded that presoaking rice seeds in a blue-green algal culture should be done with caution or avoided altogether. No study has been made in which a plant growth regulator has been isolated and characterized from blue-green algal material (Metting and Pyne, 1986).

The extent to which the blue-green algae may contribute to the nitrogen requirements of the rice crop is determined by a number of factors, the most obvious of which are the standing crop, rate of nitrogen fixation per unit area, turnover of the nitrogen fixed and the extent to which any nitrogen released becomes available to the rice plant. The relevant literature, summarized by Roger (1989), indicates that relatively little is known about nitrogen turnover or the extent to which this nitrogen becomes available to the plant. Standing crops of nitrogen-fixing blue-green algae range from a few kg to 0.5 t ha^{-1} dry weight (Roger *et al.*, 1987) and the various lines of evidence indicate a potential of approximately 30 kg N ha^{-1} per crop. Nitrogen released in the later part of the growth period of the rice crop may be too late to influence grain yield, though it may be important for the subsequent agricultural crop. Uptake of ^{15}N by rice from blue-green algae has been the subject of several studies. For instance, in pot and field trials by Tirol *et al.* (1982), 23–28% of the nitrogen fixed reached the first crop; ^{15}N from blue-green algae in a deepwater rice plot has also been shown to reach the rice plant (Watanabe and Ventura, 1982).

Methods of increasing blue-green algal biomass

The fact that different rice fields in the same region and at the same time may have markedly different blue-green algal standing crops suggests that different farming practices may influence their development. For instance, deepwater rice fields at two sites in Bangladesh shortly before the arrival of floodwater showed a marked contrast (B.A.Whitton, unpublished). Bunds (small embankments) forming the margins of fields at one site were continuous and therefore held rainwater, whereas those at another site were broken, permitting drainage from the fields. Fields at the former site had an abundant algal cover, whereas visually obvious growths were rare at the latter site. Such observations raise the possibility of deliberate manipulation of the ecosystem to favour blue-green algae by liming, phosphorus application, surface application of straw and grazer control (Watanabe *et al.*, 1981; Grant *et al.*, 1983). The addition of nitrogen fertilizer has been shown in a number of studies to decrease blue-green algal growth (Roger and Kulasooriya, 1980) or influence species composition, but the results appear somewhat erratic. Possible reasons for this are the frequent simultaneous addition of phosphate and rapid mobilization of the nitrogen in the soil. Deep placement of nitrogen fertilizer reduces its inhibitory effect on blue-green algal nitrogen fixation (Roger *et al.*, 1980).

Floating gelatinous colonies of *Nostoc* are added to some fields in China in much the same way as *Azolla* (see below), with populations allowed to develop in ponds and then released into paddy fields when the rice is planted (T.A.Lumpkin, pers. comm.). This system appears to be only local. Pantastico and Gonzales (1986) reported experimental studies with similar *Nostoc* in the Philippines which led to an increase in grain yield up to 22%. Growth of the blue-green alga was influenced markedly by grazers, but use of pot trials with a mixed system involving *Nostoc*, rice and *Tilapia* overcame this problem (Martinez *et al.*, 1978).

The effects of rice yield of soil inoculation by blue-green algae were first reported by Watanabe *et al.* (1951), with a 25% increase in yield after inoculation of poorly drained paddy with *Tolypothrix tenuis*. Several authors reported increases well over 200% from pot trials in India (Singh, 1961; Sundara Rao *et al.*, 1963). Almost all subsequent studies have indicated much lower increases in the field than pot trials, even where comparative studies have been made by the same researcher (Huang Chi-ying, 1978). Studies on the use of inocula for rice soils have been discontinued in Japan, but subsequently there have been many reports from India and a limited number from other countries. Inocula have mostly been derived from laboratory-grown strains, following the early studies with *T.tenuis* in Japan (Watanabe, 1962, 1973).

