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28 Biological nitrogen fixation in wetland rice

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Modern rice varieties have contributed significantly to total food production in developing countries. However, the high yielding varieties require heavy application of nitrogen fertilizer which is an expensive input in rice production. In many developing countries, fertilizer is subsidized in order to encourage its usage together with modern rice varieties, although subsidies put a burden on the national budget. On the other hand, a large number of farmers in Asia do not use or use low levels of nitrogen fertilizer because it is either not available or too expensive. In such a situation, the question arises: how is soil fertility in wetland paddy soils maintained over the years even though a substantial amount of nitrogen is removed by the rice crop, or is lost by leaching, denitrification, and ammonia volatilization? Long term fertility trials with rice in Japan and the Philippines suggest that as much as 50 to 75 kg N per ha. is added every year to wetland rice fields (Greenland and Watanabe, 1982). Some of this nitrogen comes from exogenous sources like rainfall, irrigation water, and atmospheric ammonia but biological nitrogen fixation is recognized to be the major source.

Biological nitrogen fixation refers to the property of a few microorganisms to utilize nitrogen from the air, to incorporate it in their cells and to release it in the soil as organic nitrogen through excretion or decomposition after dying.

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Compared with dryland soils, wetland rice soils have a high N_2 fixing potentiality. This is because the wetland rice field is a complex eco-system comprising water and soil, photic and non-photic zones oxidized and reduced environments. This diversity permits all the major groups of N_2 fixing organisms to grow in wetland rice fields. Nitrogen fixing microorganisms in rice fields comprise:

(a) heterotrophic bacteria associated with the rice plant and free living heterotrophic and phototrophic bacteria in the soil;
(b) free-living phototrophic blue-green algae; and
(c) symbiotic blue-green alga *A. azollae*, associated with *Azolla*.

Experiments have been conducted at IRRI, employing the acetylene-reduction technique, labeled nitrogen technique, and Kjeldahl technique to ascertain nitrogen balance. These experiments suggested that a significant amount of dinitrogen is fixed in rice fields by these N_2 fixing microorganisms and that a part of it is rapidly utilized by the rice plant.

Blue-green algae are photosynthetic organisms which can grow in the presence of water, inorganic salts, CO_2 from the air and sunlight. All heterocystous and few non-heterocystous blue-green algal species possess the capacity to fix atmospheric nitrogen. They are commonly found in the photic zone (floodwater, soil water interface and epiphytically on rice plant and weeds) of most wetland rice fields. Generally their growth in the rice fields is limited by low pH, P deficiency and grazer population (Roger and Kulasooriya, 1980; Grant and Alexander, 1981).

Heterotrophic N_2 fixing bacteria occur in almost all the environments of the rice field, including the submerged parts of the rice plant but are especially active and numerous in the rhizosphere of the rice plants and on the organic debris in the soil. A wide range of nitrogen fixing bacteria occur in rice fields (Watanabe and Furusaka, 1980). Ladha et al. (1982; 1983) and Barraquio et al. (1983) isolated and identified several nitrogen fixing bacteria from rice fields. They found that *Pseudomonas, Azospirillum, Enterobacter* and *Klebsiella* are N_2 fixing bacteria commonly associated with many rice varieties.

The amounts of nitrogen fixed by blue-green algae and bacteria in rice paddy soils and the extent to which nitrogen fixed by them can support rice growth are still, in general, imprecisely known. This is due to methodological problems in measuring nitrogen fixation in the field. The findings summarized by Roger and Kulasooriya (1980) suggest that annual nitrogen fixation by blue-green algae in rice fields may range from 0 to 70 kg/ha. Experiments on algal inoculation of the rice fields showing a beneficial effect on grain yield have been reported in China. Egypt, India, Japan, Philippines and the USSR. However, algal inoculation technology is still at an experimental level in most of the rice growing countries.

In the case of bacterial nitrogen fixation associated with the rice plant and rhizosphere a few studies have been made to quantify the amount of nitrogen fixed. The values vary from 5 to 15 kg N fixed/ha./crop. Several bacterial inoculation trials in grasses and upland crops with *Azospirillum* have produced variable results. In India, Subba Rao (1979) reported positive effects on rice yield after *Azospirillum* inoculation in several sites. However, more systematic and detailed long-term bacterial inoculation studies are needed.

