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Introduction

The ARC Centre of Excellence for Integrative Legume Research (CILR) is a partnership that brings together leading plant research scientists located at the University of Queensland (UQ), the Australian National University (ANU), the University of Melbourne (UM) and the University of Newcastle (UN). The Director of the Centre is Professor Peter Gresshoff, who is also Professor of Botany in the School of Biological Sciences at the University of Queensland.

The Centre was established in 2003 with an AU$ 10 million Australian Research Council (ARC) grant over five years. The Centre was awarded a further three-year extension for the period from 2008 to 2010 and received a grant for AU$ 6.9 million over the three years. Cash contributions from partner universities and State Governments, combined with in-kind contributions of staff and facilities, have generated a major AU$ 38 million research effort over eight years.

The CILR aims to drive further development of the genomics and phenomics of legumes using cutting-edge molecular biology tools, and provide a critical mass of human, intellectual and infrastructure resources to function as a world-class research centre.

Research in the Centre is providing critical insights into mechanisms of both meristem and organ differentiation and associated intercellular communication, by utilising comparative genomics on the internationally recognised model legumes *Lotus japonicus* (Lj) and *Medicago truncatula* (Mt). Studies also focus on two major crop legumes – pea (*Pisum sativum*) and soybean (*Glycine max*). Since 2007, a major translational biology research effort has developed into the development of a genomics-genetics biotechnology platform of the legume tree *Pongamia pinnata* for sustainable bioenergy production. This research has already generated significant industrial and commercial interest.

New knowledge of plant growth processes through mechanistic analysis of organ induction provides the tools to optimise legumes’ and by extension, other plants’ productivity, quality and environmental adaptation. This in turn will have a direct impact on agricultural sustainability, environmental quality and potential value-added products for human health. The Centre’s research initiatives have significant intellectual property and commercialisation potential, which will augment Australia’s international standing in scientific discovery, directly benefiting the Australian economy.
Vision and Mission

CILR’s Vision:
- To pioneer knowledge of the biology of legumes;
- To develop the knowledge of plants and their products for the benefit of health and the environment;
- To enhance recognition of the value of plant science to the Australian and global community; and
- To mentor the next generation of plant scientists.

CILR’s Mission
The CILR is committed to:
- Being the leading legume research centre in the world;
- Creating an integrated research environment;
- Developing and applying cutting-edge molecular genetic tools for research into legume genome-phenome relationships;
- Applying new and ethno-botanical knowledge of legumes for the benefit of the environment, health and agriculture;
- High quality and specialised education of undergraduate and post-graduate students;
- Developing products which have tangible benefits for human health and the environment; and
- Providing international leadership and capacity in the study of plant development.
Centre Highlights
2003-2010
The CILR has firmly established itself as one of the leading legume research centres in the world through the activities it has conducted over the last eight years. The Centre has increased awareness in Australia and overseas of legumes for agriculture, the environment and health.

The CILR has significantly enhanced the knowledge base relating to legumes in particular and plants in general, through the publication of 442 peer-reviewed scientific articles, 222 international conference presentations and 101 international poster presentations. In addition, CILR staff have made 190 presentations at national conferences and fora in Australia.

The Centre has also made a significant contribution to the future of Plant Science by graduating 61 PhD and Master’s students and 30 Honours students. Graduate outcomes are excellent.

CILR Chief Investigators were involved on the editorial boards of 30 different journals, such as Molecular Plant; Functional Plant Biology; Plant Cell Reports; and Immunology and Cell Biology and represented plant science on several national and governmental committees.

Research conducted at all four Nodes contributed to a deeper understanding of plant development by elucidating genetic and chemical bases of tissue-specific gene expression and signalling molecules. The application of mutants to analyse both nodulation and lateral branching control facilitated breakthrough discoveries in genome-phenome research. New signalling molecules (such as Strigolactones, CLE peptides, micro-RNAs, flavones and Nod factors) joined classical hormones such as auxin, cytokinins, ethylene and abscisic acid to explain complex regulatory circuits. Coordinated gene networks in shoot and root apical meristems, roots, leaves and nodules were discovered using functional genomics. User-friendly bioinformatic packages were developed to analyse complex data sets. Model legumes extended our ability to transfer technology to crop legumes such as soybean, Pongamia and pea.

The Centre developed and applied reverse genetic approaches to test functionality of newly discovered DNA sequences. Together, this body of research has helped to understand plant development, environmental adaption and plant productivity to aid plant improvement and end-user application.

The Centre has expanded its involvement with industry in the last few years, particularly with regards to the research being conducted on the legume tree Pongamia pinnata. This tree may have a significantly important role to play in the bioenergy industry. The Centre is currently communicating with a number of companies and organisations in this context. To date, the Centre has accrued $1.9 million in funding from industry sources and is actively involved in a “start-up” company with strong ties to the nursery and biofuel industry.

The work being conducted by Centre staff on aspects relating to the hormone strigolactone also has significant potential for the forestry and horticultural industries and commercial interests will be explored. A provisional patent in this area has been filed.

The Centre has actively sought to carry out its mandate to provide tangible benefits for human health and the environment. In this context, the Centre has been involved in a research program involving indigenous communities in the Northern Territory. The aim of the program was to understand Indigenous ecological knowledge by using several species of legumes as important case studies.

This document presents the Centre’s achievements for 2010. The Annual Reports for the years 2003 to 2010 can be viewed on the CILR website www.cilr.uq.edu.au.
Chief Investigators

Chief Investigators (CIs) are largely responsible for the Centre’s laboratories. The CILR incorporates 11 laboratories at four universities (nodes): The University of Queensland, Australian National University, University of Melbourne and University of Newcastle. At each node, one of the CIs also has the role of Node Leader.

The University of Queensland (Headquarters)

Professor Peter Gresshoff
Director
Associate Professor Christine Beveridge
Co-Node Leader
Associate Professor Bernie Carroll
Co-Node Leader

Australian National University

Associate Professor Michael Djordjevic
Node Leader
Professor Barry Rolfe (retired)
Dr Ulrike Mathesius
Professor Chris Parish
Dr Georg Weiller

Melbourne University

Professor Prem Bhalla
Node Leader
Professor Mohan Singh
Deputy Director

Newcastle University

Professor Ray Rose
Node Leader

Apart from its renowned researchers, the CILR is privileged to engage with many highly respected government, industry, scientific and university leaders who have made a significant contribution to the development of the Centre by agreeing to serve on the Centre Advisory Board (CAB).

The CAB provides advice on the development of strategies and vision for the future of the Centre, and its direction in finance, scientific management, commercialisation and Intellectual Property Management.
The members of the Centre Advisory Board are:

**Professor David Day**
Deputy Vice Chancellor (Research)
Flinders University
ADELAIDE   AUSTRALIA

**Professor Peter Langridge**
Australian Centre for Plant Functional Genomics
ADELAIDE   AUSTRALIA

**Professor John Irwin**
School of Biological Sciences
University of Queensland
BRISBANE   AUSTRALIA

**Professor Doug Cook**
Department of Plant Pathology
UC Davis
DAVIS   USA

**Professor Carroll Vance**
Plant Science Research Centre
University of Minnesota
ST PAUL   USA

**Professor Georgina Hernandez**
Centro de Ciencias Genomicas
UNAM
MEXICO

**Professor Satoshi Tabata**
Kazusa DNA Research Centre
CHIBA   JAPAN

**Dr T.J. Higgins**
CSIRO Plant Industry
CANBERRA   AUSTRALIA

**Professor German Spangenberg**
Plant Genetics and Genomics
Department of Primary Industries
MELBOURNE   AUSTRALIA

**Professor John Mattick**
Institute of Molecular Biosciences
University of Queensland
BRISBANE   AUSTRALIA

**Professor Noel Ellis**
Institute of Biological, Environmental and Rural Sciences
Aberystwyth University
Wales.

**Professor David Bird**
Centre for the Biology of Nematode Parasitism
North Carolina State University
RALEIGH   USA

**Professor Ueli Grossniklaus**
Institute of Plant Biology
University of Zürich
ZÜRICH   SWITZERLAND
The Node Leaders and the Chief Operating Officer attempt to meet on a regular basis for effective operation of the Centre. In addition, informal meetings, as required, are conducted at conferences and during nodal visits.

The following table lists formal meetings within the Centre for 2010

<table>
<thead>
<tr>
<th>Meeting Name</th>
<th>Dates</th>
<th>Venue</th>
<th>Attended by:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centre Advisory Board Meeting</td>
<td>2 July 2010</td>
<td>Asilomar, California, USA</td>
<td>CAB Members</td>
</tr>
</tbody>
</table>
Director’s Report

The ARC Centre of Excellence for Integrative Legume Research, with its configuration combining four nodes in Newcastle, Canberra, Melbourne and Brisbane, ceased as of December 31, 2010. Our attempts to receive continued funding in the form of two independent new Centre applications failed, despite the continued need for legume (and plant in general) research, excellent performance in terms of publications, outreach, student completions, commercial linkages, patents, and global impact in the field. One is saddened by this outcome, but at the same time one is buoyed by the history of success and contribution to several emerging fields in biological science and their application to provide solutions to emerging global problems (i.e., food security, energy sustainability, adaptation to climate change). Present and past members of the Centre are thanked for their diverse contributions, many highlighted in the outstanding and informative Annual Reports (available in electronic form on the Centre’s website (www.cilruq.edu.au).

This Annual Report celebrates the eight successful years of the CILR’s existence. Fiscal contributions came from many agencies (mainly the Australian Research Council, the member universities, and the Queensland State and NSW Governments), while intellectual and organisational inputs came from the Centre’s members, students, visitors, and employees. A large number of students, technical, administrative and research staff advanced their careers through their association with the CILR. Students graduated and moved on, while technical and administrative staff members gained knowledge and experience and are either still within the individual nodes, or other laboratories with improved positions. Many research and academic Centre members were promoted or achieved better job opportunities within Australia or abroad. A number of Centre activities and research projects are continuing within the former nodes. In short, we fulfilled one of the important goals of the CILR, namely the development of the next generation of plant scientists within Australia.

We also advanced other areas that were clear objectives of the Centre when it was formed in 2002/2003. Centre science has focussed on the regulation of lateral organ development in plants, analysing the short and long distance signals between plant parts, recognising the critical genetic and biochemical networks, and converting legume-derived concepts for the benefit of plant science as a whole.

Our productivity is evidenced by a publication record of one peer-reviewed publication per week, many in high impact journals, many leading to patentable applications, international recognition and conference speaking invitations. As part of a national research performance evaluation (ERA), Centre scientists contributed to a ranking of 5 in both Plant Science, and Genetics. This ranking is reserved for those demonstrating “outstanding performance well above world standard”. Indeed our research led to many benefits for human and environmental health, as anticipated as ‘Centre aims’ in our formative period.
As the Director of the Centre, I especially want to thank the staff and students, who, after all, made the successes possible. But also I thank the members of the Centre Advisory Committee, made up of research, government and industry leaders in the field of Molecular Plant Science, for their contribution to our success. More than ever, the need for increased knowledge and application of legume science came into focus in a world with increasing pressures on food prices, human health effects from food, soil protection and land use, need for carbon sequestration, biomass production and bioenergy sustainability.

Because of a positive response by the University of Queensland administration, mirrored in part in other former nodes, the CILR will continue, with permission to retain the CILR label, at least for the next three years at UQ. Research will evolve into new areas, but will also deepen into successful ones.

We look forward to more successes, achievements and outcomes in the future.

Peter Gresshoff
Year in Brief

Research

Breakthrough discovery
In 2008, CILR researchers were part of a major discovery in plant science viz. the discovery that strigolactones control shoot branching. Chief Investigator Christine Beveridge collaborated with scientists at INRA, Versailles and the University of Toulouse on this project. Their work was published in *Nature*, with a prominent display on the cover of the September edition. This work has continued at a rapid pace. In 2009, Associate Professor Beveridge and co-workers revealed a new genetic factor involved in regulating strigolactone. Christine Beveridge and Dr Brett Ferguson also demonstrated that strigolactone and auxin deficiency is not sufficient to induce a non-growing bud to grow into a branch. This research continued in 2010 producing very promising results (see Research Report – Christine Beveridge).

Fuelling up for the future
As part of the “Natural Science – Social Science Linkage Program”, the CILR continued researching the potential use of the legume tree *Pongamia pinnata* as a feedstock for the production of biodiesel and bioenergy in general. This program has become an important component in the Centre’s research program. In 2008, the UQ node established field trials in central Queensland and at the University of Queensland’s research farm at Gatton. In 2009 and 2010, these trials produced important results that have contributed to the development of protocols for *Pongamia* propagation. The trials also revealed the salt-tolerant nature of *Pongamia*. Professor Peter Gresshoff and Dr Paul Scott obtained a five-year ARC linkage grant to develop genetic transformation protocols for *Pongamia*, as well as funding from the federal government-funded “Caring For Our Country” scheme for a field trial on former canelands of the Sunshine Coast. The Centre was also part of a successful National and International Research Alliances Program (NIRAP) grant application involving the Queensland Sustainable Aviation Fuel Initiative. With regards to the biodiesel research, the Centre has expanded its liaison with industry and government bodies, media and the general public. A number of other cooperative arrangements with industry partners are currently being finalised.

CILR enhances its international reputation
The CILR lived up to its reputation as a world-class research facility through presentations, seminars and posters at a large number of national and international conferences. In 2010/11, CILR staff and students presented a total of 37 papers and seminars and 12 posters at international conferences, and a total of 31 papers and seminars at conferences in Australia. Ten seminars were presented by leading international scientists to CILR staff. CILR researchers also visited colleagues all over the world to discuss current and future research collaborations; in total, 21 leading laboratories were visited by CILR scientists. Research strength continues to gain momentum.

In 2010/11, CILR researchers published a total of 53 scientific papers, with 26% (14) of the papers in journals with an impact factor greater than five (according to ISI Web of Knowledge Journal Citation Reports). CILR researchers also contributed to 11 book chapters in significant scientific publications.

The CILR’s research programs have gained significant momentum during the last eight years and, unquestionably, have enhanced the Centre’s international profile. Through programs focused on differential
shoot and root apical meristem gene expression, auxin movements and pools during nodulation (for more detail see ‘Research Highlights’), the CILR is unravelling the secrets of plant development and control.

**Expanded industry initiatives**
The CILR made significant strides in 2010 in expanding its ties with industry and in particular, with the biofuels industry, with a considerable amount of interest being shown in the *Pongamia* tree for biodiesel production. The *Pongamia* pilot project, initiated in 2007, between the CILR and an industry partner continued with promising results being obtained. The aim of this project was to develop basic biological and biotechnological methods for *Pongamia pinnata* for the purpose of fatty acid and triglyceride production for biodiesel. The pilot projects success resulted in a substantial collaborative research project with the same industry partner.

