Current Developments in
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Edited by
N.S. SUBBA RAO

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Foreword

From time immemorial legumes have been of prime importance in Indian agriculture and together with millets and rice (associated with nitrogen-fixing blue-green algae), they form the staple of primary production. With this background of history and continuing recognition of the value of legumes, a book from New Delhi on nitrogen fixation is specially welcome.

The editor is exceptionally well qualified to organise and assemble the material of this volume having worked throughout his career on many aspects of nitrogen fixation in nodulated legumes and by free-living microorganisms. He has also been personally involved in a number of projects for legume improvement, notably the 'All India Coordinated Pulse Improvement Programme', the 'International Biological Programme on Nitrogen Fixation' and the 'Coordinated Project on Biological Nitrogen Fixation' of the Indian Council of Agricultural Research.

Over the last two decades there has been a remarkable resurgence of interest and research in biological nitrogen fixation caused by mounting worldwide concern over the depletion of energy resources. This was sharply accentuated by the 1973 and subsequent oil price rises and the consequent several-fold increases in the cost of nitrogen fertilizer, which has had a particularly serious impact upon the agriculture of developing countries. The research on nitrogen fixation stimulated by the energy crisis has led to a better assessment of its potential, and an appreciation of the need to promote its efficient use in agriculture by all possible means: by extending the areas of leguminous and non-leguminous nitrogen-fixing plants especially those with special relationships with free-living nitrogen-fixing microorganisms. Equally important is the need to increase the amounts of nitrogen fixed in these crops by the application of this newly gained knowledge as well as by ensuring their optimal nutrition and pest and disease control by better agronomy.

Agricultural statistics show that most crop legumes and forage plants fall far short of their potential yields and that in general those of the tropics fix appreciably less nitrogen than those of temperate agriculture. This disparity may reflect the larger environmental stresses experienced in the tropics, especially respecting temperature and water relation. It may also be partly a consequence of problems caused by symbiotic specificity. Because many tropical legumes belong to the 'cowpea miscellany', they are readily cross-infected and the consequent symbioses are not always the most effective. The widespread occurrence of naturally existing strains of *Rhizobium* of less than elite status present considerable problems in their replacement with better ones by inoculation.

Recent research has brought us to the verge of complete understanding of some of the most intractable problems of symbiosis and nitrogen fixation: the mechanism and biochemistry of the infection processes, the nature of specificity, the economy of nitrogen transportation and use within the plant and the interrelation of bacterial and host physiology and its genetic control. Some of these advances have come from the use of new techniques, notably the acetylene reduction assay for nitrogenase, the use of isotopes and the relatively new and powerful tools of microbial genetics. Some also have come from originality in outlook and interpretation.

New ideas and methodologies will doubtless promote new advances, but if the past is any guide, progress is most likely to be fostered by the multidisciplinary approach either by research teams or by individuals expert in more than one field.

This volume gives an up-to-date assessment of the present knowledge in some of these aspects of special interest, viz. the genetics, ecology, physiology and metabolism of certain nitrogen fixing systems; necessarily a single volume can cover only a part of so large a subject. As appropriate to a work of this scope the authors are cosmopolitan and do not restrict their interest to the tropical scene. Nevertheless students, research workers and agriculturalists attracted by the challenging problems of biological nitrogen fixation will find this volume valuable and stimulating.

P.S. NUTMAN, FRS
Great Hackworthy Cottage,
Tedburn, St. Mary, Exeter,
Ex 66 DIN
England

Preface

This volume on 'Current Developments in Biological Nitrogen Fixation' is an extension of the objectives with which the earlier volume on 'Recent Advances in Biological Nitrogen Fixation' was brought out a few years ago. The objective was to focus attention on the many research and developmental aspects of the unique process of microbiologically mediated reduction of dinitrogen of the atmosphere into ammoniacal form of nitrogen. In recent years, while the developing countries of the world are eagerly attempting to harness the practical aspects of the biological nitrogen-fixing processes on the farm front by field oriented technologies as a specific measure to relieve the stress on chemical fertilizers, the affluent nations are equally engaged in understanding the genetic control and the mechanism of fixation in biological nitrogen-fixing systems in general so as to pave the way for a new bio-technology to render the crop plants self-sufficient with regard to their nitrogen nutrition. As a result of these increased research activities, it was felt desirable and appropriate to take stock of the current situation again by summarizing the present state of art on some aspects of biological nitrogen fixation in the form of a book.