The so-called *Azolla* is in fact a symbiotic association between a water fern and a N_2 fixing blue-green alga. *Azolla* has world-wide distribution and comprises six species: *A. filiculoides, A. microphylla, A. caroliniana, A. mexicana, A. pinnata* and *A. nilotica* (Lumpkin and Plucknett, 1982). The algal symbiont, which is similar in all *Azolla* species, belongs to Nostocaceae and is referred as *Anabaena azollae* (Ladha and Watanabe, 1982). In some rice fields *Azolla* occurs indigenously while in others it can be introduced by inoculation.

Azolla has been used as a green manure in wetland rice culture for centuries in Northern Vietnam and Southeastern China (Lumpkin and Plucknett, 1982). However, the attention of scientists has only recently been drawn towards *Azolla. Azolla* can be grown before and/or after rice transplanting. Under favorable conditions, *Azolla* can accumulate 40-120 kg N/ha. in 30 days. Nitrogen contribution by *Azolla* can be substantially enhanced by growing a second or third crop of *Azolla* after the first crop is incorporated (see Watanabe, 1982). The effect of *Azolla* as green manure was tested in the International Network of Soil Fertility and Fertilization Evaluation for Rice organized by The International Rice Research Institute. In 1979, field experiments were conducted at 12 sites in 5 countries. Positive responses of *Azolla* incorporation over no nitrogen control were obtained in 10 sites, the effect on yield of growing *Azolla* and incorporating it before and after transplanting was equivalent to that obtained from applying 60 kg N/ha. as chemical N fertilizer. When *Azolla* is grown and incorporated 4-6 times inbetween wide rows of rice (53 cm) alternately with narrow rows (13 cm), a grain yield equivalent to that obtained from 70-100 kg N/ha. chemical nitrogen is obtained (Watanabe, 1982).

For the rapid growth of *Azolla* a continuous supply of water soluble phosphorus is required, therefore split application (1-3 kg P_2O_5 ha./4 to 7 days) is more efficient than basal application. *Azolla* damage by insects could be a serious problem in summer. The application of insecticide is recommended to control *Azolla* pests. Kikuchi et al. (1982) made a survey for the economic evaluation of *Azolla* use in rice farming in the Philippines. Their study suggested that in areas where environmental conditions are favorable for *Azolla* growth the economic return from its adoption was more than $35/ha. at 1981 prices; that the economic potential of *Azolla* could be greater in countries where the labor is cheap; and that the possible susceptibilitity of *Azolla* to insects and pest may be a problem for its adoption in some regions.

Legumes green manures, fix nitrogen through symbiotic bacteria (*Rhizobium* spp.). They are certainly the first N_2 fixing system that has been used in rice cultivation. Experiments at IRRI and in India have shown that incorporating one crop of legumes is equivalent to the application of 30 to 80 kg N fertilizers. Despite a high potential, usage of legume green manure has been abandoned for various reasons including the preference for a pulse crop and the additional work in incorporating green manures.

Table 28.1 summarizes the actual status of N_2 fixation in rice cultivation.

Table 28.1 Actual status of N_2 fixation in rice cultivation.

Nitrogen-fixing organisms in rice fields	Potentialities in Kg N/ha.*	Possible use proved	Technology available	Usage by farmers
Legumes	30-80	+	+	+
Azolla	30-60	+	+	+
Blue-green algae	30	+	±	±
N_2 fixing bacteria in soil; N_2 fixing bacteria associated with rice	10-30	+	–	–
Photosynthetic bacteria	?	–	–	–

* These values are tentative estimates.

Conclusion

In modern farming systems the importance of biological nitrogen fixation is often overshadowed by fertilizer N. However, biological nitrogen fixation is vital to subsistence farming, which does not use fertilizer N, and comprises a large part of rice cultivation in Asia. The apparent potential of N_2 fixing organisms as alternative or additional sources of nitrogen for rice are high and exceeds their present utilization. Reasons for underutilization include ecological and socioeconomical limiting factors, and lack of technology development. Research on biological nitrogen fixation is essential because nitrogen fixation is the alternative to chemical N fertilizer, which requires large manufacturing facilities, energy supplies and financial investment. Such facilities and resources will not be available in many developing rice growing countries in the immediate future. Furthermore, rising energy costs and uncertain fuel supplies may make chemical N fertilizer more expensive in future. Biological nitrogen fixation is an important, biologically safe nitrogen source that will retain economic viability in the future.

