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BIOLOGICAL NITROGEN FIXATION: RESEARCH NEEDS FOR AGRICULTURAL DEVELOPMENT IN THE TROPICS

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Nitrogen is a constituent of all proteins and is essential for all plant growth. Therefore, it is essential for all protein and carbohydrate production: both are critical in the growing world food shortage. An FAO survey for 1970 indicated that 16% of the world population consumed less protein than the lower dietary limit. In the developing countries, 25% of the population had less than adequate protein with the Far East having almost one-third of the population receiving inadequate protein. Protein shortage has always varied from chronic to acute in the tropics. Protein shortage is always more acute among the poor; the rich can always buy sufficient meat and other protein.

In crop production nitrogen is the most commonly deficient nutrient element on a worldwide basis. Nitrogen for plant growth and protein synthesis comes from fixation of atmospheric nitrogen by the rhizobia in legume root nodules, associated organisms on the roots and leaves of some non-leguminous species and some free-living microorganisms, decomposition of accumulated soil organic matter, rainfall and absorption of gaseous ammonia; animal wastes, and fertilizer.

HARDY and HAVELKA (1975) state: "In the absence of alternative technologies for producing fixed nitrogen, about 200 x 10^6 metric tons of fertilizer nitrogen will be required annually by the end of this quarter century. In 1974, 40 x 10^6 metric tons of fertilizer nitrogen with an approximate value of $8 billion was used, as opposed to 3.5 x 10^6 tons that were used annually 25 years ago." This report is concerned with these alternative technologies.

*The contributions of D.F. Weber, R.W. Weaver, F.G. Viets, H.B. Sprague, H.J. Evans and J.C. Button are gratefully acknowledged.

Alternative technologies are especially important for the developing countries and for the rural poor. Factory fixation of atmospheric N_2 into fertilizers depends on natural gas or other fossil fuels for the energy supply and the hydrogen needed in ammonia production. Petroleum is becoming more scarce and rapidly escalating in cost. So are the capital costs for construction of N-fixation plants in this capital-intensive industry. Many developing countries are so far from fertilizer manufacturing facilities that transportation and storage costs are high. Furthermore, purchase of fertilizer nitrogen involves capital outlay or credit that is not available to many small farmers in developing countries. Efficient use of fertilizer to avoid leaching and provide maximum utilization by the crop involves knowledge often not possessed or available to the small farmer. Fertilizer transport costs are not only very high, but sometimes physically difficult. In countries where the only access to fields is by foot-path, the task of carrying in the needed fertilizer is overwhelming.

Although symbiotic fixation of N_2 occurs in leguminous tropical forests, methods of capitalizing on this natural phenomenon have not been developed. The use of legumes and inoculants to promote biological nitrogen fixation involves technology that many small farmers do not have. There has been a scarcity of basic and adaptive research, of technicians trained in methods of inoculation and culture of legumes, and of dependable inoculants. These aspects of fuller utilization of natural processes in the biological fixation of nitrogen have lagged behind because of a reliance on cheap synthetic nitrogen fertilizers. This reliance now appears to be shifting in favor of agricultural systems for developing countries that will use plants both to fix nitrogen and to yield a greater proportion of the diet in the form of the grain legumes or pulses. Legumes often produce less grain per hectare than cereals, but the high quality protein and oil contribute to good nutrition. In addition to fixing enough nitrogen for the grain (up to 600 kg N/ha/season), the following crop is also provided with nitrogen (up to 100 kg N/ha).

Several recent publications provide comprehensive reviews of the various aspects of biological nitrogen fixation. These include VINCENT (1970), POSTGATE (1972), QUISPEL (1974), HARDY and HAVELKA (1975), NUTMAN (1975) and STEWART (1975). For the purposes of this review, the following principles seem pertinent.

