COMMENTARY
by Bernard Dixon

SYMBIOTES FREE MORE NITROGEN FOR RICE

Rice, cultivated in paddy fields as the staple daily food for millions of people in South East Asia, is a remarkable crop. It provides about the same share of the earth's cereal production as do wheat and maize (corn), which are much more familiar to Westerners. Indeed, it exceeds wheat in yield per acre worldwide, and surpasses maize in percentage of total acreage. But rice is not big business. As the *Encyclopaedia Britannica* points out, "the bulk of the crop is produced in relatively small quantities by large numbers of producers who are bound by customs and tradition but are shrewd, hardworking and resistant to change." What the *Britannica* does *not* say is that rice has been grown for centuries without nitrogenous fertilisers.

Look at the surface of the water in the irrigation ditches which criss-cross the paddy fields and you see why. For there you will often notice a greenish "scum"—not unlike that which occurs in neglected swimming pools, and presumably just as much a nuisance. In fact, the scum consists of a blue-green alga, belonging to the genus *Anabaena,* plus a water fern of the genus *Azolla* which also contains algal cells, living as symbiotic partners. Together with rhizobia in aquatic legumes and bacteria associated with rice roots, these two plants fix sufficient atmospheric nitrogen to support the rice crop. Partly a product of organic evolution, partly a result of human ingenuity, the process works well.

But does it work as well as it might? That question is now an urgent one for countries that will have to double their agricultural output, conventional or otherwise, by the end of the century if they are to feed their spiralling populations. Some improvements have, in fact, already been made in boosting a version of nitrogen fixation that was once a matter of chance rather than design. Many farmers in Burma, India, and Egypt now inoculate their rice soils with special strains of blue-green algae. Preparation and distribution of suitable inocula has already developed into village biotechnology, albeit on a small scale. Experiments at the International Rice Research Institute in the Philippines indicate that the *Anabaena–Azolla* partnership can generate from 50 to several hundred kg of nitrogen per hectare per year. The average increase in yield attributable to the symbiosis, compared with uninoculated control crops, is estimated at 16 percent.

The benefits are clearly substantial. But so too are the demerits. *Azolla* requires a high level of phosphorus in the soil. It is far too readily damaged by insects, and prefers low temperatures. Above all, water fern retains most of the nitrogen it fixes from the atmosphere. The vital element is released only after the plant has been ploughed-in following harvesting and its constituent proteins broken down ready for the next crop.

Working in the biological sciences department at the University of Dundee in Scotland, William Stewart and his colleagues see this as a major limitation to man's present exploitation of the fern-alga symbiosis, and are trying to develop an ingenious way around the problem. Funded by Britain's Agricultural and Food Research Council, Professor Stewart's team is seeking ways of persuading *Anabaena* to excrete some of its nitrogenous compounds directly into the environment.

Growing the blue-green alga (immobilised within calcium alginate beads) in an air-lift reactor, the Dundee researchers first confirmed that the partnership would continue to fix nitrogen under laboratory conditions. Then they applied the principle of competitive inhibition in an attempt to thwart the micro-plant in its normal activity of incorporating the nitrogen into cellular proteins. They did so by adding L-methionine-D,L sulphoximine, which blocks the enzyme (glutamine synthetase) that usually mediates the assimilation of ammonium ions. Hoping not to impair protein synthesis permanently, they added the inhibitor to the reactor in pulsed doses at six hourly intervals rather than continuously. The result: *Anabaena* constrained in this way produced satisfactory amounts of ammonium, and did so for up to 1000 hours.

As Professor Stewart and his collaborators realised from the outset, it would be quite impracticable to control such an enzyme inhibitor if it were deployed "in the field." Not only that. The inhibitor would be too expensive. And its use would probably be environmentally unwise. But the Dundee workers' success has encouraged them to pursue a closely related option, by looking for mutants of *Anabaena* which are themselves deficient in glutamine synthetase. They have now found one such strain. Cultured in the air lift reactor, it produces and liberates as much ammonium as does the alga poisoned with enzyme inhibitor. The organism appears to be particularly stable and unlikely to revert readily to its original metabolic type (possibly because the ammonium-excreting property results from a multi-point mutation). Stewart and his team are now trying to maximise the productivity of their organism by optimising pH and other cultural conditions.

In the 1981 report *Agriculture: Towards 2000,* the UN Food and Agriculture Organisation estimated that an additional production of 300 million tons of paddy rice was required between 1974–6 and the end of this century. More recently, the distinguished Indian food scientist M. S. Swaminathan, writing in *Science* (vol 218, p 967), estimated that world demand for rice would grow by 2.9 percent per year throughout the 1980s. So, while other biotechnologists pursue the tantalisingly difficult goal of transferring 17 "nif" genes into plants (and making them work), the Dundee group could be on to something with equally far-reaching implications. But they should not forget what the *Encyclopaedia Britannica* tells us about the shrewd and hardworking people whose labours are responsible for this, the most self-sufficient of the world's great crops.

Bernard Dixon, Ph.D. is a microbiologist and regular columnist for BIO/TECHNOLOGY. He is a former editor of *New Scientist*.