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Introduction

Man began to cultivate land in an organized way for food grains around 8000 B.C. Very soon, he learned that the same land cannot support the growth of plants endlessly and this led him to think about ways to improve the fertility of soil. The earliest records indicate that Romans and Aryans had many manuals for farmers to improve the cultivation of crops. For instance, Columella's treatise *Husbandry* written about 60 A.D. contains descriptions of several agricultural practices which were in use in the Roman empire for many generations. After the fall of the Roman empire, the Arabian culture flourished in the 12th century, when Ibn-al-Awan, a Moorish scholar, wrote a handbook on agriculture. This was followed by 18th century farming practices such as those of Jethro Tull (English) and the Norfolk 'four-course' system developed in Holland by years of experience based on crop rotations.

Meanwhile, many people wondered about the ingredients in soil which nourish plants. In the 16th century, Bernard Pallissy, a potter to the French royalty, argued that plant residues contained the 'salt' or the 'principle' which supported plant growth, while Jan Baptista van Helmont believed that water was the 'principle' capable of supporting the growth of plants. Later, the idea that certain ingredients in soil dissolved in water actually supported plant growth was established by experiments of John Woodward of England. Some others considered 'humus' the supporter of plant growth. However, all conjectures were set aside when the French scientist, Antoine Lavoisier, developed a table of chemical elements in 1794. He also showed that plants and animals used up oxygen by a process of respiration (USDA, 1957).

Soil Fertility

The experiments of J.B. Boussingault, a French agriculturist, in 1834 revealed the important chemical constituents of both plants and soil. This was followed by the work of Justus Von Liebig, a German chemist, who was able to prepare a balance sheet of plant nutrition in relation to soil. By about this time, Boussingault also proposed that legumes fix nitrogen in soil. One of the milestones in the area of soil fertility was the discovery of superphosphate in 1840 by John Bennet Lawes and his associate J.H. Gilbert at the Rothamsted Experimental Station in England. Further researches at Rothamsted led to a greater understanding of the chemistry of soil and the chemical requirements of plants. Boussingault's thinking on the role of legumes in soil fertility was substantiated by the discovery of legume root nodules by Hellreigel in Germany in 1886 and the discovery by Beijerinck, a Dutch scientist, in 1888 that bacteria in root nodules of legumes now called *Rhizobium* are causative agents in the fixation of atmospheric nitrogen.

Chemical fixation of nitrogen was known only several years after our understanding of the implications of biological nitrogen fixation. Fritz Haber, a German chemist, successfully synthesized nitrogen and hydrogen into ammonia during the early years of World War I. No discovery leading to soil fertility has ever equalled that of Haber. In fact, the Haber-Bosch process of ammonia synthesis, requiring temperature up to 800°F, a catalyst, and high pressures above atmospheric pressure has remained, till today, the sole method for the production of nitrogenous fertilizers in the world. Strangely, this very combination of nitrogen and hydrogen could also be accomplished by nitrogen-fixing microorganisms in soil and within the nodular tissues of certain plants at ordinary pressures and temperature mediated by an enzyme known as 'nitrogenase'.

Man-made fertilizers as we know them today, those containing N_2, P_2O_5 and K_2, increase the output of agricultural products and meet the ever increasing demands of the human population, which have been further accentuated by the limited availability of additional fertile farm land. The world's population, food production, and fertilizer consumption have increased gradually. It has been forecast that there will be a further increase in population in the developing countries by about 2.1 million people at the end of 2000 A.D. To guarantee enough food for all, either the population growth has to be stemmed or more fertilizer has to be found to meet the ever increasing demand for protein. The increase in world fertilizer requirement by

2000 A.D. would be approximately three times the rate of current consumption (Table 1).

Table 1: Population, grain production and fertilizer use, and projections for future

Year	*Population (millions)*	*Grain production (million tons)*	*Fertilizer consumption (million tons)*
World			
1980	4374	1575	113
2000	6253	2235	307
Developed countries			
1980	1145	840	83
2000	1317	1221	197
Developing countries			
1980	3229	735	30
2000	4936	1032	110
India			
1980	694	130	5.60
2000	1059	175	18.90

Source: Verghese, 1977.

