Report on a review of ACIAR-funded projects on *Rhizobium* during 1983–2004

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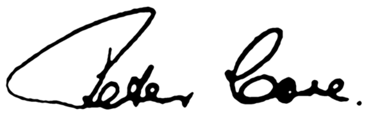
Foreword

ACIAR has long been interested in *Rhizobium* science and inoculation as a means of facilitating nodulation and nitrogen-fixation of legumes. Using legumes rather than mineral fertiliser to supply nitrogen to agricultural production systems is low-cost, environmentally benign and can provide high returns to farmers and the community. At the same time, Australia is well endowed with a legion of highly trained scientists who can oversee projects in partner countries and repeat the Australian success with *Rhizobium* and inoculation. Accordingly, *Rhizobium* science and legume nitrogen-fixation became a major part of ACIAR's plant nutrition program, and it is now 23 years since the first of the *Rhizobium* science/legume nitrogen-fixation projects was initiated. ACIAR subsequently funded, between 1984 and 2001, 13 research and development projects and one part-project on *Rhizobium* inoculants, mineral nutrition of *Rhizobium* and the legume host, and legume nitrogen-fixation in farming systems. It is timely to review the effectiveness of those projects and ACIAR's investment in them in order to guide future investments in this area.

This review provides an overview of the past *Rhizobium* research funded by ACIAR in Australia and partner countries, and attempts to identify and evaluate community impacts. It also assesses the main constraints to broad-scale adoption of *Rhizobium*-based technology in order to highlight critical research gaps requiring future investment.

The review concludes that there may be more scope for future investment options for ACIAR on the supply, rather than the demand, side of inoculant production and use. Demand is largely the product of market forces and government policy, whereas supply is driven more by technical issues that can be resolved using Australian expertise.



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Abbreviations

AIDAB	Australian International Development Assistance Bureau (now AusAID)
AusAID	Australian Agency for International Development
CARD	Collaboration for Agriculture and Rural Development (AusAID program)
CMU	Chiang Mai University (Thailand)
CU	Cantho University (Vietnam)
DOA	Department of Agriculture
IAS	Institute of Agricultural Science (Vietnam)
KKU	Khon Kaen University (Thailand)
N	nitrogen
N_2	molecular nitrogen
NSWDPI	New South Wales Department of Primary Industries
OPI	Oil Plants Institute (Vietnam)
QA	quality assurance
RRIM	Rubber Research Institute of Malaysia
UAA	University of Arid Agriculture (Pakistan)
UPM	Universiti Pertanian Malaysia
UQ	University of Queensland
UWA	University of Western Australia
VASI	Vietnam Agricultural Science Institute
WSIT	whole soil inoculation technique

Executive summary

Between 1984 and 2001, ACIAR funded 13 research and development (R&D) projects and one part-project on *Rhizobium* species as inoculants, mineral nutrition of rhizobia and the legume host, and legume nitrogen-fixation in farming systems. This review assesses the conduct and effectiveness of outcomes of the six projects that studied rhizobia as inoculants: PN/1983/006, PN/1987/031, LWR2/1988/029, PN/1992/027 (part project), LW2/1993/103 and LWR2/1998/027. The projects were conducted in China, Indonesia, Jordan, Malaysia, the Philippines, Thailand and Vietnam. It also considers constraints to the adoption of *Rhizobium* (inoculants) technology and R&D gaps, and options for future ACIAR investment.

The review had four terms of reference (TOR).

TOR 1. Compile an overview of the past *Rhizobium* research funded by ACIAR in Australia and partner countries, including the impact on capacity building in *Rhizobium* research.

The formal project reviews indicated that all of the projects were well conducted, with virtually all achieving their stated objectives. The focus of research in the *Rhizobium*/ inoculants projects changed during the almost 20 years of funding, from selection and testing of rhizobial strains and rhizobial ecology to inoculant technology. Examples of scientific outcomes were the whole soil inoculation technique (WSIT) developed in PN/1983/006 as a means of determining the nitrogen (N_2)-fixing capacity of rhizobia naturalised in soils, protocols to account for high-temperature effects on inoculant quality during transport and storage (LW2/1993/103), improved methods for determining and optimising the moisture content of inoculant carriers (PN/1983/006, LWR2/1988/029 and LW2/1993/103) and new inoculant rhizobia and inoculation procedures for *Acacia* species (PN/1992/027).

Most of the projects focused on agricultural legumes and their rhizobia in South-East and South Asia. This was logical, with the timings of projects generally reflecting periods of expansion of pulse and oilseed legume production in the partner countries. As part of the review, I travelled to and interviewed project scientists in Thailand, Malaysia and Vietnam. There was an overwhelming consensus amongst the 20 project scientists from the nine institutes visited that the ACIAR projects on *Rhizobium* and legume N_2-fixation provided substantial benefits to the individuals and institutions involved. They clearly appreciated working with the Australian project scientists and benefited from the training and resourcing that came with the projects. In some instances, research collaboration between original project scientists remains active almost 20 years after project completion, as is training in the Australian counterpart university of postgraduate students from partner countries. It is worth noting the very active interaction between Khon Kaen and Chiang Mai universities in Thailand and Murdoch University in Australia. There were also substantial impacts of the projects in Australia, principally through appointments of young scientists, employment of experienced scientists, and operational and infrastructure funding for priority R&D programs, particularly in the area of inoculant technology and quality assurance (QA).

TOR 2. Identify and evaluate community impacts (economic, social, environmental) the ACIAR-funded work on *Rhizobium* may have had in Australia and in partner countries.

Addressing this TOR involved travel to selected partner countries where there was a high likelihood of tracking impacts. The findings against this TOR were intended to serve as the basis for the possible commissioning of a follow-on formal impact assessment study.

Outcomes of the boron and iron nutrition work of PN/1883/029 and PN/1986/003 at Chiang Mai and Khon Kaen universities have had widespread adoption in northern

Thailand through changes to agronomic practices, and fertiliser recommendations and formulations. The work, originally done on soybean and groundnut, was subsequently extended to other field and horticultural crops. The ongoing capacity for production and use of *Rhizobium* inoculants in Thailand and Malaysia, and the benefits they bring, can be partly attributed to scientific training and infrastructure resourcing provided through PN/1983/005, PN/1983/006 and PN/1988/000.

In Vietnam, project LWR2/1998/027 established that replacing fertiliser nitrogen (N) with rhizobial inoculants would save Vietnamese farmers $50–60 million[1] annually in input costs. Successful field programs on the benefits of inoculation in Dong Tai province of the Mekong Delta were subsequently conducted by Cantho University collaborators. A proposal to continue the work of LWR2/1998/027 within the AusAID Collaboration for Agriculture and Rural Development (CARD) program has just been prepared and submitted ('Replacing fertiliser N with rhizobial inoculants for legumes in Vietnam for greater farm profitability and environmental benefits'). If successful, a major component of that proposal will focus on extension of LWR2/1998/027 outcomes.

In Australia, community impacts of the ACIAR projects are associated with the ongoing use by farmers of highly effective rhizobial inoculants on about 50% of the 5 million ha of legumes sown each year. The New South Wales Department of Primary Industries (NSWDPI) Gosford unit received funding through PN/1983/006, LWR2/1988/029, LW2/1993/103 and LWR2/1998/027 between 1984 and 2001, and the funds were used to improve and apply inoculants technology in Australia. The inoculant strains and a novel technique for inoculating *Acacia* species in nurseries are widely utilised in the industry (PN/1992/027).

TOR 3. Identify and evaluate the main constraints to broad-scale adoption of *Rhizobium*-based technology.

Project documents (proposals, reviews and final reports) and discussions with partner country project scientists were used to construct the following list of constraints on *Rhizobium*-based technology, related either to low demand or low/unreliable supply. On the demand side, the key constraints are as follows:

- Areas planted to legumes in, e.g. Nepal, the Philippines, Cambodia, Laos and Thailand, are small and/or declining.
- Farmer use of N on legumes. In some countries, farmers use fertiliser N on legumes, rather than inoculating with *Rhizobium*; e.g. LWR2/1998/027 determined that Vietnam's legume farmers could replace more than $50 million worth of fertiliser N with about $1 million worth of strategically applied inoculants.
- Constraints related to a lack of need for inoculants, i.e. lack of inoculation response. Clearly, inoculation is not required for all legume plantings, a situation long recognised by farmers and agronomists.
- Constraints related to poor farmer knowledge of inoculation. This remains an issue, even in countries that have had well-coordinated extension programs extolling the benefits of inoculation, e.g. Thailand, Pakistan and Malaysia. However, in other countries, e.g. Vietnam, Cambodia, Laos and Indonesia, it is likely that many farmers and agronomists are unaware of the benefits of inoculation.

[1] Unless otherwise stated all dollar values in this paper are Australian dollars.

The following are key constraints on the supply side:

- The difficulty inoculant manufacturers have in developing markets composed of smallholders operating at low input, subsistence level. This is a problem in all countries of interest.
- Technology of manufacture, QA and distribution. In many of the countries, the expertise for factory-scale inoculant production and QA either does not exist, e.g. Cambodia and Laos, or needs to be greatly strengthened, e.g. Vietnam, Myanmar, the Philippines and Nepal. In all countries, distribution and storage of inoculants is problematic, particularly in the hotter regions.
- Lack of private-sector involvement in inoculant production. It is really only the private sector that can produce and successfully distribute the large volumes of inoculant required for plantings to legumes areas greater than 100,000 ha.
- Lack of supporting R&D. Countries that have successfully embraced inoculation of legumes, such as Australia, Brazil, Canada, Thailand and the US, all have dynamic, public-sector R&D programs that feed technological advances into the manufacture, QA and application of inoculants.

TOR 4. Identify critical research gaps and assess future investment options to overcome constraints identified in TOR 3. Investment options need to be assessed in the context of ACIAR's funding priorities as stated in the ACIAR Annual Operational Plan.

There may be more scope for future investment options for ACIAR on the supply side of inoculant production and use than on the demand side. Demand is largely driven by market forces and government policy. Clearly, ACIAR could more readily respond to an increasing demand than create it. Supply, on the other hand, is driven more by technical issues that can be addressed using Australian expertise.

Future investment options

- **Increasing *Rhizobium*/inoculants demand associated with increasing legume plantings and reductions in fertiliser N use.** ACIAR could respond to existing demand in two countries in particular—Myanmar with increasing legume plantings, and Vietnam with a steady increase in legume plantings coupled with favourable government policy shifts on future legume production, biotechnology and fertiliser use.
- **Simple, rapid tests for determining the need for inoculation** leading to recommendations for strategic, rather than blanket, inoculation. The WSIT, developed in PN/1983/006 (G), needs further refinement and calibration. Widespread use of the WSIT could generate sufficient data to incorporate into predictive models for inoculation.
- **Enhancing farmer knowledge of inoculation.** Extension and training programs to educate farmers and their advisers on legume inoculation should be done in the context of legume/*Rhizobium* projects.
- **Low-income, dispersed markets.** There may be demand for inoculants in a country or region, but the fact that the market may consist of widely dispersed, smallholders operating at low-input, subsistence levels makes it difficult for suppliers of inoculants (likely an economics and policy, rather than a technical, issue).
- **Inoculant manufacturing technology, QA and distribution**. These are the linchpin issues of *Rhizobium*-based technologies—rhizobial strain selection, fermentation protocols, inoculant carriers and packaging, shelf life and storage of inoculants, death of rhizobia during inoculation, and QA.

- **Private-sector involvement in manufacture and marketing.** Ideally, public facilities should shift as quickly as possible from any role in manufacturing inoculants to providing R&D support, and perhaps external QA, leaving manufacturing and marketing to the private sector.
- **Supporting *Rhizobium* R&D capacity.** This is critical. As stated above, the countries that have successfully embraced legume inoculation also maintain dynamic public-sector R&D capacity in *Rhizobium* science. Fostering of R&D capacity in partner countries would be a natural outcome of ACIAR project funding of any of the demand and supply constraints listed above. Additionally, ACIAR could provide funding for research and training networks.
- **Other**. These principally relate to legumes and N in farming systems, e.g. legume N_2-fixation as a source of N in organic-farming systems and in land rehabilitation and forestry.

Background

Australian farmers embraced legumes and legume inoculation in the early days of the 20th century. The soils that they farmed were generally low in mineral nitrogen (N) and use of fertiliser N was not an affordable option. The mainly pasture and fodder legumes that they grew needed to be self-reliant for N, which meant fixation of molecular nitrogen (N_2) from the atmosphere by legume-associated *Rhizobium* bacteria. Australian farmers currently inoculate with rhizobia about 2.5 million ha sown to legumes annually, using certified, high-quality inoculants. This did not happen by accident, but was due largely to the country's scientific expertise in the universities, CSIRO and state departments of agriculture that was fostered throughout the 20th century. Individuals like the late Professor Jim Vincent, University of Sydney and University of New South Wales, played pivotal roles in establishing *Rhizobium* science in this country and in formulating the quality assurance (QA) system for rhizobial inoculants that eventually became a model for the rest of the world.