Several other collaborative programs are also currently under way with two City Councils in Queensland, a major national energy/gas producer and supplier, several other energy-related companies and two investment corporations. One of the City Councils, the Brisbane City Council, has provided a significant sponsorship for a PhD scholarship at the CILR (University of Queensland) to work on a *Pongamia*-related project. The recipient of the scholarship commenced her PhD program at the CILR in January 2011. In 2009, the CILR joined forces with several other UQ-based institutes and industry in submitting a National and International Research Alliances Program (NIRAP) Grant to work on developing a sustainable, feedstock source for the aviation industry. This grant application was successful and the CILR is now actively engaged in the program and meets regularly with important role-players in the aviation-fuel industry.

The CILR was successful in obtaining an ARC Linkage Grant to further the research initiative into the legume tree *Pongamia pinnata* for the biodiesel industry. A leading company in the *Pongamia* plantation/nursery industry supported this grant application. Promising results are being produced from this research.

**CILR protects its intellectual assets**
The Centre has continued to identify and exploit commercial opportunities emanating from research across all four Nodes. This aspect of the Centre’s activities is seen as very important in ensuring that the Centre maintains its status as a world-class, plant research institute.

Relevant intellectual property with commercial potential is commercialised through UniQuest Pty Ltd. UniQuest is one of Australia’s leading research commercialisation companies, specialising in global technology transfer and facilitating access for all business sectors to world class university expertise, intellectual property and facilities.
Associate Professor Christine Beveridge – Chief Investigator – University of Queensland

Being the last report for the ARC Centre of Excellence for Integrative Legume Research from the Plant Development Lab at the University of Queensland, one must first acknowledge the very important work by extremely dedicated staff in the group and who have supported the lab in countless ways over the longest time; Drs Elizabeth Dun and Philip Brewer and Mrs Kerry Condon. These and other people over the CILR years deserve great thanks as they have been an integral to the achievements great and small that have carried us through and will take us long into the future.

The ARC Centre of Excellence for Integrative Legume Research provided a wonderful opportunity to make substantial inroads into identifying long-distance signals regulating shoot architecture in plants. Senior members of CILR, across its nodes, provided very helpful mentorship throughout the years as we encountered hurdles of various kinds and magnitudes. The critical mass and collegial support of these people together with younger CIs of the Centre provided an enormous strength and basis from which we leapt into unknown scientific territory. Having previously been a lab with a physiological focus we are now proud to boast an additional molecular depth and genetic breadth which we had hitherto only dreamt of. We are therefore very grateful to all our colleagues in CILR and thank them all for their support.

At the personal and individual level it has been wonderful to see students evolving into successful scientists, graduating and moving forward into promising careers. For example, Dr Elizabeth Dun has received an independent ARC Fellowship and Dr Brewer an academic promotion. New and stronger national and international collaborations have developed.

Our greatest scientific achievement was the discovery of the plant hormone which controls shoot branching. This discovery of the function of strigolactone, published in Nature in 2008, is the sixth most highly cited plant paper from that year (Thomson ISI publications data). Since that discovery, we have realised that strigolactone affects additional agronomically important traits such as rooting, adventitious rooting, and secondary growth (wood production). We now look forward to making use of new substantial funding from ARC Discovery grants to Associate Professor Beveridge and Dr Brewer, Future Fellowship to Associate Professor Beveridge and Fellowship to Dr Dun, to further understand the role of this new hormone. This funding will be used to identify and functionally characterise genes that are involved in the response to hormones that promote or suppress axillary bud outgrowth and which act in the buds themselves. It will also be used to characterise new genes which we predict act on the strigolactone biosynthesis pathway. This work has already led to the identification of new branching mutants. Mutants in one of these genes, LBO (LATERAL BRANCHING OXIDASE) is affected in some typically strigolactone associated traits and not others. This type of mutant will be invaluable to dissect the role of different hormones, particularly auxin and strigolactone, in plant development.

Professor Prem Bhalla (Node Leader) and Professor Mohan Singh (Deputy Director) – Chief Investigators – University of Melbourne

Floral Transition in Soybean

Soybean, a major oilseed crop, remained our experimental system to investigate key regulators of floral transition. Four transcription factors identified from our floral...
transition dataset were investigated further. Ectopic expression of *G. max* bZIP and bHLH did not give any distinct phenotypes while the over-expression of ABRE and DOF transcription factors yielded some noticeable phenotypes. For example, the ectopic expression of DOF transcription factor seems to affect the floral developmental stage with elongated carpels in some lines and the absence or lack of petals in others. We are in the process of analysing these transgenic lines. Our study on novel spatial expression of soybean WUSCHEL in the incipient floral primordial was published in *Planta*.

Release of the soybean (*G. max*) genome sequence earlier this year provided us with an opportunity to undertake a genome-wide analysis of the all possible soybean homologs for the corresponding Arabidopsis genes, particularly those involved in flowering. As a paleopolyploid, soybean contains duplicated copies for most genes that may have undergone sub- or neo-functionalisation. We identified putative soybean flowering regulatory genes and further carried out phylogenetic analyses of these gene families to develop an understanding of their evolutionary relationships. We also found that paralogous soybean genes comprising the biggest orthologous group in soybean are clustered in a 1.4 Mb region on chromosome 16. Further, the soybean genes that are homologous to key flowering genes in Arabidopsis are scattered over the genome. Our genomic comparison with *Glycine soja* (wild soybean) indicated that structural variation including SNPs in flowering genes of *G. max* is not significant. Functional analyses of identified key genes will facilitate understanding of the molecular basis of floral transition in soybean.

**MicroRNAs in shoot apical meristem of soybean**

Our paper characterising the miRNA profile of the shoot apical meristem of an important legume crop, soybean, by integrating high-throughput sequencing data with miRNA microarray analysis was accepted for publication in the Journal of Experimental Botany. We also reported eight putative novel miRNAs in shoot apical meristem. Further, our study identified potential key regulators and provides vital spatial information towards understanding the regulatory circuits in the shoot apical meristem of soybean during shoot development. This work represents the first report of microRNA profile of shoot apical meristem of a major legume crop and was undertaken in collaboration with Associate Professor Bernie Carroll, (UQ, CI) and Dr Xiujie Wang, Institute of Genetics and Developmental Biology, The Chinese Academy of Sciences, Beijing.

Work is now underway to investigate the role of the identified novel miRNAs in shoot apical meristem maintenance and function.

**Proteomic reference map of the soybean root apex**

The root apex is responsible for the development of the extensive underground system of the plant. Our paper describing a proteome reference map of the soybean root apex and its comparison with the proteome of the differentiated root zone was accepted in the journal *Proteomics*. This work represents the first report of root apex proteome of a crop legume, contributing our understanding of soybean root biology. This study was the result of a successful collaboration with Dr Ulrike Mathesius (ANU CI) and Associate Professor Michael Djordjevic (CI) based at the ANU node.
Systemic spreading of gene silencing in plants

We have been using reverse and forward genetic screens in *Arabidopsis* to identify additional genes involved in graft-transmissible gene silencing. We were particularly interested in identifying genetic determinants required for transmission of mobile silencing signals from the rootstock that travel to the scion to induce systemic gene silencing. The transgenic *Arabidopsis* line we use for this research carries a transgene expressing Green Florescent Protein (GFP)-specific dsRNA from a root tip-specific promoter along with a linked 35S:GFP transgene. The GFP silencing phenotype of ungrafted plants of this line resembles the systemic silencing phenotype of grafted plants described by Brosnan et al. (2007, PNAS 104, 14741-14746), and rootstocks of this line transmit silencing to scions expressing GFP. Using this reporter system, we have shown that four known RNAi mutants, *ago1*, *rdr6*, *sgs3* and *dcl3*, are deficient in transmission of the mobile silencing signal transmitted from grafted rootstocks to scions. AGO1, RDR6 and SGS3 are components of the trans-acting siRNA (ta-siRNA) post-transcriptional gene silencing pathway, but we showed that another component of this pathway, DCL4, is not required for transmission of silencing from rootstocks. DCL3 is responsible for producing 24-nucleotide small interfering RNAs (siRNAs) that guide transcriptional gene silencing. These results indicate that the ta-siRNA pathway can act with DCL3 to produce graft-transmissible gene silencing signals in plants.

Following ethyl methane sulfonate (EMS) mutagenesis of the transgenic line described above, over 50 independent systemic silencing mutants have been identified. These new mutants fall into at least eight complementation groups including new alleles of *ago1*, *rdr6* and *sgs3*. One new mutant of interest, called EMS164, shows delayed transmission of systemic gene silencing, as well as delayed root development, increased root hair and lateral root formation. Using deep sequencing of DNA from a bulk of 500 mutant F2 plants we have mapped this mutation to a region of chromosome 4 not occupied by any known RNAi genes. Candidate genes from this region of chromosome 4 are now being used to test for complementation of the EMS164 phenotype.

Defective embryo and meristems (Dem) and plant development

We have shown that Dem proteins are required for both cell division and cell differentiation in plants. Consistent with this fundamental role at the cellular level, these proteins are also required for formation of all tissues affecting plant architecture, *i.e.* shoot and root meristems, leaves for capturing solar energy, and roots for absorbing water and nutrients from the soil. Through genetic and biochemical analysis, we discovered that Dem proteins exert their fundamental effects on cellular processes via interaction with Ran proteins involved in nucleocytoplasmic transport of proteins and RNA, and the export of microRNAs from the nucleus to the cytoplasm. MicroRNAs control the expression of many, if not all, development genes in both plants and animals. *dem* mutants have lower levels of miRNAs consistent with its phenotype and interaction with Ran. Recently, we also showed that *dem* mutant defects extend to genome-wide DNA methylation patterns and systemic spreading of gene silencing.
Recent studies have shown that Dem and Ran co-localise at the nuclear rim. Dem is also located in an unknown compartment of the cytoplasm, but is largely excluded from the nucleus. We have also recently shown that Dem is expressed in the vegetative but not the sperm cells in pollen. Expression of Dem in pollen will greatly facilitate our future research on Dem’s role in cell division, and co-localisation studies with other proteins and subcellular compartments in the cell.

Comparative analysis of microRNAs in meristems and leaves of soybean (UQ and UM)

MicroRNAs control the expression of development genes in both plants and animals. MicroRNAs have therefore played a fundamental role in the evolution of kingdoms (e.g. plants), phyla (e.g. legumes) and individual species (e.g. soybean). The aims of this project are to discover: i) microRNAs that have remained, have conserved the evolution of higher plants (by comparison of soybean to other higher plants species), ii) microRNAs that are specific to plant meristems (stem cells) and mature leaf cells (that capture solar energy via photosynthesis), and iii) microRNAs that contributed to the evolution of legumes, and more specifically, to the evolution of soybean. Hundreds of thousands of microRNAs have been sequenced from soybean meristems and leaves and many of these have been characterised for expression by microarray analysis. The majority of microRNAs are more abundant in leaves than meristems. However, we have identified small number of microRNAs that are enriched in meristems and some of these but not others are conserved between soybean and distantly related plant species.

Associate Professor Michael Djordjevic –
Node Leader and Chief Investigator –
Australia National University

2010 was an important year to establish new initiatives and consolidate existing programs. All the programs revolve around the identification of new growth regulating compounds in plants that regulate plant architecture with a focus on roots.

The biological activity of flavonoids.

We made important discoveries regarding the biological activity of flavonoids in plants and animals (in collaboration with the Parish laboratory). First, we have uncovered in vivo roles for flavonoids in regulating gravitropism and auxin transport in Arabidopsis. Second, we have shown new architectural phenotypes for Arabidopsis mutants defective in flavonoid production or accumulation using the new plant phenomics facility at CSIRO. Finally, we have also developed a novel chemical genomics approach to study the role of flavonoids in determining root architecture. Flavonoids and flavonoid-like molecules have also been screened or isolated from legumes that have new biological activities in mammalian cells. We are investigating the structure and identity of the new plant derived compounds and conducting structure activity relationship studies with purified flavonoids to help determine the mode of action.

Nod factor biological activity in mammals

New Nod factors were synthesised by GlycoSyn IRL in New Zealand (using funding leveraged through ANU Connect Ventures) and tested for biological activities in mammalian systems. These studies have defined the structural requirements for the mammalian biological activity of Nod Factors.
New regulatory peptide activities in plants
We have identified nodule specific members of the CLE gene family are regulators of root nodule number via the autoregulation pathway. These particular CLE genes encode regulatory peptides that are implicated in the long distance control of the number of root nodules formed in *Medicago*. Our results have enabled us to propose a new model linking for the first time autoregulation of nodulation to the Nod Factor and cytokinin-dependent nodule formation pathways. We have also identified another class of regulatory peptide that play a more general role in regulating root architecture. These regulatory peptides appear to act as intermediaries in pathways that allow environmental sensing by roots to be translated into developmental outcomes that control three lateral organogenesis pathways.

New regulatory microRNAs regulating root stem cells
We have used a procedure to enrich for microRNAs that regulate root stem cells. These microRNAs have been sequenced using deep sequencing strategies. Each sample returned around 25 million individual reads. We found sequences matching 48 known *M. truncatula* miRNAs and identified 22 miRNAs conserved in plants, not previously known in *M. truncatula*. We have also selected approximately 1200 novel sequences for further bioinformatic analysis (in collaboration with the Weiller laboratory). Several will be selected to investigate their biological functions.

Professor Peter Gresshoff – Director and Chief Investigator – University of Queensland

The Gresshoff Group researches two broad areas of legume genetics and genomics, namely molecular events during systemic nodule control in soybean and genome-phenome linkages in the “bioenergy” tree Pongamia.

A) Molecular genetic and biochemical analysis of processes governing nodule induction and regulation in legumes.

Nod factor perception in soybean: Arief Indrasumunar
The genes for the Nod factor receptor kinase complex of soybean were recently cloned and functionally analysed. Both receptor genes are duplicated in soybean and called *GmNFR1α/GmNFR1β*, and *GmNFR5α/GmNFR5β*. Over-expression in transgenic soybean roots of *GmNFR1α*, but not the others, significantly increased nodulation and nitrogen fixation of soybean in acid soil and also resulted in increased nodulation efficiency at ultra-low initial (10^2 cfu/mL) *Bradyrhizobium* titres. The low number of rhizobia is commonly found on soils suffering from abiotic stress (as seen in salt, drought, heat, or pH-stress conditions), or lacking prior history of compatible *Bradyrhizobium* cultivation. This discovery has been patented (see patents in Intellectual Property Management and Commercialisation section).

Analysis of soybean autoregulation: Brett Ferguson
Investigations into legume nodulation and AON identified a number of new factors acting in these processes. Three novel CLE peptide genes that regulate nodule number in soybean were discovered. The processed peptides (12 aa long) function as long- and short-distance signals during rhizobia- or nitrate-inhibition of nodulation. A number of novel root genes acting during early nodule development, or in the leaf following the leaf’s perception of the rhizobia-induced CLE peptides, were discovered via soybean transcriptome analyses (RNA-seq). Functional
characterisation of the rhizobia-induced soybean CLE genes has also begun looking at their over-expression in other legume species. Further advances were also made in characterising the SDI molecule of AON. A systemic role for acid-inhibition of soybean nodulation was established.