Chemically Fixed Nitrogen versus Biologically Fixed Nitrogen

Fertilizer nitrogen will continue to serve for increasing grain production into the foreseeable future but efforts should also be oriented towards augmenting biological nitrogen fixation mediated by microorganisms. An average acre of grain legumes like soybeans, beans, or peas provides sufficient protein for 1000–2000 days for one person, whereas an average acre of plant materials converted to animal protein like beef and poultry provides only for 75–250 days (Burns and Hardy, 1975). As the per capita income increases the demand on animal protein also increases accompanied by a several-fold decrease in the intake of vegetable protein. Therefore, in the affluent nations, where the per capita income is high, the demand for fixed nitrogen for conversion into animal protein is higher than that of less affluent nations where the per capita income is low and consequently, the intake of vegetable protein is more pronounced.

Accurate estimates of annual turnover of nitrogen in the biosphere vary from 100 to 200 million tons (Delwiche, 1970; Burns and Hardy, 1975; Burris, 1977; Subba Rao, 1977). The ratio between chemically fixed nitrogen and biologically fixed nitrogen ranges approximately from 1:4 to 1:2.5 and within biological fixation, the legume fixation is equivalent to or at least half that of industrial fixation (Table 2). The amounts of kg N_2 fixed/ha/yr for individual legumes has been roughly estimated as follows: 125–335 for alfalfa, 85–190 for red clover, 80–150 for peas, 65–115 for soybean, 65–130 for cowpea, and 90–155 for vetch (Alexander, 1977).

Table 2: Estimates of annual biological nitrogen fixation on earth

Systems	*kg N_2 fixed / (ha × yr)*	*Metric tons/yr × 10^{-6}*
Legumes	140 (80)	35 (20)
Non-legumes	35	9
Permanent grassland	15 (8)	45 (24)
Forest and woodland	10 (5)	40 (20)
Unused land	2	10
Total land		139 (83)
Sea	1 (0.5)	36 (18)
Total		175 (101)

Note: Fertilizer from chemical N_2 fixation was 40 × 10^{-6} metric tons/yr in 1976. Data extracted from Burris (1977), who has incorporated the estimates of Burns and Hardy (1975); the figures in parentheses indicate values assigned by experts at a meeting in Uppsala, Sweden, 1976.

Accurate estimates of the amounts of N_2 fixed by grain legumes have been made by the International Atomic Energy Agency (IAEA), Vienna, using labelled nitrogenous fertilizers. Some of the results obtained by the collaborating scientists in the agency's network of experiments in different countries are shown in Table 3. These values appear to be rather realistic but nervertheless do not minimize the value of legumes in the nitrogen economy of cultivated plants.

The demand for chemically fixed nitrogen is bound to be on the increase and the nitrogen gap gets doubled by 1995 (Table 4). Such a gap would be difficult to bridge in the wake of the energy crisis. Furthermore, in the area of chemical fixation, no major breakthrough is yet visible to minimize the energy requirements of the conventional Haber-Bosch process for the production of ammonia. In developing countries, the construction of new nitrogen fertilizer plants is not only expensive but time consuming. Farmers in many parts of Africa do not use inorganic nitrogenous fertilizers because they are imported and expensive. Therefore, the strategy for improving agricultural production in developing countries should take into account inexpensive, realistic, and pragmatic programmes to augment biological nitrogen fixation.

Table 3: Accurate estimates of symbiotic N_2 fixation by different legumes by the isotope dilution method — results of field trials using labelled $(^{15}NH_4)_2 SO_4$

Country	*Legume*	*Year*	*Total amount of N_2 fixation (kg/ha)*
New Delhi, India (IARI) (Mean of 3 Years' field experiments)	Chick-pea (*Cicer arietinum*)	1979–1982	61.6
Larisa, Greece	*Medicago sativa*	1980	72.8
Faisalabad, Pakistan	Mung bean (*Vigna radiata*)	1981	45.3
Piracicaba, Brazil	*Phaseolus* beans	1980	37.0
Egypt	Faba beans (*Phaseolus vulgaris*)	1980	101.0

Source: IAEA Reports, Vienna, Austria.

Table 4: The world's demand for N (million tons) in coming years which clearly shows the need for renewed efforts to augment our resource

	1980	*1985*	*1990*	*1995*	*2000*
Developed countries	37.5	47.6	58.9	71.4	85.1
Developing countries	18.1	25.4	33.9	43.6	54.5
World	55.6	73.0	92.8	115.0	139.6

Source: Verghese, 1977.

