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INTERNATIONAL BIOLOGICAL PROGRAMME 7

# **Symbiotic nitrogen fixation in plants**

EDITED BY

**P. S. Nutman**

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*Rothamsted Experimental Station,  
Harpenden, Hertfordshire, England*

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# Preface

‘... the leaven  
That spreading in this dull and clodded earth  
Gives it a touch ethereal – a new birth.’

The International Biological Programme has initiated six research programmes on particular aspects of symbiotic nitrogen fixation, and has also stimulated much work in related fields which this volume aims to bring together. The themes of the main programmes concern genetical aspects, culture collections, legume inoculants, field assessment, the environment and fixation in non-leguminous symbioses. These are dealt with here in this order except that the World Catalogue of *Rhizobium* collections, compiled by Professor O. N. Allen and Dr E. Hamatová and edited by Dr F. A. Skinner has already been published (by the IBP Central Office, London, 1973). Mention should also be made of the IBP handbook (no. 15, published by Blackwells Scientific Publications Ltd, Oxford, 1971) Prepared by Professor J. M. Vincent. Most of the chapters are based on papers read at the IBP Nitrogen Fixation Synthesis Meeting held at Edinburgh in September 1973.

A large part of the IBP programme on nitrogen fixation has profited directly from important theoretical and technical developments, especially in microbial genetics, which have occurred in the last decade, largely independently of IBP, and these in their turn have interacted with the IBP programmes and also stimulated much further research. With so much and so varied work in progress the theme co-ordinators and editors thought it unrealistic to restrict this volume to reports of IBP programmes; to have done so would have ignored interesting and important new developments.

For these reasons this volume contains a variety of chapters which when taken collectively portray the current state of knowledge relevant to the different IBP themes. Also reported is a range of other work, some entirely new, that was not even a gleam in the eye of the Scientific Director when SCIBP was conceived. Some of the more controversial aspects of recent work were discussed at an open session at the Edinburgh meeting, a transcript of which forms the volume's final chapter.

Although this approach may lack the virtues of balance, and even of uniform excellence, this volume should nevertheless provide for

## *Preface*

some years to come valuable source material in wide-ranging fields of research and starting points for future work in symbiotic nitrogen fixation, whether by individuals or in other international programmes. It has also underlined and in some respects quantified for the first time the overriding importance of symbiotic nitrogen fixation in natural and agricultural habitats, and its crucial role in bridging the protein gap.

We are all now acutely aware of the need to conserve energy and although this topic is directly referred to only occasionally in the chapters that follow, it underlies all those concerned with the efficiency of the nitrogen fixing processes, especially in agricultural legumes, and is pertinent to the relationship between fixation and fertiliser use.

*Rothamsted, Harpenden,  
Herts, 1974*

P. S. NUTMAN

# Contents

<i>List of contributors</i>	page xxi
<i>Preface</i>	xxvii

## Part I. Genetical aspects and taxonomy

1 Recent advances in the genetics of nitrogen fixation <i>R. A. Dixon &amp; F. C. Cannon</i>	3
2 Genetic analysis of nitrogen fixation in <i>Klebsiella pneumoniae</i> <i>K. T. Shanmugam &amp; R. C. Valentine</i>	25
3 Control of nitrogenase synthesis in <i>Azotobacter vinelandii</i> <i>W. J. Brill</i>	39
4 Effects of some mutations on symbiotic properties of <i>Rhizobium</i> <i>J. Dénarié, G. Truchet &amp; B. Bergeron</i>	47
5 Transduction of effectiveness in <i>Rhizobium meliloti</i> <i>M. Kowalski</i>	63
6 Genetic transformation in <i>Rhizobium japonicum</i> <i>F. Doctor &amp; V. V. Modi</i>	69
7 Plasmid control of effectiveness in <i>Rhizobium</i> : transfer of nitrogen-fixing genes on a plasmid from <i>Rhizobium trifolii</i> to <i>Klebsiella aerogenes</i> <i>L. K. Dunican, F. O'Gara &amp; A. B. Tierney</i>	77
8 The demonstration of conjugation in <i>Rhizobium leguminosarum</i> <i>J. E. Beringer</i>	91
9 Identification and classification of root nodule bacteria <i>P. H. Graham</i>	99
10 The nodulation profile of the genus <i>Cassia</i> <i>Ethel K. Allen &amp; O. N. Allen</i>	113

## Part II. Quality of legume inoculants

11 The production of high quality inoculants and their contribution to legume yield <i>R. J. Roughley</i>	125
12 Principles of <i>Rhizobium</i> strain selection <i>R. A. Date</i>	137

## Contents

13	Carriers of rhizobia and the effects of prior treatment on the survival of rhizobia <i>B. W. Strijdom &amp; C. C. Deschodt</i>	151
14	A method of making a pure-culture, peat-type, legume inoculant, using a substitute for peat <i>H. D. L. Corby</i>	169
15	Methods of inoculating seeds and their effect on survival of rhizobia <i>J. C. Burton</i>	175
16	Some studies on the necessity of legume inoculation in Serbia (Yugoslavia) <i>Z. D. Vojinović</i>	191
17	Agar and peat inoculation efficiency in Bulgaria <i>L. Raicheva</i>	199
18	Yield responses of soybean, chickpea, pea and lentil to inoculation with legume inoculants <i>J. N. Dube</i>	203