1. The legume-rhizobia system is the only system with solid data to show that the nitrogen fixation system can match the photosynthetic capability. But some legumes are known not to nodulate or fix nitrogen. Nitrogen fixation is dependent on proper selection of both host plant and rhizobia strain.
2. Nitrogen fixation is directly related to dry matter accumulation. If the legume-rhizobium association is established, the best way to increase nitrogen fixation is by increasing growth of the plant by providing adequate nutrients, pest controls, environmental conditions, etc.

3. Biological nitrogen fixation is not free. The required energy is usually supplied by ATP molecules. These ATP molecules carry energy originally trapped by photosynthesis in green plants and therefore represent solar energy. Use of this solar energy can reduce the need for fossil fuels in nitrogen fixation. Although symbiotic nitrogen fixation requires considerable carbohydrate produced by photosynthesis, recent studies show that this energy requirement is no greater than the energy needed for reduction of nitrate absorbed by the roots. Nitrogen absorbed by roots is predominately in the nitrate form even though the fertilizer may have been applied as ammonia, ammonium salts or urea. Biological nitrogen-fixing systems also require space (land or water), time, labor and management (inoculation, nutrients, pest control, tillage, water, *etc.*).
4. Through biological nitrogen fixation the production of proteins can be increased and at the same time, oil and carbohydrates are produced. These materials increase the total calories available for food, feed, fiber and fuel.
5. Legumes are a major part of the native vegetation in the tropics. The tropics are rich in legume species, but only a few have been developed for agricultural use (SPRAGUE, 1975). Many species found in the tropics are not adapted to temperate regions; many species found in the temperate regions are not adapted to tropical regions.
6. Other nitrogen fixing systems, such as the various free-living nitrogen fixing prokaryotes (for example, blue-green algae, *Azotobacter*), root and leaf nodulated plants (for example, *Myrica*, *Azolla*) appear to have limited agricultural potential, but may be useful as green manures.
7. Associations between free-living nitrogen-fixing bacteria and roots of grasses and other plants have been demonstrated in recent research. The association may occur in the root cells and appears to be genetically controlled by the host. The amount of fixed nitrogen contributed to the plant remains to be determined. The photosynthetic capability of grasses exceeds their nitrogen fixation capacity. The practical use (domestication) of grass-bacteria rhizosphere associations is still to be achieved.
8. Nitrogen fixing bacteria have been found to be active in intestinal tracts of animals, including man. The relation to nutritional status of the animals remains to be established, but a possibility exists for a symbiotic association.
9. Combined nitrogen, especially nitrate and ammonium, has been reported to almost completely inhibit biological nitrogen fixation, when present in significant amounts.
10. Transfer of nitrogen fixation genes (NIF) from bacteria possessing them to mutants and closely related bacteria defective in them was reported as early as 1971. Genetic code mapping has progressed but no significant practical transfer has yet been made, although reports of the transfer to species that never possessed NIF genes are increasing.

11. Rhizobia are capable of fixing nitrogen in the presence of root callus tissue of legumes and non-legumes. Rhizobia have produced nitrogenase in the absence of plant cells and in defined media (KURZ and LARUE, 1975; McCOMB *et al.*, 1975; PAGAN *et al.*, 1975; TJEPKEMA and EVANS, 1975).
12. The nitrogenase enzyme system has been isolated from several nitrogen fixing systems. Oxygen destroys its activity. Molybdenum and iron are metals in the enzyme complex. Cobalt is needed as a part of the B_{12} coenzyme that is essential for nucleotide reductase, methyl malonyl mutase and methionine synthesis. Ammonium prevents enzyme formation, but does not stop the activity of enzyme preparations.
13. Nitrogen fixed by combination with other elements by biological systems could reduce the need for fossil fuel.

The agents of biological nitrogen fixation are found exclusively in the non-nucleate protista – the prokaryotes. The nitrogen-fixing systems are both free-living and associated with plants (and animals).

