Enhancing biological nitrogen fixation

by S.K.A. Danso and D.L. Eskew*

Agricultural production has increased dramatically in the last few decades, but increases in world population have kept pace and still greater increases in crop production will be required in the coming decades. More than half of the increase in crop production which has been achieved can be attributed to the increased use of inorganic fertilizers.

Of the nutrient elements supplied by inorganic fertilizers, nitrogen is the most important. In some cases it accounts for 75% of the increases in crop yield which have been achieved. To meet future food requirements it is inevitable that the use of inorganic nitrogen fertilizers will continue to increase. However, such fertilizers are expensive, and they have the potential to become environmental pollutants. The industrial process by which inorganic nitrogen fertilizers are manufactured requires high temperatures and pressures, obtained by burning natural gas or other fossil fuels. The manufacture of inorganic nitrogen fertilizers therefore represents the largest single energy input in all crop production systems, from the least to the most highly mechanized: currently, 2% of all the world's fossil fuel consumption is used for this purpose. Developing countries which lack resources of fossil fuels must import either the manufactured nitrogen fertilizer or the fossil fuels themselves, creating a serious drain on limited supplies of foreign exchange.

Usually only 30 to 50% of the inorganic nitrogen fertilizer applied is used by the crop. The rest is lost by volatilization, denitrification, or leaching of nitrate into the groundwater. In industrialized countries, where high rates of inorganic fertilizers are commonly used, contamination of drinking water with nitrate has sometimes caused a significant health hazard. For these reasons, it is necessary that inorganic nitrogen fertilizers be used in the most efficient way, and only when they are necessary. Several co-ordinated research programmes (CRPs) conducted by the Soil Fertility, Irrigation and Crop Production Section of the Joint FAO/IAEA Division have concentrated on finding the most efficient way of applying nitrogen fertilizers to various crops, using nitrogen-15 (^{15}N) as a tracer. The findings of these studies have been adopted in many countries around the world, resulting in savings of nitrogen fertilizers worth many millions of dollars every year. More recently, the Section's CRPs have focused on enhancing the natural process of biological di-nitrogen fixation.

Before the development of manufactured chemical fertilizers, crop production was dependent on biological nitrogen fixation. Since the development of the industrial Haber-Bosch process, crop production has become increasingly dependent on manufactured fertilizers and according to some estimates the amount of industrially fixed nitrogen is approaching the level of biologically fixed nitrogen. A good yield of most crops contains about 200 kg N/ha; but although 78% of the earth's atmosphere is nitrogen gas and there are thus hundreds of tonnes of nitrogen over every hectare of land surface, nitrogen is the nutrient element most limiting for crop production. This paradox arises from the fact that plants are unable to directly use the di-nitrogen (N_2) gas in the atmosphere. The very stable bond between the two nitrogen atoms of di-nitrogen must be broken and the nitrogen incorporated in nitrate or ammonium compounds before it can be assimilated by plants. As mentioned above, this can be accomplished by an industrial process, or by biological di-nitrogen fixation. Although no plant is capable of biological di-nitrogen fixation by itself, nature has endowed several primitive micro-organisms with the capability to fix di-nitrogen biologically. By associating symbiotically with these micro-organisms some plants are capable of using atmospheric di-nitrogen indirectly to assist their growth. Unfortunately for mankind, such associations are either not formed or are only weakly developed in cereal crops such as rice, wheat and maize, which supply the major share of our food. Thus, to continue to feed this hungry world it is not possible for biological di-nitrogen fixation to replace manufactured nitrogen fertilizers completely. The best plant-microbial combinations, and agricultural practices which result in the most effective use of biological di-nitrogen fixation, must be found.

The most important and best known symbiotic association is the association of bacteria of the genus *Rhizobium* with legumes (See Box). Peas, soybeans and peanuts are large-seeded legumes (also known as pulses) grown for grain production. In Latin America and Africa, the common bean and cowpea are important sources of protein in the human diet. Pulses are second only to cereals in the amounts consumed. Alfalfa and clover are examples of important legumes which are used in pastures for forage production, and some leguminous trees are also used in forestry.

