The contribution of R & D on root-nodule bacteria to future cultivars of tropical forage legumes

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Abstract

The importance of the contribution of nodulated legumes to the nitrogen economy of crops and pastures is recognised. Maximising the opportunities and utilising the legume — root-nodule bacteria (RNB) symbiosis requires an understanding of the genetic or specificity relationship between legumes and RNB for effective, N-fixing nodulation. Recognition of the need to inoculate a legume and the skills required to assess this and, where necessary, to select appropriate strains is discussed. A brief description of a current project is used to illustrate the selection of new strains of RNB for a legume that does not nodulate effectively with existing soil strains.

Introduction

The major attribute of legumes that makes them important species in the crop and pasture industries is their ability to convert inert atmospheric nitrogen to an organic form that can be used by plants. The ability to make this conversion resides in the symbiosis between the legume and soil bacteria of the genera *Rhizobium* and *Bradyrhizobium*. These bacteria are referred to collectively as root-nodule bacteria (RNB) since they actively invade the root to form nodules in which the atmospheric nitrogen is reduced to ammonia before being transported to leaves as amino (asparagine, glutamine, arginine) or ureide (allantoin, allantoic acid) compounds. The reduction process is referred to as "fixation". When this process functions efficiently the symbiosis is said to be effective, and when little or no nitrogen is fixed, it is ineffective. Nitrogen added to the biosphere in this way has 2 major advantages: (i) it serves as a replacement for costly fertiliser nitrogen; and (ii) in an agricultural and environmental context, it is readily available but stable and of minimal pollution potential.

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Specificity of the legume-RNB symbiosis

The level of specificity between the legumes and RNB varies considerably. Genotype interactions for nodulation and effectiveness of nitrogen fixation occur within species (*e.g. Stylosanthes*, Date and Norris 1979; Date *et al.* 1993) and between species (*e.g. Desmodium intortum* and *D. uncinatum*, Date 1977). For practical purposes, legumes fall into 3 classes in respect of their specificity for effective nodulation (Date 1977):

(i) Those that are nodulated effectively by a wide range of strains from other hosts in the group. They are referred to commonly as being promiscuous (PE). Examples include: *Cajanus cajan*, *Macroptilium atropurpureum*, *Calopogonium mucunoides*, *Neonotonia wightii*, *Stylosanthes guianensis* (except *S. guianensis* var. *intermedia*), *S. hamata* (tetraploid accessions), *S. humilis*, *S. scabra*, *S. viscosa* and most species of *Vigna*.

(ii) Those that are nodulated effectively by strains from within the genus and generally ineffectively nodulated by RNB from other genera in the group. These are known as promiscuous ineffective (PI) and include: *Desmanthus virgatus*, *Desmodium intortum*, *D. uncinatum*, *Leucaena leucocephala*, *L. diversifolia* and *Stylosanthes guianensis* var. *intermedia*.

(iii) Those that require specific strains from the same species for effective nodulation and frequently do not even nodulate with strains from other hosts in the group. These are referred to as specific (S) and include: *Lotononis bainesii*, "Stylosanthes seabrana" and *Trifolium semipilosum*.

Resources and skills

Given the range of specificity between genotypes of RNB and genotypes of legumes and the fact that most legumes used in Australian agricultural production are either deliberate or accidental imports, the probability of matching indigenous RNB to the imported legume is less than 60%. This situation is exacerbated by the fact that the soil microbiologist is requested frequently to find a suitable strain of RNB for a legume that has already been selected, thus reducing the amount of genetic diversity that could be exploited. Ideally, selection of RNB and new cultivars of legume should be combined; however, this is not always possible. To offset this disadvantage, the ATFGRC has developed and maintains a large RNB germplasm collection, in parallel with its plant germplasm collection. The RNB collection is targetted specifically at legumes in the PI and S groups. A collection of more than 4700 strains of RNB (Table 1) is available for matching with new accessions of legume.

Table 1. Number of RNB for major tropical forage legume genera in the ATFGRC collection.

Major genera represented	Number of species	Number of *Rhizobium* and *Bradyrhizobium* strains
Acacia	10	19
Arachis	6	74
Cajanus	2	35
Calopogonium	3	41
Centrosema	9	164[1]
Desmanthus	6	146[1]
Desmodium	29	380[1]
Glycine	10	132[1]
Indigofera	15	47
Leucaena	11	255
Lotononis	6	30
Lotus	7	13
Macroptilium	8	90[1]
Macrotyloma	5	9
Medicago	8	40
Neonotonia	1	25
Phaseolus	3	48
Psoralea	6	29
Sesbania	11	51
Stylosanthes	21	1813[1]
Teramnus	4	29
Trifolium	24	154
Vigna	18	138
Others (70)	158	968
Total (94)	381	4730

[1]Targetted genera.