Discussion following

How does application of nitrogen fertilizer affect growth of nitrogen fixing organisms in the soil?

The combined forms of nitrogen are well-known inhibitors of biological nitrogen fixation. However, the application of nitrogen fertilizer enhances the growth of microorganisms, in general.

How much nitrogen can be contributed through biological nitrogen fixation from *Azolla*?

Under favorable conditions, *Azolla* can accumulate 40-120 kg N/ha. in 30 days. However, this figure can substantially be enhanced by growing a second or third crop of *Azolla* after the first crop is incorporated.

Can legumes be used in rotation with *Azolla*?

I think it can be used. However, to my knowledge there is no published information on the use of legumes in rotation with *Azolla*.

Could you elaborate on the use of *Azolla* as a technique for weed control?

Azolla rapidly forms a cover in transplanted rice fields and thereby limits light availability for the growth of weeds. Dr. Moody and his colleagues have reported a substantial reduction of weed growth in the rice fields.

What is the impact of nitrogen fixation on upland rice?

The contribution of biological nitrogen fixation in upland rice is much less than the wetland rice. However, I must say that not much attention is being given to nitrogen fixation research in upland rice.

On the west coast of India, land is sloping and soils are acidic. We find that *Azolla* and blue green algae grow very well in the laboratory, but not in the farmers' fields. What is the reason for this?

There are several limiting factors associated with the growth of blue-green algae and *Azolla* like acidic pH, phosphorus deficiency, pest problems, water availability, etc. The study of these factors might solve the question of why the *Azolla* and blue-green algae failed to grow.

References

Barraquio, W.L., Ladha, J.K. and Watanabe, I., 'Isolation and identification of nitrogen-fixing *Pseudomonas* associated with wetland rice', in *Canadian Journal of Microbiology* 29:867–873, 1983.

Grant, I.F. and Alexander, M., 'Grazing of blue-green algae (Cyanobacteria) in flooded soils by *Cypris* sp. (Ostracoda)', in *Soil Science Society of America Journal* 45:773–777, 1981.

Greenland, D.J. and Watanabe, I., 'The continuing nitrogen enigma', in *Whither Soil Research*, pp. 123–137, Trans. 12th International Congress Sci. in New Delhi, 1982.

Kikuchi, M., Watanabe, I., and Haws, D.L., 'Economic evaluation of *Azolla* use in rice production', in *Organic matter and rice*, The International Rice Research Institute, Los Baños, Philippines, pp. 569–592, 1983.

Ladha, J.K., Barraquio, W.L. and Watanabe, I., 'Immunological techniques to identify *Azospirillum* associated with wetland rice', in *Can. J. Microbiol.* 28:478–485, 1982.

Ladha, J.K., Barraquio, W.L., and Watanabe, I., 'Isolation and identification of nitrogen-fixing *Enterobacter cloacae* and *Klebsiella planticola* associated with rice plants', in *Canadian Journal of Microbiology*, Vol. 29: 1301–1308, 1983.

Ladha, J.K. and Watanabe, I., 'Antigenic similarity among *Anabaena azollae* separated from different species of *Azolla*', in *Biochem. Biophys. Res. Comm.* 109:675–682, 1982.

Lumpkin, T.A. and Plucknett, D.L., *Azolla as a green manure: use and management in crop production.* Westview Press Inc., Boulder, Colorado, 1982.

Roger, P.A. and Kulasooriya, S.A., *Blue-green algae and rice*, The International Rice Research Institute, Los Baños, Philippines, 1980.

Subba Rao, N.S., *Biofertilizers*, Oxford and IBH Publishers New Delhi, 1979.

Watanabe, I., '*Azolla-Anabaena* symbiosis – its physiology and use in tropical agriculture', in Dommergues, Y.R. and Diem, H.G. (eds),

Microbiology of tropical soils and plant productivity, Martinus Nijhoff/Dr. W. Junk, Publishers, The Hague/Boston/London, 1982.
Watanabe, I. and Furusaka, C., 'Microbial ecology of flooded rice soils', in Alexander, (ed.), *Advances in Microbial Ecology*, Vol. 4, Plenum Publishing Corporation, 1980.