CLIMA’s mandate

“To apply leading edge science to the problems and priorities identified by the Western Australian grain and pasture legume industries with the objective of creating value for the industries and the wider community.”

CLIMA’s vision

“Innovation in Legume Science and Technology”

A Centre of Excellence in grain and annual pasture legume research and development that leverages the strengths of its partners to address the problems and priorities of the Western Australian grain and pasture legume industries – to be achieved through strategic scientific research and development, linked to an applied base.

The Centre intends to be a world leader in problem-focused legume research and will achieve this by drawing on the expertise within its four partner organisations.

CLIMA’s objective is to add value to the activities of its clients, core partners and staff and in doing so, maximise the benefits of co-operation and co-ordination of research.

2005-2006 BIENNIAL RESEARCH REPORT

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Acronyms

AAAC Australian Association of Agricultural Consultants
AARI Aegean Agricultural Research Institute
ACIAR Australian Centre for International Agricultural Research
ARC Australian Research Council
ATCC Australian Temperate Field Crops Collection
ATGRC Australian Trifolium Genetic Resource Centre
AusAID Australian Agency for International Aid Development
AWA Australian Women in Agriculture
AWCC Australian Winter Cereals Collection
AWI Australian Wool Innovation
BARI Bangladesh Agricultural Research Institute
CCWA Chemistry Centre (W.A.)
CDC Crop Development Centre, University of Saskatchewan
CLIMA Centre for Legumes in Mediterranean Agriculture
CGGGO Council of Grain Grower Organisations Ltd
CSRIO Commonwealth Scientific and Industrial Research Organisation
DAFWA Department of Agriculture and Food Western Australia
DEST Department of Education, Science and Training
DNRE Department of Natural Resources and Environment, Victoria
GRDC Grains Research and Development Corporation
HAU Haryana Agricultural University, Hisar, India
IARI Indian Agricultural Research Institute, New Delhi
ICARDA International Centre for Agricultural Research in Dry Areas
ICRISAT International Crops Research Institute for the Semi-Arid Tropics
IFPRI Indian Institute of Pulses Research
INRA French National Institute for Agricultural Research
IPPR Institute of Oil and Pulse Research, Gulgarga, Karnataka, India
INOVA Jawaharlal Nehru Krishi Agricultural University
LWRDC Land and Water Research and Development Corporation
MAFF Ministry of Agriculture, Forestry and Fisheries, East Timor
NABIP National Annual Pasteure Legume Improvement Program
NARIC Nepal Agricultural Research Council
NFIP National Faba Bean Improvement Program
NSF National Science Foundation, USA
PAU Punjab Agricultural University, India
QDPI Queensland Department of Primary Industries
RARS Regional Agriculture Research Station, Ishurdi
RIRDC Rural Industries Research and Development Corporation
SARDI South Australian Research and Development Institute
USDA United States Department of Agriculture
UWA The University of Western Australia
VIDA Victorian Institute for Dryland Agriculture

Abbreviations

AFLP amplified fragment length polymorphism
BCMV Bean cucumber mosaic virus
BGM Botrytis grey mould
BWVV Beet western yellow virus
BYMV Bean yellow mosaic virus
CMV Cucumber mosaic virus
DH doubled haploid
HPLC high performance liquid chromatography
ICM integrated crop management
MFLP Microsatellite-anchored Fragment Length Polymorphism
NIR near infra-red reflectance
NIRS near infra-red spectroscopy
NLL narrow-leaved lupin
OFDTs on-farm demonstrations and trials
PCR polymerase chain reaction
RLE red-legged earth mite
RNA ribonucleic acid
The Centre for Legumes is a research alliance between the Department of Agriculture and Food Western Australia, The University of Western Australia, CSIRO and Murdoch University, formed to continue the research collaboration begun under the Commonwealth Government’s Cooperative Research Centre Program in 1992.
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In 2006, in accordance with the previously agreed rotation, I was privileged to be elected Chairman of the CLIMA Governing Board.

During the period covered by this report CLIMA flourished as an alliance of research partners focused on supporting a sustainable legume industry in Australia. This is the only example of a previous Cooperative Research Centre (CRC) that has continued its research and development post Commonwealth funding. That it has done so reflects well on the partners in the alliance and the cooperative nature of research by scientists from the partner organisations.

The Board of CLIMA has been excited to see the expansion of project funding during the period 2005/06. While continuing with many of the projects that were funded previously there have been major developments in international projects in India and Timor and an expansion of projects funded by the Australian Research Council. In these projects CLIMA researchers have partnered with international researchers to develop new crop varieties for Australia and to help solve agricultural productivity and sustainability issues in developing countries. As well as an expansion of international projects, CLIMA researchers continued to produce research on crop and pasture varieties that are of direct benefit to Australian farmers. The strong links with producers through the Industry Advisory Group and workshops with producers have maintained the focus of researchers on the need for more profitable management options for farmers.