At the outset, ACIAR was interested in *Rhizobium* science and inoculation as a means of facilitating nodulation and N_2-fixation of legumes. Using legumes rather than mineral fertiliser to supply N to agricultural production systems was low cost and high return for the farmer and community. There existed in the country a legion of highly trained scientists that could oversee projects in partner countries and mimic the Australian success with *Rhizobium* and inoculation. By the mid 1980s, *Rhizobium* science and legume N_2-fixation became a major part of ACIAR's plant nutrition program. In 1991, a two-day workshop, convened by Ian Willett, was held in Canberra to review ACIAR's investment in *Rhizobium* science/legume N_2-fixation and to plot future directions. The outcomes of that review were that future projects should capitalise on ACIAR project success in developing methods for measuring N_2-fixation by legumes in the field and focus on the role of the nodulated legumes in supplying N to farming systems (Willett 1992). New projects were duly initiated and the large portfolio of *Rhizobium*/inoculation projects somewhat reduced, although not entirely. *Rhizobium*/inoculation projects in the Philippines and Indonesia continued to be funded.

It is now 14 years since the Willett review and 23 years since the first of the *Rhizobium* science/legume N_2-fixation projects was initiated. It is timely to review the effectiveness of those projects and ACIAR's investment in them. The four terms of reference for the review are given in Appendix 1.

Addressing the review terms of reference

TOR 1. Compile an overview of the past *Rhizobium* research funded by ACIAR in Australia and partner countries, including the impact on capacity building in *Rhizobium* research

A. Overview of *Rhizobium* research funded by ACIAR in Australia and partner countries

An overview of the *Rhizobium*/legume N_2-fixation projects funded by ACIAR between 1984 and 2001 is presented as Appendix 2. Details of specific objectives of the various projects as well as a short summary of outputs and/or comments from the project reviews are presented as Appendixes 3, 4 and 5. I have divided the projects into three groups:

- projects that focused on *Rhizobium* science and inoculation of legumes
- projects that focused on the mineral nutrition of *Rhizobium* and its legume host
- projects that focused on legumes as N_2-fixing components of farming systems, including development and application of measurement methodologies.

This review will concentrate on the first group of projects, i.e. *Rhizobium* science and inoculation of legumes, although I will make reference to the other two project groups. The second group of projects dealt more with the mineral nutrition of legumes than it did with rhizobia. The third group of projects focused on the process of legume N_2-fixation and its effect on soil fertility and system productivity.

The *Rhizobium* as inoculants projects commenced in July 1984 and the last one finished in October 2001. The projects—PN/1983/006 (A–G), PN/1987/031, LWR2/1988/029, PN/1992/027 (B), LWR2/1993/103 and LWR2/1998/027—focused on:

- determining the need to inoculate legume species of particular relevance to the partner countries, e.g. groundnut, leucaena, *Pueraria* and *Calopogonium* in Malaysia, leucaena, stylo and soybean in Thailand, and *Acacia* species, soybean and mungbean in the Philippines
- improving inoculant strains through isolating *Rhizobium* strains from field soils in partner countries and Australia, adapted to local conditions and stresses, e.g. acid-tolerant strains from Peninsular Malaysia for groundnut, leucaena and the perennial cover crops, *Pueraria* and *Calopogonium*, acid-tolerant strains for tree legumes and mungbean in the Philippines, acid-tolerant strains for soybean in Indonesia, acid-tolerant strains for clover in Australia, strains for *Acacia* species in China and Australia
- understanding the ecology of rhizobia in particular soils and environments, e.g. rhizobia nodulating soybean and other tropical grain legumes in north-western Thailand, rhizobia nodulating tree legumes and mungbean in the Philippines, rhizobia nodulating soybean in rice rotations, i.e. flooded soil, in Indonesia and Australia
- developing the understanding of rhizobial ecology to the point of predicting the need to inoculate, e.g. soil test for medic rhizobia in the Middle East and Australia
- improving the quality and shelf life of inoculants, with particular emphasis on carrier material and packaging effects on numbers of rhizobia, e.g. evaluation of peats in Malaysia, carrier materials and temperatures of storage for soybean inoculants in Indonesia
- improving survival of the rhizobial inoculant once applied to the seed, e.g. effects of peat moisture potential on survival, relevant to Indonesia and Australia
- developing new procedures for inoculating *Acacia* species in nurseries, relevant to China, Vietnam and Australia
- introducing QA for inoculant production and use, e.g. in the Philippines
- conducting extension campaigns about inoculation and its benefits, e.g. mungbean and soybean in the Philippines
- conducting simple need-to-inoculate experiments and farmer surveys to provide the technical and economic bases for a follow-up project to increase production and use of inoculants in Vietnam.

There was a natural evolution of project topics during the 18 years of ACIAR funding, from the early focus on rhizobial ecology and field testing of rhizobial strains to inoculant technology. Arguably the projects got better over the years and the benefit of hindsight suggests that the early projects should have concentrated more on the practical aspects of inoculant production and use, rather than the labour- and materials-demanding strain and ecological studies. That is not to say that the early projects were lacking in useful scientific/practical outcomes. An outcome of early project PN/1983/006 was the whole soil inoculation technique (WSIT), developed by John Brockwell as a means of determining the N_2-fixing capacity of rhizobia naturalised in soils in the Middle East and Australia (Brockwell et al. 1988). The WSIT proved to be a credible and convenient test of resident populations of rhizobia in soils, and was used subsequently for a wide range of legume species and locations (e.g. Kang et al. 1991; Ballard et al. 2002, 2003; Charman and Ballard 2004). Significantly, a number of research outcomes of later projects, such as the development of protocols to account for high-temperature effects on inoculant quality during transport and storage (LW2/1993/103; Roughley et al. 1995), improved methods for determining and optimising the moisture content of inoculant carriers (PN/1983/006, LWR2/1988/029 and LW2/1993/103; Griffith et al. 1991; Griffith and Roughley 1992), and new inoculant rhizobia and inoculation procedures for *Acacia* species (PN/1992/027) have also had application in partner countries and Australia alike.

All of the projects appear to have been well-conducted and received high marks from the reviewers. There was very little criticism of, or negative comments about, the projects in the reviews (Appendixes 3–5). As an example, an excerpt from the formal review of PN/1983/006:

> … The experiments undertaken in this Project have all employed superior scientific standards. This is evident in the publications already resulting from the work and those projected. A major factor in this has been the frequency of consultation between the Australian and collaborating scientists. This is perhaps a unique feature of ACIAR, as compared to other funding agencies, and is to be encouraged … Because of the direct involvement of the collaborating scientists in the planning and execution of the Projects, and in analysing and disseminating their results, a high degree of collaboration has taken place … The Projects have clearly strengthened the research capacity of the participating institutions in the developing countries. This capacity has been enhanced through the provision of key items of equipment such as microscopes, autoclaves and facilities for growing plants etc. These facilities will be utilized not only in future research but also in the training of young scientists at institutions such as the University of Chiang Mai … The Reviewers were made very well aware of the outstanding contribution that Dr Roughley had made to the success of the Projects. Furthermore, they believe that his continuing leadership and involvement is essential to the profitable conclusion of the research that has been done. He is held in very high regard by the participating scientists, who are clearly very appreciative of the time and effort that he has devoted to their Projects …

This is not to say that the projects were without problems or frustrations. Dr Rodney Roughley, leader of PN/1983/006, LWR2/1988/029 and LWR2/1993/103, aired some of his at the 1991 Willett review, particularly those related to budget cuts during the course of PN/1983/006 and the restrictive time frame of three-year projects that terminate without provision for ongoing activities (Willett 1992).

Most of the projects focused on agricultural legumes and their rhizobia in South-East and South Asia. This was logical, with the timings of projects generally reflecting periods of expansion of pulse and oilseed legume production in the partner countries. For example, in Thailand, project activities during the 1980s coincided with increasing legume plantings (Figure 1). Project LWR2/1988/029 in Indonesia, conducted during 1990–95, coincided with the period of peak legume plantings in that country. The decline in legume areas in Thailand and Indonesia during the past 15 years reflects to a large extent the flooding of world protein markets with cheap soybean meal from North and South America. This is unfortunate because the multiple benefits of legumes to farming systems—enhanced N supply, disease/insect/weed breaks, risk management—are lost. Such benefits are usually not considered when the choice of growing locally versus importing is subjected to economic analysis.

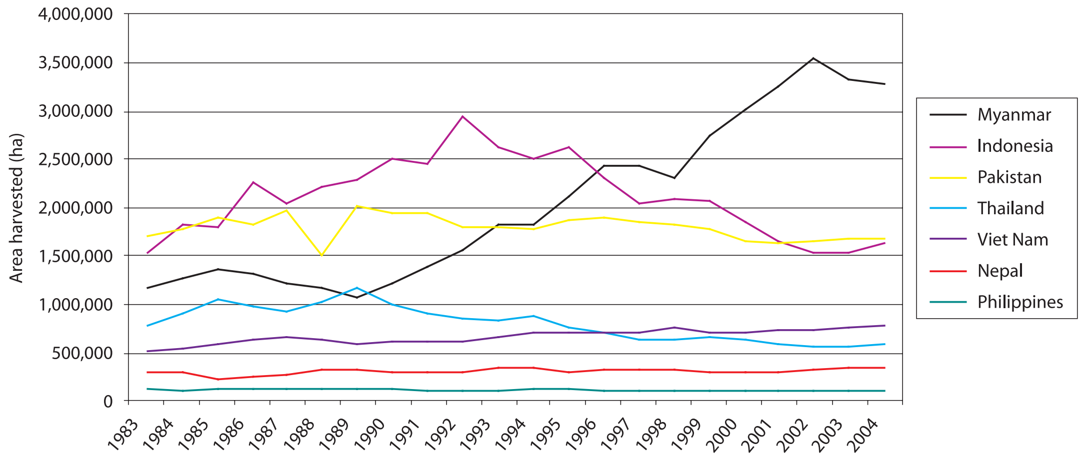

Figure 1. Area of pulse and oilseed legumes for each of the countries involved in ACIAR-funded projects on *Rhizobium* science or, in the case of Myanmar, involved in project development, but not an executed project (source: FAOSTAT data, 2006, at <faostat.fao.org>)

Malaysia was a partner country in a number of *Rhizobium* projects during the 1980s, although areas planted to pulses and oilseed legumes were very small (< 5,000 ha). Malaysia, however, cultivated large areas of cover-crop legumes, such as *Pueraria* and *Calopogonium*, in the rubber and oil-palm plantations. It was these legumes that were the primary focuses of the projects. Similarly in the Philippines, project activities, particularly those involving Peter Dart, eventually focused on the tree legumes in forestry systems, rather than agricultural legumes.

Only two countries — Myanmar and Vietnam — have shown a steady increase in legume plantings during the past 20 years. Of the two, the increase in Myanmar has been quite spectacular, from 1.2 million ha in 1990 to 3.5 million ha in 2002. The last of the *Rhizobium*/ inoculants projects was in Vietnam (1999–2001). A project was developed for Myanmar during 2003 but did not go ahead at the time because of political circumstances. Elements of the original project are currently being incorporated into a broader legume improvement–*Rhizobium* inoculants project that will likely commence in late 2006.

B. Impact on capacity building in *Rhizobium* research

In partner countries

Addressing this TOR involved travel to Malaysia, Thailand and Vietnam during January 2006 and meeting with project scientists from a subset of the projects listed in Appendix 2. Table 1 lists the *Rhizobium* scientists interviewed during the trip. Thailand, Malaysia and Vietnam were chosen for study because of the high concentration of *Rhizobium*/legume N_2-fixation project activity in those countries. Collectively, the scientists in Table 1 represent projects PN/1983/005, PN/1983/006, PN/1983/029, PN/1986/003, PN/1988/000 and LWR2/1998/027.

There was an overwhelming consensus amongst the 16 scientists from the nine institutes in Thailand, Vietnam and Malaysia that the projects were well-conducted and provided substantial benefits to the individuals and institutions involved. They clearly appreciated working closely with the Australian project scientists and benefited from the training and resourcing that came with the projects.