How to Augment Biological Nitrogen Fixation

There are both novel and conventional approaches to this problem (Subba Rao, 1976a, 1976b, 1977, 1979). Conventionally, attempts should be made: (1) to increase the area under cultivation of grain legumes by the introduction of legumes in inter-, multiple, and relay cropping; (2) to provide efficient strains of rhizobia for inoculating legumes and other non-symbiotic nitrogen fixers for inoculating cereals; (3) to evolve a technology enabling the newly introduced nitrogen fixers to successfully compete with the strains of nitrogen-fixing microorganisms already present in soil; (4) to evolve varieties of plants responsive to both biologically and industrially fixed nitrogen; (5) to overcome the inhibition of fertilizer nitrogen on biological fixation of nitrogen; (6) to define agronomic practices leading to better fixation and conservation of nitrogen on the farm; (7) to discover inexpensive nitrification inhibitors such as neem cake; and (8) to evolve simple practices to conserve water on the farm because optimum moisture is needed for successful nodulation and hence biological nitrogen fixation in legumes.

There is no limit to speculation in the unconventional approaches, which are indeed receiving considerable attention in recent years. Some of the considerations are: (1) to extend nitrogen fixation to cereals by inducing the formation of nodules on root; (2) to transfer genes from nitrogen-fixing bacteria to non-nitrogen-fixing bacteria by transformation, transduction, and conjugation; (3) to implant nitrogen fixation capacity from nitrogen-fixing bacteria into crop plants by protoplast fusion and tissue culture methods; (4) to overcome barriers in intergeneric hybridization between nodulating legumes and cereals, both at the floral level in intact plants and at the cellular level, in cell cultures; (5) to synthesize 'nitrogenase', the enzyme responsible for converting N_2 to NH_3, and harness it for use in nitrogen fixation as a catalyst in industrial processes; and (6) to extend research into new nitrogen-fixing associations among plants and harness them to agriculture.

Exciting discoveries have been made in recent decades which bring an optimistic note to many of the speculations cited above. The *nif* operon (a cluster of nitrogen-fixing genes) has been transferred from a nitrogen-fixing bacterium, *Klebsiella pneumoniae*, into a common enteric bacterium, *Escherichia coli*. At the cellular level, cultured plant tissues and cells have been made to take up nitrogen-fixing bacteria and fix atmospheric nitrogen. Roots of rice and rape plants have been made to produce nodules which seem to fix elemental nitrogen. These experiments have paved the way for eventual release of plants capable of growing without the need for chemical fertilizers. Studies on detection, isolation, and characterization of plasmids in nitrogen-fixing bacteria are in progress in many laboratories with a view to using plamids as 'vectors' in transferring *nif* genes into higher plants. The parts of the enzyme nitrogenase have been interchanged from one nitrogen-fixing microorganism into another without detriment to the property of nitrogen fixation. Biochemists have unravelled the structure and composition of nitrogenase while microbiologists have identified the existence of more plants bearing nitrogen-fixing nodules. Many fodder grasses and cereals like maize have been found to exhibit associate symbioses with nitrogen-fixing *Azospirillum* bacteria. Legumes have been shown to benefit by the dual action of obligate endophytic fungi in plant roots (endomycorrhizae) and *Rhizobium* in root nodules; the endomycorrhizal fungi appear to influence the uptake of phosphorus which helps in better fixation of nitrogen in root nodules. The biochemical basis for specificity in the legume-*Rhizobium* symbiosis has been explained as an interaction on the root surface involving the two symbionts mediated by carbohydrate-binding proteins called 'lectins'. The magnitude of loss of energy by hydrogen evolution along with N_2 reduction in nodules formed by hydrogenase-negative strains of rhizobia has been shown to be greater than that induced by hydrogenase-positive strains; field experiments have shown that hydrogenase-positive strains proved to be better inocula and helped in increasing soybean yields when compared to inocula produced by hydrogenase-negative strains. Such observations serve to point out that hydrogenase-positive strains conserve energy within the nitrogenase reactions (Dunican *et al.*, 1976; Dobereiner, 1977; Dazzo, 1980; Evans *et al.*, 1980; Islam *et al.*, 1980; Cocking *et al.*, 1990). However, at the moment it is not clear whether such research findings could be of agronomic importance to improve crop yields by better nitrogen fertilization.