Legumes have been used in agriculture for hundreds of years, but it was not until the turn of this century that is was realized that through their association with *Rhizobium* they could use atmospheric nitrogen indirectly. Since that time great advances have been

---

* The authors are staff members in the Soil Fertility, Irrigation and Crop Production Section of the Joint FAO/IAEA Division.

made in understanding the plant and bacterial genetic factors controlling symbiosis, the biochemistry of the enzymes involved, and the nutritional and environmental conditions which affect the functioning of the system. However, for two major reasons it remained difficult to measure the amounts of nitrogen actually being fixed under field conditions. The first was that symbiosis occurs in nodules on the roots of the plants beneath the surface of the soil, and to make measurements it was therefore necessary to disturb the system by digging up the plants. The second problem was that the legumes, like any other plant, use soil nitrogen and fertilizer nitrogen as well as nitrogen derived from their symbiotic association. Once soil or fertilizer nitrogen is taken up by a legume, the nitrogen from these sources cannot be distinguished from nitrogen from symbiotic fixation. Thus, it is difficult to know if a treatment which increases plant nitrogen has achieved its results by

The legume-*Rhizobium* symbiosis

Rhizobium bacteria infect the roots of legumes and induce tumor-like growths called nodules. The centre of each mature nodule is packed with billions of di-nitrogen-fixing bacteria. The host legume plant supplies the energy needed to drive di-nitrogen fixation by capturing energy from sunlight by the process of photosynthesis. The overall efficiency of this complicated symbiosis is thus dependent on the individual efficiencies of both the legume plant and the bacteria. Therefore, the genetics of both the host legume and the bacteria are extremely important, as well as the way in which the two interact. Also, any nutritional or environmental factor which affects one partner affects the overall efficiency of the symbiosis. The complexity of the symbiosis makes it absolutely essential that research be conducted under field conditions where all factors can be considered.

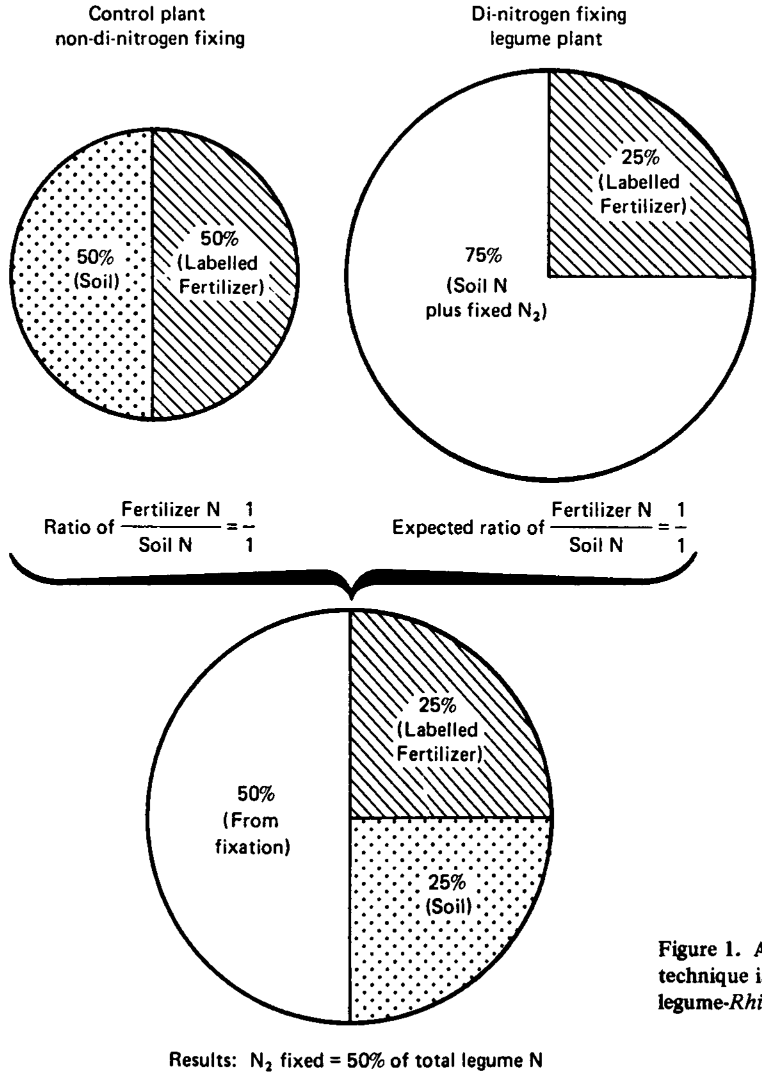

Figure 1. A simplified example of how the ^{15}N isotope technique is used to measure di-nitrogen fixation by the legume-*Rhizobium* symbiosis.

increasing nitrogen fixation, or by increasing the plant's uptake of soil or fertilizer nitrogen.