The free-living can be put into six groups:

1. Blue-green algae (*e.g.* 9 genera in the order Nostocales)
2. Facultative aerobes (*e.g. Kelbsiella*, fix N_2 only anaerobically)
3. Obligate anaerobes (*e.g. Clostridium*)
4. Obligate aerobes (*e.g.* the family Azotobacteraceae)
5. Photosynthetic facultative bacteria (*e.g., Rhodospirillum*)
6. Photosynthetic obligate anaerobic bacteria (*e.g., Chromatium*)

The plant associations can be grouped into six systems:

1. Root nodules (legumes + *Rhizobium*; non-legumes + "*Frankia*"?)
2. Algal associations (*Azolla*: water fern + blue-green algae)
3. Rhizosphere (*Paspalum* + Azotobacteraceae)
4. Bacteriorhizal (*Digitaria* + *Spirillum*)
5. Phyllosphere (leaves + Azotobacteraceae)
6. Mycorrhizal (*Podocarpus*?)

Active nitrogen fixing organisms have been reported in the intestines of man and guinea pigs, and in the rumens of sheep, cattle and deer.

Biological nitrogen fixation is best known among the legumes. Legumes are major species in the native tropical plant populations and should have a major role in agriculture in these areas. Much data indicate that the rhizobia in the root nodules have the capability of fixing enough nitrogen to match the photosynthetic capability of the legume. But many legumes are not supplied with the proper nodule bacteria. Optimal culture of legumes is dependent on the association with proper rhizobia if nitrogen fertilizer is to be conserved. Little or no nitrogen fertilizer should be needed for the production of legumes that provide protein and calories needed for human food and livestock feed. Legume genotypes that nodulate promiscuously and fix nitrogen efficiently are desired. Rhizobia that are highly effective on a wide spectrum are desired. To achieve these goals adequate supplies of the seeds of legumes and the cultures of rhizobia must be available and be *used properly* by the grower.

Legume genotypes are being developed in many places including the International Centers, but there is no comparable effort to develop rhizobial cultures for food and forage legumes. We suggest top priority for development of a laboratory at the USDA Beltsville Agricultural Research Center[1] as a center for maintenance and evaluation of a world collection of rhizobial strains for all legumes. Satellite laboratories working cooperatively with, but not administratively linked with the national laboratory should be developed. Because of the lack of information and the need for work on tropical legumes, we suggest top priority for a satellite laboratory that would concentrate on tropical legumes and their rhizobia. In the U.S.A., this laboratory is in Hawaii.[2] At least one other satellite laboratory might concentrate on the rhizobia of the temperate zone legumes. These laboratories would acquire and maintain a wide variety of rhizobial germ plasm by exchange and isolation. Improved strains would be sought. The *Rhizobium* accessions would be screened and evaluated by appropriate laboratory, green-house and field techniques. The data on each strain will be readily available either directly or through the *World Directory*.[3] Exchange and distribution of cultures to properly qualified persons should be encouraged. Evaluation of rhizobial cultures, inoculant materials and inoculated seeds is critically needed.

A major need in biological nitrogen fixation is the training of people to know legumes, to handle rhizobia and other nitrogen fixing microorganisms, to prepare inoculants, to carry out proper inoculation, and to obtain systems that can fix optimum amounts of nitrogen. Limitations of nutrient deficiencies, pathogens, parasites and unfavorable environmental conditions must be recognized and ways of correcting such limitations known. Top priority should be given to development of a short course of about 3 months duration to provide this training for persons with adequate educational background. The training staff must be experienced in the practical aspects of legume and rhizobia growth and culture with field experience an essential requisite. Facilities desired include laboratory and growth chambers, language translation opportunities, legume garden, field plot area for group experiments, pilot plant for legume inoculant production, adequate housing and food for groups at a reasonable cost. Other training opportunities include promotion of conferences in geographic regions, sponsorship of graduate student training in appropriate research projects, consultant travel and other people-to-people contacts. Cooperation with the International Centers and United Nations is sought actively.