The nitrogen nutrition of nodulated legumes is dependent upon the effective rhizobial symbiosis with the appropriate host plant. In rain-fed tropical soils where most of the grain legumes are nodulated by *Rhizobium* sp. (cowpea miscellany), the competition with native ineffective rhizobia together with the stress factors operating on both the symbionts by way of high soil temperature and drought often tend to limit the formation of effective root nodules. These aspects of rhizobial survival in tropical soils form the basis of the first chapter written by Eaglesham and Ayanaba. A related subject is the interaction of nitrogen-fixing microorganisms in soil with other soil microorganisms. The associative and antagonistic factors operating in *Rhizobium* survival and performance in soil is governed by the activity of other microorganisms which perform other functions such as the solubilization of bound phosphates, provision of auxiliary hormones and growth factors or secretion of antibacterial substances. Among the beneficial associates, the vesicular-arbuscular mycorrhizal (VAM) fungi appear to be significant. The non-symbiotic nitrogen-fixing species, *Azotobacter chroococcum* is known to secrete an antifungal antibiotic substance active against plant pathogens. Many viral infections of leguminous plants diminish the benefits of root nodulation. Therefore, an integrated view of these interactions is taken by Subba Rao who has written the second chapter on microbial interactions with reference to biological nitrogen fixation.

The meeting point between a nitrogen-fixing microorganism and the host plant benefiting from root associations is the root surface. There are biochemical events which are very significant in the pre- as well as post-penetration stages of interactions between roots of plants and nitrogen-fixing microorganisms. One of the widely discussed factors operating in the pre-penetration stages is the specific protein called lectin which binds the surface of two symbionts of legume-*Rhizobium* symbiosis and determines the attachment of nitrogen-fixing bacteria to the root surface. Recently, however, an intense controversy has emerged on the involvement of lectins in legume-*Rhizobium* infection, a process which still remains highly enigmatic. Nevertheless Dazzo and his associate, Truchet who uphold the lectin mediated recognition phenomenon explain the recent developments on this aspect in the third chapter.

Nitrogen-fixing nodules on stems of plants would indeed be a convenient way to increase the surface area for plant-*Rhizobium* interaction and stem nodulation has indeed been recorded in the genera *Aeschynomene* and *Sesbania*. The fourth chapter on stem nodules by Subba Rao and Yatazawa highlights the current status of work on this aspect and points out the importance of extending research in this area.

Ironically, nitrogen fixation is an anaerobic process even in highly aerobic systems such as *Azotobacter chroococcum*. While this is acknowledged universally, precise explanations for oxygen control mechanisms have not come forth in any of the free-living systems. Needless to say, an understanding of the oxygen control mechanisms is a necessary prelude to achieving the transplantation of nitrogen-fixing bacteria into highly aerobic plant protoplasts. A review of the current knowledge on oxygen control mechanisms has been given by Shaw of New Zealand in the fifth chapter.

Genes responsible for nodulation and nitrogen fixation situated in megaplasmids have been demonstrated in *Rhizobium meliloti*. Being extrachromosomal in occurrence, plasmids offer tremendous potentiality as vehicles for *nif* gene transfer to higher plants. Although there is yet a long way to go in this exercise, Kondorosi and his colleagues explain in the sixth chapter how plasmids work in the expression of symbiosis in legumes.

Apart from cultivated legumes, forest trees such as alder (*Alnus* spp.) and *Casuarina* spp. benefit from nitrogen-fixing symbiosis through an actinomycetous microsymbiont designated as *Frankia*. The microsymbiont has been isolated successfully in *Alnus* and *Comptonia* and many of the physiological aspects of symbiosis have now been well understood. The seventh chapter by Wheeler summarizes the recent advances in the knowledge on *Frankia* symbiosis.

Lichens are composite structures consisting of blue-green algae and fungal symbionts, the nitrogen-fixing blue-green alga helping the fungal partner in nitrogen nutrition while the fungal partner provides protection for the survival of the alga even under desiccated condition. Lichens have been recognized as primary colonizers of rocks and exhibit an extreme capacity to survive under environmental hazards. They also serve as an excellent example of symbiosis. The intricate aspects of nitrogen fixation in lichens have been covered by Millbank in the eighth chapter.