Development of Inoculant Research and Industry

Microbial inoculants are carrier-based preparations containing beneficial microorganisms in a viable state intended for seed or soil application and designed to improve soil fertility and help plant growth by increasing the number and biological activity of desired microorganisms in the root environment. Starting from modest laboratory preparations in the mid-1930s in the United States, rhizobial inoculants (also known as legume inoculants) have become industrial propositions in the United States, Europe, Australia, and India. Following the success of legume inoculants all over the world, carrier-based *Azotobacter* and *Azospirillum* inoculants for non-leguminous crops are becoming increasingly popular in India in recent years. Currently, besides, agricultural research institutes, and universities in India, the agencies also active in supplying biofertilizers are: 1. The MP State Agro Industries Dev. Corp. Ltd., Bhopal, 2. The Maharashtra Agro Industries Dev. Corp. Ltd., Bombay, 3. Department of Agriculture, Madras, (TN), 4. National Agri. Co-op. Mktng. Federation of India Ltd., Indore, 5. Gujarat State Co-op. Mktng. Federation Ltd., Baroda, 6. Gujarat State Fertilizers Co. Ltd., Fertilizer Nagar, Dist. Baroda, 7. Micro Bac India, Calcutta, 8. Indian Organic Chemicals Ltd., Bombay, 9. Madras Fertilizers, Madras.

In 1895, Nobbe and Hiltner introduced laboratory-grown cultures of rhizobia called 'Nitragin' grown on a solid medium containing extracts of leguminous plants, gelatin, sugar, and asparagine. In this maiden attempt, 17 different inoculants for important leguminous crops were produced (Fred *et al.*, 1932; Burton, 1967, 1979). In 1920, the situation in the United States was such that besides the U.S. Department of Agriculture and 20 research institutions, 17 commercial concerns were marketing inoculants. The following companies are currently active in North America in supplying *Rhizobium* inoculants: *Lipha Tech*, 3101 W. Custer Ave., Milwaukee, WI 53209, USA, ph (414) 462-7600; *Philom Bios Inc.*, 104–110 Research Drive, Saskatoon, Sask., Canada S7N 3R3, fax 306-975-1215; *Micro BioRhizogen*, Bay 5–116 103rd St. East, Saskatoon, Sask., Canada, fax 306-374-8510; *ESSO-Biologicals Canada*, 15-401 Innovation Blvd., Saskatoon, Sask., Canada S7N 2X2 fax 306-975-3750; *Titre Inc.*, 361 Rothiemay, Rd., Ryegate, MT 59074, USA or Box 220, Beachville, Ontario NOJ 1AO, Canada; *Urbana Laboratories*, P.O. Box 1393, St. Joseph, MO, 64502, USA, ph 1-800-821-7666.

In Russia and Poland non-symbiotic nitrogen-fixing bacteria of the genus *Azotobacter* gained importance in the early part of this century. A product under the trade name 'Azotobakterin' was used for soil and seed treatment and spectacular benefits were recorded on the yield of vegetables and cereals.

Reviewing the work on *Azotobacter,* Martin Alexander (1961) of Cornell University, USA, said that 'careful analysis of the Soviet experiments is difficult because of the scarcity of statistical evaluations. It is possible that the *Azotobacter* effects are not real and may be accounted for by the normal variability of field experimentation. The greater yields are difficult to reconcile with rapid decline of *Azotobacter* in inoculated soil and with the absence of the specialized structure (nodule) that permits the *Rhizobium*-legume symbiosis to operate so efficiently. For the present, therefore, reports of benefits arising from *Azotobacter* inoculation must be considered as equivocal'.

In summarizing the same work, two well-known soil microbiologists of Russia said that 'an increase in the yield of field crops of not more than 10 per cent in an agricultural experiment is not regarded as exceeding the limits of experimental error. As to the positive action of *Azotobacter*, it can only be said that statistical analysis repeatedly demonstrates the reliability of the results' (Mishustin and Shilnikova, 1969).

Field experiments have also been carried out in India on *Azotobacter chroococcum* inoculation of wheat, rice, onion, brinjal, tomato, and cabbage. Yield increases have been rather variable from simple general increases to significant increases in rice, cabbage, and brinjal (Sundara Rao *et al.*, 1963; Sundara Rao, 1964; Lehri and Mehrotra, 1968, 1972; Mehrotra and Lehri, 1971; Joi and Shinde, 1976). *Azotobacter* inoculants are popular in India because they can be applied to many non-leguminous crops and promote seed germination and initial vigour of plants due to growth substances produced by the organism (Shende *et al.*, 1977).