Another top priority area is research to remove limitations on the biological nitrogen fixation by the legume-rhizobia system. When large amounts (more than 5–10 kg N/ha) of nitrogen are used on crops such as ground-

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nuts and beans (*Phaseolus*), as is reported in Kenya and Michigan, studies are needed to determine the factors limiting biological nitrogen fixation,

Inoculation materials and methods are a major limitation due to low viability or unreliable and inconvenient methodology. Serious efforts to develop delivery systems that can provide the farmers with high potency, reliable inocula is critical to legume development, and may determine success (or failure) in introducing legumes into new lands. Survival and persistence of the rhizobia in the soil will influence the results with inoculation.

Mineral nutrition of legumes affects biological nitrogen fixation directly and indirectly. Molybdenum and iron are components of the nitrogenase enzyme. Any element that increases legume plant growth tends to increase nitrogen fixation while toxic concentrations of elements (*e.g.* aluminium) decrease nitrogen fixation. Both the rhizobia and the legume hosts have genetic variability with regard to nutrient deficiencies and toxicities. Studies are needed to select tolerant strains of rhizobia.

With regard to the immediate needs of developing countries, lower priority is given to improvement in our knowledge and understanding of the mechanisms of infection, host-rhizobia specificity, nodule formation and senescence, enzyme chemistry and kinetics, *Rhizobium* genetics, and other basic studies. Appropriately, some of these studies are being supported by other agencies (*e.g.* National Science Foundation, U.S. Department of Agriculture); the utilization of such information is unlikely to be achieved in the next few years.

Studies on the contribution of legumes and nitrogen fixation in cropping systems, including rotations, mixed cropping, multiple cropping and intercropping are needed. Because such studies tend to be site-specific, it is recommended that this area of study be encouraged at the International Centers and in institutions in various countries, especially the developing countries.

Several unexplained accumulations of nitrogen under permanent grass pastures have been reported. The reported accumulations range from less than 20 kg N/ha/year to more than 100 kg N/ha/year. Legumes were not observed to be present in the pastures. Yet, grasses in general responded dramatically to fertilization with nitrogen and no grass has been reported to fix all the nitrogen that it required for most efficient production of dry matter (VICENTE-CHANDLER *et al.* 1974).

Bacteria capable of fixing nitrogen have been isolated from the surface of leaves, stems and roots of many agronomically important grasses, *e.g.* corn, wheat, rice, sugar cane. Unfortunately, direct measurements on the rates of nitrogen being fixed are disappointing. Generally, less than 10 kg/ha have been calculated to be fixed by these bacteria that are loosely associated with plants. The bacteria are dependent on the plant for energy material in the form of leaf, stem, or root exudates needed for nitrogen fixation.

Plants have not been known to supply enough exudates to provide the bacteria with the energy required in fixing all the nitrogen needed for plant growth. Calculations show that in most situations less than 0.5% of the carbon fixed by plants may be exuded. An exception to this has been a

report that barley may exude up to 3.7% of the total plant dry weight under certain growth conditions. But even with this high rate of exudation, assuming the normal 1% efficiency of the free living bacteria in converting energy from substrate carbon into fixed nitrogen, and that all the exudates would be used by nitrogen fixing organisms, only a few kg of N per ha could be fixed. The potential for getting significant quantities of nitrogen fixed by bacteria loosely associated with plants seems very low.

Recently, two spectacular discoveries have been reported by Dr. J. Dobereiner that provide new hope for obtaining bacteria-grass associations that fix substantial quantities of nitrogen. One discovery was that *Azotobacter paspali* forms a very close association with roots of *Paspalum notatum* (DOBEREINER *et al.* 1973). The azotobacter cells were located beneath a mucilaginous sheath on the root where it would presumably have a distinct competitive advantage in obtaining root exudates. Some rather broad extrapolations indicated that more than 100 kg/ha of nitrogen may be fixed by this association.

