

# Legume Nodulation

## A Global Perspective

**Janet I. Sprent**

Emeritus Professor of Plant Biology, University of Dundee, UK  
Honorary Research Fellow, Scottish Crop Research Institute,  
Invergowrie, Dundee, UK

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*To all my friends, students and colleagues of the last 50 years, but especially to my long-suffering husband Peter.*

*'Leguminosae are one of the great lines of dicotyledonous evolution. They far exceed the idea of an order or family except within the narrow confines of nomenclature. The neap tide of modern botany never uncovers its riches. Legumes invest our lives and a feeble backwash seeps through our universities' (Corner, 1976).*





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*Color plate section between pages 86 and 87*



# Preface

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Since publishing my book 'Nodulation in Legumes' in 2001, many people have kindly suggested that I write a second edition. For various reasons I have decided not to do this, but rather attempt to put nodulated legumes into a wider context.

In the last few years several major works have appeared that have made the writing of this book easier and to which I have extensively referred. Foremost amongst these is 'Legumes of the World', edited by Gwil Lewis and colleagues at Kew. In addition to the magnificent photographs of all legume genera, and descriptions of them, the volume has extensive biogeographical coverage. A series of books has been published by Springer on 'Nitrogen Fixation, Origins, Applications, and Research Progress', under the general editorship of Bill Newton, but with individual volumes having additional editors. Unfortunately the volumes had a rather long gestation period, so that although there is a vast amount of material in them that remains important, in some cases (for example, the bacteria known to nodulate legumes), there is a large body of more recent information. On the ecological side, several important volumes have been published, one of which, 'Neotropical Savannas and Seasonally Dry Forests', edited by Toby Pennington and colleagues, has a wealth of information on the distribution of legumes in these important ecosystems. Overall, the chapters in these various volumes, all of which are written by international experts, provide a level of detail that is not attempted here. Rather I have attempted to summarise current knowledge and go into detail of aspects that are less well covered.

In trying to put a more global perspective on legume nodulation, I have had several aims. The first is to demonstrate that nodulated legumes are found in all habitats and in nature do not conform to many of the rules accepted by agronomists. With current interest in global change and in protecting fragile ecosystems, it is important to realise what nodulated legumes can and cannot do. I have also tried to counter some of the widely quoted generalizations – for example, that tropical legumes have determinate nodules and export ureides (most have indeterminate nodules and export amides) and that the polymer of  $\beta$ -hydroxybuturate (PHB) is necessarily associated with bacteroids of such genera (I argue that it is a measure of inefficiency in bacteroids of nodules from many legumes).

Deciding on a logical order for the chapters was difficult. In the end I thought that it was best to start with the plant taxonomy to give an up-to-date picture of the distribution of nodulation within the family at a generic and tribal level, which has changed somewhat since my 2001 book. I have made no attempt to list all the non-nodulated

species, but have included all the known nodulated species in appendices, with an introductory 'health warning'. Species names, where possible, have been checked against the International Legume Database and Information Service (ILDIS), but for various reasons, particularly shortage of funding, parts of this are not entirely up to date and where this is the case, I have checked with relevant taxonomic experts. In this chapter I have also given a general indication of the types of bacteria that may nodulate various genera, but it is not possible to do this in a comprehensive fashion, as new reports are coming out regularly. It is planned to produce an electronic interactive database of this information when the current volume has gone to press. Chapter 2 covers some aspects of the global distribution of legumes, largely based on the biomes described in Schrire et al. (2005). Again there are a number of systems that are not covered, usually because of insufficient information being available. Chapter 3 outlines some of the current ideas on evolution of nodulation, based on a number of recent reviews. The bacteria currently known to nodulate legumes are described in Chapter 4. New species are coming out almost weekly, with several reports being in press as this book goes to press. In this chapter I have also emphasised that isolating bacteria from nodules is not sufficient to confirm nodulating ability; Koch's postulates must be established. Reports of nodulation in some legume genera (Chapter 1) and of bacteria purporting to nodulate legumes have not always been reliable, and I have tried to indicate where this might be the case. Chapter 5 attempts to put the plants and bacteria together, discussing nodule development and functioning, but without going into the detail given in major recent reviews and chapters in other volumes. At a late stage in the book's planning I decided to end with a short chapter on possible development of new uses for legumes and for more work on underdeveloped legume crops. In spite of the noble effort of the U.S. National Academy of Sciences, there is still an overemphasis on major economic crops such as soybean and peanut, whereas in many areas of the world, other legumes may be better adapted to the local environment and also be more acceptable to local people.