Table 1. *Rhizobium* scientists interviewed in the partner countries

Country/scientist	Institution	City	Email address
Thailand (16–22 January)			
Prof. Nantakorn Boonkerd	Suranaree University of Technology	Nakhon Ratchasima	nantakon@ccs.sut.ac.th
Prof. Jukrit Homchan	Khon Kaen University	Khon Kaen	unknown
Prof. Banyong Toomsan	Khon Kaen University	Khon Kaen	banyang@kku.ac.th
Prof. Benjavan Rerkasem	Chiang Mai University	Chiang Mai	benjavan@chiangmai.ac.th
Prof. Chalermpone Sampet	Chiang Mai University	Chiang Mai	csampet@yahoo.com
A/Prof. Ampan Bhromsiri	Chiang Mai University	Chiang Mai	ampan_b@yahoo.com
Vietnam (22–27 January)			
Dr Pham Van Toan	National Institute for Soils & Fertilisers	Hanoi	pvtoan@hn.vnn.vn
Mrs Nguyen Thu Ha	National Institute for Soils & Fertilisers	Hanoi	thuhavasi@yahoo.com
Dr Nguyen Thi Lien Hoa	Oil Plants Institute of Vietnam	Ho Chi Minh City	opi.vn@hcm.vnn.vn
Mrs Tran Yen Thao	Oil Plants Institute of Vietnam	Ho Chi Minh City	yenthao@hcm.fpt.vn
Dr Phan Thi Cong	Institute of Agricultural Science	Ho Chi Minh City	congphanthi@hcm.vnn.vn
Mrs Tran Minh Hien	Institute of Agricultural Science	Ho Chi Minh City	saf@hcm.vnn.vn
A/Prof. Nguyen Huu Hiep	Cantho University	Cantho	nhhiep@ctu.edu.vn
A/Prof. Cao Ngoc Diep	Cantho University	Cantho	cndiep@ctu.edu.vn
Malaysia (27–30 January)			
Dr Norhayati Moris	Malaysian Rubber Board	Kuala Lumpur	norhayati@lgm.gov.my
Prof. Zulkifli Shamsuddin	Universiti Putra Malaysia	Serdang	zulsham@agri.upm.edu.my

In some instances, active research collaboration has been maintained between original project scientists almost 20 years after project completion. It is worth noting also the very active interaction between Khon Kaen and Chiang Mai universities in Thailand and Murdoch University in Australia in the current training of Thai postgraduate students.

Thailand

Project impacts on capacity building in Thailand can be illustrated using the experiences of Prof. Benjavan Rerkasem and A/Prof. Ampan Bhromsiri of Chiang Mai University (CMU) and Prof. Banyong Toomsan, Khon Kaen University (KKU). To a greater or lesser extent, project impacts were similar for the other Thai collaborators (Prof. Nantakorn Boonkerd, Prof. Jukrit Homchan and Prof. Chalermpone Sampet).

Benjavan was involved in ACIAR *Rhizobium*–N_2-fixation projects at the outset and stayed involved for about 10 years through projects PN/1983/029, PN/1986/003, PN/1983/005 and PN/1988/000, one AIDAB project during 1991–93 on boron in wheat, and one ACIAR small project (1994–95) on nutrient-induced sterility in wheat. Involvement in the ACIAR projects soon after returning to Thailand from PhD studies at the University of Western Australia (UWA) started Benjavan's career, leading to some critical and highly relevant work on boron nutrition of crop plants and resulted eventually in an impressive curriculum vitae containing long lists of funded projects, postgraduate students and scientific publications. She continues to work on iron nutrition of rice with Ross Welch, Cornell University, who also had previous collaboration with Jack Loneragan and Robyn Graham (formerly Adelaide University and ACIAR project leader). She continues to work also with Bernie Dell, Murdoch University. Thus, the networks established in PN/1983/029 more than 20 years ago are still solidly in place.

Benjavan is currently Professor of Agronomy, CMU, and has 15 MSc and 11 PhD students training in *Rhizobium* and legume science at CMU, Murdoch University, the University of Western Australia (UWA) and two universities in the US. Several of the students are funded through the Royal Golden Jubilee PhD program. It is relevant to this review that CMU is still sending postgraduate students to Graham O'Hara, Richard Bell and Bernie Dell at Murdoch University, all of whom started their own careers in ACIAR projects.

Benjavan was very positive about the conduct of the projects in which she had involvement. She enjoyed working with the Australian scientists and appreciated the strong support that she received from Eric Craswell and Ian Willett, coordinators of the plant nutrition program. Her only problem was to do with the lack of funding between the end of the ACIAR projects and the start of the Thai national projects. She was helped to cover the gap by Eric Craswell and Ian Willett with an AIDAB project and an ACIAR small project. She was eventually appointed as a Thailand Research Funds Senior Research Scholar in 2001, which provided her with funding for research (about $85,000/year for 3 years).

Associate Professor Ampan Bhromsiri used the techniques and knowledge of projects PN/1983/006 and PN/1988/000 for many years after the projects finished. Her involvement in PN/1988/000 and expertise in the use of the ureide methodology for measuring legume N_2-fixation facilitated mid 1990s collaboration with Alexander Hansen and other German scientists. Ampan's assistant at the time, Arawan Shutsrirung, obtained a PhD through Mie University, Japan, using the rhizobial isolates from PN/1983/006 and the ureide measurement technique from PN/1988/000. Ampan collected about 2,000 isolates of rhizobia from soybean and other species in the Chiang Mai district, as part of PN/1983/006. Extra funds were allocated by ACIAR after the completion of PN/1983/006 to freeze-dry the isolates, but only sufficient for part of the collection (300 isolates). She regrets that the full collection was not freeze-dried because in later work with Japanese scientists she started to examine the genetic diversity of the isolates using molecular techniques and the additional 1,700 isolates would have been very useful for those studies. Arawan is currently a research fellow in CMU's Department of Soil Science. The isolates and ureide methodology were also used in the projects of two other MSc students at CMU.

Ampan currently has one PhD and three MSc students. She receives funding through the Royal Project mainly for upland agronomy research, with strong emphasis on soil microbiology. Ampan retired in 2005, but continues to work with current funding.

Professor Banyong Toomsan was formerly Head of Agronomy at KKU. He has a PhD from the University of Manitoba, later worked with Dr Peter Dart and O.P. Rupela at ICRISAT and is currently collaborating with Georg Cadish (Hohenheim University, Germany) on N cycling and management in systems of north-eastern Thailand. Banyong currently supervises/co-supervises six PhD and two MSc students, including a PhD student from Laos and an MSc candidate from Bhutan.

As was the case with Benjavan, the ACIAR projects contributed substantially to the early careers of Banyong and his colleague at KKU, Jukrit Homchan, through infrastructure purchase and development, through interactions with the Australian project scientists and through publishing in international journals. Banyong has now successfully supervised about nine MSc students on *Rhizobium* science. He attributes this success largely to his involvement in ACIAR projects PN/1983/029 and PN/1986/000. He also still uses equipment bought through the project. Over the now more than 20 years since the projects commenced, he has maintained contact and collaboration with Graham O'Hara and Richard Bell, Murdoch University. The ACIAR projects also paved the way for other collaborations and internationally—e.g. CRISP (US), IDRC (Canada) and European Union—and nationally funded projects.

Thus, there were large and very positive impacts of the ACIAR *Rhizobium*-N_2-fixation projects on personnel and institutions in Thailand. A major effect of projects was to stimulate early-career research by the Thai scientists, some of whom were not long back from overseas PhD programs and working in poorly resourced institutions. The scientists not only appreciated the funding and infrastructure development that the projects brought, but also the opportunities to work closely with the Australian scientists. Significantly, a number of those relationships developed more than 20 years ago are still flourishing—e.g. Bell, O'Hara and Dell (Murdoch University) with CMU, and Bell and O'Hara with KKU—through collaborative research and postgraduate training. The other legacy was the profile that the ACIAR projects gave to the country scientists through publication in international journals and through attendance at workshops and meetings. The enhanced profiles then helped to facilitate projects with other national groups in, for example, Germany, Japan and the US.

Malaysia

ACIAR projects in Malaysia were PN/1983/005, PN/1983/006, PN/1983/029 and PN/1988/000. Dr Norhayati Moris of the Rubber Research Institute of Malaysia (RRIM) (part of the Malaysian Rubber Board) was one of the RRIM group involved in PN/1983/005 to develop and apply methods for measuring legume N_2-fixation. Work on *Rhizobium* and legume N_2-fixation declined in Malaysia during the early–mid 1990s with declining areas of rubber, coupled with the death of Dr Faizah Abdul Wahab, and retirements of Drs Chong Kewi and Mohd Noor Sudin. Faizah was the principal rhizobiologist in the group; her responsibility for producing inoculants on demand has been continued by her assistant. Faizah was also the major collaborator in the ACIAR projects, with involvement in PN/1983/005, PN/1983/006 and PN/1988/000.

Norhayati was very positive about the conduct and impact of the projects. She found that the exposure of the RRIM group to Australian and other scientists was extremely beneficial at both personal and institutional levels. The publications in international journals were also very beneficial. She commented that many of the young scientists working today at RRIM have not had this exposure and would certainly benefit from similar projects. She regretted that the projects finished after only 3–3.5 years. She thought that was far too short a time frame.

Professor Zulkifli Shamsuddin, Universiti Pertanian Malaysia (UPM), echoed the thoughts of most of the other Asian scientists—very large benefits for individuals and institutions, with community impacts tied to a large extent to other factors. The major negative was that projects were too short and ended too sharply.

Vietnam

There was only one *Rhizobium* project in Vietnam—LWR2/1998/027. Dr Nguyen Thi Lien Hoa and Mrs Tran Yen Thao, Oil Plants Institute (OPI), were involved together with personnel from Cantho University (CU), the Institute of Agricultural Science (IAS) and Vietnam National University (see last page, Appendix 3). Lien Hoa and Yen Thao were very positive about the conduct and benefits of LWR2/1998/027, citing that it:

- linked the various *Rhizobium* groups in Vietnam—Vietnam Agricultural Science Institute (VASI), OPI, IAS and CU
- gave direction to solving national issues, i.e. the need for legume inoculants in Vietnam, and the issues surrounding production of quantity and quality
- enhanced the research capability of each group through training and exposure to other Australian scientists
- provided linkages with other ACIAR projects, specifically the soybean improvement project (Andrew James and Bob Lawn)
- had good project leadership.

They saw that the follow-up of LWR2/1998/027 was good, with a detailed proposal for a new project drafted soon after LWR2/1998/027 finished. Unfortunately, the ACIAR proposal was canned and the application to the AusAID Collaboration for Agriculture and Rural Development (CARD) program in 2004, essentially based on the draft ACIAR proposal, was unsuccessful. Undaunted, Yen Thao has recently led a new consortium of Vietnamese and Australian scientists and Vietnamese agribusinesses to submit a redrafted proposal for the 2006 round of the program. This revised proposal again drew heavily on outcomes and personnel from LWR2/1998/027.

Two negative aspects of LWR2/1998/027 were identified.

- LWR2/1998/027 was a small project with limited funding and short duration. This did not allow coordination of the project with other Vietnamese Government projects, particularly seed/variety development and evaluation, or extension of results.
- Lack of funding did not allow for education and training of extension staff in inoculants, something that would need to be done in the event of any new project.

Similar comments were made by Dr Phan Thi Cong and Mrs Tran Minh Hien from IAS. Dr Cong was not directly involved in LWR2/1998/027, but indirectly as a group leader. She currently leads the IAS component of biofertiliser project directed by Prof. Ivan Kennedy (University of Sydney). Mrs Hien was part of LWR2/1998/027 and came to Australia in 1999 for training.

Associate Professors Nguyen Huu Hiep and Cao Ngoc Diep, CU, were also positive about the conduct of LWR2/1998/027 and consider that it had personal, institutional and farmer impact. They conducted a field program on the need for inoculation in Dong Tai province (Mekong Delta), which was well received by extension workers and farmers.

In Australia

Impacts of the *Rhizobium*/inoculants projects on capacity building in *Rhizobium* research in Australia were concentrated in four institutions: the NSW Department of Primary Industries (NSWDPI), Murdoch University, CSIRO Plant Industry and the University of Queensland (UQ).

For the NSWDPI, the *Rhizobium*/inoculants projects contributed to the department's priorities through the R&D programs of Rodney Roughley (PN/1983/006 (A–C), LWR2/1988/029 and LWR2/1993/103), Jack Thompson (PN/1983/006 (D)), and David Herridge (LWR2/1998/027 plus N_2-fixation/systems projects PN/1983/005, PN/1988/000, LWR2/1992/010 and LWR2/1997/062 through:

- funding of new scientists, e.g. Dr Graeme Schwenke, soil chemist, 1994–current, through LWR2/1992/010 and LWR2/1997/062.
- funding infrastructure and operations at the department's Gosford and Tamworth research institutes. The viability of the *Rhizobium* laboratory at Gosford during the 1980s and early 1990s for R&D and QA on legume inoculants relied heavily on funding through the ACIAR projects at that time. Throughout that period, the laboratory conducted independent QA on all rhizobial inoculants produced and sold in Australia. That function continues today. At Tamworth, the four N_2-fixation/systems projects provided the means for incorporating ^{15}N techniques from CSIRO Plant Industry into the R&D program for quantifying legume N_2-fixation, the contributions of below-ground N to soil N dynamics and N benefits in legume–cereal systems.