Identification of soybean short and long distance nodulation control signals: Dugald Reid (with Brett Ferguson and Julia Kehr (Spain))

We are seeking to identify long distance signals (called ‘Q’) that act as ligands for the GmNARK LRR receptor kinase. We have identified CLE-peptide encoding genes that respond to inoculation and/or nitrate treatment and which inhibit nodulation in a GmNARK-dependent manner when expressed in the root. Two Bradyrhizobium-infection induced CLE peptides inhibit nodulation systemically whereas the nitrate-induced CLE peptide acted locally to inhibit nodulation. To confirm if these, or other candidates, move systemically in AON signalling, we developed a bioassay to detect nodulation-dependent signals in xylem sap. This bioassay is reliant on differential gene expression responses in the leaves of soybean fed with xylem sap presumed to either contain Q, or be devoid of it. To identify the differential responses, complete transcriptome sequencing of soybean leaves using the Illumina GAIIx has been employed.

Characterising CLV1A, a GmNARK parologue in soybean: Saeid Mirzaei (with Brett Ferguson, Khalid Meksen and Jacqui Batley)

GmCLV1A and GmNARK (Glycine max Nodule Autoregulation Receptor Kinase; highly similar to CLV1 of Arabidopsis) are paralogous genes in soybean, sharing the same genomic environment on separate soybean chromosomes. Whereas GmNARK functions in the regulation of nodule numbers, the role of GmCLV1A remains unknown. To establish the function of GmCLV1A, a Gmclv1A (S562L) mutant was isolated from a soybean TILLING population (in collaboration with Dr Khalid Meksem (Southern Illinois) and Dr Jacqui Batley (ACPFG and CILR). Gmclv1A exhibited thicker stems, bifurcated stems and pods, increased basal branching and increased flowering with additional abortion. However, the mutant does not exhibit abnormal nodule number, nodule index and nodule size.

Deep transcriptome analysis of the soybean window of nodulation. Satomi Hayashi (with Brett Ferguson and Dave Edwards (ACPFG))

Novel responses in early soybean nodule development were identified by deep sequencing transcriptomics (RNA-seq). To best capture nodulation genes of interest, the samples were harvested from the zone of nodulation, which is the region of the root that is most susceptible to nodule initiation. Samples were harvested 48 hours following inoculation with either a compatible (wild type) or incompatible mutant (nodC-) strain of Bradyrhizobium japonicum. Approximately 3,000 differentially expressed genes were identified using this approach. This includes roughly 1,600 up-regulated and 1,200 down-regulated genes. Many of the genes identified were known nodulation genes (e.g., ENOD40, NIN1, NSP1), with numerous more being previously unknown nodulation genes. Several molecular pathways were identified as being differentially regulated, including gibberellin biosynthesis.
The shoot-derived inhibitor (SDI) in soybean nodulation: Yu-Hsiang Lin (with Brett Ferguson and Rob Capon (IMB))

During the early stages of nodule development, Q (a CLE peptide) is produced and presumably transported to the leaf veins where it is detected by the Nodulation Autoregulation Receptor Kinase (GmNARK). This leads to the production of SDI, which is transported to the root where it arrests further nodule development. A newly developed petiole-feeding bioassay allows the characterisation and determination of SDI in soybean. Feeding leaf extracts from Bradyrhizobium japonicum-inoculated wild type or super/hypernodulating mutants into additional super/hypernodulating mutant recipient plants demonstrated that suppression activity is present in wild type leaf extracts. SDI is a small, polar, NARK-induced and heat-stable molecule that is probably not an RNA or protein. The use of HPLC coupled with analytical techniques, including mass spectrometry has produced a number of potential SDI candidates.

Analysis of soil-acidity on soybean nodulation: Meng-Han Lin (with Brett Ferguson and Arief Indrasumunar)

Nodule formation is tightly regulated by the plant and can be inhibited by a number of external factors, such as soil acidity. The precise mechanism by which acidic conditions inhibit soybean nodule development remains poorly characterised. Previous investigations have typically reported the problem to be associated with disrupted plant and rhizobia growth, plant-rhizobia signalling and/or rhizobia infection. We have evidence that the inhibition of nodulation by acidic conditions is plant-regulated by the plant and that this mechanism is Bradyrhizobium-inoculation-dependent and has a systemic component. Furthermore, the inhibition of nodulation in acidic conditions likely acts in the early stages of nodule development where nodulation gene expression is affected. Soybean ‘hairy’ roots having a visual reporter system containing the red fluorescent protein, DsRed2, were generated and the nodulation-deficient mutant, nod49, was successfully complemented by over-expression of the soybean Nod Factor Receptor gene, GmNFR1α.

Computational modelling of nodulation control circuits: Liqi Han (with Jim Hanan)

Computer modelling was used to understand the complex signalling mechanisms during autoregulation of nodulation. A new modelling approach – Computational Complementation – was developed in this study. The key idea is to use functional-structural plant modelling to complement the deficiency of a loss-of-function mutant with hypothetical signalling components. If a wild-type phenotype can be restored through such complementation, the hypothesised signalling mechanism could be considered as reasonable. Concurrently, signalling-developmental processes were simulated for reconstruction of soybean root architecture and coordination.

B) Development of a genetic, genomic and molecular biology platform for the development of sustainable bioenergy production from the legume tree Pongamia pinnata:

Development of molecular, phenotypic and biochemical diversity markers in Pongamia pinnata: Qunyi Jiang (with Paul Scott)

The genetic diversity of Pongamia pinnata at the level of individual trees was investigated using PISSR (Pongamia Inter-Simple Sequence Repeat) primers. We demonstrated: 1) an extensive number of polymorphic bands generated by this approach, enabling detailed analysis of genetic
relatedness of local and overseas accessions; 2) reproducible generation of PISSR polymorphisms in clonal *Pongamia* trees; and 3) population diversity of mature *Pongamia* trees using over 100 polymorphic markers generated by 10 PISSR DNA primers. Currently, work is underway to characterise the genomic regions that encompass the polymorphic markers via sequencing and annotation of relevant DNA fragments excised from polyacrylamide gels.

The experimental field trial site (~300 trees) on the UQ Gatton campus continues to be a source of valuable data. These trees have been used to study morphological phenotypes that might be established as useful biomarkers. The relevant phenotypes include precocious flowering, biomass yield, seed and leaf shape, tree height and seed oil composition.

The analysis of *Pongamia* seed oil composition demonstrated that seed size correlates with both seed oil and oleic acid content.

**Transformation and tissue culture of *Pongamia pinnata*: Bandana Biswas (with Paul Scott)**

Transformation protocols are being developed to enable future genetic enhancement of *Pongamia pinnata* as part of a 5-year ARC Linkage grant in collaboration with BioEnergy Research Pty Ltd. The development of reliable transformation protocols will not only allow improvement in oil content and composition, but also insect resistance, herbicide tolerance, improvement in nodulation efficiency, alteration of plant architecture and physiological responses to stress. An important step towards genetic transformation is an efficient regeneration system that will produce transgenic plants. We have also been able to form somatic embryos as well as rooted seedlings through organogenesis from green immature cotyledons of *Pongamia* trees. Our first attempts at transformation with *Agrobacterium tumefaciens* (strains AGL-1 and EHA 101) containing a GFP-GUS+ plasmid (Vickers et al. 2007) formed callus expressing GFP. So-called “hairy root transformation”, via *Agrobacterium rhizogenes*, is also starting to show success with early results of new roots showing GFP expression. Work is currently underway to confirm the transgenic nature of these roots.

**Deep sequencing and annotation of the *Pongamia* genome: Stephen Kazakoff and Jasper Koehorst (with Paul Scott and Dave Edwards (ACPFG))**

The *Pongamia* deep DNA sequence database, generated via Illumina GAIIx technology, continues to be a valuable resource. We successfully identified and characterised the DNA sequences of many genes relating to seed oil yield and composition, nodulation and nitrogen fixation, and photosynthesis. The sequence database was constructed from two-insert size libraries derived from total *Pongamia* DNA (i.e., nuclear, mitochondrial and chloroplast DNA). The inclusion of mitochondrial and chloroplast DNA in library construction enabled the assembly and annotation of the genomes for these two organelles. This was achieved utilising the novel software (SASSY; by Michael Imelfort, ACPFG) that assembles short DNA TAG sequences. We continue to have a strong collaborative association with Dr Edwards and his group, and plan to continue this functional genomics approach to characterise further the nuclear genome of *Pongamia*.

**Field trials of *Pongamia* plantings: Paul Scott (with all *Pongamia* research group)**

Decisions on future planting of *Pongamia* on a commercial scale in northern Australia will rely to an
extent on the results of field trials. These field trials will provide important data on the performance of this legume tree under relevant agricultural landscapes and climatic conditions. The trial of approximately 300 trees on the UQ Gatton campus site continues to provide important data on the growth of *Pongamia*, including the accumulation of biomass, the sequestration of carbon and nitrogen, flower and seed development and the response of trees to local biotic and abiotic factors. Since the initial planting in December 2008, these trees have, on average, increased approximately 1000 fold in biomass dry weight. The past year has seen these trees challenged by severe climatic (e.g., above average rainfall) and biotic (e.g., insect) stresses and yet they have survived and prospered. The success of the other major field trial site on the Origin Energy site at Spring Gully has been vindicated by the decision to extend beyond the initial 2 hectare trial to a planting of 450 hectares. The vigorous and robust growth of *Pongamia* under conditions throughout Queensland has also been seen through plantings on the acid-sulphate soils of ex-canelands on the Sunshine Coast, a project in collaboration with Maroochy Landcare and private landholders and funded by the Federal Caring for Our Country scheme.

**Dr Ulrike Mathesius – Chief Investigator – Australian National University**

**The role of flavonoids in nodulation and root development**

The objective of the project has been to define the role of flavonoids during plant development. The main developmental process under investigation was the development of symbiotic root nodules in the model legume *Medicago truncatula*. Studying nodule development has the advantage that this organ can be induced externally at a predictable place and time by spot-inoculating rhizobia onto the root.

We showed previously that flavonoids are necessary for nodule development and that silencing the flavonoid pathway by RNA interference prevents rhizobia manipulating auxin transport (Wasson *et al*. 2006 Plant Cell 18, 1617-1629). Because nodules are developmentally similar to lateral roots and root galls induced by parasitic root knot nematodes, we then investigated whether flavonoids are also necessary for these other organs. Surprisingly we found that flavonoids are not required for correct lateral root or gall development, but that lack of flavonoids alters the structure of root galls (Wasson *et al*. 2009 New Phytologist 183: 167–179). This led to a new model for the regulation of auxin transport by flavonoids during nodule symbiosis and nematode parasitism (Grunewald *et al*. 2009 Plant Cell 21: 2553-2562).

The requirement for flavonoids during nodule development prompted the question as to which regulatory pathways are targeted by flavonoids, in addition to the auxin transport machinery. We analysed gene expression during early time points of nodule development in the inoculation zone of roots in which flavonoids were abolished by RNAi of chalcone synthase and compared this to roots containing flavonoids. We analysed gene expression by Affymetrix microarray and real time PCR and found major changes in the expression of defence-related pathways during nodulation in roots lacking flavonoids. In addition, cell wall modifying enzymes, nodulins and other enzymes of the flavonoid pathway were affected. We are currently investigating whether these gene expression changes lead to altered defence responses or defects in the infection process of rhizobia that could contribute to the failure to nodulate in flavonoid-deficient roots.
In collaboration with Dr Florian Frugier’s group in France, we also investigated the role of flavonoids during root development in a new *M. truncatula* mutant identified by Dr Frugier’s group, *cra* (*Compact Root Architecture*). This mutant is characterised by short thick roots with altered lignifications but still forms nodules and lateral roots. We showed that this mutant accumulates specific flavonoids that can regulate auxin transport. The mutant phenotype could be mimicked by external treatment of the wild type with these flavonoids, whereas reducing flavonoid content in the mutant by RNAi against chalcone synthase restored its auxin transport phenotype (Laffont et al. 2010 Plant Physiology 153: 1597-1607).

Further collaborative work with Dr Frugier’s group, who isolated a cytokinin signalling-defective mutant (*cre*), showed that cytokinin signalling partially acts through auxin signalling during nodulation, although we did not find any convincing involvement of flavonoids downstream of cytokinin signalling in this mutant (Plet et al. 2011 Plant Journal, in press).

Altogether this project showed convincing evidence for the involvement of flavonoids in nodule and root development. The mechanism of action of flavonoids includes the regulation of auxin transport and auxin accumulation during these developmental processes. The microarray experiments have further demonstrated that flavonoids are involved indirectly in the alteration of defence related pathways that could also affect nodule development and invasion of rhizobia.

**Proteome analysis of root development and nodulation**
Over the last few years, we have developed proteomics techniques to characterise protein changes occurring in legume roots during development and during the interaction with rhizobia (Mathesius, 2009 J. Proteomics 72: 353-366). Previous work characterised the proteome changes in *Medicago truncatula* occurring during early stages of nodule initiation and how this is altered in the nodulation mutants *sunn* (van Noorden et al. 2007 Plant Physiology 144: 1115-1131) and *sickle* (Prayitno et al. 2007 J. Proteome Research 5: 3084-3095).

In collaboration with CILR chief investigators Michael Djordjevic, Georg Weiller, Mohan Singh and Prem Bhalla, we recently investigated the proteome changes occurring during root development in soybean. We were particularly interested in the proteome of the root apical meristem and compared this to the proteome of the differentiated root zone (Mathesius et al. 2011). After establishing an extensive proteome reference map for the root, we identified 73 differentially accumulated proteins, many of which were involved in redox homeostasis and flavonoid metabolism. Visualisation of flavonoid and redox markers in the root apical meristem supports a role for these metabolites in regulating meristem activity.

**Professor Chris Parish – Chief Investigator – Australian National University**

In collaboration with Professor Barry Rolfe, Professor Peter Gresshoff and Associate Professor Michael Djordjevic, work has continued on legume-associated molecules and their variants that can either enhance, or inhibit angiogenesis (new blood vessel growth) in mammals. These molecules, which are generically known as Nod factors, are released by *Rhizobium*-bacteria and initiate nodulation in legumes. The Nod factor-related molecules have been tested for their pro- or anti-angiogenic activity using a number of basic *in vitro* assays and, more recently, *in vivo* assays for angiogenesis.
These studies have resulted in the identification of novel compounds with either pro- or anti-angiogenic activity, with very subtle changes in the molecular structure of the molecules dramatically changing their biological activity. Remarkably, a disaccharide structure has been identified that exhibits pro-angiogenic activity. Industrial Research Limited (IRL), New Zealand, has synthesised three derivatives of the pro-angiogenic disaccharide. Two of these modified disaccharides exhibited in vitro anti-angiogenic activity and one displayed modest pro-angiogenic effects. These studies confirmed that small changes in the structure of the Nod factor derivatives can result in dramatic changes in the biological activity of the molecules.