The interest generated in India led in 1977 to the All-India Coordinated Project on

Algae, which involves the production and distribution of inocula. Books based on the results give details of practical methods (Venkataraman, 1972, 1981). Inocula are derived from a mix of strains isolated originally from rice-fields 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 blue-green algal mats which develop are allowed to dry and the dried flakes are stored in bags for use at 10 kg ha^{-1} in farmers' fields. Algalization, the term widely used for the addition of such inocula, has received considerable publicity. Some reviews (Agarwal, 1979) have accepted the success of the method in raising grain yield as a well-established fact. Many studies have reported increased grain yield, grain nitrogen content or straw nitrogen content (Venkataraman, 1981; Singh and Singh, 1987), with the effects of blue-green algae being equivalent to the addition of 20−30 kg ha^{-1} nitrogen provided phosphorus fertilizer is added (Sharma and Gupta, 1983). Reports on field experiments available to Roger and Kulasooriya (1980) showed that on average, algal inoculation, where effective, causes about 14% increase in grain yield, corresponding to about 450 kg grains ha^{-1} per crop. However, Roger (1989) concludes that the effects of inoculation of rice-fields by free-living blue-green algae seem often to be erratic and limited. It is difficult to find a clear-cut example which shows the increase to be statistically significant. Firm data also appear to be lacking to support the statement from Venkataraman (1981) and quoted by other authors (Metting, 1988): 'A conservative estimate suggests that about two million hectares under rice are currently covered with algal biofertilizer technology'. Roger *et al.* (1985) reported that algalization was adopted in only two states of India and there the inoculated fields comprise only a few percent of the total area under rice.

A deliberate increase in blue-green algal population density is likely to be much more important where there are marked seasonal changes in use of land, such as when the ground is ploughed many times before planting a winter crop other than rice. Under such circumstances the natural blue-green algal population density may be low at the beginning of the subsequent rice season, leading to a lag of several weeks before it can make a significant contribution to nitrogen fixation. Under some circumstances, inoculation may be more effective if carried out after the rice has been planted. For instance, a multivariate analysis of data from West African sites (Reynaud, 1987) showed that the best time to inoculate is at the beginning of tillering. There are other ways in which algalization may help. For instance, added inocula may have an advantage over the *in-situ* algal population at the start of the rice season because of the use of phosphorus fertilizer during the production of the inoculum. The effect is, however, only likely to be more important if the inoculum is derived from local populations (Bisoyi and Singh, 1989). Cells in the inoculum are probably phosphorus-rich and can therefore divide rapidly and perhaps increase in density by an order of magnitude independently of soil phosphorus. This is likely to be more important in the case of those species which survive desiccation as whole filaments, as akinetes (spores) are apparently not characterized by a high phosphorus content (Whitton, 1987).

Most algalization trials have been carried out using inocula developed from mixes of laboratory isolates. The data appear to be lacking to support the claim by Agarwal (1979) that the (introduced) blue-green algae can establish themselves almost permanently if inoculation is done repeatedly for 3−4 cropping seasons. The only 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 1 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 blue-green algae and did so only when the growth of indigenous nitrogen-fixing species was poor or after population declines of indigenous species. The soils were dried at the end of the period and then resubmerged, together with neem (*Azadirachta indica*), to control grazers. Two of the inoculated strains did not reappear, but one (*Aulosira fertilissima*) developed an agronomically significant population on two soils. In field situations with a rich natural flora, it seems likely that indigenous strains will usually rapidly outgrow populations derived from the original laboratory isolates. Where farmers increase their inocula in the shallow trays, it is probable that strains present in the added local soil may outgrow the original laboratory isolates even before inocula are added to the fields.

Efforts have been made to obtain strains with especially high nitrogen-fixing ability, so that these can be incorporated into the inocula. The approaches used have included screening of a range of strains obtained from enrichment cultures and attempts to obtain mutants in strains already in culture. The former approach has provided strains which are fast-growing in the laboratory (Antarikanonda and Lorenzen, 1982), but there is as yet no evidence that such strains have a particular advantage in the field. Although there are few quantitative data, many rice field blue-green algae probably often double every 1–3 days. It seems unlikely, therefore, 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. It may prove useful to introduce fast-growing strains, if there is a sharp change in fertilizer practice, such as the use of high phosphorus, but no added source of nitrogen at a site which was not previously fertilized.