Projects PN/1983/029 and PN/1986/003 contributed to personnel and infrastructure at Murdoch University. Dr Graham O'Hara and Prof. Richard Bell were appointed as postdoctoral researchers at Murdoch University through project funding in the early 1980s—both remain as academics at the university. Similarly, the *Rhizobium* program of Peter Dart at UQ was heavily reliant on ACIAR project funding.

Dr Mark Peoples was appointed as PN/1983/005 and PN/1988/000 project scientist in 1984 and located at the CSIRO Plant Industry laboratories, Canberra. Mark is now assistant chief at CSIRO Plant Industry. Project PN/1992/027 provided the impetus and basis for CSIRO Plant Industry's current R&D of *Acacia* and their rhizobia in landscape management.

TOR 2. Identify and evaluate community impacts (economic, social, environmental) the ACIAR-funded work on *Rhizobium* may have had in Australia and in partner countries

The intention of this review was to identify findings against this TOR that would serve as the basis for the possible commissioning of a follow-on formal impact assessment study.

A. Partner countries

Thailand

Thailand maintains effective capacity for *Rhizobium*/inoculants R&D and for production of legume inoculants. The latter are used to inoculate part of a declining, but still substantial, area of legume plantings in the country (about 600,000 ha). To some degree, this can be attributed to the involvement of the institutions (KKU, CMU, Department of Agriculture (DOA)) and personnel in ACIAR projects PN/1983/005, PN/1983/006 and PN/1988/000. Not all project outcomes were positive for inoculation. For example, PN/1983/006 (D) established that inoculation was not necessary for soybean production in the Chiang Mai valley because naturalised populations of rhizobia infective and effective for soybean had developed. This information was disseminated to the farmers through local extension programs.

Outcomes of the boron and iron nutrition work of PN/1883/029 and PN/1986/003, involving Benjavan (CMU) and Banyong and others (KKU), have had widespread adoption in northern Thailand through changes to agronomic practices and fertiliser recommendations and formulations. The work, originally done on soybean and groundnut, was subsequently extended to other field and horticultural crops.

Malaysia

Community impacts in Malaysia are likely similar to those described above for Thailand. ACIAR projects PN/1983/005, PN/1983/006, PN/1983/029 and PN/1988/000 contributed scientific outcomes, training and resources to collaborating institutions that continued to be involved in supply and R&D of *Rhizobium*/inoculants once the projects had finished. Inoculant use in Malaysia is minor, but what is used is largely supplied by the Malaysian Rubber Board from the late Dr Faizah's laboratory.

Vietnam

Major outcomes of LWR2/1998/027 related to the strong responses of legumes to inoculation in the hostile soils of the Mekong Delta (average of 42% and 30% for groundnut and soybean, respectively) and the large economic benefits of replacing fertiliser N with inoculation. In the 23 inoculation experiments conducted in the Mekong Delta and provinces around Ho Chi Minh City, profitability was increased by as much as $760/ha (average profitability increase was over $100/ha) through inoculation and reduced fertiliser N inputs. The project established that replacing fertiliser N with rhizobial inoculants would save Vietnamese farmers $50–60 million annually in input costs. Field programs on the benefits for inoculation in Dong Tai province of the Mekong Delta were subsequently conducted by Cantho University collaborators, and were well received by extension workers and farmers. A proposal ('Replacing fertiliser N with rhizobial inoculants for legumes in Vietnam for greater farm profitability and environmental benefits') to continue the work of LWR2/1998/027 within the CARD program has just been prepared and submitted. If successful, a major component of that proposal will focus on extension of LWR2/1998/027 outcomes.

B. Australia

Impacts of the *Rhizobium*/inoculants projects on the community were principally through the production and use of high-quality inoculants for both agricultural and forestry legumes. Funding and research outcomes from PN/1983/006, LWR2/1988/029 and LW2/1993/103 contributed to the effective operation of the NSWDPI *Rhizobium* unit led by Rodney Roughley at Gosford. As stated at the beginning of this review, Australian farmers embraced legume inoculation about 100 years ago and currently inoculate some 50% of the 5 million ha of legumes sown each year. The Gosford unit has a national mandate to conduct and oversee inoculants and pre-inoculated seed testing, mother-culture supply and R&D related to inoculant technology, all of which help to ensure that those 2.5 million ha of legumes are inoculated with highly efficacious products. The economic benefits of past and current inoculation in Australia are substantial, with nodulated legumes growing on 25 million ha land fixing $3–4 billion worth of N annually.

The impact of PN/1992/027 is also substantial, with rhizobial strains identified in the project now incorporated into commercial inoculants (Brockwell et al. 1999a, b), and methods of inoculating nursery and field plantings of *Acacia* widely adopted by industry (e.g. Wattle Grow™).

TOR 3. Identify and evaluate the main constraints to broad-scale adoption of *Rhizobium*-based technology

Project documents (proposals, reviews and final reports) and discussions with Australian project leaders and partner-country project scientists were used to construct the following list of constraints to adoption of *Rhizobium* inoculants.

LOW DEMAND for inoculants:

- **Related to legume area**. A key constraint. In Thailand, for example, demand for inoculants has more than halved in the past 10 years because of declining plantings of soybean and groundnut and establishment of rhizobial populations in the Thai cropping soils. The reduced plantings, referred to in TOR 1, are to a large part due to the dumping of cheaper protein meals by the US, Brazil and Argentina. Other countries of interest with small legume areas and therefore low inoculant demand are Nepal, the Philippines, Cambodia and Laos.

- **Related to the use of fertiliser N.** A second key constraint. Vietnam and China are the benchmarks where inoculants have been outcompeted in the marketplace by fertiliser N. We determined in LWR2/1998/027 that Vietnam cultivates about 800,000 ha of legumes annually, virtually none of which are inoculated but all of which are fertilised with N at rates of 50–150 kg N/ha (Hiep et al., 2002; Hoa et al., 2002; Herridge 2002). This represents an annual cost to the farmers of more than $50 million, a cost that could be reduced by 98% to about $1 million by replacing fertiliser N with inoculation and using inoculants strategically. Now, in 2006, government policy is shifting from support (subsidy) of urea usage to developing biofertilisers, including rhizobial inoculants, for N supply.

Data from China are even more alarming. In 1994, it was estimated that about 540,000 tonnes fertiliser N was applied to 10.5 million ha of soybean and groundnut, equivalent to 50 kg N/ha (P.W. Singleton, pers. comm.). In 2004, areas of groundnut and soybean planted in China were 14.5 million ha (FAOSTAT data, 2006, at <faostat.fao.org>). If these crops were fertilised with N at the same rate as in 1994, total fertiliser N applied would have been 750,000 tonnes, valued at around $1 billion. The cost of inoculants for the same area would be in the order of $20–30 million.

- **Related to a lack of need for inoculants, i.e. lack of inoculation response.** Clearly, inoculation is not required for all legume plantings, a situation long recognised by farmers and agronomists. It is likely that inoculation would not be required for the majority of legume cover-crop plantings in Malaysia (PN/1983/006-A) and for legumes in all countries grown in non-hostile soils in established legume areas (e.g. groundnut in Malaysia (PN/1983/006-B) and Vietnam (LWR2/1998/027)).
- **Related to poor farmer knowledge of inoculation**. This remains a problem, even in countries that have had well-coordinated extension programs extolling the benefits of inoculation, e.g. Thailand (Chanaseni and Kongngoen 1992), Pakistan and Malaysia. However, in other countries, e.g. Vietnam, Cambodia, Laos and Indonesia, it is likely that many farmers and agronomists are unaware of the benefits of inoculation. Farmers may also lack confidence in inoculant quality or efficacy, resulting from past experience with poor-quality inoculants, or experience of technical difficulties with inoculant application.

LOW or UNRELIABLE SUPPLY of high-quality inoculants:

- **Related to the difficulty that inoculant manufacturers have in developing markets composed of smallholders operating at low input, subsistence level**. This is a problem in all countries of interest.
- **Related to issues of manufacture, QA and distribution**. In many of the countries, the expertise for factory-scale inoculant production and QA either does not exist, e.g. Cambodia and Laos, or needs to be greatly strengthened, e.g. Vietnam, Myanmar, the Philippines and Nepal. In all countries, distribution and storage of inoculants is problematic, particularly in the hotter regions.
- **Related to lack of private-sector involvement in inoculant production**. It is really only the private sector that can produce and successfully distribute the large volumes of inoculant required for plantings of legumes exceeding 100,000 ha.
- **Related to a lack of supporting R&D**. Countries that have successfully embraced rhizobial inoculation of legumes, e.g. Australia, the US, Canada, Brazil and Thailand, all have dynamic, public-sector R&D programs that feed technological advances into the manufacture and application of inoculants. As an example, Australian public-sector institutions have been involved in *Rhizobium* R&D for more than 110 years and the *Rhizobium* researchers and commercial inoculant manufacturers meet at least annually to discuss technical issues.

TOR 4. Identify critical research gaps and assess future investment options to overcome constraints identified in TOR 3. Investment options need to be assessed in the context of ACIAR's funding priorities as stated in the ACIAR Annual Operational Plan

There may be more scope for future investment options for ACIAR on the supply side of inoculant production and use than on the demand side. Demand is largely driven by market forces and government policy. Clearly, ACIAR could more readily respond to an increasing demand than create it. Supply, on the other hand, is driven more by technical issues. As was recognised by ACIAR management at the outset, Australia was, and continues to be, a country with a heavy reliance on legume inoculation and has maintained technical expertise in that scientific discipline. Essentially, that expertise remains available for collaborative projects.

A. Investment options in R&D related to DEMAND for rhizobial inoculants

Legume area and fertiliser N use

Globally, it is significant that inoculant manufacturing and use has frequently been created in concert with the large-scale introduction of exotic, rhizobia-specific legumes. Examples of the success stories are soybean in Brazil, where the outcomes of *Rhizobium* science and rhizobial inoculation equate to more than $7 billion worth of nitrogen-fixation annually (Alves et al. 2003), soybean in the US, pea and lentil in Canada (Vessey 2004), and pasture and pulse legumes in Australia (Brockwell 2004). The legumes in those countries needed exotic rhizobia to flourish, thereby creating the demand for inoculants and *Rhizobium* technology. Fertiliser N was used sparingly, if at all, on the legumes, and extension campaigns kept pace with expansion of the legume cropping. Thus, the criteria were met for healthy demand for inoculants.

In Myanmar and Vietnam, there is demand for inoculants and potential for that demand to increase (see Figure 1). In the case of Myanmar, this is because of the rapid expansion of legume cultivation during the past decade, coupled with a very low usage of fertiliser N. In fact, the annual application of N fertilisers to all crops of 0.12 million tonnes is just 10% of that of Thailand (1.02 million tonnes), although total production of grains is very similar in the two countries (28.9 million tonnes for Myanmar and 32.2 million tonnes for Thailand (FAOSTAT data, 2006, at <faostat.fao.org>)). An ACIAR-funded project, 'Increasing food security and farmer livelihoods through enhanced legume cultivation in the central dry zone of Burma', is currently under development for a late 2006 start. The project will have a strong focus on production and application of inoculants.

In Vietnam, there has been a steady increase in legume planting during the past two decades (from 500,000 ha in 1983 to almost 800,000 in 2004). As well, government policy on legumes, imported fertilisers and biofertilisers appears to have dramatically changed in the past 2 years. The ministries of Agriculture and Rural Development (MARD) and Industry (MOI) now have ambitious plans for further expansion of legume plantings in Vietnam and replacement of rice with soybean and groundnut. The legumes are to be grown for industrial products as well as food. The ministries also have plans for the development of biotechnology in agriculture and the replacement of urea with biofertilisers. Farmers have traditionally applied fertiliser N to their legume crops (Herridge 2002), but this may now have to change because of the very rapid and substantial increase in fertiliser N prices associated with the price of oil.

As stated in TOR 3, other countries in the region either have declining areas of agricultural legumes (Pakistan and Thailand) or maintain small areas (Nepal, the Philippines, Cambodia and Laos). Although legume plantings in Indonesia have declined by about 50% in the past two decades, the country remains a significant producer of grain legumes (about 1.5 million ha in 2004).

Need for legume inoculation

Legumes will respond to inoculation if nodulation by the resident soil rhizobia is insufficient to meet the N demand of the growing plant. If there are no resident rhizobia in the soil, then legumes will almost certainly respond to inoculation. Published reports (e.g. Rupela et al.