The mode of action of the pro- and anti-angiogenic Nod factor derivatives has also been investigated in some details. The molecules have little or no effect on endothelial cell proliferation or migration but have dramatic effects on endothelial cell tube formation, the pro-angiogenic molecules enhancing and the anti-angiogenic compounds inhibiting the rate of tube formation. In fact, the molecules can act within 1 – 2 hours to either enhance or inhibit endothelial cell tube formation. The current working hypothesis is that the Nod factors modify, either directly or indirectly, the adhesive activity of endothelial cell integrins. This work is being prepared for publication.

Professor Ray Rose – Node Leader and Chief Investigator – University of Newcastle

Research at the University of Newcastle Node is centred on *Medicago truncatula*, a genetic and genomic model for legumes (Rose, 2008). Non-legume models such as Arabidopsis are used in some cases. There are three areas of focus carried out with Research Associates Drs Kurdyukov, Nolan and Sheahan.

1. **The molecular mechanism of the induction of somatic embryogenesis**
2. **Regulation of zygotic embryogenesis and oil body biogenesis.**
3. **Organelle dynamics in regenerating plant cells**

1. **The molecular mechanism of the induction of somatic embryogenesis**

Somatic embryogenesis (SE) is the asexual production of embryos from differentiated somatic cells. SE has important application to biotechnology — for the production of transgenic plants, clonal propagation and in the production of haploids and doubled haploids during plant breeding. Furthermore, because somatic embryos are more readily accessible than zygotic embryos, studying SE has provided valuable insights into the process of zygotic embryogenesis (ZE). Although understanding of the regulation of SE is increasing, a detailed knowledge of the underlying molecular mechanisms is absent. Accordingly, an ability to induce SE in many species, or cultivars within a species, is lacking. The goal of this project is therefore to provide an understanding of the molecular regulation of SE that will enable the application of SE to a range of economically important legumes.

In addition to the utility of somatic embryogenesis this work increases the understanding of plant development, stem cells and apomixis.
**Signalling in the induction of embryo formation from somatic cells in Medicago truncatula.**

Based on our studies using 2HA and Jemalong we have developed a model of the signalling events that drive SE in *M. truncatula* (see Figure below). Incubating leaf explants on a basal medium containing auxin + cytokinin causes mesophyll cells to dedifferentiate (Rose et al. 2010; Wang et al. 2011). Production of reactive oxygen species (ROS) occurs rapidly and stimulates ethylene biosynthesis, which in turn drives 

**MtSERF1** (**SOMATIC EMBRYO RELATED FACTOR 1**) expression just before embryo induction (Mantri et al. 2003; Rose et al. 2010). Expression of **MtSERK1** (**SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE 1**) and **MtWUS** (**MtWUS**) are important during both cell proliferation and embryo formation (Chen et al. 2009; Nolan et al. 2009). Expression of **MtWUS** and **MtCLV3** is associated with the shoot apical meristem (SAM) and **MtWOX5** with the root apical meristem (RAM) (Chen et al. 2009). Note that **MtWUS** is associated with totipotent (can produce all cell types) and pluripotent stem cells (can produce a limited number of cell types). **MtEIL1**, a homologue of Arabidopsis **ETHYLENE INSENSITIVE 3** (**EIN3**), is downregulated in 2HA compared to Jemalong (Imin et al. 2008) but the closely related **MtEIL2** is expressed (Kurdyukov et al., data presented at ICLGG V Conference). The thioredoxin, **MtTrxH** (identified by a proteomic study) is upregulated in 2HA (Imin et al. 2005) and is related to the stress response. The stress-related hormoneABA appears to modulate the ROS- and ethylene-mediated regulation of stress. ABA stimulates SE when applied at culture initiation and improves embryo quality when applied at the time of embryo formation. **MtSK1** (**STRESS KINASE 1**), an SnRK2 stress kinase possibly mediates ABA signal transduction (Nolan et al. 2006).

**Characterisation of the legume SERK-NIK gene superfamily including splice variants: Implications for development and defence**

SERK genes were initially demonstrated to play a role in somatic embryogenesis, but the view of their role in plant development has broadened. **MtSERK1** expression is associated with developmental change in *Medicago truncatula* (Nolan et al. 2003, 2009), including somatic embryogenesis, nodule and lateral root formation. Moreover, current evidence shows SERK proteins function both in developmental and defence signalling pathways, which occur in response to both peptide and steroid ligands. SERKs are generally present as small gene families in plants, with five SERK genes in Arabidopsis. Knowledge gained primarily through work on Arabidopsis SERKs indicates that these proteins probably interact with a wide range of other receptor kinases and form a fundamental part of many essential signalling pathways. Genes in the *M. truncatula* SERK family and other legumes are largely unidentified and their functions unknown. We have identified and sequenced the mRNAs of five more SERK and three SERK-like genes in *M. truncatula* (in addition to **MtSERK1**), and used these sequences to identify homologous genes in soybean. Phylogenetic analysis shows that some of these genes fall distinctly in the SERK family, while others are **SERK-like**, which include NIK genes and other LRRI subgroup RLK-LRR family members. The *M. truncatula* SERK3/4/5 subfamily genes have undergone a gene duplication event that is not present in orthologous genes in soybean or Lotus. One of these duplicated genes apparently encodes a number of sequences, consistent with the existence of splice variants, which is a novel finding for a SERK gene. The gene duplication event and the presence of splice variants may be indicative of a role in defence, similar to that observed in NBS-LRR genes.
(Nolan et al. 2011). Other members of this replicated SERK3/4/5 gene cluster are upregulated in embryogenic tissue cultures implying a similar developmental role to that previously observed for MtSERK1.

Are the changes leading to the super-embryogenic Jemalong 2HA mutant epigenetic?
The well-known phenotype of the Medicago truncatula 2HA line has the ability to form large numbers of somatic embryos in culture. Using the Medicago Affymetrix Gene Chip with four-week cultures of Jemalong wild type (WT) and 2HA (just prior to embryos being visible to the naked eye in 2HA) we found two down-regulated transcripts with nearly identical expression profiles after qRT-PCR. Subsequent PCR amplifications and sequencing revealed that the two transcripts (Mtr.10439.1.S1 and Mtr.1670.1.S1) were part of the same gene, which we deposited in GenBank (accession number GQ914771). Sequence alignments and phylogenetic analysis showed this gene to be a homolog of Arabidopsis EIN3 and we called the gene MtEIL1. This gene was found to be substantively down-regulated (differences up to 30 times) in all organs of 2HA compared to WT. In the ethylene triple response test at low levels of ACC (0.5 and 1 µM) the bending of cotyledons was different between 2HA and WT. Root growth was retarded by ACC in both Jemalong and 2HA. However roots of seven-day seedlings grown without addition of ACC were more elongated in 2HA than in WT and were similar to Sickle (an EIV2 mutant). Lateral root emergence was delayed compared to WT, with an intermediate phenotype between WT and Sickle. Under standard conditions, 2HA formed the same number of nodules as WT and AVG similarly stimulated nodulation in 2HA and WT. However addition of ACC, which completely inhibits nodulation in WT, did not abolish nodulation in 2HA plants. Other differences were noted, consistent with a weak ethylene insensitivity of the 2HA line, such as the delayed senescence of some flowers. To investigate the nature of the Mt-eil1 mutation we checked MtEIL1 levels in segregating populations of F2 plants after 2HA x WT crosses. This segregation was about 1:8 indicating that this recessive mutation did not segregate as a one or two Mendelian gene model. There were no large scale differences in the 2HA karyotype compared to WT. With AMP (amplified methylation polymorphism) analysis, a PCR-based marker protocol, we found methylation differences between 2HA and WT but no evidence of DNA sequence differences. Sequencing of the MtEIL1 gene and its promoter showed no sequence differences between 2HA and WT. We also found no differences in methylation of the MtEIL1 promoter between wild type and 2HA. However, in the case of 2HA the coding region showed strong methylation. These data are suggestive of an epigenetic change resulting in a weak ethylene phenotype (Kurdyukov et al. 2010).

2. Regulation of zygotic embryogenesis and oil body biogenesis.
This research is particularly aimed at understanding early embryo development as well as protein and oil accumulation, and partitioning between the two. Legume embryos develop into the major part of the seed.

From embryo sac to oil and protein bodies in the embryo of the model legume Medicago truncatula
Medicago truncatula produces cotyledons rich in oil and protein bodies and as a genetic and genomic model for legumes has been used to study seed development. However, the cell and developmental biology of embryo development in M. truncatula, has received little attention.
We have studied embryo development in relation to oil and protein body biogenesis in *M. truncatula*. Embryo sac and zygote development is similar to classical models with an initial asymmetric division of the zygote. Early in development and before the globular stage, a distinctive multicellular epiphysis and suspensor develops. By the heart stage an extensive suspensor exists and a substantial procambium connects the shoot and root apical meristems. Using electron microscopy to examine oil and protein body organization in the mature cotyledon revealed a distinctive cellular arrangement, with ovoid oil bodies (0.1-0.5 µm in diameter) lining the periphery of protein bodies and the plasma membrane. Biochemical analysis showed that fatty acids accumulate as cotyledons begin to develop with the major fatty acids present being palmitic, oleic, linoleic and linolenic acid (linolenic acid alone accounting for 36% of total fatty acid). *OLEOSIN* genes, which encode integral oil body membrane proteins that prevent oil body coalescence, exhibit an expression profile that complements fatty acid accumulation. The storage protein genes *VICILIN* and *LEGUMIN* commence expression at a similar time to *OLEOSIN* genes, but peak in expression earlier. These data will assist in linking the biochemistry and cell biology to the genetic regulation of oil and protein body biogenesis in legumes (Wang et al. 2010).

Oil bodies (single arrow) surround the protein bodies (P) and are also aligned along the plasma membrane (double arrow). This image is from a mature dry *M. truncatula cv. Jemalong* seed hydrated at 4°C.

3. Organelle dynamics in regenerating plant cells

Provides insights into dedifferentiation and the biology of DNA containing organelles (chloroplasts, mitochondria) as well as non-DNA containing organelles such as peroxisomes. Organelles are linked to many fundamental plant processes. Dr Michael Sheahan had an Australian Research Council Postdoctoral Award for much of the work in this area.

**Organelle dynamics and the initiation of cell division**

Previous work on the dynamics of DNA-containing organelles (chloroplasts and mitochondria –Sheahan et al. 2004; 2005) and non-DNA containing organelles (vacuoles, lytic vacuoles, endoplasmic reticulum) and the cytoskeleton has shown how collectively these cytoplasmic organelles prepare for the re-initiation of cell division and their partitioning to daughter cells. Newer information on peroxisomes, and P-bodies provides an integrated picture of the cell as it reprograms into cell division.
RBOH-dependent ROS production in regeneration and somatic embryogenesis

One of the earliest responses to stress is increased production of reactive oxygen species (ROS) at the site of stress. ROS can be highly damaging to cells, but can also act in signalling pathways critical for normal plant development and environmental perception. A number of experimental strategies indicate that ROS production is required for regeneration. Probing NADPH oxidase (NOX; superoxide generating flavoprotein) expression in Medicago revealed strong up-regulation of two genes, *MtRBOH-1* and -2, in explants. Analysis of regeneration in Arabidopsis NOX mutants for orthologs of these genes (*AtRBOHA* and *AtRBOHB*) suggest that these genes have an important role in the initiation of cell division and callus production. RNAi experiments are now in progress in Medicago and evidence is accumulating that controlled ROS production is necessary for the re-initiation of cell division in *Medicago* leaf explants, supporting our model of somatic embryogenesis.

Peroxisome dynamics and ROS homeostasis

Plants exhibit a remarkable ability to initiate morphogenic adaptations in response to environmental stress so as to enhance survival outcomes. Regeneration initiated from somatic cells represents an important form of morphogenic adaptation. Regeneration reflects the acquisition of a totipotent state; dedifferentiation, followed by cell proliferation and subsequent differentiation of cells into organised tissues and organs. We have shown that dedifferentiation and re-initiation of the cell cycle in response to wounding and culture involves signalling by reactive oxygen species (ROS). While ROS signals are necessary for initiation of cell division, it is accepted that excessive ROS like hydrogen peroxide (H$_2$O$_2$) induce cell death and therefore ROS homeostasis should be restored before cell division. Plants have a general antioxidant capability mainly found in chloroplasts, mitochondria and peroxisomes. While the major antioxidants, ascorbate and glutathione, are found in all three organelles, only peroxisomes possess catalase, an enzyme capable of extremely high H$_2$O$_2$ turnover. The sole localisation of this antioxidant enzyme in peroxisomes suggests they might play an important role in the restoration of ROS homeostasis. Here, using mesophyll protoplasts as an experimental system to study wound-dependent regeneration, we show that peroxisomes proliferate during culture and before cell division. Further, we show that mutation of genes encoding peroxisome-localised antioxidant machinery and moreover, peroxisome biogenesis (*PEX*) genes, greatly decreased capacity to remove excess H$_2$O$_2$. Our findings (Tiew *et al*. 2010) suggest that peroxisomes are important for the efficient re-initiation of cell division in plants.

P-Bodies and re-programming for regeneration

Regeneration initiated from somatic cells of plants represents an important mechanism of morphogenic adaptation to stress and entails the acquisition of a totipotent state. The acquisition of totipotency requires dedifferentiation, a process that reprograms somatic cells to a stem cell-like state. Inherent to this reprogramming are massive changes in gene expression. Here we investigated processes that influence transcriptome reprogramming during the regeneration of tobacco mesophyll protoplasts. Processing bodies (P-bodies) are cytoplasmic RNA-protein complexes that both store and degrade mRNA and play an important role in developmental transitions. Moreover, P-bodies are dynamic structures that can change in response to environmental stress. We therefore investigated P-body dynamics during dedifferentiation and
cell division initiation of tobacco mesophyll protoplasts. To visualise P-bodies, we created stable tobacco transformants expressing a YFP fusion to the C-terminal domain of VARICOSE (VCsc), a P-body localised protein that facilitates mRNA decapping. YFP-VCsc labelled spherical structures that ranged in size from 7 to 150 μm in leaf epidermal cells. In protoplasts, P-bodies exhibited variable size but also abundance per cell. While average P-bodies size in protoplasts remained relatively constant during culture, the number of P-bodies per cell increased significantly during early (24 h) culture and then again preceding cell division. P-body size and abundance was significantly reduced by cycloheximide and actinomycin D treatments suggesting P-bodies require continual recruitment of translationally-repressed mRNA. Transiently expressing a fusion of DCP2 (a decapping enzyme) to CFP, revealed an apparent heterogeneity in the P-body population with co-expression of VCsc promoting localisation of DCP2 to P-bodies. Our results suggest P-bodies are dynamic structures that potentially participate in reprogramming the transcriptome of mesophyll cells (Bhullar et al. 2010).