The Soil Fertility, Irrigation and Crop Production Section, in collaboration with the Soils Section of the FAO/IAEA Agricultural Biotechnology Laboratory at Seibersdorf, have made important contributions to the development of a field-scale technique, based on the use of ^{15}N, which allows simpler and more accurate estimates of biological di-nitrogen fixation than was possible using previous methods [1]. A ^{15}N-labelled fertilizer is applied to a non-nitrogen-fixing "control" plant to determine the utilization of soil and fertilizer nitrogen, then a nitrogen-fixing legume plant is grown with the same ^{15}N-fertilizer application to determine fixation. A simplified example is shown in Figure 1. In the top left part is shown the hypothetical case in which a non-fixing control plant derives half of its nitrogen from the soil and half from the ^{15}N-labelled fertilizer. It is then necessary to assume that the legume will use soil and ^{15}N-labelled fertilizer in this same 1:1 ratio. In the extreme case, in which no nitrogen is fixed, the ^{15}N enrichment of the control and legume would be the same. The top right part of Figure 1 illustrates the case in which the di-nitrogen fixing legume plant derives part of its nitrogen from atmospheric di-nitrogen. By combining the information in the top two diagrams it is possible to determine the relative contribution of soil, fertilizer and atmospheric nitrogen to the total nitrogen in the legume, as shown in the bottom part of the diagram. The magnitude of the decrease in the proportion of the legume nitrogen derived from the ^{15}N-labelled fertilizer is thus directly proportional to the amount of nitrogen derived from the atmosphere; in this case, it is 50%. This method has now become widely accepted around the world as the most direct, practical and useful method for quantifying nitrogen fixation under field conditions.

The ^{15}N isotope technique for measuring nitrogen fixation has been and is being used in several of the Soil Fertility, Irrigation and Crop Production Section's CRPs. It has been used in a programme on grain legumes to measure the effects of nitrogen and phosphorus fertilization on nitrogen fixation, and to compare the nitrogen-fixing capabilities of different varieties of grain legume. It is currently being used to measure the nitrogen input from several grain legumes in multiple cropping systems, in which legumes are grown either in a mixture with maize, sorghum or other crops, or with these crops grown in alternate cropping seasons. This practice is based on the concept that nitrogen fixed by the legume can be used by the second crop. By using ^{15}N techniques the amount of nitrogen made available can be quantified, and cropping practices modified to maximize the benefit. The ^{15}N isotope technique is also being used in a programme supported by the Italian Government to improve pasture management. Legumes are commonly mixed with grasses in pastures to increase the quantity of protein in the forage. Using the ^{15}N technique it is possible to identify management practices which enhance nitrogen fixation. Most recently, a programme has been initiated on the use of the *Azolla-Anabaena* symbiosis for supplying nitrogen-flooded rice cultivation.

Table 1. Variation in nitrogen-fixing capability between varieties of *Phaseolus* (common bean) in Brazil and soybeans in Greece

Phaseolus variety	**kg N/ha fixed**
Goiana precoce	24.6
Moruna	37.1
Carioca precoce	46.2
Costa Rica	57.9
Carioca	65.0
Soybean variety	**kg N/ha fixed**
Chippewa	54.0
Williams	215.6
Amsoy-71	247.4

The value of the ^{15}N isotope technique for measuring nitrogen fixation is illustrated by the fact that many of the results already obtained in the grain legume programme could not have been obtained with other methods. A co-ordinated research programme on di-nitrogen fixation in grain legumes was initiated in 1979 with the support of the Swedish International Development Agency (SIDA). The scientists participating in this programme came from 19 Member States. The most important result obtained was the finding that different species of legume have greatly different nitrogen fixation capacities, and that substantial differences exist between varieties within a species. Further, by conducting experiments in many different countries, the programme has shown that these differences persist under a wide range of environmental conditions. For example, the common bean (*Phaseolus vulgaris*) which is widely grown in Latin America was clearly identified as a poor nitrogen fixer, with rates of 30 to 60 kg N/ha reported in Mexico, Kenya and Brazil. At the other end of the scale the field or horse bean (*Vicia faba*) was clearly shown to be an outstanding nitrogen fixer with rates of 150 to 300 kg N/ha in Egypt and Austria. Although other techniques had earlier indicated similar results, there was only a limited amount of information available and from only a few locations. With the data obtained from this programme it is now possible to concentrate efforts to increase nitrogen fixation on those crops where it is most needed. A programme is now being initiated to improve the nitrogen-fixing capacity of *Phaseolus*.