I have intentionally allowed some overlap in content between chapters, as I believe that this will make for easier reading for those wanting to concentrate on particular topics. Measurement of nitrogen fixation in the field is fraught with difficulties and in many areas effectively impossible. The fact that I have not attempted to do this does not mean that I do not think that legumes nodules do not have a vital role to play in nutrient cycling.

In recent years, there has been a tendency for both the legume and rhizobial fraternities to be isolated from each other. This has been an inevitable consequence of current research conditions and the need to publish in either highly specialised journals or in the top group of journals such as *Nature* and *Science*. This has had some unfortunate consequences, as journals tend to use referees with a rather narrow focus, in the case of nodulated legumes with either plant or microbial specialists who may not appreciate the other's disciplines. Some disciplines such as whole plant physiology and soil science have been neglected in favour of new molecular studies. Careful, old-fashioned fieldwork, especially in difficult terrains, gets few brownie points these days, as it may take years of work to produce a set of statistically significant data. However, with the recognition that the climate really is changing and that food supplies cannot be guaranteed, there is a move to bring people of different disciplines together. I hope that

this book may be of some help, by being comprehensible and sufficiently jargon free to interest soil microbiologists, plant physiologists, taxonomists and ecologists and well as agronomists. A basic understanding of biology is assumed and there are extensive references to the literature so that readers can indulge their interests in particular topics. To do justice to them all would have required a very large volume, so I have erred on the cautious side.

It is impossible without writing another book on the subject to thank the many people who have helped me over the years. Just to give an indication, at one end of the age scale is my old friend 'Tom' (H.D.L.) Corby, whose pioneering work on nodule morphology when he was working in what is now Zimbabwe inspired me and many others to expand on this topic. I still have Tom's collection of nodules and was delighted to catch up with him, now 95 and firing on all cylinders, in Cape Town in 2008. At the other end of the age scale I count the many young students, for example in Murdoch University in Western Australia, who are a joy to talk to. In between there are new contacts, working in some of the extreme conditions that so urgently need study, such as H.S. Gehlot, working in the Thar desert in India and Wenfeng Chen from Beijing working in the Qinghai Tibetan plateaux at 4000 to 5000 m and who, though I have not yet met them, have been generous in supplying information and photographs. More recently Ben-Eric van Wyk has introduced me to the wonderful world of South African legumes. I hope the hundreds more that I could have included will forgive their absence from this list, but I must mention Euan James, an unfortunate casualty of the reorganization of Life Sciences in Dundee, whose expertise and collaboration has been invaluable for years, but especially in recent research and publications. Euan and many others have generously supplied me with photographs, and my husband Peter constructed the diagrams. Kate Nuttall and Nigel Bamforth at Blackwell/Wiley have patiently coped with my various idiosyncrasies and steered the volume through its gestation period.





## Chapter 1

# Nodulation in a Taxonomic Context

---

In 2005, Lewis et al. published their comprehensive and beautiful book 'Legumes of the World', in which all genera then known are described and at least one species of each illustrated. In addition, the book contains a very interesting account of legume biogeography, which will be the subject of Chapter 2. The general terminology of Lewis et al. (2005) will be followed here, i.e. the family Leguminosae (or Fabaceae as some prefer) is divided into three sub-families, each of which is divided into tribes. Table 1.1 summarises these tribes and the numbers of genera and species within them. Since, with a very few known exceptions, detailed where appropriate, nodulation is a generic characteristic, for the purposes of the following discussion all species within a genus are presumed to nodulate, even though the number recorded as nodulated may be far less than the total (Tables 1.4 to 1.17). At various times when genera have been divided, it has unwittingly also been on presence or absence of nodulation (Table 1.2). Many of the more recently described genera in sub-families Mimosoideae and Papilionoideae have been segregated from others that can nodulate, but often there is no information on the nodulation status of the new combinations. Whilst, because of their taxonomic position, many of these are likely to be able to nodulate, in view of the examples given in Table 1.2, it certainly cannot be taken for granted.