In order to improve the probabilities of matching RNB to plants, the first phase in selecting RNB for a new legume cultivar involves growing plants aseptically in N-free culture. Large numbers of strains are screened and the number reduced to a manageable level for further testing. Current studies include the use of molecular marker (PCR-RAPD) techniques (Liu and Date 1997) and strain provenance information (R.A. Date, unpublished data) to help in this aspect by identifying strains with similar genetic and ecological backgrounds before committing resources to plant assays. The most effective strains from this phase are next assessed in soil-pot assays with several representative soils to determine ability to colonise the soil and rhizosphere and to form nodules in competition with any existing soil strain(s) (Phase 2). The response of the best strains is confirmed in field trials to ensure a high level of saprophytic competence, and the ability to nodulate in successive growing seasons and increase plant yield (Phase 3).

Need-to-inoculate and selection of RNB

From a series of trials completed over the last 20 years, we have developed a database of response information that indicates which legumes require inoculation with selected strains of RNB in order to achieve effective symbiosis. We refer to this series as need-to-inoculate trials. They are simple 3-treatment experiments that have an uninoculated control, a treatment in which seed is inoculated with a known effective strain of RNB and finally an added-nitrogen control (Date 1977; 1982). The information from such trials provides essential basic information about the RNB requirements for the legume and about the presence or absence and performance of indigenous soil RNB. Thus:

- The uninoculated control will determine whether native rhizobia are present, and if so, their potential for nitrogen fixation by comparison with plant yield of the inoculated and +N control treatments.
- The inoculated treatment provides information on the performance of the inoculum strain relative to the +N control and, if nodules are examined, some indication of its competitive ability to form nodules when native strains are present. If growth is poor and nodules present, then the selected strain fails to colonise and

compete with native soil strains, indicating a need to select more competitive strains of inoculum.

- The +N control ensures that the legume will grow in the selected situation when provided with adequate nitrogen; a failure to grow would indicate a limitation of a factor other than nitrogen.

Using this procedure, we have been able to recommend effective strains of RNB for all the currently used cultivars of crop and pasture legumes used in tropical and subtropical Australia. Of the 16 strains used commercially, we are responsible for the isolation and testing of 14. The remaining 2 are strains from other laboratories but selected by ATFGRC for use in Australia. In this way, we have provided strains to the legume-inoculant industry as new cultivars have been released. Our most recent releases have been a new strain of *Rhizobium* for *Desmanthus virgatus* and 2 new strains for *Calliandra calothyrsus.*

A current example

What can be offered for the future? By way of example, I should like to relate briefly what we refer to as the "aff. *scabra* story". *Stylosanthes* sp.aff. *S. scabra* ("Stylosanthes seabrana", caatinga stylo) is a forage plant of high potential for the clay soil regions of central and southern Queensland (Date *et al.* 1996). It originates from Brazil and has been a consistently good performer in Mr L.A. Edye's work on the evaluation of a collection of *S. scabra* and *S. hamata* germplasm (Edye and Hall 1993). Several accessions from this collection have been identified as a distinct taxon, with some morphological characteristics of diploid *S. hamata* and some more similar to *S. scabra.* They are referred to as *S.* sp.aff. *S. scabra*, "Stylosanthes seabrana". Two selections from this material, CPI 92838B (cv. Primar) and CPI 110361 (cv. Unica) were endorsed for release by the Queensland Herbage Plant Liaison Committee in 1996.

Paralleling Edye's agronomic evaluation, the ATFGRC microbiology group has screened the same accessions for their RNB requirements. This work revealed that the caatinga stylo accessions were nodulated effectively by the same 2 strains which formed effective nodules on the diploid *S. hamata* lines (Date and Norris 1979; R.A. Date, unpublished data). In multisite field trials, L.A. Edye (personal communication) has demonstrated improved plant yield and increased plant numbers (recruitment) compared with *S. scabra* cv. Seca but, at some sites, caatinga stylo plants appeared to become nitrogen-deficient. This occurred after 2–3 seasons on fertile clay soils and immediately in the establishment year where the caatinga stylo was sown on less fertile, lighter-textured soils.

Samples from plants in Edye's trials confirmed that the plants were either not nodulated or, if nodulated, only sparsely so. Isolation and testing of the RNB from these nodules confirmed that they were ineffective in N fixation and were not contributing to the growth of the plant despite the fact that plants had been inoculated with a mixture of 2 of the strains of RNB selected from earlier screening of *S. hamata.* Serological testing confirmed that none of the isolates corresponded with the 2 inoculum strains.