Blue-green algae play a role in the nitrogen economy of tropical rice soils (De, 1939; Singh, 1961; Subramanyam and Sahay, 1964; Venkataraman, 1972). The nitrogen-fixing algae can be cultured in open-air tanks and used for rice cultivation. The results obtained by algal inoculation of rice fields in India have shown the possibility of using blue-green algae as biofertilizer in rice cultivation.

Microorganisms convert bound phosphates such as superphosphate and rock phosphate into forms which are easily assimilated by plants (Gerretsen, 1948; Sperber, 1957; Sethi and Subba Rao, 1968; Sundara Rao, 1968). The Russian microbiologists introduced a product called 'phosphobakterin' containing cells of *Bacillus megatherium* var. *phosphaticum* for soil and seed application. Since then, other microorganisms have been tested in India and the results are quite promising.

Cellulose is the main component of organic matter in plant residues. Numerous microorganisms in soil degrade cellulose to different degrees and the products containing either the cells of cellulolytic microorganisms or their enzymic extracts are being produced in Europe and the United States and marketed for quick composting of agricultural wastes. Some of the brand names of such products from Europe are 'Cofuna', 'Agromax', and 'Eokomit'. In India, similar products are yet to be produced and marketed although considerable research on cellulolytic and lignolytic microorganisms has been done.

Thiobacilli (sulphur-dissolving bacteria) were used in the preparation of 'biosuper' in Australia by mixing rock phosphate and sulpur. The suphuric acid produced in the mixture dissolves the rock phosphate and thereby enhances phosphorus nutrition of plants. Whether this kind of biofertilizer is worthwhile and economical in India depends on the availability of sulphur and rock phosphate for this purpose and the utility of the product on the farm for increasing yields of crop plants.

The re-discovery of *Azospirillum brasilense* in Brazil (Dobereiner *et al.*, 1976) led to field testings of Indian isolates of the bacterium on a large scale in India (Subba Rao *et al.*, 1979a, 1979b) which have shown beneficial inoculation effects of this organism on ragi, bajra, sorghum, maize, barley, and oats. Field trials point out significant increase in yields of crops in many locations and also indicate savings on the application of inorganic nitrogenous fertilizers from 20 to 30 kg N/ha, depending on the crop and location.

The future for the inoculant industry in developing countries is bright. In the whole of Africa, there are very few places where fertilizer factories exist and the average farmer scarcely uses fertilizer nitrogen for crop production. Therefore, agronomists all over the world should take cognizance of developments in the area of organic fertilization of soil, especially biofertilizers.

The energy crisis has necessitated the search for renewable resources for nitrogen inputs in crop cultivation. One way to achieve partial self-sufficiency is to recycle animal and plant residues by scientific methods of composting. Using sunlight, which is abundant in the tropics, the cultivation of tiny water-borne *Azolla* ferns in aquatic nurseries on the farm has been continuously practised in Thailand, Vietnam, and China with the object of using the biomass as a nitrogen-rich green manure in rice cultivation. The *Azolla* fern, which has the nitrogen-fixing blue-green alga *Anabaena azollae* in its fronds, grows profusely as a floating plant in flooded rice fields and can fix 100–150 kg N/ha/yr in approximately 40–60 t biomass. The tremendous potential of this fern is being harnessed in other countries including India. Next only to nodulating legumes in its N_2-fixing potentialities the *Azolla-Anabaena* association appears to hold promise for the future. It is this kind of technology which can be taken as an example in planning future strategies in agriculture to save on costly inputs which come from non-renewable sources of energy.

Tropical rain forests are fast dwindling at a rate of 142,000 sq km/yr, primarily due to logging. The total area cleared annually is about 12.01 million ha (FAO sources). India alone contributes to this devastation to the extent of 1.5 million ha/yr and the highest is in Indonesia to the extent of 9 million ha/yr. Other factors which diminish forest cover are conversion of land to farming and fuelwood gathering. In fact, felling of trees for cooking energy is inescapable in third world countries and the only solution to stem this decline lies in extensive replanting or afforestation. Scientifically managed nurseries are needed for fast-growing trees. The use of nitrogen-fixing bacteria, especially rhizobia, for nitrogen-fixing leguminous trees in the nursery stage together with inoculation with vesicular-arbuscular mycorrhizal (VAM) fungi to provide facility for greater absorption of phosphorus have been considered desirable microbiological practices all over the world. The application of beneficial nitrogen-fixing and phosphate-mobilizing *Rhizobium* and VAM fungi is an integral part of modern nursery technology. Similarly, inoculation of seedlings of alder (*Alnus* spp.) and *Casuarina* spp. with appropriate *Frankia* cultures has been advocated.