During 2006 the Director of CLIMA, Professor Kadambot Siddique, resigned to take up a new position as Chair in Agriculture and Director of the University of Western Australia’s Institute of Agriculture. The Board wishes to record its sincere appreciation of the outstanding work done by Professor Siddique in maintaining and lifting the performance of the centre during 2002/06. The Board has been fortunate in recruiting Professor Neil Turner as Interim Director to lead and manage the centre until the current CLIMA Memorandum of Understanding ceases in mid-2007. The year 2007 will be another period of change for CLIMA as the Board is challenged with the future arrangements for the centre.

Alistar Robertson
CHAIRMAN, GOVERNING BOARD
The Industry Advisory Group (IAG) continued to play a major role in providing a conduit for the flow of information between the industry and researchers during 2005/2006.

The IAG held four meetings during the period, with excellent industry participation (on average 13 of the 16 IAG members) on all occasions. Activities of the IAG included reviewing CLIMA’s progress, developing themes for annual Industry Forums hosted by CLIMA, the construction of a table of drivers of annual pasture legume research, discussion and advice on research themes and sources of funding, and individual input throughout the year to CLIMA researchers and managers.

Over the two years, the 6-monthly IAG business meetings were held on a rotational basis at facilities run by each of the CLIMA alliance partners, providing the participants with the opportunity to visit glass house, laboratory and field plots to meet with researchers and view the work being conducted in legume research and development at each institute. More than 60 researchers were involved in the visits to Murdoch University (February 2005), CSIRO Floreat (October 2005), Department of Agriculture and Food western Australia (DAFWA) (March 2006) and The University of Western Australia’s Shenton Park Field Station (November 2006). On average, 11 industry representatives stayed on for each interactive tour of research facilities.

In 2005, IAG meetings covered drivers for annual pasture legume research, research project possibilities, new variety releases, progress with the establishment of the umbrella organisation that has now been named Agricultural Research Western Australia, the continuation of CLIMA and the IAG, attendance at field days, and ideas for a 2005 Industry Forum. The tour of research activities at Murdoch University including viewing the work of the State Agricultural Biotechnology Centre and the Australian Centre for Necrotrophic Fungal Pathogens, looking at fungal and viral disease resistance, identification of new diseases, genetic mapping, molecular marker identification and commercial biotechnology diagnostics (Saturn Biotech). At CSIRO, IAG viewed work on aphid and disease resistance in legumes, improved pulse adaptation, improved yield and stability in lupin and pasture/livestock interactions.

The topic proposed by the IAG for the 2005 Industry Forum was ‘Maintaining crop profitability whilst lifting animal performance on mixed farms’, hosted jointly by CLIMA and the CRC for Plant-Based Management of Dryland Salinity. The venue was full, with 62 researchers (animal, crops and pasture), economists, farmers, advisers, feed suppliers and other industry members addressing the issues facing mixed farms. The most recent research results on animal production and feed rations, cropping systems and farm profitability were conveyed to the industry. The future industry needs of mixed farms and animal production in relation to sustainability and profitability were identified.

In March 2006, researchers from DAFWA discussed with growers, industry representatives and researchers from other institutions topics ranging from lupin breeding to de-hulling, fungal disease, molecular technology and pasture breeding and selection.

During the March meeting, the ideas and theme for the 2006 Industry Forum were considered and an action plan was instigated. The theme for the forum was ‘Grow your nitrogen, don’t buy it?’ With the decline in the uptake of grain legumes by growers and the increasing costs associated with the buying of nitrogen fertiliser, the topic was deemed very appropriate. This proved to be correct with the forum over-subscribed and many were unable to participate due to restrictions on the capacity of the function facility. The forum was well covered by the media, with several articles appearing over the next few weeks in ‘The Countryman’. All those involved in organising the forum agreed that it was a very satisfying result.

Interest in the workings of the group continued throughout the year. Some members about to retire by rotation were already suggesting nominations for their replacement prior to the next meeting. Retirees also offered to assist the Group in the future, wherever they were able to.

The second regular meeting of 2006 was held in November, at The University of Western Australia Field Station at Shenton Park. This meeting resolved to assist wherever it could to encourage continued funding in the area of pasture research and variety development. IAG members saw the need and importance of legume research to assist the future farming system developments.
Following the November meeting, members were given a comprehensive tour of the Shenton Park facilities, including the field trials, laboratory and enclosed trial plots to view first-hand the work being conducted in herbicide tolerance in pulse and lupin crops, plus some new work on variety development of lupin and oilseed crops.

The Group continues to see a significant benefit in the linkage between researchers and the industry, particularly the growers. This provides the forum for healthy interchange with new developments in the field of legume research.

