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**Biological Nitrogen Fixation in  
Agriculture and Forestry**

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## BIOLOGICAL NITROGEN FIXATION IN AGRICULTURE AND FORESTRY

Nitrogen though an essential nutrient for plant growth, is often not available in adequate quantities in most ecosystems, as it is easily lost through a number of processes. Frequently, therefore, nitrogen is a nutrient limiting crop production in many farming systems.

The industrial production of nitrogenous fertilizer commonly utilizes the Harber-Bosch process which uses fossil fuel resources as feedstocks to provide energy and reducing power. For developing countries like Sri Lanka which do not have fossil fuel resources, the chances of success of industrial manufacture are somewhat uncertain and this in fact was our own experience. The one and only urea factory in Sri Lanka had to be closed down within the first 10 years of its operation and all our nitrogenous fertilizer requirements are now imported.

On the one hand it is unwise to base crop production entirely on imported resources, in a country whose economy is based on agriculture. On the otherhand, it is a continuous drain on our valuable foreign exchange. An average of 181,000 m.t. of urea and 74,000 m.t. of sulphate of amonia has been imported in 1986. The total cost of fertilizer imports to Sri Lanka in 1987 is reported to be Rs.1,269,882,000/- (about 38.5 million US\$) of which about 60% was on nitrogenous fertilizers. To encourage the use of fertilizer by farmers the Government gave a heavy subsidy on this commodity. This amounted to 1000 million rupees in 1986 but was reduced 700 million in 1987 and 600 million in 1988. On the advice of International Donor Agencies, the Government removed this subsidy altogether this year, as a consequence of which fertilizer prices have increased nearly 5-fold.

Under these circumstances, it has become imperative that we look for alternative methods of sustaining soil fertility under various crops. A very attractive alternative is Biological Nitrogen Fixation (BNF) and today everyone from H.E. the President of Sri Lanka downwards, is anxiously looking forward to the maximum utilization of BNF for

crop production.

BNF is a microbial process and only certain prokaryotic microorganisms are capable of converting the inert dinitrogen into a combined utilisable form. This process is carried out autotrophically by certain photosynthetic microorganisms such as cyanobacteria and photosynthetic bacteria, (the latter only under anaerobic conditions). Heterotrophic bacteria fix  $N_2$  either aerobically (*Azotobacter*), microaerobically (*Klebsiella*) or anaerobically (*Clostridium*). Both autotrophic and heterotrophic organisms form symbiotic associations that fix  $N_2$ . The better known examples are: *Azolla-Anabaena* (photosynthetic); legume-rhizobium, and non-legume - *Frankia* associations. In addition there are a number of bacteria that fix  $N_2$  in association with members of the grass family including major cereals such as wheat, rice, barley & oat. Example of such associative  $N_2$  fixing organisms are species of *Azospirillum*, *Alcaligenes faecalis*, and members of the Enterobacteriaceae. More recently some unique associations have been discovered e.g.  $N_2$  fixing stem nodulated plants like *Sesbania rostrata* with *Azorhizobium caulinodans*; sugar cane and *Acetobacter nitrocapta* which live & fix  $N_2$  inside the vascular system of the plant.

With such diverse organisms and associations fixing  $N_2$ , BNF accounts for 60% of the nitrogen fixed on a global scale and this is nearly 2 1/2 times that of industrial fixation which produces chemical fertilizer. Concerted research efforts should be directed to utilize this natural process for production of food, fuel, fertilizer, fibre and forests, in order to improve the quality of life among mankind.

We should also be conscious of another potential negative aspect of the use chemical N-fertilizer. Most modern high yielding varieties of crops (notably cereals) respond well to chemical fertilizer and produce dramatic results. However, a good part of the chemicals added is not taken up by the plants. Even under the most favourable field conditions provided experimentally at the International Rice Research Institute in the Philippines, it has been reported that fertilizer use efficiency rarely exceeds 50%. Under farmers' field conditions this would be even less. What happens to the rest of the fertilizer added? It is lost either by ammonia volatilization, leaching, percolation, denitrification etc. Chemical

nitrogen thus lost increase the  $\text{NO}_3$  content of water which could be a health hazard.  $\text{N}_2$  lost to the air forms gaseous oxides of nitrogen that affect the ozone layer around the atmosphere. There is thus an inherent danger in the indiscriminate use of chemical N-fertilizer that could result in environmental pollution.