A further use for selected strains might be the addition of inocula resistant to pesticides. There are marked differences in the relative sensitivity of blue-green algae and weeds to widely used herbicides; the former are sometimes relatively insensitive. Overall, however, it is clear that the growth and activities of blue-green algae are affected adversely by some commonly used pesticides (Singh and Singh, 1983; Padhy, 1985b). A nitrogen-fixing strain of *Gloeocapsa* isolated from a rice-field was reported by Singh *et al.* (1986) to be highly resistant to the herbicides Machete and Basalin, whereas *Nostoc muscorum* from another source was quite sensitive. Repeated laboratory culture with increasing levels of pesticide led to increased resistance of three nitrogen-fixing strains to four fungicide and insecticides (Sharma and Gaur, 1981). Artificially induced mutants resistant to Blitox have been obtained for two *Nostoc* strains, and other blue-green algal mutants have obtained for resistance to Carbaryl, Zineb and Mancozeb (Padhy, 1985a). Most studies on pesticide tolerance in blue-green algae have been laboratory-based and without regard to the source of the strains tested. It would be useful to have more data based directly on field observations and assays on strains of particular species taken from sites with a known pesticide history. This would indicate whether or not genetic tolerance to particular pesticides is acquired by blue-green algae easily under field conditions.

Azolla

Occurrence and agronomic aspects

Abundant growths of *Azolla* not only make a useful addition of combined nitrogen to the ecosystem but can also provide a 'green manure'. In contrast to free-living blue-green algae, however, *Azolla* usually needs to be inoculated and cultivated to develop a significant biomass in rice fields (Roger, 1989). According to Lumpkin and Plucknett (1980, 1982), there is a long history of *Azolla* cultivation in China and Vietnam, records for the latter going back to the 11th century. There was a research thrust in both countries in the 1960s, combined with a considerable expansion in cultivation, and this interest has spread to a number of other countries since the 1970s. The *Azolla* can be intercropped with other plants besides rice, such as *Sesbania* and *Colocasia* (Kannaiyan, 1987), but most interest has been focused on rice. The recent introductions to other countries have usually commenced with trials at research institutes or similar organizations. In the South Cotabato region of the Philippines, *Azolla* was used on 84 000 ha in 1985 (Mabbayad, 1987) and in some other countries, such as India (Kannaiyan, 1987; P.K.Singh, pers. comm.), mixed *Azolla*–rice cultivation also appears to be sufficiently well established in a few regions to exist without the support of agricultural extension workers, but in others such as Brazil (Fiore and Gutbrod, 1987), it is probably still almost entirely dependent on such support.

How important is mixed *Azolla*–rice cultivation on a global scale? Data in the literature for China, by far the most important country for *Azolla*, include markedly different values, but Lumpkin and Plucknett (1982) gave an estimate of 2% of the 34 million ha of rice. The use of *Azolla* in China as a green manure is decreasing (Liu Chung-chu, pers. comm. to P.A.Roger). *Azolla* is also widely used for other purposes, such as feeding pigs, ducks and cattle, in China and some other countries in the region (Moore, 1969; Edwards, 1980) and there is increasing interest in its potential as food for fish (Antoine *et al.*, 1986), with an integrated rice–*Azolla*–fish system developed in China (Liu Chung-chu, 1987). However, it seems probable that *Azolla* is currently used on less than 2% of the global 150 million ha under rice cultivation (Roger, 1989).

The system of utilizing the *Azolla* differs slightly from place to place, but whatever is done is labour-intensive (Lumpkin and Plucknett, 1982). Typically the plant is inoculated into flooded fields before the rice is transplanted, but this may also be done at the same time as transplanting. After about 2 weeks the water may be partially drained away and the *Azolla* heeled or ploughed into the soil. In some cases part of the *Azolla* crop is removed and placed in piles to develop into organic fertilizer, which is subsequently incorporated into the soil. This process may be repeated, but eventually the field is left undisturbed, the rice planted, if not already done, and the *Azolla* grows intermingled with the rice and any weeds. Inocula are developed in ponds, which typically received phosphorus fertilizer, sometimes other nutrients, and usually an insecticide. More fertilizer may be added to the intercrop *Azolla*.