In Brazil, sugar cane is grown on virgin soil requiring very little mineral nitrogen fertilizer. Biological nitrogen fixation has been demonstrated by finite methods in this crop and several non-symbiotic nitrogen-fixing bacteria have been implicated in this process. The role of bacteria in nitrogen fixation in sugar cane has been dealt with by Ruschel and Vose in the ninth chapter.

Wetland rice fields provide an ideal microhabitat for photosynthetic as well as heterotrophic nitrogen-fixing microorganisms which live and fix nitrogen under submerged conditions. The International Rice Research Institute, Manila, Philippines has carried out intensive work on this system and Watanabe and Roger provide an integrated information on this subject in the tenth chapter.

Graminaceous plants have nitrogen-fixing bacteria in their root system among which *Azospirillum* has been highlighted in recent years. The role of *Azospirillum* and other diazotrophic nitrogen-fixing bacteria in the nitrogen nutrition of grasses such as sorghum and millets has been substantiated from more than one laboratory in recent years. Many of the recent developments in this exciting area have been elucidated by Boddey and Döbereiner in the eleventh chapter.

The genetics of *Azotobacter* and *Azospirillum* is receiving increasing attention in recent years, more particularly on plasmid controlled molecular biology. From the world famous Pasteur Institute, Claudine Elmerich explains the methodology and results connected with this area of research in the twelfth chapter.

Admittedly, it is difficult to bring home all the developments in biological nitrogen fixation in a single handy volume but nevertheless, the present exercise highlights potential areas where significant advances are being currently made. In this task, I owe a great deal to the various contributors for their prompt compliance with my request in providing the manuscript. I wish to express my indebtedness to Dr. O.P. Gautam, Director-General, Indian Council of Agricultural Research, Dr. N.S. Randhawa, DDG(SAE), and Dr. H.K. Jain, Director, Indian Agricultural Research Institute, New Delhi for their kind encouragement. Finally, I will be failing in my duty if I do not say a word of thanks to my wife Gowri Subba Rao and my daughters

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N.S. SUBBA RAO
Microbiology Division,
Indian Agricultural
Research Institute,
New Delhi 110012

Contents

Contributors

A. Ayanaba, International Institute of Tropical Agriculture, Ibadan, Nigeria

R.M. Boddey, Programma Fixacao Biologica de Nitrogenio, EMBRAPA/ SALCS Seropedica, 23460, Rio de Janerio, Brazil

F.B. Dazzo, Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48824, USA

J. Döbereiner, Programma Fixacao Biologica de Nitrogenio, EMBRAPA/ SALCS Seropedica, 23460, Rio de Janerio, Brazil

I. Dusha, Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, H-6701, Szeged, P.O. Box 521, Hungary

A.R.J. Eaglesham, Boyce Thompson Institute, Tower Road, Ithaca, NY, 14853, USA

C. Elmerich, Physiology and Genetics Department, Pasteur Institute, 28, Rue Du D'Roux, 75724, Paris Cedex 15

G.B. Kiss, Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, H-6701, Szeged, P.O. Box 521, Hungary

A. Kondorosi, Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, H-6701, Szeged, P.O. Box 521, Hungary

J.W. Millbank, Department of Pure and Applied Biology, Imperial College of Science and Technology, London, SW7 2BB, UK

P.A. Roger, Office de la Recherche Scientifique et Technique Ontre Mer, France

A.P. Ruschel, CENA, Piracicaba, Sao Paulo, Brazil

B.D. Shaw, Plant Physiology Division, DSIR, Private Bag, Palmerston North, New Zealand

N.S. Subba Rao, Microbiology Division, Indian Agricultural Research Institute, New Delhi 110012, India

G.L. Truchet, Institut de Cytologie et Biologie Cellulaire, Faculte des Sciences Marseille—Lumimy L.A./C.N.R.S. 179, Marseille Cedex 2, 13288, France

P.B. Vose, CENA, Piracicaba, Sao Paulo, Brazil

I. Watanabe, Soil Microbiology Department, The International Rice Research Institute, P.O. Box 933, Manila, Philippines

C.T. Wheeler, Department of Botany, University of Glasgow, Glasgow, G12 8QQ, UK

M. Yatazawa, Faculty of Agriculture, Nagoya University, Chikusa, Nagoya, 464 Japan