In all three sub-families, there is active research on tribal and generic details. The authors of the various chapters in Lewis et al. (2005) present the current situation as they see it, pointing out anomalies without taking a position, because one of their aims is to stimulate research. In this sense, their book can be described as covering 'work in progress'. In some cases nodulation characteristics may add some clarity, and this will be attempted in the present chapter. A further complication is that the current ideas of taxonomy, phylogeny and evolution are not entirely congruent with known major alterations in the chloroplast genome. Doyle (1995) outlined the major changes of importance for legumes, of which two are relevant here. Chronologically the first is the inversion of a 50kb section of chloroplast DNA. This is absent from Caesalpinioideae and Mimosoideae, but present in most Papilionoideae. The second is the loss of a large duplicated and inverted section of the genome, present in nearly all land plants, and known as the inverted repeat (IR). Liston (1995) carried out an extensive survey of this feature of legumes, which is largely confined to the more advanced papilionoid

**Table 1.1** Summary of tribes, genera and species in the three subfamilies of Leguminosae, mainly as given in Lewis et al (2005), except that Mimosoideae is included here in Mimoseae and some recent changes in Crotalariaeae are included. Numbers of species are approximate

Caesalpinioideae			
Tribe	Genera	Species	Comments
Caesalpinieae	56	429	7 gen. known to nodulate 171 spp.
Cassieae	21	73	One nodulating genus 330 spp.
Cercideae	12	335	May be a separate branch
Detarieae	82	747	None known to nodulate
Mimosoideae			
Tribe			
Acacieae	1	1450+	Subdivision in hand
Ingeae	36	950	~300 in <i>Inga</i>
Mimoseae	41	870	~500 in <i>Mimosa</i>
Papilionoideae			
Tribe			
Abreae	1	17	
Amorpheae	8	246	165 in <i>Dalea</i>
Bossiaeeae	6	72	
Brongniartieae	10	151	
Cicereae	1	43	
Crotalariaeae	12	1120	Subject to revision
Dalbergieae	49	1324	250 in <i>Dalbergia</i>
Desmodieae	30	527	275 in <i>Desmodium</i>
Dipterygeae	3	22	Non-nodulating
Euchrestieae	1	4	
Fabeae	5	328	Formerly Vicieae
Galegeae	24	2929	2300+ in <i>Astragalus</i>
Genisteae	25	561	225 in <i>Lupinus</i>
Hedysareae	12	426	140–180 in <i>Hedysarum</i>
Hypocalypteae	1	3	
Indigoferae	7	768	700 in <i>Indigofera</i>
Loteae	22	282	Includes Coronillieae
Millettieae	45	908	350 in <i>Tephrosia</i>
Mirbelieae	25	687	
Phaseoleae	89	1576	230 in <i>Rhynchosia</i>
Podalyrieae	8	125	
Psoraleae	9	185	
Robinieae	11	71	
Sesbanieae	1	60	
Sophoreae	45	295	Polyphyletic
Swartzieae	17	258	Polyphyletic
Thermopsidaeae	6	45	
Trifolieae	6	425	

**Table 1.2** Generic changes that have unwittingly included nodulation. Nodulating genera in bold type. They include one genus from each sub-family