Quantitative studies on *Azolla* biomass and its contribution to the nitrogen economy of rice-fields have been reviewed by Roger and Watanabe (1986) and Watanabe (1987). The reported maximum standing crop of *Azolla* ranged from 0.8 to 5.2 t ha^{-1} and averaged 2.1 t ha^{-1}. International field trials conducted for four years at 37 sites in 10 countries (Watanabe, 1987) showed that incorporation of one crop of *Azolla* grown before or after transplanting is equivalent to a split application of 30 kg fertilizer N; incorporation of two *Azolla* crops grown before or after transplanting is equivalent to split application of 60 kg nitrogen.

Environmental factors and use of Azolla

Successful growth of *Azolla* is more demanding than that for a blue-green algal cover. The environmental factors which restrict the area over which *Azolla* is used are reviewed by Lumpkin (1987). The most obvious factor is water, because *Azolla* plants can survive only a few days on paddy soil that dries during intermittent rains (Lumpkin, 1987). This is in marked contrast to rice field blue-green algae, almost all species of which are tolerant of drying, often without the need for akinete formation (authors' unpublished data). *Azolla* can form sporocarps which survive drying, but the factors which lead to sporulation are not well understood, although it is now possible to produce sporocarps routinely with *A.filiculoides* (Shuying, 1987) and the 'red duckweed' *Azolla* sp. (Xiao Qing-yuan, 1987). Conditions favouring germination of sporocarps are much better known (Xiao Qing-yuan *et al.*, 1987) than are those which lead to their formation.

Azolla has a relatively high requirement for light. When it is grown as an intercrop with rice, its growth will start to be influenced by the rice leaf canopy 2–3 weeks after transplanting and it will stop growth 45 days after transplanting in most *Azolla* species (Lumpkin, 1987). Temperature is an especially important limiting factor. The optimum temperature for most species is within the range 20–35°C (Lumpkin, 1987), though some *Azolla* strains can grow at temperatures of 40°C or more (Watanabe and Berja, 1983). The poor growth of *Azolla* at higher temperatures is at least in part due to increased effects of grazing, parasites and competition with free-living algae. *Azolla* appears to be most successful in the subtropics, with the optimum temperature for most species in the 20–30°C range. Lumpkin (1987) comments that because high temperatures are not a direct limitation, *Azolla* has an excellent potential for successful cultivation in irrigated deserts where humidity is relatively low and alternate host plants for insects are limited; he mentions the northern border of Senegal as a region where it already does well. This suggests its possible use in many countries for which there are at present few or no reports, such as parts of North Africa and the Middle East.

Phosphorus is typically the major limiting element in the field (Roger and Watanabe, 1986; Roger, 1989). As the plant can accumulate about 10 times the amount of phosphorus required to support its normal nitrogen concentration (Lumpkin, 1987), the use of phosphorus fertilizer in ponds used for developing inocula permits the plant to increase in biomass when transferred to paddy fields, even if water in the field is low in phosphorus (Watanabe *et al.*, 1988).

Possible new symbiotic associations

The most direct method to enhance blue-green algal nitrogen fixation in rice fields would be to produce a new symbiotic association combining rice and a suitable nitrogen-fixing strain. Is this approach plausible? Although the *Azolla–Anabaena azollae* association is at present the only symbiotic association important in rice culture, examples of symbiotic associations between blue-green algae and other organisms are widespread in nature. There are records from most phyla, both plant and animal, and more and more examples are being found. The blue-green alga may be intra- or extracellular and in most cases is a nitrogen-fixer. This has led to attempts over the past 20 years to develop new symbiotic associations combining a blue-green alga with a crop plant. Several research groups have succeeded in incorporating a unicellular blue-green alga into higher plant protoplasts, though apparently not yet a nitrogen-fixing strain and not yet in a long-term stable relationship (Gamborg and Bottino, 1981). An attempt to produce tobacco protoplasts incorporating the filamentous nitrogen-fixer, *Anabaena variabilis*, was relatively unsuccessful (Meeks *et al.*, 1978); the protoplasts disintegrated in five days, although some blue-green algal filaments remained intact.