Chloroplasts (red autofluorescence) are distributed across the entire cell area (0-48 h), however they cluster around the nucleus (72 h) before the onset of cell division, as earlier described (Sheahan et al. 2004). P-bodies (small green spheres) exhibit two phases of proliferation in regenerating protoplasts, one related to dedifferentiation and one related to cell division.

Dr Georg Weiller – Chief Investigator – Australian National University

In the bioinformatics laboratory, we develop software programs and web servers that aid the analysis of large sets of expressed sequences and other gene-expression data. These software solutions are available to experimentalists in the CILR and the wider scientific community on the web. We also support the analysis of data obtained from labs associated with the CILR.

Software updates and development

We extended and updated our PathExpress web server, in particular the Enzyme Neighbourhood method, which can detect the coordinated expression of interacting genes even if these are not part of the same classical pathway.

We developed the Web-CLUEs system to facilitate access and analysis of local expression data without requiring the installation of expensive or unwieldy software. The service is run through a web-interface, providing an easy means for scientists to share their data and basic analytical capabilities with other labs across the internet.

We have continued work on extending our nucleotide motif search algorithm MciP to protein motifs and developed the MotifDraw web server.

Gene expression atlas

We developed specialised software and provided bioinformatics support for global gene expression atlases for Lotus japonicus and Phaseolus vulgaris. For Lotus japonicus we presented an integrated genome-wide analysis of transcriptome landscapes in wild-type and symbiotic mutant plants and showed the effect of transcripts on organ development. For Phaseolus vulgaris we presented the effect of abiotic stress on global changes in gene expression that affect overall metabolism.
Novel initiatives
We have started developing a novel bioinformatics software suite that assists the analysis and interpretation of next-generation sequencing data. We also began the development of a fast and efficient motif finding algorithm that is able to utilise large amounts of sequence data to find motifs that are specific for large groups of sequences (such as all legumes or all plants). This method has already found genes that are specific for legumes.

Natural Science - Social Science Linkage Programs

Miles Holmes – PhD Student – University of Queensland

As part of the CILR’s Natural Science – Social Science linkage program, PhD student Miles Holmes has investigated Warlpiri ecological knowledge. Miles Holmes is an anthropologist who has been working in the Northern Territory for the last eight years. He conducted his PhD research on Indigenous Ecological Knowledge to answer the question “What is the structure of Warlpiri Plant Knowledge?” Miles Holmes was co-supervised by Professor Peter Gresshoff and Dr Mary Laughren (UQ Faculty of Arts).

Significant research findings, included the articulation of a Warlpiri system of ecological knowledge through a concept called Ngurra-kurlu, which means “from country” or “with country” and is a template for the interrelationships between the five key elements of Warlpiri culture. Ecological knowledge about any given plant or animals is a function of these elements which are: Country, Skin (kinship), Law, Language and Ceremony. In 2008, Miles published a monograph about Ngurra-kurlu in conjunction with a Warlpiri man named Wanta Pawu-kurlpulunu Jampijinpa. The paper achieved high readership and continues to deliver significant benefits to Warlpiri people, which is an important ethical component of this research. The monograph is now compulsory reading for new teachers at the Lajamanu Community Education Centre and Wanta and the other authors have been invited to present at several conferences.

1 The Warlpiri are Indigenous Australians who reside in the Tanami Desert in the Northern Territory.
QLD weather plays havoc
The CILR is committed to the development of the next generation of plant scientists and pursues a comprehensive Education and Outreach strategy to reach this goal. The CILR’s output in terms of the Education and Outreach program was unfortunately curtailed due to the absence of an Education and Outreach officer for much of 2010 (position not filled). However, the Centre continued to play an important role in promoting plant science amongst school pupils and teachers.

The association with the Brisbane Boys’ College (BBC) was further enhanced with six boys from BBC involved in research projects at the UQ Node of the CILR. This is the third year that the program has been run and provides an exceptional opportunity for talented science students to gain first-hand knowledge and insight in plant research. At the completion of their research projects, each student is required to compile a research report and present it to a wide audience at BBC. This program will be continued in 2011.

Training tomorrow’s scientists

In 2010, five students graduated with a PhD and three students with an Honours degree. Many of the graduates continue to work at the CILR as Post-Doctoral Fellows. The four nodes also had undergraduate students working in their laboratories on different research projects.

Australia China Young Scientists Exchange Program

In September 2010, Dr Jacqueline Batley was one of eight Australian Scientists invited as alumni to the Australian China Young Scientists Exchange Program, held at the Australian Pavilion, Shanghai. This visit was followed by alumni from China and Australia commenting on their previous exchange visit and outcomes arising from their time in China. This included discussions on Chinese research and researchers in their specific research field; what needs to be done to develop effective collaboration between China and Australia; the benefits of the exchange and any new insights that will assist with their research and institutional linkages. The alumni took part in panel discussions on how they subsequently have capitalised on the exchange and held discussions on the success of past programs, impacts opportunities and future activities. The formal presentations were followed by enjoyable tours of the Australian pavilion and the Expo site, providing opportunities for further networking.

STEP IN LABS — Science Teacher Education Program

(Science Teachers Education Partnership IN Legumes And Biotechnology Studies)

STEP IN LABS was originally conceived within the Centre and developed in partnership with Education Queensland as part of the ‘Queensland Government’s Spotlight on Science’ initiative. The program was initiated in 2006. The program had two primary objectives:

1. To promote an increased awareness of the importance of legumes, showcase the sophistication of plant biotechnology research, and inspire teachers to take their new enthusiasm back to the classroom, and
2. To provide opportunities for high school teachers to gain real scientific experience, in a research laboratory, so that they would be able to take back the knowledge gained and use it to create interesting and exciting scientific experiences for their students.
In 2006, eleven teachers from around Queensland attended the week-long program. The initiative was an immediate success, with all teachers animatedly discussing how they would adapt their newly acquired skills and knowledge to high school biology practical classes, particularly with regards to the extended experimental investigation (EEI) required under the new Queensland Biology Syllabus.

STEP IN LABS has been presented each year subsequent to 2006 with the program becoming more and more professional and sophisticated. The response from teachers throughout Queensland has been very encouraging. To date (2006 to 2010) a total of 65 teachers have attended the STEP IN LABS program at the CILR. Several important milestones have been achieved along the way. After successful discussions with Education Queensland (EQ) at the end of 2006, EQ agreed to provide sufficient funding to support a teacher release scheme. This scheme has contributed significantly to the success of the program, but equally importantly, it has allowed teachers from more rural areas to attend the STEP IN LABS program. For example, the majority of teachers attending STEP IN LABS 2006 were from south east Queensland and Brisbane, in particular. However in 2007, the majority of teachers attending STEP IN LABS were from outside greater Brisbane. Teachers were from districts such as Roma, Warwick, Kepnock (Bundaberg), Proserpine and Charters Towers. A Rural Scholarship initiative, subsidised by EQ, was another important milestone in the development of STEP IN LABS. Rural Scholarships were made available to enable rurally-located teachers to attend STEP IN LABS by providing funds for three teachers towards travel and accommodation. This program continued through to 2009.

In 2009, for the first time, the professional development program STEP IN LABS was organised by the CILR independently. Demand for the STEP IN LABS program from the private school sector enabled the CILR to advertise the program more widely and oversee the registration process. The program was shortened to two days to accommodate teachers’ busy schedules. Within days of the advertising email being sent out the 15 available places were filled. However, in light of the demand, the CILR decided to offer a second round. The 15 places of the second round were also filled within days. 15 high school teachers from all over Queensland attended both rounds of the two-day program. Teachers travelled to Brisbane from as far as Cairns and Mackay to attend the STEP IN LABS.

Topics such as Nodulation and Nitrogen Fixation, Tissue Culture, Apical Dominance, and DNA Extraction and Sequencing, have enabled attending teachers to gain an insight into the world of plant biotechnology research. Using leguminous examples such as pea (Pisum sativum) and soybean (Glycine max), teachers developed an appreciation for the integrative and cross-disciplinary nature of plant biology and biotechnology.

In all cases workshops have been designed to be simple, cost-effective and can easily be completed within a classroom, keeping in mind teachers’ accessibility to consumables and resources by utilising chemicals and products readily available from either supermarkets or plant nurseries. The CILR’s Seeds for Schools program provides teachers with different soybean seeds (wild type and two mutants), to enable them to conduct the nodulation experiment at school. The new ideas and resources proved to be useful in developing Extended Experimental Investigations (EEIs).
Each year, based on teacher feedback, the modules have been revised and modified in order to render them more effective and reflect the syllabus. In order to assist teachers once they are back at their respective schools, the protocols for all workshops have been made available on the CILR website: (www.cilr.uq.edu.au). A recall day, where teachers return to the CILR for discussions with the research staff and where necessary, to brush up on any areas which they have identified as problems, or where they require additional information, have proved very successful. Teachers were in regular contact with the Education and Outreach Manager (EOM) at the CILR.

Apart from the EOM, there is a significant commitment by the scientific staff at the Centre to the success of the program. Experienced CILR post-doctoral research scientists deliver all workshops with additional help by technical staff and PhD students.

To date, teachers have been exposed to the following techniques:

- Tissue culture;
- DNA extraction;
- Visualising DNA on an agarose gel;
- Decapitation of plants;
- Hormone application to plants;
- Plant bud, node and root identification;
- Legume nodule identification;
- Plant stem, bud, node and root measurements;
- Staining of biological specimens;
- Microscope techniques;

In addition to these techniques, teachers are able to learn more complex techniques such as grafting and petiole drip-feeding during the time they spend with CILR PhD students. Teachers particularly enjoyed this opportunity to learn more about the life of a PhD student and how each student came to the CILR.

There has been an overwhelmingly positive response from teachers who have attended the STEPS IN LABS program. They have all indicated that they were previously unaware of the importance of legumes and the exposure to the biotechnology research and
techniques in the field has broadened their minds immeasurably. In all cases teachers have stated that they looked forward to imparting their knowledge to their students. This can only be good for science education, students, teachers, schools and the broader scientific community.

The STEP IN LABS program would not have achieved the level of success that it has if it were not for the dedication and enthusiasm of the Centres EOM’s. Special mention must be made of Ms Lisette Pregelj who was involved in the program in 2006 and took full responsibility for running the program in 2007. In 2008 and 2009 Ms Charlotte Camerer was responsible for the program and made a number of important and significant modifications.

The Centre research staff, most notably, Dr Paul Scott, Dr Brett Ferguson, Dr Bandana Biswas, Dr Qunyi Jiang, Dr Liz Dun, Dr Phil Brewer and PhD student Yu-Hsiang Lin need to be recognised for their considerable efforts in making the program such a success.
Intellectual Property Management and Commercialisation

The CILR has given priority to best practice management in intellectual property that will be generated by its research activities. The Centre Management Committee decides upon protection of IP generated by the Centre. All Centre IP is channelled through UniQuest Pty Ltd, a recognised leader in university commercialisation.

**Patent applications**

A total of 15 disclosures were registered with UniQuest Pty Ltd for the period 2008 to 2010.

Two patent applications that were filed in 2008 were advanced in 2009 and 2010. The first application relates to Soybean Nodulation Factor Receptor Proteins and the second relates to the research done on the Strigolactone hormone in collaboration with French colleagues.

The Soybean patent was registered at a National phase in the US, Argentina, Brazil and China. Some commercial interest has already been shown in this work and discussions have been conducted with a large multinational organisation to commercialise this work.

This patent describes the sequence of the soybean *Glycine max*) Nod Factor Receptor. The researchers have found that, in soybean, two copies of the Nod Factor Receptor gene, *GmNFR1*, exist. This gene is mutated in the soybean mutant *nod49*, and when overexpressed in transgenic roots, results in both an increase in nodule numbers (nodulation) and nitrogen assimilation (nitrogen fixation). The increased nodulation appears to stem from the attenuation of the internal Autoregulation Of Nodulation (AON) system. This discovery could potentially provide the means of increasing soybean nitrogen fixation, increasing seed and oil production, and enhancing establishment in low Bradyrhizobium soils.

A provisional patent on the strigolactone work was filed by the Institut National de la Recherche Agronomique (“INRA”) in France on behalf of all the collaborators in this project including the researchers at the CILR.

A provisional patent (Prov. 61/398854) - Soybean nodulation regulatory peptides and methods of use (Gresshoff, P M, Reid, D and Ferguson, B) was filed in 2010.

**Existing patents**

In May 2006, a provisional patent on Nod Factors as modulators of angiogenesis was filed. It was found that compounds derived from the early *Rhizobium*-legume signalling for the onset of nodule development, and their derivatives, have the ability to modulate angiogenesis in mammalian systems (human and rat). These compounds, generically known as Nod Factors, have the potential for therapeutic benefit in the treatment of a range of cancers...
and other disease. Due to the role that angiogenesis plays in cancer therapy and chronic inflammatory diseases this market is a major focus of the pharmaceutical industry. This project received an additional $72,000 in funding from the commercialisation company at the Australian National University (ANU Connect) to further progress this work.

A provisional patent was filed in September 2006 and describes technology indicating that a homologous transgene that produces single-stranded RNA, in addition to a homologous transgene producing double-stranded RNA or another homologous RNAi-inducing molecule, enhances induction of RNAi-based virus resistance in plants (we have demonstrated this with one plant virus), and induction of RNAi against endogenous genes. The patent also describes identification of several genes that play a key role in long-distance transmission of RNA silencing to newly-formed shoot apices of plants.

**Start-up Company**

A start-up company called Bio Energy Solutions (BES) Pty Ltd was initiated through UniQuest Pty Ltd. BES received substantial financial backing from an industrial partner. Most of the cash injection into BES was earmarked for research and development in the CILR. The major research thrust has centred on the legume tree *Pongamia pinnata*, which is now recognised as being highly suitable as a feedstock for the biofuel industry. At present, BES is the IP holding company for Pongamia with various licenses in place to ensure that the industry benefits from the research at the CILR. There has been growing interest from other industrial players to become involved with Pongamia research and development.
Research Publications 2010 and 2011*

*Many publications were printed in 2011 but appeared electronically in 2010.