1994) suggest that 50–70% of agricultural legumes in Asia and Africa could benefit from inoculation. The likely level of the response appears to be about 0.2 tonnes/ha grain (30%), equating to about 6 million tonnes of grain with a value of $200 million, for Asia and Africa. Additional economic benefits would be derived from fertiliser-N reductions and soil-N enrichment. There are two possibilities for approaching the question of when to inoculate—either inoculate all legumes at sowing or take a more strategic approach. The only risk of the first option is over- or unnecessary inoculation and the small additional cost of production. The risk of the second option is that inoculants are not used when they are needed, resulting in N-deficient crops and a substantial reduction of product and revenue, i.e. the status quo.

It is technically simple but demanding of time and resources to establish the need for inoculation using traditional experimentation in the field. Results are site- and, to some degree, season-specific, making it difficult to extrapolate too widely. For that reason, Brockwell in PN/1983/006 (G) broke ranks with the traditional approach (e.g. PN/1983/006 (A–D)) and developed the glasshouse-based WSIT (Brockwell et al. 1988). The WSIT remained site-specific and was never properly calibrated as a need-to-inoculate test. It was certainly quicker and far less demanding of time and resources than field testing and gave a good indication of the N_2-fixing capacity of the rhizobia in test soils for particular legumes. There is scope to further develop the WSIT as a quick, reliable test for the need to inoculate, by calibrating it against field tests. A calibrated WSIT would have immediate application in future ACIAR-funded research. Predictive models on the population dynamics of soil rhizobia and the need to inoculate could eventually be developed once sufficient information on the factors affecting numbers and effectiveness of the rhizobia was gathered (e.g. Herridge 2006).

Farmer knowledge of inoculation

Extension and training programs for farmers and their advisors on legume inoculation and to stimulate interest should be done in the context of legume/*Rhizobium* projects. It is interesting that only one of the suite of *Rhizobium*/inoculants projects, i.e. LWR2/1993/103, contained extension as an objective. Extension and training are major components of the new project proposal for Myanmar and of the proposal to the CARD program in Vietnam. It is worth noting that development of extension and training packages for a particular country, e.g. Myanmar, would also have substantial application and potential benefits to other countries of the region and to Australia.

B. Investment options related to SUPPLY for rhizobial inoculants

Low-income, dispersed markets

There may be demand for inoculants in a country or region, but the fact that the market may consist of widely dispersed, smallholders operating at low-input, subsistence levels makes it difficult for suppliers of inoculants. This constraint may well fall under the policy (and economic) umbrella but is also a part of extension and training and inoculants distribution.

Inoculant manufacturing technology, QA and distribution

The biggest issues with legume inoculation are whether there is a demand for inoculants in the marketplace (see above) and whether efficacious, high-quality inoculants are available in that same marketplace. The following factors affect the production, distribution and QA of inoculants:

- Rhizobial strains. In all countries, including Australia, there is an ongoing need to identify and use highly effective and adapted strains.

- Fermentation protocols and other production issues. These may need to be developed as part of project activities. Specific issues include fermentation capacity and/or type of fermenters, as well as broth formulations, sterility maintenance, procedures for dispensing broths into the inoculant carrier etc.
- Inoculant carriers and packaging. In most instances, inoculants are delivered to farmers in small packets of peat or similar material or in liquid or granular form. The efficacy and cost-effectiveness of the different formulations and their packaging need to be established for any particular country or region.
- Shelf life and storage of inoculants. Because rhizobial inoculants contain living things, shelf life and storage conditions are critical issues. Shelf life should be as long as possible to account for the often extended periods between manufacture and use. Factors that affect shelf life are temperature and humidity during storage and transport, strains and carrier formulation, and the use of protectants, e.g natural and synthetic polymers, incorporated into the inoculant.
- Death of rhizobia during the process of inoculation. This is a universal problem (e.g. LWR2/1993/103), particularly for pre-inoculated seed in Australia.
- Distribution of inoculants. This is not so much an R&D issue but a matter of utilising existing networks, e.g. extension networks, or creating new ones in the country or region.
- QA of inoculant production and use. QA protocols should be applied to all aspects of inoculant production and use. For most countries, the protocols would need to be developed then implemented. Strain verification (serology, PCR etc.) and maintenance (freeze-drying, paraffin etc.) are major issues with rhizobia. Because of the slow growth rates, particularly in the case of the tropical species, contamination of cultures is an ever-present problem.

Private-sector involvement in manufacture and marketing

The desirability of public (departments of agriculture, universities) and private-sector involvement in inoculant R&D, QA, production and marketing has been recognised for some time. In practice, public and private-sector roles are often blurred; many countries, for example, have at least rudimentary inoculant production units in public institutions, often called ‘pilot production’. They usually aim to establish the technical feasibility of production, to demonstrate inoculants and inoculation to farmers and to raise awareness of the benefits of inoculation amongst users and policy makers. Few pilot facilities develop into larger private-sector operations or significantly increase market penetration, perhaps because of the research, rather than commercial, focus of public institutions. The performance indicators of research (new knowledge, scientific publications etc.) differ from those of a private-sector company (product development and quality, market acceptance, sales and especially profit). Ideally, public facilities should shift as quickly as possible from manufacturing inoculants to providing R&D support, and perhaps external QA, leaving manufacturing and marketing to the private sector (Singleton et al. 1997).

It is worth noting the commitments of the Fitohoocmon Fertilizer Joint Stock Company and Cu Chi Bio-Chemical Fertilizer Joint Stock Company to the 2006 OPI-led CARD proposal to develop inoculant production in Vietnam. The companies have committed to participate in manufacturing, marketing and extension through contributions of cash, personnel, equipment and fertiliser.

Supporting* Rhizobium *R&D capacity

This is critical. As stated above, the countries that have successfully embraced legume inoculation also maintain dynamic public-sector R&D capacity in *Rhizobium* science. Fostering of R&D capacity in partner countries would be a natural outcome of ACIAR project funding of any of the demand and supply constraints listed above. Additionally, ACIAR could provide funding for research and training networks, i.e. linking individuals in Australian and country institutions. During my travel for this review, both Thai and Malaysian scientists expressed interest in such funding. For example, Zulkifli (UPM, Malaysia) would like to see a research network/training project that facilitated collaborative research and postgraduate training in inoculants. Funding would come jointly from Australian and Malaysian institutions. Training would be most cost-effectively done as a Malaysian university MSc/PhD with time spent in one of the Australian universities.

Other opportunities for R&D investment

In Thailand, constraints to adoption of *Rhizobium* technology relate to constraints on legume production (demand), rather than to the technology itself. Thailand has a history of large-scale inoculant use and *Rhizobium* technology is well-developed in this country, with considerable expertise in the laboratories at Suranaree (Nantakorn), Khon Kaen (Banyong) and Chiang Mai universities (Rerkasem, Ampan and Arawan) as well as in the DOA Biological Nitrogen Fixation Centre, Bangkok. It became clear from discussions with these scientists that the rising cost of urea N has prompted an upsurge in interest in legumes and N supply in farming systems in, for example, the following areas:

- Legume N_2-fixation as a source of N in organic-farming systems. There is a growing interest in Thailand, stimulated by government policy, for production of organically farmed food. Driving forces are concerns for human health and both national and export markets. Clearly, there is a parallel need for *Rhizobium* inoculants for the various legume species used in the organic-farming systems.

- Legume N_2-fixation in land rehabilitation and forestry. Currently, the DOA supplies *Rhizobium* inoculants for tree and forage legumes. According to Nantakorn Boonkerd, this is a growing market, responding to concerns about flooding, rising watertables and salinity caused by clearing of the native vegetation and its replacement with annual crops (sounds familiar).

- Legume N_2-fixation as a source of N in conventional farming systems. Banyong Toomsan (KKU), in particular, is now almost 100% focused on integrating legumes and legume N_2-fixation into the farming systems of north-east Thailand, e.g. groundnut in cassava and sugarcane systems. Both Ampan and Chalermpone at CMU are focused on legumes and N in the upland and lowland systems of north-west Thailand.

Thus, it would appear that there has been a similar evolution of thinking about legumes and legume N_2-fixation in Thailand to the evolution of thinking by ACIAR and Australian scientists in the early 1990s (Willet 1992); i.e. that legume N_2-fixation should be considered in the context of the farming system, rather than just for the growing of the legume itself.

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Appendix 1

Terms of reference

1. Compile an overview of the past *Rhizobium* research funded by ACIAR in Australia and partner countries, including the impact on capacity building in *Rhizobium* research.
2. Identify and evaluate community impacts (economic, social, environmental) the ACIAR-funded work on *Rhizobium* may have had in Australia and in partner countries. Addressing this TOR will involve travel to selected partner countries where there is a high likelihood of tracking impacts, and the findings against this TOR will serve as the basis for the possible commissioning of a follow-on formal impact assessment study.
3. Identify and evaluate the main constraints to broad-scale adoption of *Rhizobium*-based technology.
4. Identify critical research gaps and assess future investment options to overcome constraints identified in TOR 3. Investment options need to be assessed in the context of ACIAR's funding priorities as stated in the ACIAR Annual Operational Plan (ACIAR 2006).

Key deliverables

A report documenting the findings and providing recommendations on where ACIAR might target impact assessment studies (if any) and potential future investments. Depending on the information collated, this report might lend itself to the production of an ACIAR publication for broader public circulation.

Appendix 2

Overview of projects on *Rhizobium* science and legume N_2-fixation funded by ACIAR

Personnel - Australia	Personnel - overseas	Project/Year	1984 1985 1986 1987 1988 1989 1990 1991 1992 1993 1994 1995 1996 1997 1998 1999 2000 2001
Rhizobium/inoculation projects			
Roughley, Date,	Sundram	PN/1983/006 – A	Malaysia
Roughley, Date,	Faizah	PN/1983/006 – B	Malaysia
Roughley, Date,	Homchan	PN/1983/006 – C	Thailand
Thompson	Bhromsiri	PN/1983/006 – D	Thailand
Dart	Almendras, Umali-Garcia	PN/1983/006 – E (1985/073)	Philippines
Dart	Paterno	PN/1983/006 – F (1985/074)	Philippines
Brockwell	Daoud	PN/1983/006 – G (1985/075)	Jordan
Roughley	Simanungkalit	LWR2/1988/029	Indonesia
Roughley	Paterno, Inciong	LWR2/1993/103	Philippines
Dart	Almendras, Umali-Garcia, Paterno	PN/1987/031	Philippines
Gibson, Burdon, Brockwell	Bai Jiayu, Le Dinh Kha	PN/1992/027 – B	China, Vietnam
Herridge	Hiep, Diep, Lien Hoa, Yen Thao, Hien, Hong	LWR2/1998/027	Vietnam
Mineral nutrition of *Rhizobium* and its legume host			
Loneragan, Dilworth	Boonkerd, Parkpian, Ratanarat	PN/1983/029 and PN/1986/003	Thailand, Malaysia
O'Hara, Bell	Keerati-Kasikorn, Toomsan Rerkasem, Mahmood		
Legume N_2-fixation, cropping systems projects			
Bergersen, Herridge	Sudin, Faizah, Kewi, Norhayati, Rerkasem	PN/1983/005	Malaysia, Thailand
Peoples	Rerkasem, Sampet, Bhromsiri, Nurhayati, Faizah	PN/1988/000	Thailand, Indonesia, Malaysia
Herridge, Peoples	Shah, Shah, Ali, Aslam, Bhattarai, Maskey, Hong	LWR2/1992/010	Pakistan, Nepal, Vietnam
Schwenke	Shah, Shah, Ali, Aslam, Bhattarai, Maskey	LWR2/1997/062	Pakistan, Nepal
	Rupela (ICRISAT)	LWR2/1995/710	ICRISAT (India)
	Sanginga (IITA)	LWR2/1995/712	IITA (Nigeria)

Appendix 3

Summary of ACIAR-funded projects on *Rhizobium* and inoculation of legumes

PN/1983/006 – Ecological studies of root nodule bacteria and the use of legume inoculants

Part A – Nodulation and nitrogen fixation with *Arachis hypogaea* and *Leucaena leucocephala*

Commissioned organisation

NSW Agriculture & Fisheries

Project personnel

Dr R.J. Roughley	NSW Agriculture & Fisheries, Gosford, Australia
Dr R.A. Date	CSIRO Tropical Crops & Pastures, Brisbane, Australia
Mrs Joy Sundram	MARDI, Kuala Lumpur, Malaysia

Project duration

July 1984–December 1987

Project objectives

- To determine the nodulation status of groundnut and leucaena in a range of soil types
- If inoculation is required, to select adapted strains and assess their success

Project conduct/achievements

Extracted from the Henzell, Saono, Graham, Craswell and Wallis review in August 1987:

> …This Sub-project has achieved its major objective of determining the nodulation status of groundnut and Leucaena in Peninsula Malaysia. Inoculation, which resulted in reasonable establishment of inoculant strains, did not significantly increase peanut yields at any of six sites where the crop had not previously been grown; neither did the application of 100 kg N per ha. In this, as in other Sub-projects, it was considered that other mineral nutrient deficiencies may have limited the response to inoculation and/or N fertilizer.
>
> With Leucaena, inoculation gave a significant early response, but had a longer-lasting effect only on the peat and bris soils, where the legume grew relatively poorly. A very interesting feature of the results obtained with Leucaena was the marked cycling of nodules, with good populations at 98 and 423 days and virtually none at 205 days. The xylem sap analysis method has not yet been calibrated for use with leucaena. If that can be done in time, it would be desirable to use it on leucaena plants in parallel with determination of the presence or absence of nodules by excavation and sieving.
>
> Effective acid-tolerant strains of Rhizobium for both groundnut and leucaena have been isolated, including a super-tolerant strain for groundnut …

PN/1983/006 – Ecological studies of root nodule bacteria and the use of legume inoculants

Part B – Improvement of the existing inoculant production and inoculation technique for perennial cover crops

Commissioned organisation

NSW Agriculture & Fisheries

Project personnel

Dr R.J. Roughley	NSW Agriculture & Fisheries, Gosford, Australia
Dr R.A. Date	CSIRO Tropical Crops & Pastures, Brisbane, Australia
Dr Faizah A. Wahab	Rubber Research Institute of Malaysia, Kuala Lumpur

Project duration

July 1984–December 1987

Project objectives

- To select suitable carriers of rhizobia for legume inoculants and to determine suitable methods of preparation, sterilisation and packaging
- To investigate the establishment of inoculum strains for *Pueraria phaseoloides* and *Calopogonium caeruleum* in young rubber plantations

Project conduct/achievements

Extracted from the Henzell, Saono, Graham, Craswell and Wallis review in August 1987:

> …All three objectives have been achieved.
>
> (a) Malaysian sources of peat that are suitable as carriers of inoculant have been selected, and revised methods developed for the preparation, sterilisation and packaging of legume inoculants. Using these methods counts of rhizobia in peat of as high as 9×10^9 per gram have been achieved. A simple procedure for determining the water potential of peat has also been devised.
>
> (b) acid-tolerant strains of rhizobia have been isolated from Malaysian soils. Attempts to develop antibiotically marked strains of *Bradyrhizobium* for use in inoculation studies were discontinued when test strains were found to have high natural levels of resistance to streptomycin and other antibiotics. Instead fluorescent antibody procedures were used in subsequent ecological studies.
>
> (c) two bradyrhizobia isolated from *Calopogonium* were used to inoculate *Pueraria* and *Calopogonium* in field studies in young rubber plantations. There was little evidence of response to inoculation when normal rates of inoculation were used. As with *Leucaena* in Peninsular Malaysia (Sub-project A) there was evidence of nodule cycling. The reasons for this remain to be determined but could be reflection of mineral nitrogen levels in the soils. Sap analysis samples taken over the next several months together with further field sampling for the presence or absence of nodules, might resolve these problems …

PN/1983/006 – Ecological studies of root nodule bacteria and the use of legume inoculants

Part C – Rhizobial ecology of forage legumes

Commissioned organisation

NSW Agriculture & Fisheries

Project personnel

Dr R.J. Roughley	NSW Agriculture & Fisheries, Gosford, Australia
Dr R.A. Date	CSIRO Tropical Crops & Pastures, Brisbane, Australia
Dr Juckrit Homchan	Khon Kaen University, Khon Kaen, Thailand

Project duration

July 1984–December 1987

Project objectives

To study *Rhizobium* population dynamics in the soil and rhizospheres of *Leucaena leucocephala* and *Stylosanthes* spp. and its effect on yield

Project conduct/achievements

Extracted from the Henzell, Saono, Graham, Craswell and Wallis review in August 1987:

> … The objectives of this Sub-project have been achieved conclusively. The failure of two species of Stylosanthes to respond to inoculation at some sites on the main soil series of NE Thailand was attributed to failure of the inoculant to form a significant proportion of the nodules. With leucaena, the inoculant formed a high proportion of nodules and increased yield in the establishment year but

> this response was not maintained into the second year. It appears also that there are other nutritional constraints limiting N_2-fixation by leucaena on these Thai soils.
>
> The Reviewers were informed by Dr Juckrit Homchan that the University of Khon Kaen has ceased research on herbaceous forage legumes, though forage work is continuing in the Thai Livestock Department. Fuelwood is very scarce in NE Thailand and there is likely to be a strong demand in the future for nitrogen-fixing trees that provide both wood and forage …

PN/1983/006 – Ecological studies of root nodule bacteria and the use of legume inoculants

Part D – Soybean–rhizobium compatibility and the need for inoculation

Commissioned organisation

NSW Agriculture & Fisheries

Project personnel

Dr J.A. Thompson	NSW Agriculture & Fisheries, Tamworth, Australia
Mrs Ampan Bhromsiri	University of Chiang Mai, Chiang Mai, Thailand

Project duration

July 1984–December 1987

Project objectives

- To examine the compatibility of naturally occurring rhizobium in traditional soybean growing areas of northern Thailand with soybeans from local and overseas sources
- To determine the need for inoculation and examine the need for selection of host germplasm for compatibility with the local rhizobium populations
- To integrate these studies with other workers in Asia to provide a better understanding of the relationships of the soybean host germplasm and the rhizobia which nodulate them. Such understanding could ultimately result in the definition of zones where seed inoculation is not necessary.

Project conduct/achievements

Extracted from the Henzell, Saono, Graham, Craswell and Wallis review in August 1987:

> … Parts (i) and (ii) of the objective have been answered very elegantly. The existing *Rhizobium* populations in the traditional soybean growing areas of northern Thailand are compatible with a wide range of soybean genotypes. With current farming practices the inoculation of soybeans in northern Thailand is neither beneficial nor necessary.
>
> The 1,500 strains of *Rhizobium* obtained from this region, which is close to the centre of origin of soybean, are a very valuable genetic resource.
>
> Part (iii) of the objectives has been achieved to some extent through conference presentations and personal contacts. The Reviewers see benefit in closer links being developed between this Sub-project and ACIAR Project 8405 'Improvement of Soybeans and mungbeans in the Tropics', especially to evaluate the inoculation response of major lines of soybean parental material in use in Thai breeding programs …

PN/1983/006 – Ecological studies of root nodule bacteria and the use of legume inoculants

Part E (PN/1985/073) – Ecology of *Rhizobium* nodulating tree legumes

Commissioned organisation

NSW Agriculture & Fisheries

Project personnel

Dr P. Dart	Australian National University, Canberra, Australia
Ms Angela Almendras	Visayas College of Agriculture, The Philippines
Dr Mercedes Umali-Garcia	University of the Philippines at Los Baños

Project duration

January 1986–December 1987

Project objectives

- To determine the interrelationships between *Rhizobium* strains and promising tree legume species and cultivars in different regions of the Philippines, and to select effective and competitive *Rhizobium* strains suitable for use as inoculants
- To select strain–host cultivar combinations tolerant of soils with low pH–high aluminium levels and/or find other economic management practices to ameliorate this stress
- To determine the response to microbial inoculation of promising tree species in different field environments

Project conduct/achievements

Extracted from the Henzell, Saono, Graham, Craswell and Wallis review in August 1987:

> … Good progress has been made in a year and a half in achieving the objectives of:
>
> (a) selecting *Rhizobium* strains suitable for use as inoculants: 120 strains from the Philippines and elsewhere have been purified and authenticated,
>
> (b) determining the inter-relationship between *Rhizobium* strains and legume tree species and cultivars: glasshouse pot trials have been conducted to test cross infection of *Rhizobium* from other *Acacia* spp. on *A. mangium* and *A. auriculiformis*; seven field sites have been established to assess natural nodulation of legume trees, and
>
> (c) determining the response to inoculation: nursery and field trials have been established with two inoculant strains on *A. mangium*.
>
> In addition, equipment has been provided, culture techniques worked up and a start made on studying soil acidity problems. The effective interaction with ACIAR Project 8320 'Australian hardwoods for fuelwood and agroforestry' was noted …

PN/1983/006 – Ecological studies of root nodule bacteria and the use of legume inoculants

Part F (PN/1985/074) – Ecology of *Rhizobium* nodulating grain legumes in the Philippines

Commissioned organisation

NSW Agriculture & Fisheries

Project personnel

Dr P. Dart	Australian National University, Canberra, Australia
Dr Erlinda S. Paterno	University of the Philippines at Los Baños

Project duration

January 1986–December 1987

Project objectives

- Assess the need to inoculate soybean and mungbean in different farming systems in the Philippines
- Determine the type of *Rhizobium* nodulating local and improved soybean and mungbean germplasm

- Select strains suitable for use as inoculants for soybean and mungbean and assess their field performance
- Advise soybean and mungbean breeders on the strategy to adopt to ensure good nodulation and nitrogen-fixation from their material when grown in farmers' fields

Project conduct/achievements

Extracted from the Henzell, Saono, Graham, Craswell and Wallis review in August 1987:

> … Despite delays in finalising the MOU and in providing funds to the ANU and UPLB, commendable progress has been made in a year and a half, building on work already being supported by other agencies. Specifically, the following objectives have been addressed:
> (a) assessing the need to inoculate: field trials have been set up in the Philippines to assess the long-term persistence of inoculant strains on soybean and the cultivar x strain and inoculation x fertilizer interactions on mungbeans (research supported by other agencies showed large responses to inoculation of soybean at seven sites in the Philippines),
> (b) determining the type of *Rhizobium* nodulating soybean and mungbean: *Rhizobium* populations in some Philippine soils have been enumerated by the MPN technique and,
> (c) selecting strains suitable for inoculants: more than 200 isolates have been made and strain x cultivar tests set up in the ANU glasshouse and in the field in the Philippines (mungbeans only).
> The research has not yet advanced to the point where the 4th objective can be addressed …

PN/1983/006 – Ecological studies of root nodule bacteria and the use of legume inoculants

Part G (PN/1985/075) – Ecology of rhizobia as related to vegetation, soil, climate and land management

Commissioned organisation

NSW Agriculture & Fisheries

Project personnel

Mr J. Brockwell	CSIRO Plant Industry, Canberra, Australia
Mr Deifallah M. Daoud	University of Jordan, Amman, Jordan

Project duration

January 1986–December 1987

Project objectives

- To develop a non-microbiological procedure for predicting the number and symbiotic capacity of populations of *Rhizobium* spp. that occur naturally in soil

Project conduct/achievements

Extracted from the Henzell, Saono, Graham, Craswell and Wallis review in August 1987:

> … This Project has made substantial progress in a year and a half. The populations of *Rhizobium* capable of nodulating *Medicago* spp. in the field have been sampled over a wide area in Australia, and also in Jordan where the intensity of cultivation is greater. Tests have been carried out to estimate the effectiveness of this material and this information will be used to assess the hypothesis that is the main objective of the Project.
>
> Research in Australia and West Asia has shown that *Medicago rigidula*, a potentially useful legume for farmers in West Asia, is highly strain specific.
>
> Good links have been developed with ICARDA and the Reviewers see value in continuing contact between Mr Brockwell and the forage legume scientists at this Centre, especially if it is possible to examine *Rhizobium* populations in the large collection of soils that ICARDA has made in West Asia. Provision should also be made for a further visit by Mr Brockwell to the University of Jordan in 1988.
>
> The Reviewers noted the advice of the Dean, Faculty of Agriculture, University of Jordan (Appendix III) that the lentil–faba bean system has more relevance to Jordan's agriculture than the medic system …

PN/1987/031 – Biological nitrogen-fixation in food and tree legume production systems in the Philippines

Commissioned organisation

University of Queensland

Project personnel

Dr P. Dart	Faculty of Agriculture, University of Queensland
Dr Erlinda S. Paterno	University of the Philippines at Los Baños
Ms Angela Almendras	Visayas College of Agriculture, the Philippines
Dr Mercedes Umali-Garcia	University of the Philippines at Los Baños

Project duration

January 1988–June 1991

Project objectives

Tree legumes

- Assess natural nodulation of the tree legume species, *Acacia mangium, A. auriculiformis, Gliricidia sepium, Enterobolium cyclocarpum, Calliandra callothyrsus, Sesbania grandiflora, Albizia falcataria, Pterocarpus indicus* and determine the response to inoculation with *Rhizobium*
- Select *Rhizobium* inoculant strains for use in nurseries and with direct seeding. Develop inoculation methods. Determine the seasonal pattern of nodule development.
- Determine nutritional factors limiting biological nitrogen-fixation in the field and develop fertiliser recommendations for use in the nursery and field. Select strain and species/provenances tolerant of acid-soil-related stresses.
- Measure biological nitrogen-fixation by tree species

Food legumes

- Examine the need to inoculate soybean and mungbean in different sites and cropping systems. Select *Rhizobium* strains for use in inoculants.
- Assess inoculum build-up in soil and the requirements for continued inoculation
- Select *Rhizobium* strain and cultivar combinations for acid soils. Determine nutrient amendments for soil which maximise soybean and mungbean nitrogen-fixation
- Estimate the amount of nitrogen fixed by soybean and mungbean under field conditions, and made available to subsequent crops

Project achievements

Rhizobium strains nodulating tree legumes in the Philippines were isolated and tested for effectiveness in nitrogen-fixation in the nursery and field. A large, diverse collection of tree-legume rhizobia was assembled and a computerised database with search capability for different characters was developed. The response to inoculation, nodulation and N_2-fixation of a range of *Acacia* species was tested in acid soils with high aluminium concentrations. The response by *Acacia mangium*, *A. auriculiformis* and *Gliricidia sepium* to fertiliser addition was assessed in a range of soils. Amounts of N_2 fixed were quantified using ^{15}N abundance.