The period under review saw the retirement of the Director of CLIMA, Professor Kadambot Siddique, in order to fulfill the position of Director of the Institute of Agriculture at UWA. The Industry Advisory Group places on record its appreciation for the tireless work undertaken by Professor Siddique and at the same time welcomes Professor Neil Turner to the position. The IAG is encouraged by Professor Turner’s enthusiasm and the knowledge he has already brought to his interim posting.

Robert Sewell, AM
CHAIRMAN, INDUSTRY ADVISORY GROUP
Director’s Report

Although I have been associated with CLIMA from the time of the first bid for Cooperative Research Centre (CRC) funds, I have had the privilege of being its Director for only five months of the reporting period of 2005-06.

As a research alliance CLIMA has now successfully continued for six and a half years from the time that the CRC phase (and Commonwealth funding) ended, longer than that of any other CRC. In part this is due to the vision, drive and enthusiasm that Professor Kadambot Siddique brought to the centre in the more than five years that he was Director. On 1 August 2006, Professor Siddique was appointed Chair in Agriculture and Director of the newly re-established Institute of Agriculture at The University of Western Australia. He continues his interest in CLIMA, but in his new position has a broader mandate than grain and pasture legumes. I was appointed to be the Director of CLIMA on a part-time basis from 1 August 2006 to 30 June 2007 when the present Memorandum of Understanding between the research partners (CSIRO, Department of Agriculture and Food Western Australia, Murdoch University and The University of Western Australia) concludes.

It has indeed been a privilege to serve as Director because CLIMA is highly regarded nationally and internationally for its contribution to legume science and because the staff and associates of CLIMA work effectively together for the benefit of industry. In the short time that I have been Director, I have been very pleased with the great support from industry and the support and advice that the Industry Advisory Group (IAG) gives to CLIMA. The Chair of the IAG reports separately on their activities, but I wish to acknowledge the loyal support of the members of the IAG to me personally, and to CLIMA generally.

Over the past two years, CLIMA continued to contribute to legume science as the research reports and publications record in this biennial report demonstrate. CLIMA has also contributed strongly to industry with the continued release of new pasture and grain legume cultivars. Five PhD students successfully completed their projects on legumes during the reporting period.

West Australian Premier, Mr Alan Carpenter (second from right) and Dr Dyno Keatinge, ICRISAT’s Director of Research (right) receive an update from Dr Vincent Vadez, Plant Physiologist (left) on CLIMA/ICRISAT/COGGO collaborative chickpea projects at ICRISAT Hyderabad, India.
Industry contributes strongly to CLIMA through research funding. Over the past two years, CLIMA’s funding base has broadened with strong funding from the Council of Grain Grower Organisations (COGGO) and the Australian Centre for International Research (ACIAR) in addition to funding from the Rural Industry Research Corporations. Over the past two years, CLIMA has been successful in attracting funding from the Australian Research Council (ARC) through its Linkage Grants program, in which CLIMA researchers link with an industry partner to bring basic and strategic research to the benefit of industry. Examples of these projects are reported in this biennial report. The Grains Research and Development Corporation (GRDC) continues to be an important partner through a number of research projects.

CLIMA has further strengthened its international linkages, particularly with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India and the International Centre for Agricultural Research in the Dry Areas (ICARDA) in Syria. Indeed, ICRISAT is now a research partner in fast-tracking chickpea breeding for Western Australia and in selecting chickpea germplasm with salinity tolerance and boron toxicity tolerance. CLIMA partners have strong links to ICARDA in identifying chickpea and pasture germplasm for Australian farming systems. Several international scientists visited CLIMA during the reporting period.

CLIMA is active in communicating its research outcomes not simply through publication in journals, but through regular press releases, a newsletter ‘Beanstalk’ and its website and fortnightly seminars. The Biennial Research Report is widely distributed and provides an excellent summary of current research at CLIMA.

The Board of CLIMA has been busy over the past two years, particularly over the past year as the conclusion of the present Memorandum of Understanding draws near. In February 2007, the Board agreed that from 1 July 2007, CLIMA will function as a centre within the Faculty of Natural and Agricultural Sciences at The University of Western Australia. While the full implications of this change are still being worked through, CLIMA will continue to function as a centre for pasture and grain legume science and will maintain its links with our partners in the research alliance (CSIRO, Department of Agriculture and Food Western Australia and Murdoch University), national and international collaborators and industry partners.

The focus of CLIMA research in its third phase will be the enhancement of pasture and grain legume germplasm for yield and quality characteristics that are required by breeders and industry. It is anticipated that researchers will use the modern techniques of molecular genetics, cell biology, molecular biology, tissue culture, embryo rescue, mutagenesis, transformation, and DNA fingerprinting to identify and develop germplasm with desirable traits (such as cold tolerance, herbicide/disease/insect pest resistance, drought and salinity tolerance) and to develop wide crosses, double haploids, interspecific hybrids and transgenic plants for important grain and pasture legume species. The research will be conducted in close collaboration with national and international researchers and breeders in the public and private arena so that the germplasm with desirable traits will be quickly incorporated into superior cultivars.