The estimated area under nonforest waste lands in India is around 93,700,000 ha, where limiting factors such as salinity, alkalinity, lack of water, waterlogging, and unknown parameters operate in any programme of afforestation. While we are aware of the soil microbiology of arable soils, we are completely ignorant of the microbiological aspects of wastelands, especially with regard to the ability of these denuded soils to support nitrogen-fixing rhizobia and phosphate-mobilizing mycorrhizae. This knowledge is important since self-propelling nitrogen-fixing tree species are ideal candidates for afforestation of barren lands. The future objectives should be to identify and develop suitable microbial inoculants for selected nitrogen-fixing tree species. Some of the species recommended for planting are shown in Table 5.

Table 5: List of recommended firewood species

For Humid Tropics	*Eucalyptus citriodora*
Acacia auriculiformis	*Eucalyptus grandis*
Albizia lebbek	*Eucalyptus microtheca*
Alnus jorullensis	*Eucalyptus saligna*
Anogeissus latifolia	*Eucalyptus tereticornis*
Anogeissus leiocarpus	*Gliricidia maculata*
Avicennia spp.	*Gliricidia sepium*
Azadirachta indica	*Gmelina arborea*
Bruguiera spp.	*Grevillea robusta*
Cajanus cajan	*Guazuma ulmifolia*
Calliandra calothyrsus	*Inga edulis*
Cassia siamea	*Inga vera*
Cassia spectabilis	*Leucaena leucocephala*
Casuarina cunninghamiana	*Muntingia calabura*
Casuarina equisetifolia	*Parkinsonia aculeata*
Casuarina lepidophloia	*Pithecellobium dulce*
Eucalyptus camaldulensis	*Pongamia glabra*

Table 5 continued

Table 5 Contd.

Rhizophora apiculata	*Acacia seyal*
Rhizophora mangle	*Acacia tortilis*
Rhizophora mucronata	*Albizia lebbek*
Sesbania grandiflora	*Anogeissus leiocarpus*
Syzygium cummii	*Anogeissus pendula*
Terminalia spp.	*Azadirachta indica*
Trema guineensis	*Cajanus cajan*
Trema micrantha	*Cassia siamea*
Trema orientalis	*Casuarina cristata*
Other *Trema* spp.	*Casuarina decaisneana*
For Tropical Highlands	*Casuarina equisetifolia*
Acacia dealbata	*Casuarina glauca*
Acacia decurrens	*Casuarina stricta*
Acacia mearnsii	*Colophospermum mopane*
Alnus glutinosa	*Eucalyptus camaldulensis*
Alnus jorullensis	*Eucalyptus citriodora*
Alnus nepalensis	*Eucalyptus gomphocephala*
Alnus rubra	*Eucalyptus microtheca*
Casuarina cunninghamiana	*Eucalyptus occidentalis*
Casuarina equisetifolia	*Eucalyptus tereticornis*
Casuarina junghuhniana	*Eucalyptus viminalis*
Casuarina luehmannii	*Gmelina arborea*
Eucalyptus bicostata	*Halozylon aphyllum*
Eucalyptus camaldulensis	*Halozylon persicum*
Eucalyptus citriodora	*Parkinsonia aculeata*
Eucalyptus globulus	*Pinus brutia*
Eucalyptus gomphocephala	*Pinus eldarica*
Eucalyptus grandis	*Pinus halepensis*
Eucalyptus macarthuri	*Pithecellobium dulce*
Eucalyptus maidenii	*Prosopis alba*
Eucalyptus saligna	*Prosopis caldenia*
Eucalyptus viminalis	*Prosopis chilensis*
Grevillea robusta	*Prosopis cineraria*
Trema orientalis	*Prosopis farcta*
For Arid and Semiarid Regions	*Prosopis juliflora*
Acacia auriculiformis	*Prosopis pallida*
Acacia brachystachya	*Prosopis tamarugo*
Acacia cambagei	*Tamarix* spp.
Acacia cyanophylla	*Tamarix aphylla*
Acacia cyclops	*Tamarix articulata*
Acacia decurrens	*Terminalia glaucescens*
Acacia holosericea	*Zizyphus jujuba*
Acacia mollissima	*Zizyphus mauritiana*
Acacia nilotica	*Zizyphus nummularia*
Acacia raddiana	*Zizyphus spina-christi*

REFERENCES

Alexander, M. (1961). *Introduction to Soil Microbiology*. John Wiley and Sons, New York.