Twelve sites were established to determine inoculation responses of mungbean and soybean. Acid tolerant (in vitro) mungbean cultivars and rhizobial strains were selected and tested in acid soils in the field. Effects of NPK and Ca and lime on nodulation, N_2-fixation and yield of mungbean were assessed. Amounts of N_2 fixed were quantified using ^{15}N abundance and ureide methodologies, in collaboration with CSIRO Plant Industry (PN/1988/000) and funded by an AIDAB–ACIAR Special Project.

Laboratory and plant and soil processing equipment was purchased and shipped to the Philippines.

LWR2/1988/029 – Biological nitrogen-fixation by soybeans in rotation with rice

Commissioned organisation

NSW Agriculture & Fisheries

Project personnel

Dr R.J. Roughley	NSW Agriculture & Fisheries, Gosford, Australia
Mr J. Brockwell	CSIRO Plant Industry, Canberra, Australia
Dr Robert D.M. Simanungkalit	Bogor Research Institute for Food Crops, Bogor, Indonesia

Project duration

Phase 1: October 1990–September 1993
Phase 2: October 1993–September 1995

Project objectives

Phase 1 (1990–93)

- To study the symbiotic response of local cultivars and those of US origin with rhizobia of Indonesian and international origin
- To study the performance of strains in current use with ones selected as 'presumptive tolerant' of acid-soil factors and a standard strain known to perform well in a range of environments
- To compare the population dynamics of rhizobia in soil and in the rhizospheres of rice and soybean in different rice management systems including, in Australia, high-watertable cultures for soybean
- To evaluate carrier materials from Indonesia and to determine the optimum moisture potential for strains of soybean rhizobia with respect to numbers produced and their survival on seed

Phase 2 (1993–95)

- To test the performance of 24 acid-tolerant strains of bradyrhizobia selected in the first phase of the project at three field sites in Indonesia with acid soils
- To determine whether or not the application of molybdenum would increase N_2-fixation
- To compare methods for selecting strains of rhizobia tolerant to acid soils
- To freeze-dry the germplasm bank of soybean rhizobia collected and tested in the original project
- To re-assess the effect of moisture potential of peat-based inoculants on survival of rhizobia when inoculated onto seed

Project conduct/achievements

The Boonkerd, Dar, Saono review of the Phase 1 of the project in April 1993 was positive:

> … The project … has substantially achieved all major project objectives. The scientific methodology employed in the project appears to be sound and up to date.

> There was evident high degree of co-operation and collaboration…. which resulted in achieving quality of the reports and publications.
>
> The project is extremely relevant to Indonesian agriculture, especially in increasing soybean production which is highly in demand in the country. It has great impact on the environment by maintaining soil fertility including the reduction of the use of chemical fertiliser. Men, women and children are also benefited by having adequate protein in their diet.
>
> We have no reason to criticise the administration of the project which was done efficiently. We also believe that there will be real high cost benefit from this project. The project has significant potential spillover benefits not only to local inoculant producers but also to producers of other countries in Southeast Asia as well as Australia.

LWR2/1992/027 (B) – Australian acacias for sustainable development in China, Vietnam and Australia

Commissioned organisation

CSIRO Forestry and Forest Products

Project personnel

Dr A.H. Gibson	CSIRO Plant Industry, Canberra, Australia
Dr J.J. Burdon	CSIRO Plant Industry, Canberra, Australia
Mr J. Brockwell	CSIRO Plant Industry, Canberra, Australia
Mr Bai Jiayu	Research Institute of Tropical Forestry, China
Professor Le Dinh Kha	Forest Science Institute of Vietnam, Vietnam

Project duration

January 1994–June 1999

Project objectives

Phase 1 (1994–1996)

- To isolate, test and characterise strains of rhizobia suitable for inoculating a range of *Acacia* species and provenances; to determine their ability to fix N in the glasshouse, nursery and field and to persist in the nursery and field
- To develop inoculant technologies and silvicultural practices to ensure that nursery-grown seedlings are healthy and well nodulated at outplanting
- To apply molecular techniques to the characterisation of rhizobia from various hosts and different sites as a means of defining relationships between molecular characters and (a) soil type of origin and (b) symbiotic performance; to appraise these techniques as a means of strain identification

Phase 2 (1997–1999)

- To develop a reliable method for rhizobial inoculation of nursery-grown *Acacia* seedlings
- To select strains of rhizobia that can be used in multi-strain inoculants for inoculating multiple, symbiotically diverse species of *Acacia*
- To devise a technique for accurate enumeration of *Acacia* rhizobia

Project conduct/achievements

Progress in Phase 1 was compromised somewhat by the sad and untimely death of subproject leader Alan Gibson. The subproject was reviewed by John Brockwell in 1996 and objectives in Phase 2 revised as a result of that review. John Brockwell then led the subproject until termination in 1999. Phase 2 appears to have been very successful in achieving objectives, as the following excerpts for the project termination report of John Brockwell indicate:

> The subproject has accomplished a good deal in a relatively short time and on a modest budget … developed a simple procedure in which peat culture of appropriate nitrogen-fixing rhizobia is incorporated in nursery soil immediately prior to sowing *Acacia* seed. This delivers inoculant at a rate of 10 million cells per seedling at a cost of $0.10–0.25 (Australian) per *Acacia* tree. It ensures that each seedling is abundantly nodulated and vigorously fixing nitrogen at the time of outplanting … selected six strains of rhizobia that between them have the capacity to nodulate and fix nitrogen with 20 species of Acacia that are in use in farm and plantation forestry … developed a serial dilution, nodulation frequency, plant infection technique, using siratro (*Macroptilium atropurpureum*) as a test plant, that accurately enumerates *Acacia* rhizobia … the microbiology laboratory at RITF is now equipped to produce peat inoculant for nursery-grown *Acacia* seedlings. Part of ACIAR's cash contribution to RITF was used to buy a rotary flask shaker of large capacity. This can be used to produce enough broth culture to prepare peat inoculant for one million nursery-grown *Acacia* tree seedlings per year at a cost to RITF not exceeding $0.25 (Australian currency) per tree. This presents RITF with the opportunity of commercializing inoculant production for acacias and other tree legumes. This might be a significant consideration in terms of the Institute's budget …

LWR2/1993/103 – The production, quality control and use of legume inoculants

Commissioned organisation

NSW Agriculture

Project personnel

R.J. Roughley	NSW Agriculture, Gosford, Australia
Prof Erlinda S. Paterno	University of the Philippines at Los Baños
Dr Nora Inciong	Department of Agriculture, Manila, the Philippines

Project duration

April 1994–March 1996

Project objectives

- To determine the most suitable methods of introducing improved strains of rhizobia for soybean and mungbean into field soils
- To demonstrate the selected method(s) to extension workers and thence to farmers
- To determine the most suitable local carrier material for legume inoculants and to optimise the survival of rhizobia during transport and on seed
- To introduce a comprehensive quality control system for inoculants available in the Philippines

Project conduct/achievements

This was an ACIAR small project with a two-year duration and budget of $150,000. The project was not formally reviewed. The following assessment was taken from the project final report (dated 1996), prepared by the project leader, Dr R.J. Roughley:

> … In practice it proved difficult to achieve all the objectives. The trials were usually located far from the base laboratory and were influenced by the skills of the local operators. By far the most important limiting factor was weather. While some trials were severely affected by drought, others sown in the dry season were washed by heavy rain. Nevertheless some promising results were obtained in what was a short, intensive program. Many questions for which answers were sought for the extension package must necessarily be answered after the project has ended and the necessary trials will be detailed in the follow-up section of this report …
>
> The project probably achieved what it could, given the limited time-frame and budget. The final report outlined three areas of follow-up work, which continued the themes of the original project. It is not known whether the follow-up activities were completed or not.

LWR2/1998/027 – Increasing yields and nitrogen-fixation of soybean, groundnut and mungbean in Vietnam through rhizobial inoculation

Commissioned organisation

NSW Agriculture

Project personnel

Dr D.F. Herridge	NSW Agriculture, Tamworth, Australia
Dr N.H. Hiep	Cantho University, Cantho, Vietnam
Dr C.N. Diep	Cantho University, Cantho, Vietnam
Dr N.G. Lien Hoa	Oil Plants Institute, Ho Chi Minh City, Vietnam
Ms T. Yen Thao	Oil Plants Institute, Ho Chi Minh City, Vietnam
Ms T.N. Hien	Institute of Agricultural Science, Ho Chi Minh City, Vietnam
Mr P.N. Quy	Institute of Agricultural Science, Ho Chi Minh City, Vietnam
Mr H.H. Tien	Institute of Agricultural Science, Ho Chi Minh City, Vietnam
Mr N.X. Hong	Vietnam National University, Hanoi, Vietnam

Project duration

April 1999–October 2001

Project objectives

- Quantify N_2-fixation by legumes in farmers' fields in Vietnam and identify farmer management practices affecting N_2-fixation
- Evaluate the quality of inoculants, currently produced in Vietnam
- Complete multi-location inoculation experiments to determine the most-effective strains for each species and benefits of inoculation in terms of yield and N_2-fixation
- Enhance the capacity in Vietnam to produce and distribute high-quality rhizobial inoculants through research and training and infrastructure development
- Lay the foundation for a (non-ACIAR) development project to produce large volumes of high-quality rhizobial inoculants in Vietnam to satisfy an expanded legume industry

Project conduct/achievements

This project was not formally reviewed; rather it terminated with a workshop in Hanoi highlighting project activities and published as ACIAR Proceedings No. 109 (Herridge D. (ed.) 2002. Inoculants and nitrogen fixation of legumes in Vietnam. ACIAR Proceedings No. 109e at <www.aciar.gov.au>.

The project was essentially conducted as outlined in the proposal document. In 1999, soon after the project commenced, five Vietnamese scientists were trained in inoculant production technology and QA procedures for a period of 4 weeks at the NSW Agriculture laboratory, Gosford. Surveys of 155 farmer crops of soybean, mungbean and groundnut in Vietnam were conducted during 1999–2001 to determine current levels of N_2-fixation for those species and farmer attitudes to and use of rhizobial inoculants and fertiliser N for legumes. At the same time, effective strains of rhizobia were assembled for these species and the benefits of inoculation evaluated in 23 field trials during 2000–01 in the south of the country. The quality of inoculants produced in Vietnam was evaluated during 2001. Finally, a review and planning workshop was held in Hanoi during October 2001.

Appendix 4

Summary of ACIAR-funded projects on mineral nutrition of *Rhizobium* and its legume host

PN/1983/029 – Nutrient requirements in relation to symbiotic nitrogen-fixation

PN/1986/003 – Boron and other micronutrients for food legume production

Commissioned organisation

Murdoch University

Project personnel

Professor J.F. Loneragan	Murdoch University, Perth, Australia
Professor M.J. Dilworth	Murdoch University, Perth, Australia
Dr G.W. O'Hara	Murdoch University, Perth, Australia
Dr R. Bell	Murdoch University, Perth, Australia
Dr Nantakorn Boonkerd	Department of Agriculture, Bangkok, Thailand
Dr Preeda Parkpian	Department of Agriculture, Bangkok, Thailand
Mr Suwapan Ratanarat	Department of Agriculture, Bangkok, Thailand
Dr Pirpoon Keerati-Kasikorn	Khon Kaen University, Khon Kaen, Thailand
Dr Banyong Toomsan	Khon Kaen University, Khon Kaen, Thailand
Dr Benjavan Rerkasem	University of Chiang Mai, Chiang Mai, Thailand
Dr Marziah Mahmood	Universiti Pertanian Malaysia, Serdang, Selangor, Malaysia

Project duration

March 1984–June 1987

Project objectives

PN/1983/029

- To develop and test for general use, techniques and standards for diagnosis of nutrient deficiencies of soybean and peanut
- To define the relationship of symbiotic associations and environmental conditions to nutrient requirements and deficiency diagnosis in soybean and peanut
- To define the nutrient deficiencies of selected soils in Thailand for the growth of soybean and peanut
- To develop physiological and biochemical diagnostic tests for the copper status of legumes in Malaysia

PN/1986/003

- To examine the requirement of iron for nodulation and nitrogen-fixation in peanut

Project conduct/achievements

The Craswell, Blair, Schiller, Boonkerd and Wallis review of the project in February 1986 was very positive:

> The review team was impressed by the high standard of the scientific investigations carried out by the collaborators in the project. The problem addressed by the project appears to be a major limitation to the production of peanuts, soybean and mungbean which are important crops in

Thailand. Considerable progress was made in identifying and defining boron deficiency as a limitation to crop production but further research is necessary before fertiliser recommendations can be made confidently ...