A major focus of this third phase of CLIMA will be postgraduate research training in legume science. As a university centre CLIMA is expected to enhance postgraduate research training in genetics, physiology and pre-breeding technologies through collaboration within UWA with the School of Plant Biology, the ARC Centre of Excellence in Plant Energy Biology and the Western Australian Institute of Medical Research.

At the time of writing this biennial report, the search for a new Director to lead the third phase of CLIMA is under way. As the present Director, I am sure that CLIMA will continue as a strong and viable centre of legume science over the next biennial reporting period. In my semi-retirement, I look forward to continued involvement as a researcher in the third phase of CLIMA.

Profesor Neil C. Turner  
DIRECTOR
GOVERNING BOARD

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Research Highlights

Over the period 2005/06, CLIMA has continued to undertake outstanding research on pasture and grain legumes as evidenced by the list of publications in internationally-recognised journals and books, by the release of new cultivars and by the research reports that follow.

The Pasture Legume Program has continued to comb the Mediterranean Basin for new species of pasture legumes that could benefit Australia’s Mediterranean climatic regions. Collection trips were undertaken to Israel and Morocco and a collection of *Biserrula pelecinus* was acquired from Spain, Morocco, the Canary Islands and Eritrea through a visiting student. Native legumes were collected in Western Australia.

These collections are currently being evaluated using molecular and traditional techniques to identify useful traits and to ensure that the maximum diversity of traits is retained in the collections. The entire collection of *Trifolium spumosum*, which was characterised in 2004, is being used to authenticate a core collection based on eco-geographic and molecular data in order to aid in future germplasm selection. The success of previous collections and evaluations has led to the release of two new pasture species – Electra™ purple clover (*Trifolium purpureum*) and Flamenco™ sulla (*Hedysarum coronarium*). Other new pasture legumes are close to release.

While the release of a new cultivar is a milestone in itself, the Pasture Legume Program continues to evaluate releases for their benefits in the farming system. Electra™ purple clover was released because of its high seed yield and ease of harvesting, and has also been shown to have tolerance of clover scorch disease (*Kabatiella caulivora*), while Flamenco™ sulla has been shown by further evaluation to have high forage and seed production.

“...the Grain Legume Program is orientated to the genetic improvement of the major cool season grain legumes grown in Australia.”
Research Highlights

However, further evaluation of biserrula (*Biserrula pelecinus*) has shown that it causes photosensitivity in sheep grazing biserrula pastures. In a year such as 2006 which was very dry in Western Australia, the drought tolerant biserrula was one of the few pasture legumes to thrive causing concern that photosensitivity could become a serious problem. This led to research to identify the secondary plant compounds that are inducing the photosensitivity.

A component of the evaluation is the role of newly developed annual pasture species and cultivars in water use and water extraction. The research has shown that the new species and cultivars with deeper roots and longer season growth are better than subterranean clover in water use and water extraction, but not as good at extracting summer rain as the perennial species, lucerne.

Pastures are primarily used for feeding livestock and the Pasture Biotic Interactions Sub-program aims to understand livestock–pasture interactions. Stable isotopes of carbon in the faeces, wool, rumen, plasma and urine have been used to identify the selection of plants in mixed pastures. The research showed that all tissues allowed differentiation between diets, but only faecal and rumen samples equilibrated quickly enough to be used in short-term studies and were accurate in predicting diet selection with less than 5% error.

The vast bulk of the Grain Legume Program is orientated to the genetic improvement of the major cool season grain legumes grown in Australia. All projects are aligned to high priority industry needs, but many have a medium- to long-term horizon for payback to the industry. During 2005/06 the Grain Legume Program continued to play an important role in expanding the germplasm base, particularly in *Lupinus* and *Cicer*. As with the pasture legumes, collection trips of grain legumes have been organised to Peru and Chile to collect *Lupinus mutabilis* and to Central Asia and the Caucasus, the origin of several grain legume species. *L. mutabilis* (Andean or pearl lupin) is high in oil and a potentially high-value lupin species for Australia.

The projects utilise the most up-to-date molecular techniques alongside the best traditional approaches to genetic enhancement. Molecular techniques have been used to analyse the lupin germplasm collection to identify a core collection of wild and landrace accessions of *L. angustifolius* that represent most of the diversity for important traits in the complete collection.

A similar approach has been used to identify valuable traits in the wild *Cicer* collection assembled in Australia. Here, for example, molecular techniques have been used along with traditional methods for identifying cold tolerant accessions.