The second discovery was that a nitrogen fixing bacterium *Spirillum lipoferum* had infected some stem and root cells of *Digitaria decumbens*, var. *transvaala* (DAY *et al.* 1975); this organism was found to be highly efficient in fixing nitrogen under certain conditions in pure culture. Nitrogen fixation is very low when the temperature is below 28°C. Further investigations on nitrogen fixation by the *Azotobacter paspali-Paspalum notatum* and *Spirillum lipoferum-Digitaria decumbens* systems may yield significant practical returns from relatively short term efforts. But neither of these systems has been adequately defined. Research is needed to determine how the N_2-fixing microorganisms can be established in the grass roots. Proper experiments in the field are necessary to determine the amount of nitrogen contributed to the grass plant, using comparisons with added fertilizer nitrogen. After successful nitrogen-fixing associations have been established, the transport of photosynthetic to nitrogen fixation sites needs to be investigated. Additional studies to find other nitrogen-fixing systems in grasses that are known to be accumulating nitrogen may provide an additional potential for increasing biological nitrogen fixation.

The most immediate and likely return from nitrogen fixation work is with the tropical grasses. The evidence available indicates nitrogen accumulations in pastures have occurred. Consistent additions of nitrogen for the cereal crops, except rice, have not been reported. Nitrogen fixation research on grain grasses offers little immediate potential of return. However, long term research in this area has potential for tremendous payoff, even though it is high risk.

Although nitrogen fixation is known by blue-green algae, free-living bacteria and some root nodulated non-legumes, the contribution of these systems to food production has usually been indirect. They may be used as green manures or manures, but harvesting costs and competition for nutrients and light have often limited their potential. In crops grown on soils submerged in water, *e.g.* rice or cranberries, the blue-green algae have been

shown to contribute nitrogen, but the use of relatively costly fertilizer nitrogen has often increased economic return. Further studies on these systems appear to have limited potential for increasing food production, but they may provide some additional nitrogen materials for use as manures in a labor intensive agriculture. These studies would also contribute to our knowledge of the total nitrogen economy of our environment.

Other nitrogen fixing systems may contribute appreciable amounts of nitrogen, but research on these systems would get lower priority, due to limited application or to low probability of utilization. Some of these systems may be valuable in localized situations. These include the leaf nodule and root nodule nitrogen fixing non-agricultural plants, composting of waste materials, culturing of *Azolla* or blue-green algae, inoculation with *Azotobacter* or *Clostridium*, and establishment of nitrogen fixing bacteria in animal digestive systems

In suggesting priorities in developing a program in biological nitrogen fixation, the committee used the following criteria:

1. The urgent need for food with high quality protein as well as adequate calories and protein. Countries in the tropics and subtropics have the greatest needs.
   a. Highest priority on projects with potential for payoff in the very near future by increased protein and food production, *e.g.*, legume inoculation
   b. Productive capacity and food preference for cereals and grasses promote consideration of nitrogen fixing systems with these plants, even if the risk of failure is very high.
2. The small farmer in developing countries should be provided as quickly as possible with the best available technology to use biological nitrogen fixation.
   a. Transfer and use of the best currently available technology to farmers was a top priority consideration.
   b. Additional training, research and ways of utilizing technology in biological nitrogen fixation is needed urgently, especially in LDC's.
3. The extremely limited resource base of both knowledge and personnel in biological nitrogen fixation in the U.S and other countries, but recognizing Australia as a leader in this area.
   a. The building of institutional capabilities, state-of-the-art studies and linkage of present resources were top priority considerations.
   b. Projects complementing programs at the International Centers and other institutions.
4. Wise utilization of resources, such as energy, soils, crops, labor and management.
5. Other factors, including scientific feasibility, location of LDC's, technology available and the need for greater understanding of the biology and use of nitrogen-fixing bacteria and plants.

Based on the premise that funds should be utilized to develop knowledge and technology of immediate help to the small farmer in developing countries, approximately 80% of the overall effort should be aimed at

developing the legume-rhizobia system and utilization of the technology related to nitrogen fixation by the legumes. Only about 20% of the overall effort should be directed to evaluation and possible domestication of grass-rhizosphere bacteria associations and non-symbiotic nitrogen fixation systems. About 60% of the available financial support should be used in research to provide better materials and delivery systems for the farmer to use, with about 20% in training and 20% to increase utilization of techniques and materials available.

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