The project enhanced considerably the capacity of Thai and Malaysian scientists to undertake micronutrient research. However. the developing country collaborators have relied heavily on Murdoch University for analytical back-up and further strengthening of the developing country partners in this area is necessary. The project was well organised and collaboration between Murdoch University and the Thai and Malaysian institutions was generally excellent …

Appendix 5

Summary of ACIAR-funded projects on legumes as N_2 fixers in farming systems and methods for measurement of N_2-fixation

PN/1983/005 – Development and evaluation of methods to measure biological nitrogen-fixation

Commissioned organisation

CSIRO Plant Industry

Project personnel

Dr F.J. Bergersen	CSIRO Plant Industry, Canberra, Australia
Dr M.B. Peoples	CSIRO Plant Industry, Canberra, Australia
Dr D.F. Herridge	NSW Agriculture, Tamworth, Australia
Dr Mohd Noor Sudin	Rubber Research Institute of Malaysia, Kuala Lumpur, Malaysia
Dr Faizah Abdul Wahab	Rubber Research Institute of Malaysia, Kuala Lumpur, Malaysia
Dr Chong Kewi	Rubber Research Institute of Malaysia, Kuala Lumpur, Malaysia
Dr Norhayati Moris	Rubber Research Institute of Malaysia, Kuala Lumpur, Malaysia
Dr Benjavan Rerkasem	University of Chiang Mai, Chiang Mai, Thailand
Dr Kanok Rerkasem	University of Chiang Mai, Chiang Mai, Thailand

Project duration

July 1984–June 1988

Project objectives

- To develop suitable methods for measuring N_2-fixation by peanuts and soybeans which are promising grain-legume intercrops in rubber smallholdings. As it seems unlikely that one single method will be applicable to all of these systems, research will proceed along three fronts:
  - methods using application of ^{15}N-labelled materials
  - methods based on natural abundance of ^{15}N
  - specific translocate methods.
- Evaluate these methods after a suitable period of development along with other commonly used methods for measurement of N_2-fixation (difference method), specifically for applications in developing countries
- Define the role of nodulated legumes, in particular rice bean (*Vigna umbelata*) in intercropping systems with maize and make a comparison with appropriate monocrop systems

Project conduct/achievements

The Chalk/Myers review of the project in February 1987 was very positive:

> … impressed with the organisation and administration of the project, and the high standard of the scientific investigations carried out by all participating scientists …
>
> … both the quality and the quantity of the scientific endeavour is to be commended …
>
> … The investigators have been successful in achieving the stated objectives of the project …
>
> … The reviewers are confident that the project has considerably enhanced the research capacity of the scientists in the collaborating institutions in Malaysia and Thailand …

… too early to assess the direct economic benefit to the developing countries of the research conducted. However, we are confident that benefits will ultimately accrue to countries, including Australia, which adopt the technology developed in this project to evaluate and optimise N inputs via biological nitrogen-fixation …

PN/1988/000 – Measurement of nitrogen-fixation in legume production systems

Commissioned organisation

CSIRO Plant Industry

Project personnel

Dr F.J. Bergersen	CSIRO Plant Industry, Canberra, Australia
Dr M.B. Peoples	CSIRO Plant Industry, Canberra, Australia
Dr D.F. Herridge	NSW Agriculture, Tamworth, Australia
Dr Faizah Abdul Wahab	Rubber Research Institute of Malaysia, Kuala Lumpur, Malaysia
Dr Benjavan Rerkasem	University of Chiang Mai, Chiang Mai, Thailand
Mr Chalermpone Sampet	University of Chiang Mai, Chiang Mai, Thailand
Mrs Ampan Bhromsiri	University of Chiang Mai, Chiang Mai, Thailand
D.P. Nurhayati	Balai Penelitian Ternak, Bogor, Indonesia

Project duration

July 1988–December 1991

Project objectives

- To develop and calibrate methods for the measurement of N_2-fixation for selected food legumes and key herbaceous forage and tree legumes based on xylem solute and ^{15}N analysis
- To apply these methods in legume production systems in Thailand, Indonesia and Malaysia
- To promote the concept of, and techniques for, the measurement of N_2-fixation as a fundamental component of all ACIAR research involving legumes

Project conduct/achievements

The Chalk review of the project in September 1992 was very positive:

1. Project 8800 was a successful replacement project to its precursor, Project 8305.
2. All three objectives of the project as set out under Section 4.1 were met.
3. The scientific merit of the project was high, as assessed by the amount of new information provided.
4. The project had particular relevance to the wider adoption of legumes into farming systems.
5. The project provided both immediate and long-term benefits in terms of an enhanced ability to assess the role of legumes in the N economies of farming systems.
6. Effective and mutually beneficial collaboration occurred between the Australian scientists and their Malaysian, Thai and Indonesian counterparts, and between ACIAR projects involving legume research.
7. The project strengthened the research capacities of all participating scientists and their respective institutions.
8. There was an effective transfer of new knowledge to the scientific community through refereed journal publications, presentations at national and international conferences, seminars and newsletters.
9. There was effective transfer of the new technology to developing countries through workshops in Malaysia and Thailand and the publication of a methodology monograph.

LWR2/1992/010 – Management of legume N_2-fixation for rainfed cereal production in Pakistan, Nepal, Vietnam and Australia

Commissioned organisation

NSW Agriculture

Project personnel

Dr D.F. Herridge	NSW Agriculture, Tamworth, Australia
Dr G.D. Schwenke	NSW Agriculture, Tamworth, Australia
Mr W.L. Felton	NSW Agriculture, Tamworth, Australia
Dr M.B. Peoples	CSIRO Plant Industry, Canberra, Australia
Dr Muhammad Aslam	National Agricultural Research Centre, Islamabad, Pakistan
Dr Safdar Ali	University of Arid Agriculture (UAA), Rawalpindi, Pakistan
Dr Zahir Shah	North West Frontier Province Agricultural University, Peshawar, Pakistan
Dr Sabir Shah	North West Frontier Province Agricultural University, Peshawar (Agricultural Research Institute), Tarnab, Pakistan
Dr Surya Maskey	National Agricultural Research Institute, Kathmandu, Nepal
Ms Shanti Bhattarai	National Agricultural Research Institute, Kathmandu, Nepal
Mr Nguyen Xuan Hong	Vietnam National University, Hanoi, Vietnam

Project duration

January 1994–December 1997

Project objectives

- Quantify amounts of N_2 fixed by commercially grown legumes through surveys of farmer crops in all countries
- Develop legume–cereal cropping sequences that maximise production of the cereals in the sequence as well as economic productivity
- Develop farming practices that maximise the inputs of N_2 fixed by the legumes
- Assess effects of cropping sequences on the mineral, biological and organic fertility of the soil and therefore on long-term sustainability
- Synthesise the data on soil water and nitrate and yields of both legumes and cereals, and legume N_2-fixation, to develop quantitative relationships between these factors to be used as management tools

Additional objectives, specific for Vietnam:

- Quantify N_2-fixation by soybean, groundnut and mungbean intercropped with upland mulberry
- Determine the N benefit to the mulberry through isotopic measurements and measurement of soil and plant parameters
- Evaluate management practices on amounts of N_2 fixed and identify those practices that contribute most to high inputs of fixed N by the legumes
- Compare economic returns of mulberry–legume intercrop systems with mulberry alone and relate to other food and cash cropping systems in Vietnam

Project conduct/achievements

The Myers, Ladha review of the project in August 1996 was very positive. The reviewers were:

> … highly impressed with the research conducted in this project. Although not all objectives were achieved, what was achieved represents a considerable achievement, and what was not achieved represents unrealistic objectives. As a result of this project, a range of scientists in three Asian countries now have enhanced skills at quantifying N_2-fixation in legume crops, and they have also collected a comprehensive and unique data set on N_2-fixation of legume crops growing in farmers' fields. We believe that this data set is the first of its kind. As well as providing new information on N_2-fixation in these environments, the data have potential value in improving global estimates of N inputs and balance. The project scientists have reported that rotation experiments have been established at all sites, but that it is too early for substantial results to be available. If these experiments are conducted with the same skill and enthusiasm as the N_2-fixation surveys, the researchers should be able to go on to achieve the remainder of the objectives …

LWR2/1997/062 – Sustainable grain legume–cereal production systems through management of N_2-fixation

Commissioned organisation

NSW Agriculture

Project personnel

Dr D.F. Herridge	NSW Agriculture, Tamworth, Australia
Dr G.D. Schwenke	NSW Agriculture, Tamworth, Australia
Mr W.L. Felton	NSW Agriculture, Tamworth, Australia
Dr M.B. Peoples	CSIRO Plant Industry, Canberra, Australia
Dr Muhammad Aslam	National Agricultural Research Centre, Islamabad, Pakistan
Dr Safdar Ali	UAA, Rawalpindi, Pakistan
Dr Zahir Shah	North West Frontier Province Agricultural University, Peshawar, Pakistan
Dr Sabir Shah	North West Frontier Province Agricultural University (Agricultural Research Institute), Tarnab, Pakistan
Dr Surya Maskey	National Agricultural Research Institute, Kathmandu, Nepal
Ms Shanti Bhattarai	National Agricultural Research Institute, Kathmandu, Nepal

Project duration

January 1998–June 2001

Project objectives

- Develop highly productive, profitable and sustainable legume–cereal rotations for rain-fed systems in Pakistan, Nepal and the northern grains belt of Australia through effective utilisation of legume N_2-fixation
- Evaluate effects of legumes and legume N_2-fixation on soil N fertility, particularly related to short-term fertility and sustainability indices, and on soil N processes such as mineralisation
- Enhance existing information sources on legumes (simulation growth models) and legume N_2-fixation (multivariate, regression analysis of N_2-fixation of chickpea in PN 9210) to utilisation of tools such as the APSIM framework involving both chickpea and other species

- Develop management and information packages appropriate for all three countries on legume growth and production, legume N_2-fixation and legume benefits and N management in cereal-production systems and hand over to collaborating research institutes
- Complete calibration of the xylem solute method for measuring legume N_2-fixation, commenced in previous ACIAR-funded projects, for both ureide (e.g. mungbean, common bean) and non-ureide (e.g. groundnut) species
- Quantify N_2-fixation by legumes in farmers' fields in Nepal, using natural ^{15}N abundance and xylem ureide methods, and identify farmer management practices in all project countries that contribute most to high inputs of fixed N by the legumes
- Enhance the quality assurance of procedures in collaborating laboratory and field programs through training, properly documented protocols and cross checking
- Maximise the effectiveness of the project for collaborating groups as well as the spillover to other groups and individual researchers through workshops, meetings and, where possible, analytical assistance via formalised and informal arrangements.

Project conduct/achievements

There was no formal review of this project. The final report reads:

> … The Project was conducted according to the Specific Objectives, and the Timetable of Activities and Outputs Table. Operationally, LWR2/97/62 was built around 16 key field rotation experiments in Pakistan, Nepal and Australia, 5 field physiological experiments in Australia, 8 glasshouse experiments in Australia and surveys of farmer legume crops in Nepal … There was a strong emphasis on publication of outputs of LWR2/97/62. The publication list includes: 37 published or submitted scientific papers, 4 scientific papers in preparation, 10 published scientific abstracts, 6 workshop manual, 8 internal project and trip reports, 6 articles of project publicity … Operationally, the Project ran smoothly and much of the credit goes to the principal scientists in Pakistan and Nepal. The project team was formed in 1992 and did not change during the lifetimes of LWR2/97/62 and its predecessor LWR2/92/10. The stability of the team had great benefits for the running of the projects. It did not seem to matter that the stability of the scientific team was almost matched by instability in a number of the administrations …