Alexander, M. (1977). *Introduction to Soil Microbiology* (Second Edition). John Wiley and Sons, New York.

Burns, R.C., and Hardy, R.W.F. (1975). *Nitrogen Fixation in Bacteria and Higher Plants*. Springer Verlag, New York.

Burris, R.H. (1977). Overview of nitrogen fixation. In *Genetic Engineering for Nitrogen Fixation*. Ed. A. Hollaender. Plenum Press, New York. pp. 8–18.

Burton, J.C. (1967). *Rhizobium* culture and use. In *Microbial Technology*. Ed. H.J. Peppler, Reinhold Publishing Corporation, New York. pp. 1–29.

Burton, J.C. (1979). New developments in inoculating legumes. In *Recent Advances in Biological Nitrogen Fixation*. Ed. N.S. Subba Rao. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi. pp. 380–405.

Cocking, E.C., Al-Mallah, M.K., Benson, E., and Davey, M.R. (1990). Nodulation of non-legumes by rhizobia. In *Nitrogen Fixation: Achievements and objectives*. Eds. P.M. Greshoff, L.E. Roth, G. Stacey and W.E. Newton. Chapman and Hall, London.

Dazzo, F.B. (1980). Determinants of host specificity in the *Rhizobium*-clover symbiosis. In *Nitrogen Fixation*, Vol. II. Eds. W.E. Newton and W.H. Orme-Johnson. University Park Press, Baltimore. pp. 165–189.

De, P.K. (1939). The role of blue-green algae in nitrogen fixation in rice fields. *Proc. R. Soc.*, **127 (3)**, 121–139.

Delwiche, C.C. (1970). The nitrogen cycle. In *The Biosphere*. A Scientific American book. W.H. Freeman and Co., San Francisco. pp. 69–80.

Dobereiner, J. (1977). N_2 fixation associated with non-leguminous plants. In *Genetic Engineering for Nitrogen Fixation*. Ed. A. Hollaender, Plenum Press, New York. pp 451–461.

Dobereiner, J., Marriel, I.E., and Nery, M. (1976). Ecological distribution of *Spirillum lipoferum*, Beijerinck. *Can J. Microbiol.* **22**, 1464–1473.

Dunican, L.K., O'Gara, R., and Tierney, A.B. (1976). Plasmid control of effectiveness in *Rhizobium:* Transfer of nitrogen-fixing genes on a plasmid from *Rhizobium meliloti* to *Klebsiella aerogenes*. In *Symbiotic Nitrogen Fixation in Plants*. Ed. P.S. Nutman. Cambridge University Press, London. pp. 77–90.

Evans, H.J., Emerich, D.W., Ruiz-Argueso, Maier, R.J., and Albrecht, S.L. (1980). Hydrogen metabolism in the legume-*Rhizobium* symbiosis. In *Nitrogen Fixation*, Vol. II. Eds. W.E. Newton and W.H. Orme-Johnson. University Park Press, Baltimore. pp. 69–86.

Fred, E.B., Baldwin, I.L., and McCoy, E. (1932). *Root Nodule Bacteria and Leguminous Plants*, Univ. Wisconsin, Madison, Wisc.

Gerretsen, F.C. (1948). The influence of microorganisms on the phosphate intake by the plant. *Plant and Soil*, **1**, 51–81.

Islam, R., Ayanaba, A., and Sanders, F.E. (1980). Response of cowpea (*Vigna unguiculata*) to inoculation with VA-mycorrhizal fungi and to rock phosphate fertilization in some unsterilized Nigerian soils. *Plant and Soil*, **54**, 107–117.

Joi, M.B., and Shinde, P.A. (1976). Response of onion crop to azotobacterization. *Maharashtra Agric. Universities*, **1** (2–6), 161–162.

Lehri, L.K., and Mehrotra, C.L. (1968). Use of bacterial fertilizers in crop production in U.P. *Curr. Sci.*, **37**, 494–495.