Publications funded by, or related to the core activities of the Centre

Publications in journals with impact factor greater than or equal to 5 (according to ISI Web of Science Journal Citation Reports)


**Publications in journals with impact factor less than 5**


Book Chapters


Other publications by CILR staff


## Major Conference Presentations, Seminars and Workshops

<table>
<thead>
<tr>
<th>Presenter/s</th>
<th>Title</th>
<th>Venue</th>
<th>Date</th>
<th>Approx attendance</th>
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<tbody>
<tr>
<td><strong>International Presentations, Seminars &amp; Workshops</strong></td>
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<tr>
<td>Bhalla, P.</td>
<td>Male Germ Line Cell Development And Histone Variants In Lilium Longiflorum</td>
<td>International symposium on Genus Lilium, Pescia, Italy</td>
<td>Jan. 14 – 19 2010</td>
<td>100</td>
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<tr>
<td>Brewer, P., Mason, M.G., Frickey, T., Dun, E., Meyers, E., Filardo, F., Cremer, J. and Beveridge, C</td>
<td>Transcriptional Evidence Points To Highly Active RNA Silencing Machinery in Plant Sperm Cells</td>
<td>International Conference on Arabidopsis Research, Yokohama, Japan</td>
<td>June 2010</td>
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<tr>
<td>Name(s)</td>
<td>Title</td>
<td>Conference/Event</td>
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<tr>
<td>Djordjevic, M.A.</td>
<td>Proteolytic cleavage of CLE peptides by secreted proteases.</td>
<td>Donald Danforth Plant Science Institute, St Louis, Missouri, USA</td>
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<tr>
<td>Gresshoff, P.M.</td>
<td>Biotechnology and Molecular Genetics of Legumes for Climate Change Response: Food and Biofuel Production Issues</td>
<td>International Atomic Energy Agency (IAEA), Vienna Austria</td>
<td>May 2010</td>
<td></td>
</tr>
<tr>
<td>Gresshoff, P.M.</td>
<td>Seminar: Biotechnology and Functional genomics of Biofuel Production from Pongamia</td>
<td>Qatar Foundation, Qatar</td>
<td>May 17th 2010</td>
<td></td>
</tr>
<tr>
<td>Gresshoff, P.M.</td>
<td>Legume tree Pongamia pinnata – feedstock for the biofuel industry</td>
<td>Qatar University, Qatar</td>
<td>May 19th 2010</td>
<td></td>
</tr>
<tr>
<td>Gresshoff, P.M.</td>
<td>Seminar: Systemic regulation of nodulation in legumes</td>
<td>Cambridge University, UK</td>
<td>May 7th 2010</td>
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</tbody>
</table>

2010 Annual Report 46
<p>| Gresshoff, P.M | The Role of CLE Peptides in Long- Distance Regulation of Nodulation in Legumes. | MOLECULAR PLANT Symposium in Shanghai | June 22, 2010 | 400 |
| Gresshoff, P.M | Plant Stem Cell Biology and Organ Differentiation Opportunities for Australia and China. | The Queensland-Shanghai Science Collaboration Workshop and Symposium in Shanghai | June 22, 2010 | 100 |
| Gresshoff, P.M | Research seminars on legume biology, Pongamia-based biofuel production, and Systemic regulation of nodulation in legumes. | Guangzhou, China | June 24, 2010 | |
| Gresshoff, P.M | Chaired a session on Symbiosis | Vth International Congress on Legume Genetics and Genomics. Asilomar, CA, USA. | 2 – 8 July 2011 | 250 |
| Gresshoff, P.M. | Seminar: Pongamia-based biofuel production. Presentation made as part of his Chinese Academy of Science Senior Scientist Professorial Fellowship. | Guangzhou, China | June 24th 2011 | |
| Gresshoff, P.M. | Seminar: Systemic regulation of nodulation in legumes. Presentation made as part of his Chinese Academy of Science Senior Scientist Professorial Fellowship. | Guangzhou, China | June 25th 2011 | |
| Hayashi, S., Reid, D., Lorenc, M., Stillier, J., Edwards D., Ferguson B.J. and Gresshoff, P.M. | Early legume nodulation responses identified using RNA-seq to generate complete transcriptomes of soybean roots. | Plant and Animal Genome Conference XIX, San Diego, CA, USA | Jan 15 – 19 2011 | 100 |
| Indrasumunar, A., Reid, D., Ferguson, B.J. and Gresshoff, P.M. | Nitrate Sensing And Signaling During Soybean Nodulation. | Plant and Animal Genome Conference XIX, San Diego, CA, USA | Jan 15 – 19 2011 | 100 |
| Imin, N., Mohd Radzman, N. and Djordjevic, M.A. | Molecular Control of Root Organogenesis and Its Relationship to Crop Productivity | Xinjiang Agricultural University, Urumqi, China. | 2010 | |</p>
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Title</th>
<th>Conference Details</th>
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<tbody>
<tr>
<td>Parish, C.R.</td>
<td>Destruction of Heparan Sulfate by Heparanase Mediates Loss of Insulin Producing Beta Cells in Autoimmune Diabetes</td>
<td>14th International Conference of Immunology, Kobe, Japan</td>
</tr>
<tr>
<td>Rasmussen, A. and Beveridge, C.</td>
<td>The new plant hormone, strigolactones, control adventitious root formation.</td>
<td>International Plant Growth Substances Association Conference, Tarragona, Spain, June 2010</td>
</tr>
<tr>
<td>Reid, D.E., Li, D., Hayashi, S., Ferguson, B. and Gresshoff, P.M.</td>
<td>CLE peptide induction of soybean nodule regulation,</td>
<td>Vth International Congress on Legume Genetics and Genomics. Asilomar, CA, USA, 2 – 8 July 2010</td>
</tr>
<tr>
<td>Brewer, P., Mason, M.G., Frickey, T., Dun, E., Meyers, E., Filardo, F., Cremer, J. and Beveridge C.</td>
<td>Mining for novel strigolactone-related branching genes.</td>
<td>International Plant Growth Substances Association Conference, Tarragona, Spain, June, 2010</td>
</tr>
<tr>
<td>Buer, C.S. and Djordjevic, M.A.</td>
<td>In vivo role for flavonoids in auxin transport and gravity responses</td>
<td>Plant Vascular Biology, Columbus, Ohio, USA, 2010</td>
</tr>
<tr>
<td>Authors</td>
<td>Title</td>
<td>Conference/Event</td>
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<tr>
<td>Gursanscky, N., Brosnan, C. Bowman, J.L. and Carroll, B.J.</td>
<td>Genetic determinants involved in transmission of gene silencing from rootstocks of Arabidopsis</td>
<td>Keystone Symposium, RNA Silencing Mechanisms in Plants. Santa Fe, New Mexico, USA</td>
</tr>
<tr>
<td>Kurdyukov, S., Mathesius, U., Nolan, K. E., Goffard, N., Carroll, B. and Rose, R. J.</td>
<td>Molecular analysis of Root Organogenesis in Medicago truncatula.</td>
<td>Vth International Congress on Legume Genetics and Genomics, Asilomar USA</td>
</tr>
<tr>
<td>Rasmussen, A. and Beveridge, C.</td>
<td>The 2HA line of Medicago truncatula has characteristics of an epigenetic mutant that is weakly ethylene insensitive</td>
<td>International Plant Growth Substances Association Conference, Tarragona, Spain</td>
</tr>
<tr>
<td>Wang, X., Song, Y., Garg, M. and Rose, R. J.</td>
<td>From embryo sac to oil and protein bodies in the seed of the model legume Medicago truncatula</td>
<td>Vth International Congress on Legume Genetics and Genomics, Asilomar USA</td>
</tr>
</tbody>
</table>

**Australia – Presentations, Seminars & Workshops**

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<thead>
<tr>
<th>Authors</th>
<th>Title</th>
<th>Conference/Event</th>
<th>Date</th>
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<tbody>
<tr>
<td>Bhalla, P.</td>
<td>Unlocking the secrets of male germline development in plants</td>
<td>Botany, LaTrobe University Invited seminar</td>
<td>19 May 2010</td>
</tr>
<tr>
<td>Djordjevic, M.A.</td>
<td>The Molecular Dialogue of Rhizobium Interactions with Eukaryotes.</td>
<td>Australian Society for Microbiology, Sydney</td>
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<tr>
<td>Authors</td>
<td>Title</td>
<td>Conference/Event Details</td>
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<tr>
<td>Gresshoff, P.M.</td>
<td>Seminar: Sustainable production of Biofuel from the legume tree Pongamia pinnata</td>
<td>Sunshine Coast Council, Brisbane, Australia</td>
<td>22 March 2010</td>
</tr>
<tr>
<td>Gresshoff, P.M.</td>
<td>Seminar: Sustainable Biofuel Production’</td>
<td>Queensland Chem. Engineering Association</td>
<td>May 25th 2010</td>
</tr>
<tr>
<td>Gresshoff, P.M.</td>
<td>Seminar; Pongamia &amp; aviation jet fuels</td>
<td>Avalon Airshow, Melbourne</td>
<td>March 2011</td>
</tr>
<tr>
<td>Gresshoff, P.M.</td>
<td>Seminar/Lecture: Food for a Healthy Planet</td>
<td>Melbourne University</td>
<td>April 2011</td>
</tr>
<tr>
<td>Nolan, K.E. and Rose, R.J.</td>
<td>Characterisation of the SERK gene family in legumes involved in development and defence</td>
<td>International OzBio 2010 Conference, Melbourne, VIC, Australia</td>
<td>26 Sep. – 1 Oct. 2010</td>
</tr>
<tr>
<td>Parish, C.R.</td>
<td>Cancer Immunotherapy with Dendritic Cell Targeting Vaccines</td>
<td>3rd Australasian Vaccines and Immunotherapeutics Development Conference, Melbourne</td>
<td>5-7 May, 2010</td>
</tr>
<tr>
<td>Parish, C.R.</td>
<td>Preservation of Islet Heparan Sulfate as a Therapy for Type I Diabetes</td>
<td>IgV-Mitenyi Winter Seminar, Melbourne</td>
<td>28 July 2010</td>
</tr>
<tr>
<td>Parish, C.R.</td>
<td>Emerging Cancer Therapies</td>
<td>Boots Course on Translational Medicine: The Pathway from Discovery to Healthcare, Canberra</td>
<td>17-20 Aug. 2010</td>
</tr>
<tr>
<td>Parish, C.R.</td>
<td>The Role of Heparanase and Heparan Sulfate in Health and Disease</td>
<td>Centre for Vascular Research, University of NSW, Sydney</td>
<td>24 Feb. 2010</td>
</tr>
<tr>
<td>Parish, C.R.</td>
<td>New Approaches to Cancer Immunotherapy</td>
<td>Centre for Vascular Research, University of NSW, Sydney</td>
<td>6 Oct. 2010</td>
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<tr>
<td>Parish, C.R.</td>
<td>Antigen Receptor Sharing: A New Immunological Paradigm</td>
<td>Herston Research Centre, University of Canberra, Canberra</td>
<td>17 Nov. 2010</td>
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<tr>
<td>Name</td>
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<td>Kordbacheh, F., Hocart, C., Du Fall, L., Oakes, M., Bezos, A., James, G., Parish, C. and Djordjevic, M.A.</td>
<td>Characterisation of pro-angiogenic molecules from legumes</td>
<td>The John Curtin School of medical research, ANU</td>
<td>1 – 4 Nov. 2010</td>
</tr>
<tr>
<td>Tiew, T.W.Y., Rose, R.J. and Sheahan, M.B.</td>
<td>Peroxisome dynamics: importance to the restoration of reactive oxygen species (ROS) homeostasis during plant cell regeneration</td>
<td>International OzBio 2010 Conference, Melbourne, VIC, Australia</td>
<td>26 Sep. – 1 Oct. 2010</td>
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<tr>
<td>Wong, A.</td>
<td>Floral Initiation Process in Soybean</td>
<td>Bio21 Symposium, Melbourne (Seminar)</td>
<td>19 Mar. 2010</td>
</tr>
<tr>
<td>Wong, A.</td>
<td>Small RNAs Profiling of Shoot Apical Meristem</td>
<td>Bio21 Symposium, Melbourne</td>
<td>19 Mar. 2010</td>
</tr>
</tbody>
</table>
External Collaborations and Linkages

International visitors – presentations and seminars

Dr Sofia Costa, University of Coimbra, Portugal visited Dr Uli Mathesius (ANU) - 5 February 2010 to 24 April 2010; Joint project on legume-Rhizobium-nematode interactions funded by the Foundation for Science and Technology, Portugal;

Pek Lan Chan visited Professor Ray Rose (Newcastle Node) from May-August 2010, Research Officer, Advanced Biotechnology and Breeding Centre, Malaysian Palm Oil Board, Selangor, Malaysia;

Dr P. Balasubramanian, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore, India; April 13, 2010; India-Queensland Biotechnology Workshop, University of Queensland (Carroll Lab);

Dr Shudhanshu Vrati, National Institute of Immunology, New Delhi, India; April 13, 2010; India-Queensland Biotechnology Workshop, University of Queensland (Carroll Lab);

Dr Bhaskar Saha, National Centre for Cell Science, Ganeshkhind, Pune, India; April 13, 2010; India-Queensland Biotechnology Workshop, University of Queensland (Carroll Lab);

Dr Atul Kumar Johri, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India; April 13, 2010; India-Queensland Biotechnology Workshop, University of Queensland (Carroll Lab);

Dr Jiri Friml, University of Ghent, Belgium; August, 2010, Presented a seminar and discuss collaboration on auxin transport and plant development;

Dr Eva Benkova, University of Ghent, Belgium, University of Queensland (Beveridge Lab);

Dr Jenny Aitken, Director of The Tree Lab visited the UQ Node of the CLLR with regards to the Pongamia project. University of Queensland (Gresshoff Lab);

Dr Claire Kinlaw and Naveen Sikka from Terviva Bioenergy in the US visited Professor Peter Gresshoff, Dr Paul Scott and Dr Alvin van Niekerk with regards to setting up a cooperative biofuel project. University of Queensland (Gresshoff Lab);

Dr M.K. Bhan, Secretary to the Government of India, Department of Biotechnology. Topic of discussion: India-Queensland biotechnology collaboration. University of Queensland;

Professor M S Swaminathan widely recognized as the Father of the Green Revolution. Business and Food Security meeting – Melbourne;

Professor David Bird visited the Djordjevic lab Canberra 18th August 2010 to promote collaboration between the two labs;

Professor Brian Staskawicz, UC Berkeley, San Francisco, USA; April 15-16, 2010; seminar, University of Queensland (Carroll Lab);

Professor Sunil Mukherjee, International Centre for Genetic Engineering and Biotechnology, New Delhi, India; April 12-14, 2010; India-Queensland Biotechnology Workshop, Brisbane, and UQ seminar. University of Queensland (Carroll Lab);

Professor Kailash Bansal, Indian Agricultural Research Institute, New Delhi, India; April 12-14, 2010; India-Queensland Biotechnology Workshop, Brisbane, and UQ seminar. University of Queensland (Carroll Lab);

Professor Murray Grant, University of Exeter, Exeter, UK; Aug 26-27, 2010; seminar. University of Queensland (Carroll Lab).
Visits to International Laboratories

Professor Prem Bhalla visited Dr Pierre Ricci at Agrobiotech, Sophia Antipolis, France in September 2010;