It should not be implied however that we should abandon the use of chemical fertilizer, without which the full yield potential of many modern crop varieties cannot be realised. The most suitable compromise is the judicious combination of chemical and natural fertilizers and it is certainly worthwhile to exploit BNF for this purpose. One should therefore not view BNF as a substitute for chemical fertilizer, but rather as a supplement that could improve fertilizer use efficiency and sustain long term soil fertility in many farming systems.

The paddy rice sector consumes the highest share of N-fertilizers used in Sri Lanka and this accounts for 43% of the total consumption. The flooded field conditions in the rice ecosystem offer an environment congenial for BNF by cyanobacteria (blue-green algae), heterotrophic bacteria, associative bacteria and Azolla. There is thus a tremendous potential to exploit BNF to improve and sustain soil fertility in paddy rice.

Records of N-fixation by cyanobacteria in rice fields range from a few to 85 kg N/ha, but a conservative estimate is 25-30 kg N/ha/season. The Azolla-Anobaena system under favourable conditions could perform better, with an average rate of around 50kg N/ha. Rice root associated bacteria do fix  $\text{N}_2$  but their N-input is generally below 20 kg/ha. However they benefit the rice plants by stimulating root growth by way of producing growth promoting substances. Stem nodulated, flood-tolerant legumes like *S. rostrata* could also be utilized to fertilize rice fields, but their integration into a rice production system has still to be worked out. To obtain an average yield 5t/ha of brown rice, it is necessary to supply 100kg of N. None of the natural systems alone could therefore provide the entire requirement. They could therefore be used either as a supplement to chemical fertilizer or as a substitute to realise a part of the yield potential if that is economically beneficial.

Improvement of N<sub>2</sub>-fixation by cyanobacteria in rice fields had been attempted by inoculation with selected species. This type of algalization does not always succeed because very often the introduced algae fail to colonize the soil due to various limitations. It has often been found that the indigenous algal populations of rice fields are high but they are unable to grow due to certain constraints. Removal of such constraints could stimulate the blooming of natural populations with very desirable results.

A better known area of BNF utilization is the legume-rhizobium system which man has used almost since civilization. A number grain legumes provide nutritive grains to the rural population in developing countries like Sri Lanka. In the poverty alleviation programme of the present government, a major thrust is directed at overcoming malnutrition particularly among the rural children. For this purpose, the extensive cultivation of indigenous grain legumes such as mung bean, cowpea and black gram is encouraged. Very recently a school based programme to popularize Dambala or Winged bean (*Psophocapus tetragonolobus*) as a protein rich food crop was launched by the IFS. All these legumes crops are potentially N<sub>2</sub> fixing plants, but no concerted effort is made in Sri Lanka to use rhizobium inoculation on them. At present it is presumed that these plants form root nodules with a diverse population of indigenous rhizobia belonging to the cowpea group and it is not necessary to inoculate them. This was the situation with soyabean in the early part of this century, where the wild varieties of this crop fixed N<sub>2</sub> in association with non-specific rhizobia. However, years of dedicated research to improve the crop yield together with the perfection of the host-rhizobial symbiosis has resulted in the production of plant types giving very high yields with little or no chemical fertilizer additions, but with inoculation with specific rhizobia. For instance a tropical country like Brazil annually produces 12 million tons of soyabean without the addition of N-fertilizer, thereby saving something equivalent to 5 million tons of N-fertilizer. Shouldn't we try to achieve something like this with our local food legumes?

The use of leguminous cover crops such as *Pueraria phaseoloides*, *Calopogonium* and *Stylosanthus* has been an age old practice in rubber plantations in Sri Lanka. These covers are expected to prevent soil erosion and improve soil fertility through N<sub>2</sub>-fixation.

However, it is only during the past five years that some systematic research has been done on the BNF of these cover crops. Such work has already shown several ways by which natural  $N_2$ -fixation may be improved. In fact the traditional planting pattern of these cover crops permit rather sparse nodulation and it has been shown that this could be increased by changing the planting pattern.