Lehri, L.K., and Mehrotra, C.L. (1972). Effect of *Azotobacter* inoculation on the yields of vegetable crops. *Indian J. Agric. Res.*, **9(3)**, 201–204.

Mehrotra, C.L., and Lehri, C.K. (1971). Effect of *Azotobacter* inoculation on crop yields. *J. Indian Soc. Soil Sci.*, **19(3)**, 243–248.

Mishustin, E.N., and Shilnikova, V.K. (1969). Free-living nitrogen fixing bacteria of the genus *Azotobacter*. In *Soil Biology: Reviews of Research*, UNESCO. pp 72–109.

Sethi, R.P., and Subba Rao, N.S. (1968). Solubilization of tricalcium phosphate and calciu phytate by soil fungi. *J. Gen. Appl. Microbiol.*, **14**, 329–331.

Shende, S.T., Apte, R.G., and Singh, T. (1977). Influence of *Azotobacter* on germination of rice and cotton seed. *Curr. Sci.*, **46**, 675.

Singh, R.N. (1961). *The Role of Blue-green Algae in Nitrogen Economy of Indian Agriculture.* Indian Council of Agricultural Research, New Delhi.

Sperber, J.I. (1957). Solution of mineral phosphates by soil bacteria. *Nature*, Lond., **180**, 994–995.

Subba Rao, N.S. (1976a). Field response of legumes in India to inoculation and fertilizer application. In *Symbiotic Nitrogen Fixation in Plants.* Ed. P.S. Nutman. Cambridge Univ. Press, London. pp 255–268.

Subba Rao, N.S. (1976b). Is Nitrogen Deficient? In *A Treatise on Dinitrogen Fixation,* Eds. R.W.F. Hardy and A.H. Gibson, Wiley Interscience, New York. pp. 1–32.

Subba Rao, N.S. (1977). *Soil Microorganisms and Plant Growth*, Oxford & IBH Publishing Co., New Delhi.

Subba Rao, N.S. (1979). Chemically and biologically fixed nitrogen — Potentials and prospects. In *Recent Advances in Biological Nitrogen Fixation.* Ed. N.S. Subba Rao, Oxford & IBH Publishing Co., Pvt. Ltd., New Delhi. pp. 1–7.

Subba Rao, N.S., Tilak, K.V.B.R., Singh, C.S., and Lakshmi Kumari, M. (1979a). Response of a few economic species of graminaceous plants to inoculation with *Azospirillum brasilense. Curr. Sci.*, **48**, 133–134.

Subba Rao, N.S., Tilak, K.V.B.R., Lakshmi Kumari, M., and Singh, C.S. (1979b). *Azospirillum* — A new bacterial fertilizer for tropical crops. *Sci. Rep.*, CSIR, (India), **16(10)**, 690–692.

Subramanyam, R., and Sahay, M.N. (1964). Observation on nitrogen fixation by some blue-green algae and remarks on its potentialities in rice culture. *Proc. Indian Acad. Sci.*, **60B**, 145–154.

Sundara Rao, W.V.B. (1964). Soil organic matter and microorganisms. *Bull. INSA*, **26**, 221–226.

Sundara Rao, W.V.B. (1968). Phosphorus solubilization by microorganisms, *Proc. All India Symp. on Agricultural Microbiology,* Univ. Agric. Sci. Bangalore, India. pp. 21–29.

Sundara Rao, W.V.B., Mann, H.S., Pal, N.B., and Mathur, R.S. (1963). Bacterial inoculation experiments with special reference to *Azotobacter. Indian J. Agric. Sci.*, **33**, 279–290.

Sundara Rao, W.V.B., Mann, H.S., Pal, N.B., and Mathur, S.P. (1966). Bacterial inoculation experiments with special reference to *Azotobacter. Ind. J. Agric. Sci.*, **33**, 279–290.

USDA (1957). *The Year Book of Agriculture.* The US Government Printing Office, Washington, DC.

Vekataraman, G.S. (1972). *Algal Biofertilizers and Rice Cultivation.* Today and Tomorrow Printers and Publishers, New Delhi.

Verghese, M.C. (1977). *Issues Facing the World Fertilizer Industry,* Proceedings of the FAI-IFDC Fertilizer Seminar 1977, Trends in Consumption and Production. The Fertilizer Association of India, New Delhi PS-1/1–41.