The utilisation of wild germplasm for cold tolerance (and for other important traits such as insect resistance) will depend on the success of another project to develop interspecific crosses between wild and cultivated chickpea. The project, a collaboration between CLIMA and ICRISAT, has generated hybrids between cultivated chickpea and accessions of *C. bijugum*, *C. pinnatifidum* and *C. judaicum*. This achievement was made possible using *in vitro* technology to rescue very young hybrids in the laboratory before they aborted from the mother plant in the glasshouse. Without rescue, most hybrids of *C. pinnatifidum* and *C. judaicum* abort from the mother plant 14 to 21 days after pollination, whereas *C. bijugum* abort as early as seven days. Hybridity of *in vitro* plantlets has been confirmed using DNA technology.

To increase the genetic diversity in *L. angustifolius*, interspecific crossing between *L. angustifolius* and *L. luteus*, *L. albus* and *L. mutabilis* has been initiated. While this project is in its infancy a few putative hybrids have been identified and their actual hybridity will be tested by MFLP analysis, while the development of the embryo is being followed to identify the appropriate time for embryo rescue.

Mutagenesis has been used to develop lines of *L. angustifolius* with tolerance to several herbicides. The most promising are two mutants of the cultivar Tanjil that are tolerant to the herbicide metribuzin and tolerant of the disease anthracnose. They are now in the national breeding program and are being fast-tracked for release as anthracnose resistant, metribuzin tolerant cultivars.

A number of other grain legumes have been evaluated for their tolerance of several groups of herbicides. Among 200 accessions of field pea, faba bean and lentil, a wide range of tolerance was discovered to the herbicides isoxaben, isoxaflutole and carfentrazone-ethyl.
Ascochyta blight in chickpea devastated the industry in the 1990s, but rapid progress has been made in the development of ascochyta tolerant cultivars. The genetics of ascochyta blight resistance has been shown to be controlled by two recessive genes in chickpea and wild Cicer. Two new kabuli cultivars, Almaz and Nafice, that are ascochyta resistant, high quality and high yielding, have been released. In a collaborative program with ICRISAT, rapid progress is being made with improving the ascochyta resistance and cold tolerance in desi chickpea. A kabuli variety, Kimberly Large, has been released for the Ord River Irrigation Area that aims at the high value market.

Improving the adaptation of grain legumes to the agro-ecological environment is an important theme of the Grain Legume Program. In a collaborative project with the Indian Institute of Pulses Research, phenology was shown to be central to the adaptation of chickpea to the diverse environments of India and Australia. While physiological traits were evaluated, surprisingly, osmotic adjustment was shown to be poorly inherited and did not correlate with higher yield under terminal stress conditions. However, a six-fold range of salinity tolerance has been discovered in chickpea and the tolerance has been shown to occur at the reproductive stages of yield development.

Resistance to pests and diseases is another major focus of the Grain Legume Program. Progress is being made at the molecular level in the Medicago truncatula model species in the development of mapping populations for the genetic analysis of resistance against eight foliar and three root pathogens. Molecular markers for anthracnose in L. angustifolius have been identified and an integrated crop management package for the control of Botrytis grey mould in chickpea developed for both Bangladesh and Australia.

Ascochyta blight in chickpea devastated the industry in the 1990s, but rapid progress has been made in the development of ascochyta tolerant cultivars. The genetics of ascochyta blight resistance has been shown to be controlled by two recessive genes in chickpea and wild Cicer. Two new kabuli cultivars, Almaz and Nafice, that are ascochyta resistant, high quality and high yielding, have been released. In a collaborative program with ICRISAT, rapid progress is being made with improving the ascochyta resistance and cold tolerance in desi chickpea. A kabuli variety, Kimberly Large, has been released for the Ord River Irrigation Area that aims at the high value market.
Genetic improvement of seed quality continues to be researched with the view of enhancing value for premium end-users in the food and feed sectors. A project involving the Department of Fisheries and the Chemistry Centre (W.A.) has contributed to the development of new aquaculture markets for lupins through the active involvement of grain marketers and commercial end-users.

While the focus in CLIMA is on pasture and grain legumes, new oilseed options for Australian farmers and industry are also being pursued. Camelina oil, mustard and golden linseed have been developed to the stage where markets for these products are being assessed and the possibility of alternate oilseeds for the developing biodiesel market is being explored.

CLIMA is pleased to be involved with two large international projects, the Seeds of Life in East Timor and the ‘Better crop germplasm and management of wheat and barley and pulse and forage legumes in Iraq’ project. Both projects aim to improve food security and to re-establish viable crop production and capacity building in countries that have been devastated by internal conflict. Despite the continuing difficulties and lack of security, progress has been made in identifying suitable cultivars of food crops and the development of associated technologies for improved food and fodder production.