Systems for mixed culture of a blue-green alga and higher plant tissue have been developed by a group at Moscow State University (Gusev *et al.*, 1984), though rice has apparently not been tested. Mixed cultures of tobacco callus and *A. variabilis* were shown (Gusev *et al.*, 1986) to be capable of nitrogenase activity (acetylene reduction assay), and regenerated tobacco plants have been obtained with *A. variabilis* in the intercellular spaces of the primary cortex (Pivovarova *et al.*, 1986). It is not essential for the blue-green alga to act as an autotroph in such relationships, since *Chlorogloea* (*Chlorogloeopsis*) *fritschii* is able to grow in mixed culture in the dark (Gusev *et al.*, 1980). In the naturally occurring symbiotic associations involving *Gunnera* and *Cycas*, the nitrogen-fixing blue-green alga exists entirely or largely in the dark, presumably obtaining all its fixed carbon from the host plant. This indicates an important advantage that a symbiotic blue-green algal—rice system might have over the use of free-living nitrogen-fixers. Fixation would not be limited by the lack of light, as the leaf canopy increased during the growth of the crop but would take photosynthate from the crop.

When considered together, the evidence summarized above suggests that it is quite feasible that some form of artificial symbiotic association will one day be created between a rice plant and a nitrogen-fixing blue-green alga and that the alga need not necessarily be located in the light. Whether or not it proves to have any practical relevance is another matter. In view of the importance of input of combined nitrogen to rice-fields and the fact that blue-green algae are often abundant in this ecosystem, there would seem to have been plenty of opportunity for symbiotic associations to have evolved naturally. The abundance of blue-green algae on aquatic roots of wild deepwater rice in well-illuminated situations (authors' observations) indicate that the close association of rice plants with blue-green algae occurred long before the plant was cultivated. However, the only report of endophytic algal growth is for *Nostoc* and *Calothrix* inside senescent leaf sheaths of cultivated deepwater rice plants (Kulasooriya *et al.*, 1980; Whitton *et al.*, 1989). Even if all the nitrogen fixed by these organisms passed to the rice plant, it would probably supply less than 0.05% of the plant's requirements (B.A.Whitton, unpublished). This suggests that there has been no marked pressure favouring the evolution of a rice—blue-green algal symbiotic association.

Conclusions

We will summarize by stating what we believe is the situation. It should be possible to influence the amount of blue-green algal nitrogen fixation in many rice fields. The response is likely to be most evident in non-acidic soils not treated with nitrogenous fertilizer with moderate to high phosphorus availability. One of the ways of achieving an increase in the blue-green algal population is by addition of inocula, a method which is certainly sometimes effective. However, in contrast to the many excellent studies on *Azolla*, such as those carried out by the Central Rice Research Institute in India, many of the studies on algalization have been uncritical. Further, they have often appeared to be concerned only with grain yield and to lack any interest in the microbial and ecological processes that might influence this. The extent to which algalization will eventually prove worthwhile will depend on local circumstances. In general, free-living blue-green algae have less potential in terms of nitrogen fixed than *Rhizobium*, legumes or *Azolla* (Roger and Watanabe, 1986), but need less input of time and materials.

The dearth of quantitative studies on the fate of introduced inocula of free-living blue-green algae is unfortunate, not only because it has hindered the evaluation of algalization studies, but also because of the current interest in the fate of added microbial inocula to ecosystems associated with the release of genetically engineered organisms. Blue-green algae are potentially one of the most useful groups for such studies, because it is possible to use strains with easily detectable morphological features such as spore coats.

Although the spread of *Azolla* culture resulting from recent research has been quite slow, we suggest that it will eventually spread throughout the subtropics, except for countries like Australia and the USA where labour costs are high. Expansion is likely to be favoured by the breeding of strains which overcome environmental problems, and the increasing use of the plant for purposes other than green manure, such as animal feed. However, the area incorporating *Azolla* cultivation is unlikely much to exceed 2% of the total rice area, unless the costs of nitrogen fertilizers increase markedly or strains can be obtained which thrive under fully tropical conditions.

It seems quite probable that success will one day be achieved in bringing about a symbiotic association between a rice cultivar and a nitrogen-fixing organism and that the latter may be a blue-green alga. However, as suggested above, the question is whether such an association would have much practical relevance for rice culture, although it might have for other crops. If it was adopted, it might still be worthwhile to encourage the growth of nitrogen-fixing blue-green algae or *Azolla* before the rice leaf canopy thickens.

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