Professor Prem Bhalla visited Tohoku University Sendai, Japan; 25-26 Jan 2010;

Professor Prem Bhalla visited Nara Institute of Science and Technology, Nara, Japan, 28 Jan 2010;

Associate Professor Michael Djordjevic visited Dr. Toni Kutchan, Donald Danforth Plant Science Institute, St Louis, Missouri, USA;

Associate Professor Michael Djordjevic visited Dr. Tom Smith, Donald Danforth Plant Science Institute, St Louis, Missouri, USA;

Associate Professor Michael Djordjevic visited Dr Jan Jaworski, Donald Danforth Plant Science Institute, St Louis, Missouri, USA;

Associate Professor Michael Djordjevic visited Prof Ann Hirsch, Plant Sciences, UCLA, California, USA;

Associate Professor Michael Djordjevic visited Prof Zhang laboratory Lab of Natural Products School of Pharmacy Second Military Medical University Shanghai, China;

Associate Professor Michael Djordjevic visited Prof Wang Liang-Sheng laboratory, Beijing Botanical Garden Institute of Botany, The Chinese Academy of Sciences Beijing, China;

Associate Professor Michael Djordjevic visited Oliver Yu, Donald Danforth Plant Science Institute, St Louis, Missouri, USA;

Associate Professor Michael Djordjevic visited Dr. Jan Jaworski, Donald Danforth Plant Science Institute, St Louis, Missouri, USA;

Associate Professor Michael Djordjevic visited Oliver Yu, Donald Danforth Plant Science Institute, St Louis, Missouri, USA;

Professor Peter Gresshoff and Dr Alvin van Niekerk visited The Tree Lab in Rotorua, New Zealand;

Professor Peter Gresshoff attended the Queensland-Shanghai Science Collaboration Workshop and Symposium in Shanghai on the 22nd of June 2010 and presented a lecture on: Plant Stem Cell Biology and Organ Differentiation Opportunities for Australia and China;

Professor Peter Gresshoff presented research seminars on legume biology, Pongamia-based biofuel production, and Systemic regulation of nodulation in legumes in Guangzhou, China on the 24th and 25th of June 2010, as part of his Chinese Academy of Science Senior Scientist Professorial fellowship;
Professor Peter Gresshoff visited Professor Julio Lucinio, Director of the John Curtin School of Medical Research at ANU, Canberra, in October 2010;

Dr Mike Mason (Post-doctoral researcher - UQ) visited collaborator Junko Kyozuka University of Tokyo, Japan;

Dr Ulrike Mathesius visited Prof Tom Beeckman and Prof. Sofie Goormachtig; Ghent University, Belgium; 25-26 May 2010;

Professor Chris Parish visited Eijkman Institute, Jakarta, Indonesia 3 August 2010;

Professor Chris Parish visited PT. Bio Farma, Bandung, Indonesia 4 August 2010;

Professor Mohan Singh visited Tohoku University Sendai, Japan; 25-26 Jan 2010;

Professor Mohan Singh visited Nara Institute of Science and Technology, Nara, Japan, 28 Jan 2010.

**Industry organisational contacts**

- Bioenergy Plantations Pty Ltd;
- Bio Energy Solutions Pty Ltd;
- Boeing Aircraft Corporation;
- Mitsui & Co. (Australia) Ltd;
- Monsanto;
- ORIGIN Energy CSG Ltd;
- Pacific Renewable Research;
- Origo Resource Partners;
- Primary Holdings;
- Qantas;
- Tarong Energy;
- Terviva BioEnergy
- Virgin Airlines.
Successfully Submitted Honours, Masters and PhD Theses

PhD:

ANU
Lucia Kusumawati – Secreted Proteins in Medicago species: Identification, Gene Expression and Functional Analysis;
Karsten Oelkers – Identification and Analysis of CLE Signalling Peptides in Root and Nodule Development;
Euan McNaughton – The Presentation of Multivalent Antigens to CD4+ T Cells.

UQ
Miles Holmes – An Anthropological Study of the Structure and Contemporary Application of Warlpiri Ecological Knowledge;
Liqi Han – Computational Modelling for Studying Signalling Mechanisms Behind Autoregulation of Nodulation.

Honours:

ANU
Liang Pin Jason Ng – Regulation of Auxin Location and Transport During Nodulation by the Cytokinin Receptor, CRE1, in Medicago truncatula;

Newcastle
Dilbag Singh Bhullar (Honours Class 1) – Identification of Plant P-Bodies and Their Role During the Re-Initiation of Cell Division.

CILR Centre and Staff Achievements

Peter Doherty award
The Centre (UQ) received the 2010 Peter Doherty Science Education Partnership and Community Science Award, from the Queensland Government for its contribution to science education. This award was made based on the teacher science education program – Step in Labs that has been conducted at the UQ Node since 2006.

Professor Ray Rose received the “Recognising our Authors” award. The award was based on publications in the journals Plant Physiology and Plant Cell. The American Society of Plant Biology posted a list on both journal sites of authors publishing the most influential plant science based on 2004-2008 publications. This was for CILR work.
• Dr. Kim Nolan received the Excellence in OH&S award from the University of Newcastle Faculty of Science and IT award for Faculty leadership in OGTR (gene technology) and AQIS (quarantine) compliance based on work in CILR labs.

• Professor Peter Gresshoff was honoured and recognised with a Chinese Academy of Science Senior Scientist Professorial Fellowship.

• Assoc. Prof. Adrienne Nicotra, Dr. Gonzalo Estavillo, Dr. Ulrike Mathesius, Dr. Elizabeth Beckman, Ms Amy Davidson and Assoc. Prof. Michael Djordjevic received the ANU Vice Chancellors award for excellence in education. May 2010.

• Dr Ulrike Mathesius was awarded an ARC Future Fellowship.

• Associate Professor Christine Beveridge was awarded an ARC Future Fellowship.

• Dr Elizabeth Dun was awarded an ARC Postdoctoral Fellowship.

• Dr Jacqui Batley was elected to the steering committee for the Brassica SNP consortium.

• Dr Jacqui Batley was invited to China as an alumnus of the Australia-China Young Scientist Exchange Program. Dr Batley has subsequently been invited to join the “next step program – making collaboration happen” and will again visit China in 2011 to further the collaborations.

CILR staff involvement on editorial boards of refereed journals or international advisory committees:

**Associate Professor Christine Beveridge**

• Editorial Board Member of Plant Growth Regulation
  Prof. Prem Bhalla

• Editorial Board Member of Recent Patents of Biotechnology;

• Editorial Board Member of Biomedicine and Biotechnology;

• Editorial Board Member of Journal of Botany;

• ARC College of Experts;

• BBRC Grant Review Assessor.

**Associate Professor Michael Djordjevic**

• Editorial Board Member of Genomic Insights;

• Managerial Board Member of the Australian Proteomics Computational Facility;

• Academic Board Member of SunFix University of Sydney.
Prof. Peter Gresshoff
- Editorial Board Member of Symbiosis;
- Editorial Board Member of BioEnergy Research;
- Editorial Board Member of Molecular Plant;
- Editorial Board Member of the Journal of Plant Physiology;
- Member of the RIRDC Granting Committee;
- Member of the Advisory Committee to the Office of Gene Technology Regulator (GGTAC).

Dr Ulrike Mathesius
- Advisory Board Member of Functional Plant Biology;
- Chair of the International Medicago truncatula Steering Committee.

Prof Chris Parish
- Editor-in-Chief of Immunology and Cell Biology;
- Member of the Medical Research Advisory Committee, Australian Cancer Research Foundation;
- Councillor, Council of the International Union of Immunological Societies;
- Founding Member of the World AllergoOncology Task Force (Vienna-based).

Prof. Barry Rolfe
- Managerial Board Member of the Australian Proteomics Computational Facility.

Prof. Ray Rose
- Editor of Plant Cell Reports;
- Editorial advisory Board Protoplasma.

Prof. Mohan Singh
- Editorial Board Member of International Journal of Food Agriculture and Environment;
- Editorial Board Member of Indian Journal of Aerobiology.

Dr Georg Weiller
- Chair of the Scientific Advisory Committee of the Australian Proteomics Computational Facility.
### Financial Report

#### INCOME (to 31 December)

<table>
<thead>
<tr>
<th></th>
<th>2009 $ per Node</th>
<th>2009 $ Centre Total</th>
<th>2010 $ per Node</th>
<th>2010 $ Centre Total</th>
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<td><strong>ARC Centre Grant</strong></td>
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<td><strong>Headquarter Strategic Funds</strong></td>
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<td>Funds carried forward</td>
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<td>EXPENDITURE (to 31 December)</td>
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<td>2009 $ Centre Total</td>
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### EXPENDITURE (to 31 December) (continued)

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<td><strong>CENTRE TOTAL EXPENDITURE</strong></td>
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<td><strong>CENTRE CARRY FORWARD</strong></td>
<td>-453,802</td>
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Staff, Students and Associates
(To December 31, 2010)

CENTRE STAFF
- Professor Peter Gresshoff – Director
- Dr Alvin van Niekerk – Chief Operating Officer
- Charlotte Camerer – Education and Outreach Manager (Jan. 2010 only)
- Susan O’Brien – Personal Assistant to the Director

ANU Node
Chief Investigators
- Associate Professor Michael Djordjevic – Node Leader
- Dr Ulrike Mathesius
- Professor Chris Parish
- Dr Georg Weiller

Post Doctoral Staff
- Dr Charles Buer
- Dr Tancred Frickey
- Dr Stephanie Hueber
- Dr Britta Winterberg

Research Officers/Technical Officers
- Anna Bezos
- Anna Browne
- Dr Han Cai Chen
- Cassandra Harris
- Marie Oakes

Research Students
- Greg Bodulovic (Masters)
- Lauren du Fall (Honours)
- Rod Eyles (PhD)
- Chooi Hua Goh (Masters/Honours) (from August)
- Samira Hassan (Masters/Honours) (from August)
- Peta Holmes (PhD)
- Furzaneh Kordbachen (PhD)
- Lucia Kusumawati (PhD – graduated)
- Jason Ng (Honours – completed)
- Karsten Oelkers (PhD)
- Susanti Susanti (PhD)
- Chen Xiaodong

Centre Associates
- Dr Charles Hocart
- Dr Nijat Imin

University of Melbourne Node
Chief Investigators
- Professor Prem Bhalla – Node Leader
- Professor Mohan Singh – Deputy Director
Post Doctoral Staff
- Dr Raza Gluam, Research Fellow
- Dr Chol-Hee Jung, Research Fellow
- Dr Maarten Kooiker, Research Fellow
- Dr Annie Wong, Research fellow

Research Officers/Technical Officers
- Neslihan Goc
- Nick Henderson
- Melani Hutchins
- Alexis Soo
- Rory Wilson

Research Students
- Paul Knight (PhD)
- Nha Uyen Phan (PhD)
- Niharika Sharma (PhD)

University of Newcastle Node
Chief Investigators
- Professor Ray J Rose – Node Leader

Post Doctoral Staff
- Dr Sergey Kurdyukov
- Dr Kim Nolan
- Dr Michael Sheahan (ARC Postdoctoral Fellow)
- Dr Xi Yi Zhang (part-time)

Research Officers/Technical Officers
- Dr Xin-Ding Wang

Research Students
- Dil Bhullar (Hons student)
- Youhong Song (PhD student)
- Terence Tiew (PhD student)

UQ Node
Chief Investigators
- Associate Professor Christine Beveridge – Co-Node Leader
- Associate Professor Bernie Carroll – Co-Node Leader
- Professor Peter Gresshoff – Director

Post Doctoral Staff
- Dr Bandana Biswas
- Dr Phil Brewer
- Dr Elizabeth Dun
- Dr Brett Ferguson
- Dr Arief Indrasumunar
- Dr Alice Hayward
- Dr Thierry Lonhienne
- Dr Paul Scott
- Dr Qunyi Jiang
ARC QEII Fellow
- Dr Jacqui Batley

Research Officers/Technical Officers
- Olga Berking
- Dr Ning Chen
- Kerry Condon
- Dr Jessica Dalton-Morgan
- Dr Uwe Dressel (From July)
- Dongxue (Snow) Li

Research Students
- Michael Christie (PhD)
- Nial Gursanscky (PhD)
- Liqi Han (PhD – graduated))
- Satomi Hayashi (PhD)
- Miles Holmes (PhD – graduated)
- Stephen Kazakoff (PhD)
- Meng Lin (PhD)
- Yu-Hsiang Lin (PhD)
- Emma Myers (Honours)
- Jonathan Peterson (PhD)
- Amanda Rasmussen (PhD)
- Dugald Reid (PhD)
- Saeid Mirzaei (PhD)
- Stacey Cook (Honours)
- Shang Yen (Honours)
- Jessica Vogt (Honours – graduated)

Occupational Trainees
- Iris Elsen – Han University, The Netherlands
- Jasper Koehorst, Han University, The Netherlands
- Aubree Morigi, Polytech’ Clermond-Ferrand in France
Acknowledgements

The Director and Chief Investigators would like to express their appreciation for the cash and in-kind support provided to the CILR by the following organisations, groups and people:

- The Australian Research Council.
- The Vice-Chancellors and Deputy Vice-Chancellors (Research) of the four partner universities.
- Faculty of Science, University of Queensland.
- School of Molecular and Microbial Sciences, University of Queensland;
- School of Biological Sciences, University of Queensland;
- Research School of Biological Sciences, Australian National University;
- School of Biochemistry and Molecular Biology, Australian National University;
- John Curtin School of Medical Research, Australian National University;
- School of Agriculture and Food Systems, University of Melbourne;
- Faculty of Land and Food Resources, University of Melbourne;
- School of Environmental and Life Sciences, University of Newcastle;
- The Australian Government (Caring For Our Country and COMET grants);
- The State Government of New South Wales;
- Origin Energy CSG Ltd;
- Bio Energy Solutions Pty Ltd;
- Bioenergy Plantations Pty Ltd.