“The Hon. Minister for Agriculture, Kim Chance, chats with CLIMA researchers Margaret Campbell and Clive Francis during a visit to Shenton Park field station.”
Communication and Training

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CLIMA’s communication activities aim to:

• inform clients of CLIMA products and technical expertise and facilitate their use, including new varieties, management packages, decision aids, research technology and general legume information
• provide information to decision makers
• ensure good information flow between researchers within the CLIMA alliance, and with other researchers and support networks nationally and internationally
• provide professional development opportunities where appropriate.

Clients include agribusiness (growers, advisors, other service providers), funding bodies and research colleagues (local, national and international).

CLIMA’s scientific achievements and international and local activities are publicised through:

• regular media releases (about 10 annually through Brendon Cant and Associates), resulting in around 90 news articles annually in national, local, rural and international newspapers and newsletters, as well as radio interviews
• articles in scientific journals, magazines, Crop Updates (about 40 refereed publications each year)
• CLIMA newsletters available electronically through the website (three annually)
• CLIMA’s website (updated weekly)
• presence at relevant field days, forums, conferences, Expos and workshops
• seminars and presentations at CLIMA and when CLIMA researchers are visiting other institutes
• CLIMA research facility tours for visiting scientists, farmer groups, industry guests.

CLIMA Industry Advisory Group members interact with CLIMA researchers during a tour of CSIRO’s Floreat Park facilities in October 2005.

STANDING (L-R): Kadambot Siddique (CLIMA), Mary Nenke (AWA), David Sermon (AAAC), Keith Alcock (DAFWA), Hans Lambers (UWA), Rory Coffey (ALOSCA), Merv McDougall (Pulse Australia), Jairo Palta (CSIRO), Jens Berger (CSIRO), Merrie Carlshausen (GRDC), Neil Young (WANTFA), Trevor Flugge (Chair) and Robert Sewell (CBH-GPWA)

FRONT (L-R): John Carstairs (COGGO) and Karam Singh (CSIRO)
Communication and Training

Communication activities throughout 2005 and 2006 also included:

- CLIMA’s fortnightly seminar series; over 40 individual presentations each year by CLIMA students, scientists, research associates and visitors, with an average attendance of 26 people per seminar
- biannual business meetings with CLIMA’s Industry Advisory Group, followed by research updates
- industry workshops: CLIMA and CRC Salinity Forum 2005 ‘Maintaining crop profitability whilst lifting animal performance on mixed farms’ and CLIMA Forum 2006 ‘Grow your nitrogen, don’t buy it’
- research and professional development workshops: ‘Scientific writing’, GL3 sub-program meeting ‘From collection to characterization and breeding – applied agro-ecological adaptation from a lupin perspective’, 2006 CLIMA discussion forum ‘Benefits and constraints’
- responses to requests from industry groups for CLIMA speakers or visits to CLIMA facilities.

Media releases during 2004/06 covered a wide range of topics including new chickpea varieties, new pasture species for Australia, identification of molecular markers for herbicide resistance, screening and breeding for disease resistance in chickpeas, germplasm collection and characterisation, development of core collections for pasture species, lupin protein products for aquaculture, mutation breeding for herbicide tolerance in lupin and pulses, visitors to CLIMA, the results from students’ projects, and the change of leadership at CLIMA.

CLIMA’s website hosts information on the centre’s structure, the current seminar series and abstracts of past series, newsletters (CLIMA, Lathyrism and Botrytis Grey Mould newsletters), media releases, lists of publications, variety information, lists of research projects, research highlights, forum summaries, staff, student and research associate directories, information for schools on health and nutrition, and links to related websites.

CLIMA hosts numerous visits to the centre and its alliance partners, by individuals and industry groups who wish to meet researchers, see research facilities, hear research updates, or learn about products in the pipeline. In 2006, six industry groups were hosted by CLIMA: Beacon and Wialki farmers; Pulse Association of the South East; Lower Eyre Agricultural Development Association; South African Protein Research Foundation; East Timor Seeds of Life project and Ningham Farm Focus Group. In September the Minister for Agriculture and Food, Hon Kim Chance viewed CLIMA activities at UWA’s Shenton Park field station. In addition, CLIMA’s Industry Advisory Group meets twice a year for a formal business meeting followed by presentations from CLIMA researchers or a tour of research projects at one of CLIMA’s partner institutes. These meetings foster information exchange and provide feedback to researchers.

CLIMA’s staff, associated students at UWA and Murdoch University and research associates from partner institutions not only attend CLIMA workshops and other events but also regularly suggest and facilitate them. Their continued support for the co-operative ethos has been truly inspiring in maintaining CLIMA’s high profile in legume research and ensuring open lines of communication between researchers, the agricultural industry and funding providers.
Western Australian industry position

The global demand for vegetable protein for animal feed and traditional pulse and processed food ingredients continues to rise with European and Asian countries the largest importers. However, this demand is being balanced by increasing supply of soybean meal from North and South America. Forecasts of increasing production of biofuels from corn, canola and soybean in Europe and North America will result in even larger volumes of protein meal by-products for trade. Therefore, it seems likely that prices received by Australian growers may not rise at a time when the costs of production continue to do so.