Recognising that we had a problem of poor colonisation and/or survival of the introduced strain, we examined the ATFGRC RNB germplasm collection for likely alternative strains. Of some 25 strains selected on the basis of originating from nodules of *Stylosanthes* spp. growing in the same geographic areas as the caatinga stylo in Brazil, several were found to be effective in soil-pot assays in a glasshouse study, but, like the earlier 2 strains, they were not competent in the field and failed to form nodules. Provenance information from the ATFGRC plant germplasm database on caatinga stylo suggested that this taxon has a narrow distribution centred on the state of Bahia in Brazil.

With assistance from the Meat Research Corporation (MRC), new accessions of caatinga stylo and its associated RNB were collected in mid-1994. More than 1200 new isolates of RNB for "Stylosanthes seabrana", *S. macrocephala* and *S. capitata* were obtained from the nodule samples. Of these, 988 were authenticated as RNB and effective on either "Stylosanthes seabrana", *S. macrocephala* or *S. hamata* cv. Verano, in aseptic tube-assay. Based on the effectiveness rating on caatinga stylo in the tube-assay, the best 130 of these isolates were evaluated for ability to nodulate caatinga stylo effectively in soil-pot assays, using soils from the field sites where poor and ineffective nodulation had been noted previously.

Of these 130, 18 have been selected on the basis of plant dry matter yield and nitrogen concentration for further evaluation in field trials at 4 sites: CSIRO, Narayen Research Station (red

earth and granitic soils); "Hollyrood" near Roma (red earth); and Lansdown Research Station (yellow podzolic soil). The first of the new strains was used in field trails sown in January 1995 and the remainder in similar trials in January 1996. The 1995 trials have demonstrated that at least one of the new strains has high potential as an inoculum. It formed only a few nodules and increased yield by only 10–20% in the growing season of 1994–95 but, in the second season (1995–96), there has been a 5-fold increase in plant yield compared with that of controls (Table 2), and most nodules on the plants were formed by the original inoculum strain, CB3481.

Table 2. Second growing season plant dry matter yield (g/10 plants) of "Stylosanthes seabrana" at Narayen and Lansdown inoculated with strains of *Bradyrhizobium* or uninoculated.

Strain of *Bradyrhizobium*	Narayen	Lansdown
CB3053	32	104
CB3481	98	194
Uninoc. control	20	40

Prospects and opportunities

A major limitation to the use of this new strain, and maybe to the success of the new cultivars, is that producers have a preference, because of the heavy clay soils, to sow such small-seeded species into hot dry soils in anticipation of germinating rains. During the period of the field trials, we have been recording soil temperatures at the surface in excess of 60°C for several hours each day during summer and of 45–50°C at 2 cm depth for up to 8 h/d. Such temperatures are lethal to RNB. Thus, it is necessary to find either an alternative method of introducing the RNB to the soil or to develop a technique that significantly improves the viability of the RNB on the inoculated seed when exposed to such conditions.

Both these avenues of research form part of the ATFGRC program. Preliminary trials are encouraging. When RNB were introduced as an inoculum on winter-sown cereal seed and caatinga stylo seed sown without inoculation the following summer, some nodulation with the inoculum strain occurred. Such a technique is suited to arable land situations, but for sowing legumes into native grass pasture, an improved seed inoculation technique will be needed. Preliminary work has shown that CB3481 survives moderately well on seed stored at 35°C (R.A. Date and J. Baker, unpublished data) but the new strain for *Desmanthus* is very susceptible to such temperature conditions (A. Becerra *et al.* 1998). Developing a protective mechanism that will promote survival of RNB on seed is a high priority project and one for which we seek industry funding. Exposure of the RNB on inoculated seed to oxygen and low relative humidity (RH) combined with high ambient temperatures is known to cause rapid death of RNB, but if the oxygen and RH can be controlled, the effect of temperature is much less severe. Our research is exploring possibilities in this area.

The successful resolution of the nodulation of caatinga stylo highlights the benefit of having a research capacity in RNB and legume-nodulation specificity allied with plant evaluation and cultivar release. It is important if we are to maximise the use of new cultivars added to the list of economically important legume plants. Collection of new RNB germplasm in Argentina, Brazil and Uruguay in 1992 and again in Brazil in 1994 has yielded many new strains for legumes with potential, for which we have extensive and representative collections of germplasm (B.C. Pengelly, personal communication). We already know that the new RNB for caatinga stylo is highly effective on diploid *S. hamata*, a species highly regarded but eliminated from testing until now because of poor nodulation in the field. The same collection of RNB contains strains for *S. macrocephala* and *S. capitata*, also previously considered to lack agronomic potential because of lack of a satisfactory strain of RNB. Similarly, we have new strains for accessions of *Macroptilium bracteatum*, *M. martii* and *M. panduratum*, which could have potential as forages.

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