Nearly 10% of the total land area in Sri Lanka is under upland rainfed, farming which has traditionally used shifting cultivation. This production has supplied 40% of the demand for "other food crops" in Sri Lanka. This production is successful only if there is plenty of forest to maintain a safe fallow period of 10 - 15 years between cultivation so as to allow the forest to regenerate and the land regain its natural fertility. This is not the case at present and farmers tend to cultivate land until its productivity declines beyond recovery. Increased population pressure during the past few decades has accelerated the degradation and made shifting cultivation almost impossible to continue. Hence, farmers have become an underprivileged group in terms of their quality of life. Under this situation, the development of sustainable farming systems for rainfed upland is considered a top priority in Sri Lanka. The Department of Agriculture has embarked on a major research programme to develop suitable farming systems using agroforestry techniques to increase the production of this land. The central government had itself taken up the conservation farming recommendations as a policy in developing the rainfed lands given to landless people in the dry zone of Sri Lanka. Although there are various agroforestry techniques practised in the tropics, alley cropping appears to be the most promising alternative for shifting cultivation. In alley cropping, arable crops are grown between hedgerows of planted shrubs and trees, preferably N-fixing leguminous species which are periodically pruned to prevent shading the companion crops. The prunings are added as a mulch and it also acts as a green manure to improve soil fertility. In areas like Kandy where the terrain is not flat, systems like sloping Agricultural Land Technology (SALT) has been recommended as a sustainable farming system. Here again, fast growing,  $N_2$ -fixing trees and shrubs are grown as hedgerows along the contours. These rows are expected to minimise soil erosion by reducing the water flow and to improve soil fertility through BNF. A tremendous amount

of research work is still necessary to really ascertain  $N_2$ -fixation in these systems.

Yet another area that is currently drawing our attention is reforestation. Natural forest ecosystems when undisturbed remain stable with their nutrient cycles more or less in equilibrium. The moment man interferes with them, either to utilise the forest or to clear the land for some other purpose this stability is lost. It takes a long, long time of carefully nurtured conservation to restore a disturbed forest. Even so, the forest may never reached its original grandeur as can be seen with the disturbed dry zone forests which have turned into non-productive scrublands of grasses and weeds. The use of fast growing,  $N_2$ -fixing trees as pioneer members in rapid reforestation schemes has paid rich dividends in temperate regions. While  $N_2$ -fixing tree legumes such as *Acacias* and *Leucaenas* have been used, more success have been achieved by the use of  $N_2$ -fixing non-leguminous tree species such as alder which produce perennial root-nodules with endosymbiotic *Frankia*. Unfortunately such temperate tree species do not grow well under tropical conditions and the only tree of this type with which we are familiar is *Casuarina*, which is also an exotic plant. Another research project to be launched soon by the IFS together with the collaboration of the Catholic University of Louvain, Belgium, is to search for lesser known  $N_2$ -fixing trees in Sri Lanka, including non-legumes. It is hoped that this project would provide us with a few  $N_2$ -fixing indigenous tree species that could turn out to be indispensable for rapid reforestation programme.

While it is pertinent to emphasize research on the practical utilization of BNF, it may perhaps be more rewarding to focus ones attention to basic aspects of  $N_2$ -fixation.

Certain organisms are able to fix  $N_2$  because they possess are enzyme called nitrogenase. Actually it is an enzyme complex composed of two main protein components; the Fe-protein and the Fe-Mo-protein. For this complex to function, energy in the form of ATP (Adenosine tri Phosphate), reducing power and anaerobic conditions are essential. In addition, a ready supply of carbon skeletons is needed to assimilate and remove ammonia, the key intermediate of  $N_2$ -fixation, which would otherwise result in end-product repression. All these functions are under the control of a genetic system that is now fairly well

understood. Studies on such nitrogen-fixing genes or 'nif-genes' have given hopes of genetic engineering that could produce crop plants which may have the ability of supplying their own nitrogen. In fact during the past two decades we have seen the transfer of 'nif-genes' from  $N_2$ -fixing microorganisms, not only to non-fixing other prokaryotic microorganisms, but even to higher plant cells. However, the establishment and sustenance of the  $N_2$ -fixing ability in new organisms is not at all easy and has yet to be achieved.

Another approach in modern biotechnology is to attempt protoplast fusion between a  $N_2$ -fixing bacterial cell and a higher plant cell. This could be a more accessible goal, because certain  $N_2$ -fixing bacteria live within higher plant cells and it is also believed that cellular organelles such as chloroplasts & mitochondria in eukaryotic cells may have originated by the fusion of primitive prokaryotic and eukaryotic cells. One of the research projects in the BNF programme at the IFS is directed towards this objective. If successful, it may result in the production of  $N_2$ -fixing rice plants!

What I have attempted is to make you aware of a natural, biological system which has the unique ability to convert inert dinitrogen to a combined form, under ambient conditions. There are several ways to harness this natural system for the benefit of mankind. With the knowledge so far available it is possible to apply some of these systems to achieve more profitable crop production and forest regeneration. However, research efforts on the more fundamental aspects of BNF may lead to breakthroughs that could revolutionise agriculture.