The desire of the Western Australian grains industry to have more legumes in the rotation remains high as growers realise the risks associated with a high frequency of wheat in monoculture. The challenge for the industry to improve efficiency is huge against the backdrop of the cost–price squeeze.

Lupin production has declined dramatically since 1999, mostly in low rainfall areas, as break-even yields approach 1.5 t/ha. Weed control issues are currently the number one priority issue for lupin growers in Western Australia. The recent completion of a large lupin de-hulling plant in Perth is a welcome investment that promises the development of new markets and value-adding opportunities.

Field pea production is stabilising at higher levels, mainly in the less frost-prone southern cropping areas of Western Australia. With the advent of more erect and easy-to-harvest varieties, the area is likely to climb further, which will increase the pressure from the main disease, blackspot.

Chickpea production is expected to return to and should exceed the levels of the late 1990s in northern areas, as higher levels of ascochyta resistance reduce the need for fungicide application.

Grain Legume Program research portfolio

The vast bulk of the Grain Legume Program’s research is orientated to the genetic improvement of the major cool season grain legumes grown in Australia. All projects are aligned to high priority industry needs but many have a medium to long-term horizon for payback to the industry. The portfolio includes projects which ultimately aim to boost industry profitability through increasing and
stabilising yield, decreasing input costs or increasing returns by lifting grain value. The program has striven to understand the needs of breeders, growers and the end-users of grain legumes and where possible to orientate research in a way that will lead to rapid adoption. There are also an increasing number of overseas projects that aim to benefit developing countries while providing Australia with valuable knowledge or new germplasm.

During 2004/06 the Program continued to play an important role in expanding the germplasm base, particularly in *Lupinus* and *Cicer*, and in researching new technologies through our linkages with national breeding programs. This research should help to underpin those programs well into the future. Our projects continue to utilise the most up-to-date molecular techniques alongside the best traditional approaches to genetic enhancement. An example is the effort going into inter-specific crosses in *Lupinus* and *Cicer* to generate novel germplasm with a unique blend of economically important traits unavailable in a single species. Transgenic research and induced mutation are also being used to broaden the range of traits available to breeders.

Improving adaptation to our agro-ecological environments remains an overarching theme. We have ongoing research on drought tolerance, cold tolerance, winter vigour and a new initiative on understanding regional adaptation in lupin in relation to crop architecture and phenology.

Resistance to pests and diseases remains a major focus with progress at the molecular level in the *Medicago truncatula* model species and at the specific crop level with the major necrotrophic fungal pathogens. These pathogens are the most difficult to control in our targeted Mediterranean environment.

Genetic improvement of seed quality continues to be researched with the view of enhancing value for premium end-users in both the food and feed sectors. A project involving the Department of Fisheries and the Chemistry Centre (W.A.) has contributed to the development of new aquaculture markets for lupins through the active involvement of grain marketers and commercial end-users in the project.

The Program’s scientists are to be commended on the excellence of their research as well as their commitment to develop and maintain national and international linkages which has broadened our funding base. We also thank our institutional and industry stakeholders for their active involvement and financial support.
The past two years have seen continued evolution of CLIMA as a one-stop shop for grain legume research and development. The molecular technology work of previous years, now routinely applied to disease resistance in lupin breeding on a scale with few parallels in the grain legume breeding elsewhere in the world, is also being extended to the domestication traits. Genetic mapping of the Lupin genome and comparative studies with the model legume species Medicago truncatula promises a wealth of genomic resources for crop improvement. Characterisation of the core lupin germplasm collection and of wild Cicer species will lead to improved utilisation of these resources.

The development of novel germplasm through studies in inter-specific hybridisation in Lupinus and Cicer is being pursued. Genetic transformation work showed promise for control of some necrotrophic fungi for which resistance is not currently available in the germplasm. An exciting new development in the past two years is a project on herbicide tolerance that not only located germplasm with improved tolerance to herbicides but is also developing new variation through mutation breeding.

The application of research to grain legume improvement has been further strengthened through work on lupin end product utilisation looking at allergenicity, food processing and food product development in a range of projects in lupin species. The CLIMA-based chickpea breeding international alliance has shown excellent promise in less than two years with breeding lines ready to enter regional trials and the projected release of new high yielding, high quality and ascochyta resistant desi varieties in the near future. This international alliance has helped to identify partners overseas that are working with CLIMA to develop new related projects on abiotic stresses.