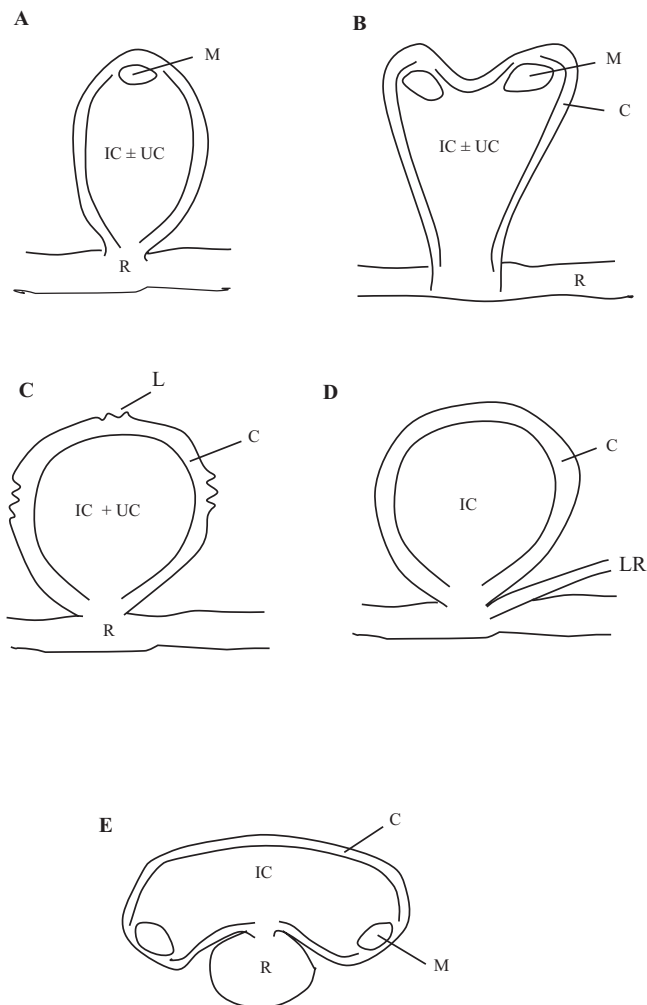
Old genus	New genera	Reference
<i>Cassia</i>	<i>Cassia</i> , <i>Senna</i> , <b><i>Chamaecrista</i></b>	Irwin & Barneby, 1982
<i>Newtonia</i>	<i>Newtonia</i> , <b><i>Pseudopiptadenia</i></b>	Lewis & Lima, 1991
<i>Sophora</i>	<b><i>Sophora</i></b> , <i>Styphnolobium</i>	Sousa & Rudd, 1993

tribes, but which also occurs in some others, causing some difficulties for classification, discussed later. As each of these two changes is thought to have occurred only once, it is hoped that when more genera have been analysed for them, their presence/absence will help clarify some anomalies. Although very important for legume phylogeny, there are no known nodulation characteristics involved in these chloroplast genome changes (or, indeed, in the chloroplast genome at all).

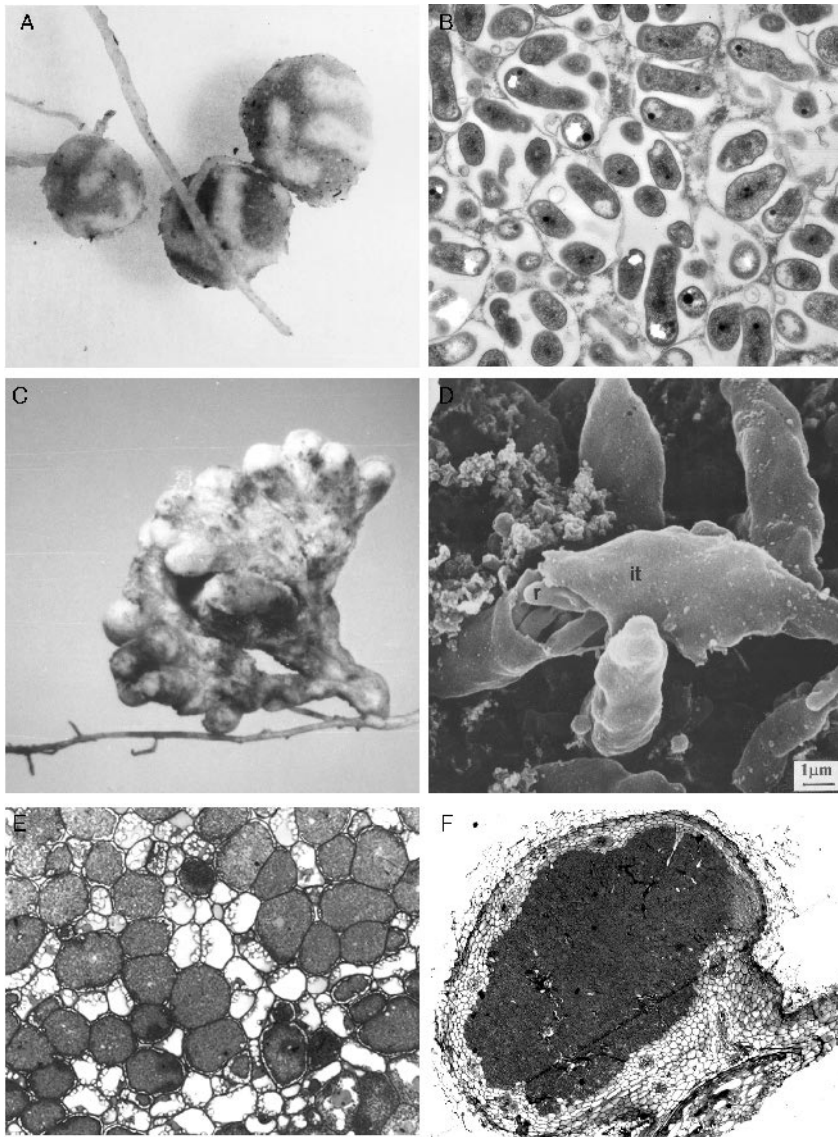
In the following sections, brief reference will be made to nodule morphology and structure (Figs. 1.1 and 1.2), and to the bacteria inducing nodules, detailed in Chapter 4. Basically, bacteria nodulating legumes are known collectively as rhizobia, and they fall within several families of two branches ( $\alpha$  and  $\beta$ ) of phylum Proteobacteria. Earlier they were often categorised in terms of fast or slow growth and these terms will also be used here.