## Part III. Field experiments on nitrogen fixation by nodulated legumes

19	IBP field experiments on nitrogen fixation by nodulated legumes <i>P. S. Nutman</i>	211
20	Influence of inoculation, nitrogen fertilizers and photosynthetic source-sink manipulations on field-grown soybeans <i>G. E. Ham, R. J. Lawn &amp; W. A. Brun</i>	239
21	Field response of legumes in India to inoculation and fertiliser applications <i>N. S. Subba Rao</i>	255
22	Effect of inoculation and fertiliser application on the growth of soybeans in Rumania <i>C. Hera</i>	269
23	Inoculation and nitrogen fertiliser experiments on soybeans in Cuba <i>E. Sistachs</i>	281
24	Field and greenhouse experiments on the response of legumes in Egypt to inoculation and fertilisers <i>Y. A. Hamdi</i>	289

- 25 Application of the acetylene reduction technique to the study of nitrogen fixation by white clover in the field  
*C. L. Masterson & P. M. Murphy* 299

**Part IV. Legume nitrogen fixation and the environment**

- 26 Symbiotic specialisation in pea plants: some environmental effects on nodulation and nitrogen fixation  
*T. A. Lie, D. Hille, Ris Lambers & A. Houwers* 319
- 27 Physiology of the reaction of nodulated legumes to environment  
*J. S. Pate* 335
- 28 Symbiosis in tropical grain legumes: some effects of temperature and the composition of the rooting medium  
*P. Dart, J. Day, R. Islam & Johanna Döbereiner* 361
- 29 Recovery and compensation by nodulated legumes to environmental stress  
*A. H. Gibson* 385
- 30 Nitrogen fixation by legumes subjected to water and light stresses  
*Janet I. Sprent* 405
- 31 Photosynthate as a major factor limiting nitrogen fixation by field-grown legumes with emphasis on soybeans  
*R. W. F. Hardy & U. D. Havelka* 421

**Part V. Nitrogen-fixing symbioses in non-leguminous plants**

Preface

*G. Bond*

- 32 The results of the IBP survey of root-nodule formation  
*in non-leguminous angiosperms*  
*G. Bond* 443
- 33 Symbiotic interactions in non-leguminous root nodules  
*A. F. Angulo, C. van Dijk & A. Quispel* 475
- 34 Ultrastructural studies of non-leguminous root-nodules  
*Isobel C. Gardner* 485
- 35 Nitrogen fixation in root nodules of alder and pea in relation to the supply of photosynthetic assimilates  
*C. T. Wheeler & Ann C. Lawrie* 497

## Contents

36	The formation and nitrogen-fixing activity of the root nodules of <i>Alnus glutinosa</i> under field conditions <i>A. D. L. Akkermans &amp; C. van Dijk</i>	511
37	Endophyte adaptation in <i>Gunnera-Nostoc</i> symbiosis <i>W. B. Silvester</i>	521
38	Nitrogen fixation in some natural ecosystems in Indonesia <i>J. H. Becking</i>	539
39	Bacterial leaf symbiosis and nitrogen fixation <i>C. van Hove</i>	551
40	A discussion of the results of cross-inoculation trials between <i>Alnus glutinosa</i> and <i>Myrica gale</i> <i>C. Rodriguez-Barrueco &amp; G. Bond</i>	561
41	The possibility of extending the capacity for nitrogen fixation to other plant species. A summary of proceedings at an open session held during the Edinburgh meeting. Chairman: Professor G. Bond	567
	<i>Index</i>	573

**PART I**

**Genetical aspects and taxonomy**





# 1. Recent advances in the genetics of nitrogen fixation

R. A. DIXON & F. C. CANNON

Although biochemical studies of nitrogen fixation have become well-established in the past decade, only in recent years has it been possible to apply molecular genetics to studies involving nitrogen fixation. Because of the importance of symbiotic nitrogen fixation in agriculture, many workers have studied the genetics of the microsymbiont *Rhizobium*, but due to the complexity of the root nodule symbiosis, genetic studies of nitrogen fixation have been restricted to the phenotypic level. The recent development of methods of transfer of the nitrogen fixation genes in free-living bacteria and analysis of mutants defective in nitrogenase has facilitated a molecular genetic approach that will no doubt be extended to symbiotic systems.

This account will be primarily concerned with genes which determine synthesis of the enzyme nitrogenase (*nif* genes) and with associated determinants which allow nitrogen fixation to occur *in vivo*; it does not aim to provide a complete synthesis of data concerning the genetics of nitrogen fixation as more comprehensive reviews of this subject have been published elsewhere (Brill, 1974; Schwinghamer, 1974). In symbiotic systems such as legume root nodules, the interaction between *Rhizobium* and host requires both bacterial and plant gene products. The genetics of the host plant have been discussed by Nutman (1969) and will not be considered here.

## Isolation of *nif*<sup>-</sup> mutants

This section will relate specifically to mutants deficient in the enzyme nitrogenase. Undoubtedly, many other mutations will result in a *Nif*<sup>-</sup> phenotype; these will be discussed later. Since rhizobia have not been observed to fix nitrogen in the absence of the host plant, screening methods for isolating *nif*<sup>-</sup> mutants in this system must involve plant tests. Thus, to date it has not been possible to determine whether *Rhizobium* strains with altered symbiotic activity have mutations specifically in *nif* genes; indeed the origin of *nif* genes in this system has not yet been determined (see later section). The first *nif* mutants characterised at the biochemical level were of *Azotobacter vinelandii* (Fischer & Brill, 1969;