The ^{15}N isotope technique was instrumental in establishing that there is also variability between legume varieties, as well as between different legumes, in their ability to support nitrogen fixation. Examples of varietal comparisons for *Phaseolus* in Brazil [2] and for soybeans in Greece are shown in Table 1. Even with the limited

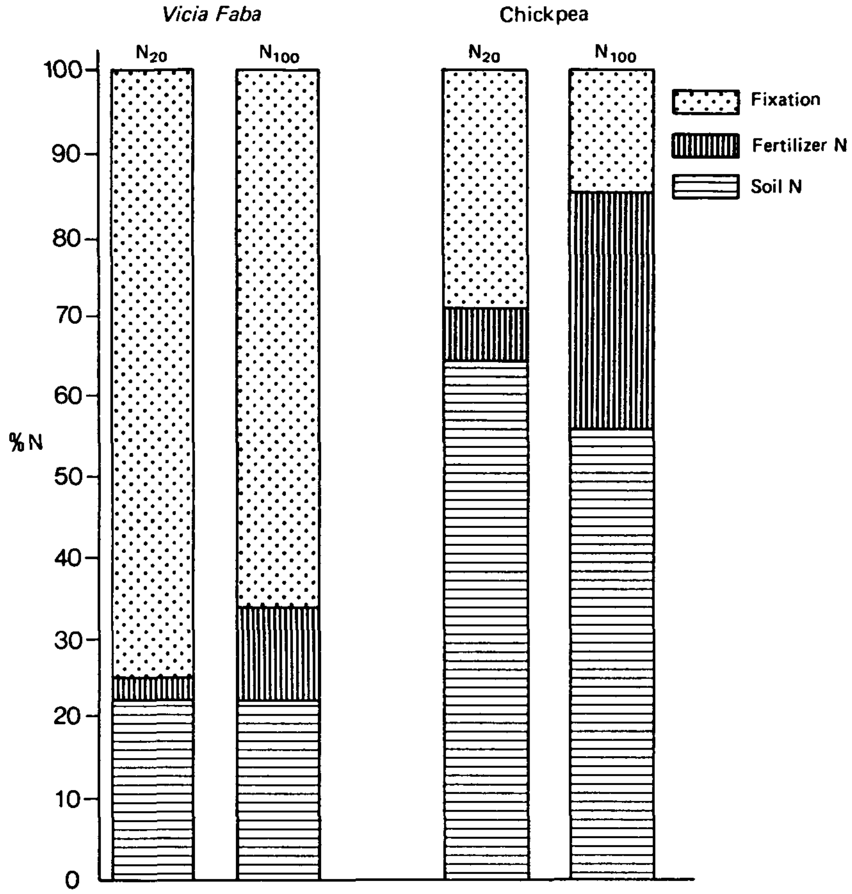

Figure 2.
Sources of nitrogen in *Vicia faba* and chickpeas grown in Egypt at two nitrogen levels.

number of varieties used in these experiments, a 2.6 fold variation of fixation was revealed in *Phaseolus* and a 4.6 fold variation between soybean varieties.

The amount of nitrogen actually fixed by a legume depends not only on the genetics of the bacteria and host plant but also on the environment and agricultural practices. Among the common agricultural practices, fertilization with P and N have important effects in nitrogen fixation. In Kenya, fertilizing *Phaseolus* beans with 150 kg/ha of P increased seed yield by 62% and increased nitrogen fixation from an average of 8 kg/ha to 60 kg/ha. In an experiment with mungbeans in Pakistan, increasing the P fertilizer rate from 25 to 35 kg P/ha resulted in an increase in N fixation from 20 to 48 kg/ha. In this case, the ability of the ^{15}N isotope technique to differentiate between soil nitrogen and nitrogen fixed from the atmosphere was particularly important, since total nitrogen content increased only slightly. Thus, mungbeans at the higher rate of P fertilization in fact used less soil nitrogen because of increased fixation.

It is a well-established fact that, when legumes are grown in soils high in available nitrogen, the nitrogen fixation rate is reduced. However, when legumes are grown in mixed or multiple cropping systems, it is often still necessary to add nitrogen fertilizers to the non-leguminous component of the systems. Thus, it is necessary to identify legume species or varieties which continue to fix atmospheric nitrogen even when fertilizer nitrogen is added. Comparing the effects of 20 and 100 kg N/ha on the field bean (*Vicia faba*) and chickpea in Egypt, it was found that the nitrogen fixation by chickpea was reduced much more than that of *Vicia faba* (Fig.2). This suggests that *Vicia faba* may be a much better choice in multiple cropping systems than chickpeas.