## 1.1 Caesalpinioideae

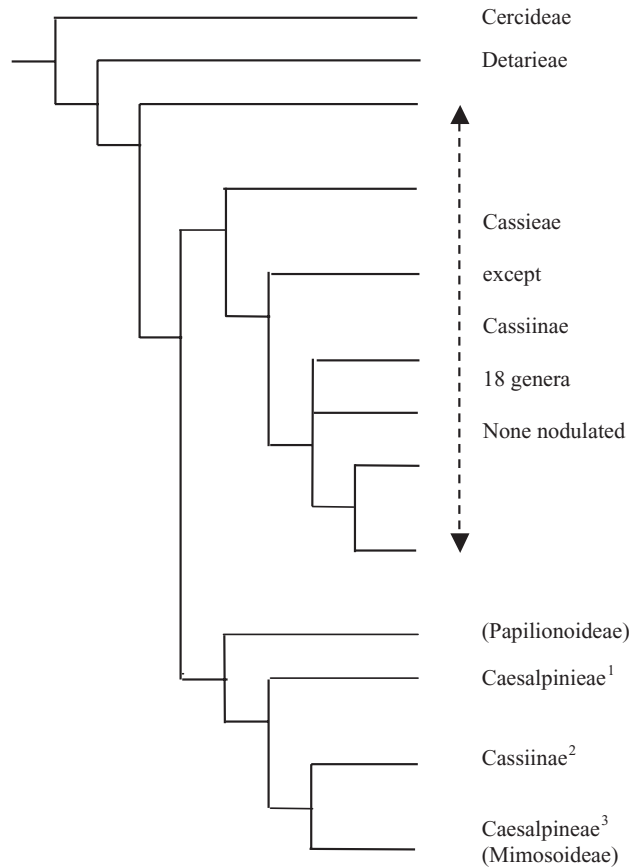
This sub-family has long been known to contain the smallest proportion of nodulated species (Allen & Allen, 1981). However, it is worth re-examining the distribution of nodulation in the light of currently described tribes. Cercideae and Detarieae are basally branched from the rest of Caesalpinioideae (Fig. 1.3): neither has known nodulated members and Detarieae is uniformly ectomycorrhizal. Most legumes are arbuscular mycorrhizal (AM) or, in some cases, have both types. Tribe Cassieae has one nodulating genus, *Chamaecrista*. However, this has 330 species, a significant number of which have been recorded as nodulated and none as non-nodulated. This genus represents nearly half the species in the tribe. Further, sub-tribe Cassiinae, which contains *Chamaecrista*, appears to fall within the confines of tribe Caesalpinieae (Lewis, 2005a; Fig. 1.3), which contains all other known nodulating genera. Caesalpinieae has been divided into a number of groups, some of which contain only non-nodulating genera. Nodulated genera are scattered among several groups, with no apparent logic. In a more recent study, Bruneau et al. (2008) sampled all but one of the caesalpinoid genera, but with varying levels of rigour because of the availability and quality of DNA. This analysis clarified the relations among many of the genera. Unfortunately the nodulated genera remain scattered and the hope expressed by Haston et al. (2005) that, with the inclusion of more molecular characteristics, the nodulating genera may emerge as more closely related than generally thought has not yet been fulfilled. One generic change