Images supplied by:
CILR Staff

Editor:
Dr Alvin van Niekerk

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# Key Performance Indicators

<table>
<thead>
<tr>
<th>Key</th>
<th>Performance Measure</th>
<th>Target</th>
<th>Progress to 31 December 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research findings and competiveness</td>
<td>Quality of Publications. Peer reviewed journals, invited reviews, monographs and publications with broad readership for public education. The goal is to achieve 6 publications per annum in journals with impact factor &gt; 5.</td>
<td>14 publications (CILR researchers) in journals with an impact factor greater or equal to 5.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number of Patents. Filing an average of two provisional patent applications per annum. Target of one PCT level patents per annum.</td>
<td>Two (one prov and one national phase)</td>
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<tr>
<td>Invitations to address and participate in international conferences</td>
<td>Members will address the international meetings of the International Society of Plant Molecular Biology (ISPMB), Plant and Animal Genome Conference, International Society of Molecular Plant Microbe Interactions (ISMPMI) and national meetings of Combined Biological and Biochemistry Societies of Australia (COMBIO), and the Australian Society of Plant Scientists. Target: five per annum.</td>
<td>CILR researchers made 37 international presentations and 31 national presentations at conferences and workshops. Conferences include: International Plant Growth Substances Association Conference, Tarragona, Spain; Keystone Symposium on RNA Silencing, US; 9th European Nitrogen Fixation Conference, Geneva, Switzerland; International Conference on Plant Vascular Biology in Columbus Ohio; Vth International Congress on Legume Genetics and Genomics. Asilomar, CA, USA; Plant and Animal Genome Conference XIX, San Diego, CA, USA</td>
<td></td>
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<tr>
<td>Invitations to visit leading international laboratories</td>
<td>Members of the centre will visit international laboratories to conduct research and learn technologies. Target: six per annum.</td>
<td>In 2010 &amp;2011, CILR researchers visited a total of 21 international laboratories, such as: The National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, China; Xinjiang Agricultural University, Urumqi, China; Ecole Normale Superieure, Sophia Antipolis, France; UCLA, Riverside, USA</td>
<td></td>
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Additional competitive grant income

ARC Linkage Grant (Scott & Gresshoff -UQ);
Discovery project (Mathesius - ANU);
Discovery Project (Weiller – ANU);
Discovery project (Djordjevic - ANU);
Discovery project (Rose - NU).

ARC Linkage Grant ($670,000);
ANU ($241,000);
ANU ($250,000);
ANU ($390,000);
Newcastle ($82,000).

Number and nature of commentaries about the Centre's achievements.

The activities of the centre will be widely recognised in speciality and general publications. The electronic media will recognise the achievements through interviews and invited commentary to programs such as Landline. Target: 3 per annum.

15 media reports on Centre activities were recorded in 2010. Articles appeared in both National and International news media.

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<table>
<thead>
<tr>
<th>Research training and professional</th>
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<tbody>
<tr>
<td>Number of postgraduates recruited.</td>
<td>4 per annum.</td>
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<tr>
<td>A total of 35 postgraduate students plus three international Occupational trainees worked at the CILR during 2010.</td>
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<tr>
<td>Number of postgraduate completions.</td>
<td>4 per annum.</td>
</tr>
<tr>
<td>In 2010, 5 PhD students graduated from the CILR. (61 PhD and Masters students have graduated form the Centre since 2003).</td>
<td></td>
</tr>
<tr>
<td>Number of honours students.</td>
<td>4 per annum.</td>
</tr>
<tr>
<td>Three Honours student graduated during 2010. (30 since Centre was established).</td>
<td></td>
</tr>
<tr>
<td>Number of professional courses.</td>
<td>1 per annum.</td>
</tr>
<tr>
<td>Achieved</td>
<td></td>
</tr>
<tr>
<td>Participation in professional courses.</td>
<td>2 per annum.</td>
</tr>
<tr>
<td>Staff and students participated in a UniQuest-organised workshop on commercialisation.</td>
<td></td>
</tr>
<tr>
<td>Number and level of undergraduate and high school courses in the priority area(s).</td>
<td>Host 2 per annum (high school courses).</td>
</tr>
<tr>
<td>CILR staff were involved in:</td>
<td></td>
</tr>
<tr>
<td>1. Lab Experience, Feb-Apr, ANU;</td>
<td></td>
</tr>
<tr>
<td>2. Experience Science Workshops, July, UQ;</td>
<td></td>
</tr>
<tr>
<td>3. Science Immersion, September, UQ.</td>
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</tr>
</tbody>
</table>
## International, national and regional links and networks

<table>
<thead>
<tr>
<th>Description</th>
<th>Details</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of papers published with international co-authors/ reports for international bodies</td>
<td>8 per annum.</td>
<td>Nine.</td>
</tr>
<tr>
<td>Number of international visitors.</td>
<td>10 per annum.</td>
<td>21 international visitors across all nodes. See External Collaborations and Linkages for detailed information.</td>
</tr>
<tr>
<td>Number of national and international workshops.</td>
<td>Attend 6 per annum; provide 6 per annum.</td>
<td>Attended: Workshop: Post-NARK Analysis During Regulation of Plant Stem Cell Proliferation in Legume Nodulation, XVII Plant and Animal Genome Conference, San Diego, CA, USA (P. Gresshoff - UQ); Herston Research Centre, University of Canberra, Canberra; Indo-Queensland Workshop on Biotechnology Collaborations, April 13 2010, The University of Queensland (B Carroll – UQ); Dr Jenny Aitken, Director of The Tree Lab (New Zealand) held a workshop for the UQ Node of the CILR with regards to Pongamia tissue culture. University of Queensland (Gresshoff Lab).</td>
</tr>
<tr>
<td>Number of visit to overseas laboratories</td>
<td>These visits are mainly for information transfer, collaboration arrangements, centre marketing and research perspectives. 12 per annum.</td>
<td>CILR researcher visited a total of 20 overseas laboratories in 2010.</td>
</tr>
<tr>
<td>Number of memberships of national and international professional committees</td>
<td>20 per annum.</td>
<td>Achieved</td>
</tr>
</tbody>
</table>
Examples of relevant Social Science and Humanities research supported by the Centre.

The centre will conduct numerous activities related to Social Science and Humanities.

PhD student Miles Holmes researched the knowledge transfer amongst Aboriginal communities (Walpiri tribe) in central Australia. The Pongamia research program at UQ has significant social (job creation) and environmental benefits (carbon sequestration, reduced nitrogenous fertiliser, high saline tolerance, drought resistance) which the biodiesel industry has already noted.

### End-user links

<table>
<thead>
<tr>
<th>Number and nature of commercialisation activities.</th>
<th>Linkages will be established through:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Meristomics is registered nationally as a trading name for the commercialisation activities of the Centre;</td>
<td></td>
</tr>
<tr>
<td>2. The four partner universities of the Centre have approved the formation of a virtual company through UniQuest Pty Ltd (the main commercialisation company of the University of Queensland) to commercialise plant research discoveries;</td>
<td></td>
</tr>
<tr>
<td>3. Start-up Company – Bio Energy Solutions with industry partner;</td>
<td></td>
</tr>
<tr>
<td>4. Cooperative Pongamia research program being negotiated with large oil/gas company;</td>
<td></td>
</tr>
<tr>
<td>5. Discussion with potential industry partners for commercial development of each of the Centre’s provisional patents, ongoing;</td>
<td></td>
</tr>
<tr>
<td>6. Ongoing discussions with major airline companies with regards to the use of Pongamia in the aviation biofuels area;</td>
<td></td>
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<tr>
<td>7. Discussion currently being held with US Biofuel company with regards to international collaboration;</td>
<td></td>
</tr>
<tr>
<td>8. Negotiations ongoing with large biotech company regarding cooperative research relating to the patent on Soybean nodulation factor receptor proteins;</td>
<td></td>
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<tr>
<td>9. 3 CDAs;</td>
<td></td>
</tr>
<tr>
<td>10. 24 MTAs;</td>
<td></td>
</tr>
</tbody>
</table>
Throughout the year the Centre Director, Chief Operating Officer and certain CIs had numerous meetings and discussions with the Brisbane City Council, the Sunshine Coast Regional Council, SEQ Catchments, Origin Energy, Origo Sino-India Plc, Mitsui & Co, Primary Holdings, Boeing Aircraft Corporation, RM Williams and other institutions with regards to the Pongamia biodiesel research.

<table>
<thead>
<tr>
<th>Number of government, industry and business briefings.</th>
<th>Target: 5 per annum.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Centre associates trained/ing in technology transfer and commercialisation.</td>
<td>The Centre will facilitate training through third parties in technology transfer. Target: 2 per annum and maintaining.</td>
</tr>
<tr>
<td>Number and nature of Public Awareness programs.</td>
<td>1. Significance of legumes to society; 2. Significance of legumes to health; 3. Australian Science Teacher Association; 4. Science Fairs. Target: 3 per annum.</td>
</tr>
<tr>
<td>Cash contributions from end-users to the Centre, including research contracts</td>
<td>1. Research contract (over 3 years); 2. One additional cash contribution per annum.</td>
</tr>
<tr>
<td>In-kind contributions from end-users to the Centre</td>
<td>Plants, cuttings, seeds, know-how, advice.</td>
</tr>
</tbody>
</table>

The COO and Postdoctoral Fellows and PhD students participate regularly in UniQuest training seminars on the commercialisation of university research. The COO also runs a seminar on commercialisation at the Centre Symposium each year for Centre staff.

The CILR has had to curtail its school teacher education program due to the vacant Education and Outreach position. However, the Centre has maintained its support for science education programs and the UQ Node continues to host grade 12 boys from a prominent, private Brisbane Boys’ High School.

The Centre was awarded the prestigious Peter Doherty award for science education.

| | 1. Significant contribution for Pongamia research through the start-up company – Bio Energy Solutions. 2. Research collaboration with Sunshine Coast Regional Council. |
| Plants, cuttings, seeds, know-how, advice. | | 1. Land & infrastructure for Pongamia trials; 2. Seeds – for Pongamia research; 3. Know-how form industrial partners. |
## Organisational Support

| Annual cash contributions from Collaborating Organisations. | 2010 - UQ: $602,500 per annum, ANU: $225,000 per annum, UoN: $70,000 per annum, UoM: $136,000 per annum. | $500,000 received from UQ; $225,000 received from ANU; $136,000 received from MU; $70,000 received from NU. |
| Annual in-kind contributions from Collaborative Organisations. | UQ: $353,100, ANU: $291,141, UoN: $200,000, UoM: $98,197. | Each partner university has met its promised in-kind contribution commitments. |
| Number of new Organisations recruited to be involved in the Centre. | The Centre will initiate new linkages as demanded by research advances both within the Centre and overseas. Target: 4 in 2003/4, increasing by 1 per annum. | The CILR has initiated collaborations/communications with the following organisations: Brisbane City Council, the Sunshine Coast Regional Council, SEQ Catchments, Origin Energy, Origo Sino-India Plc, Mitsui & Co, Primary Holdings and other institutions with regards to the Pongamia biodiesel research. |
| Level and quality of infrastructure provided to the Centre. | 1. An efficient centre administration at UQ; 2. Provision of quality laboratory space at all nodes; 3. Access to high quality research infrastructure across the nodes; 4. Quality greenhouse space for controlled plant growth under PC2 (transgenic) containment; 5. Direct access to electronic journals and other library facilities for centre scientists. | 1. The Centre administration is housed in modern offices at UQ; 2. Substantial laboratory facilities are available at all nodes; 3. Equipment and facilities are state-of-the-art and are maintained by trained personnel; 4. Scientists at all nodes have direct access to university library facilities, including electronic and in-print journal publications. |
### Governance

<table>
<thead>
<tr>
<th>Breadth and experience of the Advisory Board.</th>
<th>The Centre Advisory Board and Scientific Expert Advisory Committee were amalgamated into a single Centre Advisory Board during 2009. A number of world-class scientists agree to sit on the CAB. The Centre will maintain its compliance with OGTR and AQIS regulations. The laboratories at UQ are certified quarantine facilities – maintain this status. Two of the UQ Node personnel are certified QAP’s.</th>
<th>See Centre Advisory Board (CAB) membership. OGTR and AQIS regulations and standards have been maintained; Laboratory staff regularly attend lab-related courses.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency and effectiveness of Advisory Board meetings.</td>
<td>Yearly, for entire Centre Advisory Board; quarterly update from Director and COO to Board and subsequent teleconference.</td>
<td>The Centre Advisory Board Members (International) met at the Vth International Congress on Legume Genetics and Genomics, Asilomar, CA, USA, together with the Director, the COO and some of the Centre’s CI’s.</td>
</tr>
<tr>
<td>Quality of the Centre strategic plan.</td>
<td>The Centre’s strategic plan was established on the combined knowledge of the applicants and their consultants within the partner universities.</td>
<td>The Centre’s Strategic Plan is up-dated annually.</td>
</tr>
<tr>
<td>Effectiveness of arrangements to manage Centre nodes.</td>
<td>The nodes will communicate through an already established website. Additional interactions to occur through: 1. Monthly nodal leader phone-conference; 2. Quarterly rotational visits to the nodes; 3. Ad hoc meetings of CIs and nodal leaders at research conferences; 4. Annual research coordination meetings with CIs, Scientific Expert Advisory Committee and Advisory Board. Centre participants and line managers at each node report satisfactory arrangements during ARC reviews. Node research is featured in the Centre’s Annual Report.</td>
<td>1. Chief Investigators regularly discuss research and Centre items during phone-conferences.</td>
</tr>
<tr>
<td>The adequacy of the Centre’s key performance indicators.</td>
<td>1. International benchmarking to research in top international plant research Centres such as the MPI, John Innes Centre and the Danforth Centre;</td>
<td>The KPI’s are regarded as an important instrument to gauge the effectiveness and relevance of the Centre as an international Plant Science Centre of Excellence.</td>
</tr>
</tbody>
</table>
### National Benefit

**Measure of expansion of Australia's capability in the priority area(s).**

1. Widespread involvement of genomic and phenomic technology in Australian industry and academia as evidenced by linkages and research expansion;
2. Improved dialogue between social and life sciences in areas of overlap (i.e., GMO, health, environmental ethics);
3. Quality publications in world-class journals in the priority area;
4. Development of patents and commercialisation;
5. The functioning of the Centre as a focal point for related commercial development.

Collaborations with industry (e.g. Origin Energy, Pacific Renewable Energy, GlycoSyn), government (e.g. Brisbane City Council, NSW Agriculture) and academia (e.g. Chinese Academy of Sciences) are constantly being expanded. Especially the biodiesel research project created many new linkages in 2010;

1. 14 publications in journals of impact factor greater or equal to 5, and 28 publications in journals with an impact factor of between 1 and 5;
3. Increased national and international interest in biofuel research.

**Case studies of economic, social, cultural, or environmental benefits.**

1. Increased awareness of biomedical benefits of legumes in diets and impact to human health (preparation of a ‘Legume Cookbook’; possible information on cereal boxes; ‘Sanitarium’ sponsorship);
2. Increased teaching content on Systems Biology and genome/phenome relations for high school/undergraduate/graduate education;
3. Increased awareness and access of Science Teachers to Genome/Phenome technology and understanding. Work through existing linkages as well as new programs such as the ‘Bright Minds’ project at UQ;
4. Popularise the history of legumes in Australia; e.g., lupins and the west; clover and the sheep; effects of GMO soybean in Australia.

1. Legume recipes available for download on the Centre website;
2. Development of new workshops for high school teachers and student; 3) Involvement in the Bright Minds “Science Immersion” program;
3. Seeds for School Program;
4. Development of new fact sheets on Pongamia and biodiesel;
5. Development of a biodiesel display for public events;
6. Update of the CILR website.