The ^{15}N isotope technique has proven to be very valuable in studies of the legume-*Rhizobium* symbiosis, allowing many more experiments than before to be done and yielding much new practical information. The Soils Section is now working to extend the use of the technique to other nitrogen-fixing symbioses.

The symbiosis of the free-floating water fern *Azolla* (of which there are six known species) with the nitrogen-fixing blue-green algae (also known as cyano-bacteria) *Anabaena azollae* can supply large amounts of nitrogen for flooded rice [3]. The nitrogen-fixing blue-green algae live in a cavity in the fern leaf and supply the host

Figure 3. The rapid growth of which *Azolla* is capable is shown by the plants in these two trays: those on the right are two weeks older.

plant with nitrogen. This symbiosis is capable of very rapid growth in media which do not contain chemically-combined nitrogen (Fig.3). Under good conditions, 30 kg N/ha can be fixed in two weeks. Although the potential of the *Azolla* symbiosis has only recently gained international attention, it has been used for centuries in southern China and Vietnam as a source of nitrogen for flooded rice. Since the fixed nitrogen is assimilated into the fern biomass it is not directly available to the rice plant. The *Azolla* plants must be incorporated into the soil and allowed to decay before the nitrogen becomes available for rice growth. A new co-ordinated research programme will use ^{15}N isotopic techniques to quantify the amount of nitrogen fixed by the *Azolla* symbiosis under realistic field conditions, and seek to develop optimum practices to enhance the efficiency with which nitrogen fixed by *Azolla* is used by the rice plant.

References

[1] **M. Fried and H. Broeshart.** *An independent measure of the amount of nitrogen fixed by a legume crop.* Plant and Soil **43,** pp 707–711 (1975).

[2] **A.P. Ruschel, P.B. Vose, E. Matsui, R.L. Victoria, and S.M.T. Saito.** *Field evaluation of N_2-fixation and N-utilization by* Phaseolus *bean varieties determined by ^{15}N isotope dilution.* Plant and Soil **65,** 397–407 (1982).

[3] **I. Watanabe, B. Kezhi, N.S. Berja, C.R. Espinas, O. Ito, and B.P.R. Subudhi.** *The* Azolla-Anabaena *complex and its use in rice culture.* IRRI Research Paper Series 69 (1981).

◆ ◆ ◆ ◆ ◆ ◆

Forthcoming IAEA conferences...

Date	Subject	Place
1984		
10–14 September	NEA/IAEA Symposium on Nuclear Third Party Liability and Insurance-Status and Prospects	Munich Fed.Rep.of Germany
12–19 September	10th International Conference on Plasma Physics and Controlled Nuclear Fusion Research	London
8–12 October	International Symposium on High-Dose Dosimetry	Vienna
22–26 October	International Conference on Radiopharmaceuticals and Labelled Compounds	Tokyo
29 October–2 November	International Symposium on Safety Codes and Guides (NUSS) in the Light of Current Safety Issues	Vienna
19–23 November	International Symposium on Assessment of Radioactive Contamination in Man	Paris
1985		
14–18 January	International Conference on Neutron Scattering in the 90's	Jülich Fed.Rep. of Germany
4–8 March	FAO/IAEA International Symposium on Food Irradiation Processing	Orlando Fla., USA
6–10 May	International Symposium on Advances in Nuclear Power Plant Availability, Maintainability and Operation	Munich Fed.Rep. of Germany

...and seminars

Date	Subject	Place
1984		
16–27 July	IAEA/UNESCO Seminar on the Use of Isotope Techniques in Water Resources Development in Latin America	Buenos Aires
2–5 October	IAEA/NEA Seminar on Remote Handling Equipment for Nuclear Fuel Cycle Facilities	Harwell UK
22–26 October	INIS Regional Training Seminar for Asia and the Pacific	Tokyo
5–8 November	Seminar on Practical Problems Encountered in the Transport of Radioactive Materials for Safeguards-Related Activities	Vienna
12–16 November	FAO/IAEA Seminar on the Use of Isotopes in Studies of Biological Nitrogen Fixation for developing countries in the Middle East and Africa	Ankara
26–30 November	Seminar on Nuclear Power Plant Operations Management	Vienna

---

Further information on these conferences can be obtained from the IAEA, or the appropriate body in each Member State: the authority responsible for nuclear matters, or the Ministry of Foreign Affairs.