**Figure 1.1** The arrangement of tissues in the major types of nodule. **A** and **B** are indeterminate forms (see also Fig. 1.2C), with a single or a branched apical meristem. Nodules that appear similar in morphology may have uniform infected tissue or a mixture of infected and uninfected cells, according to taxonomic position. **C**, desmodioid (determinate) nodule (Fig. 1.2A), with infected tissue always containing uninfected cells. **D**, aeschynomenoid nodule as found in legumes from the Dalbergioid clade. These are always associated with lateral or adventitious roots and have uniform infected tissue. **E**, a variant of an indeterminate nodule containing only infected cells in the infected region, but with two lateral meristems, resulting in a ‘collar’ or lupinoid nodule that encircles the subtending root (occasionally stem). Only known from a few Genistoid legumes. (M, meristem; C, nodule cortex, containing vascular tissue; R, subtending root; LR, lateral root; IC infected cells; UC uninfected cells.) (Modified from Sprent, 2007.)



**Figure 1.2** Major types of legume nodule. **A**, desmodioid nodule as found in the phaseoloid tribes (Table 1.15, and some members of tribe Loteae (Table 1.16). Lenticels are characteristic. Nodules vary from 2 to 5 mm in diameter and the infected tissue contains both infected and uninfected cells (Fig. 1.1C). **B**, symbiosomes; these membrane-bound structures may contain 1 to 8 bacteroids (the nitrogen-fixing form of rhizobia). **C**, a much-branched indeterminate nodule, as found in many species from all three subfamilies. Other indeterminate nodules may be much less branched or unbranched, and the infected tissue may contain only infected cells or a mixture of infected and uninfected cells (Fig. 1.1A, B), a taxonomic characteristic. Size varies from 3 mm to several cm in length. **D**, a broken modified infection thread (IT), often called a fixation thread, showing bacteroids. This is considered a primitive state in which bacteria are not released into symbiosomes and is found in caesalpinoid and some papilionoid nodules. **E**, infected tissue containing a mixture of infected (darkly stained) and uninfected cells (see Fig. 1.1). **F**, infected tissue containing only infected cells. Infected cells in either **E** or **F** arrangements may be highly vacuolate in some species. (From Sprent, 2007.)



<sup>1</sup> Pro parte; 7 genera, none nodulated

<sup>2</sup> 3 genera, including *Chamaecrista*

<sup>3</sup> Pro parte; 49 genera, including all 7 nodulated ones

**Figure 1.3** Possible relationships between nodulated and non-nodulated groups in the Caesalpinioideae. The position of Papilionoideae in this diagram can be ignored. (After Lewis, 2005a.)

since Sprent (2001) is that *Sclerolobium* has been incorporated into *Tachigali*. Both can nodulate, although this was not one of the properties used in the reclassification. The seven genera known to nodulate (*Campsiandra*, *Chidlowia*, *Dimorphandra*, *Erythrophleum*, *Melanoxydon*, *Moldenhawera* and *Tachigali*) account for 171 of the 429 species in tribe Caesalpinieae. Again, this is a significant proportion, equal or exceeding that in some tribes of the Papilionoideae. All nodulating caesalpinoid genera, except *Chidlowia*, a monotypic genus from tropical Africa, and *Erythrophleum*, which is found in Africa and Australia, together with some herbaceous pan-tropical species of *Chamaecrista*,