Grain legume variety development continued with the release of two new kabuli varieties, Almaz and Nafice, that are high yielding, high quality and ascochyta resistant. Another kabuli variety, Kimberly Large, was released for the Ord River Irrigation Area that aims at the high value market. Development of new grain legumes continued with studies in pearl lupin (Lupinus mutabilis) showing promise of a new industry for southern Australia.

The sub-program continued its presence in international efforts to explore and conserve germplasm resources with participation in missions to Central Asia and the Caucasus.
Plant genetic conservation, documentation and utilisation in Central Asia and the Caucasus

**PRINCIPAL INVESTIGATORS:** Dr Ken Street (ICARDA), Dr Michael Mackay (AWCC), Prof. Clive Francis (UWA) Dr Bob Redden (ATFCC) with nominees from Georgia, Armenia, Azerbaijan, Kazakhstan, Kyrgyz republic, Turkmenistan, Uzbekistan, Tajikistan, in collaboration with the Vavilov Institute St Petersburg

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This ACIAR-funded project lead by Dr Ken Street of ICARDA is designed to benefit genetic resource in the participating countries. Australia does not have adapted endemic genetic resources of most important crop species. By contrast Central Asia and the Caucasus is a rich centre of genetic diversity and crop origin of a large number of globally important agricultural species including cereals and legumes. In two collections in Armenia in which CLIMA has participated, over 1000 accessions have been lodged with the ICARDA, Armenian and Vavilov collections and several hundred returned to Australia, most recently 43 Trifolium accessions featuring perennial species like *Trifolium ambiguum* and *T. hybridum*.

While a number of these legumes such as clover, vetch, faba bean and pea are readily usable by plant breeders, the old wheats are a longer term proposition. We only want specific genes, like the drought- or frost-tolerance genes. We don’t want genes that might undo the highly developed agronomic traits of modern varieties. However, it is difficult to control for unwanted genes with normal cross-pollination techniques, and at present, gene transfer technologies are not permitted in Australia in food crops.

The work by Ken Street and Clive Francis also involves trying to save or rebuild the once pre-eminent plant collections housed in the crumbling, neglected, botanical institutes of the former Soviet republics in Central Asia. To quote Ken Street, “The world is losing irreplaceable seed from these collections simply because the local people can’t afford to replace water pumps, or stored seed is being eaten by mice. This is an absolute tragedy; doubly so because it is avoidable. The rate of deterioration is very advanced so we are desperately trying to collect, store, document and manage as much diversity from old varieties and wild relatives before they are gone forever. We don’t know what challenges future farmers will face, but we do know the answers to those challenges are held in the genes of the plants we are collecting.”

Collecting missions like these, in countries such as Armenia, are now part of an international program developed under the auspices of the new Global Crop Diversity Trust, set up as an instrument of the International Treaty on Plant Genetic Resources for Food and Agriculture. This was formed only a year ago, with considerable Australian support, to try to arrest the erosion of the world’s plant genetic resources.

**This research is supported by the Australian Centre for International Agricultural Research (ACIAR) – (CIM/2004/004).**
International *Lupinus mutabilis* (pearl lupin) collaboration with Peru and Chile

**PRINCIPAL INVESTIGATORS:** Dr Jon Clements (UWA), Dr Roger Jones (DAFWA), Mr Geoff Thomas (DAFWA)

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*Lupinus mutabilis* (pearl or Andean lupin) is a lupin species with great potential for Australia because its seed has extremely good quality (oil and protein content). A second small GRDC-funded project based at CLIMA to develop pearl lupin for Australian agriculture has just begun.

Good linkages have been established with lupin researchers in Peru (the centre of origin of pearl lupin) and in Chile. The Peruvian researchers at Cuzco and Puno are responsible for the two of the largest germplasm collections of the pearl lupin in the world. Their collections contain seeds collected from throughout South America from very diverse climatic, edaphic and geographic zones. They include an enormous wealth of useful traits of interest to Australia, including early flowering, drought resistance, anthracnose resistance and virus resistance.

These resources will be an invaluable contribution to the first major effort to produce early flowering, low alkaloid and high yield pearl lupin varieties for Australia and collaborating countries.

In order to pursue a collaborative, international project discussed and agreed with the Peruvians and Chileans, financial resources are needed. This international project aims to evaluate a common set of genotypes of pearl lupin, many of which will be provided by Peru, for agronomic, yield, disease (anthracnose, CMV, BYMV) and seed quality traits.

Some extremely promising germplasm will be included in the evaluation set and many good traits are expected to be highlighted through these studies. A better understanding of degree of genotype x environment interaction and the range of variation available to a breeding program for this species will be attained along with access to new lines.

The project is currently at the phase of increasing seed of lines to be sent among the collaborators. We hope that all parties will have the set of accessions by May 2007. A Crawford Fund grant has facilitated this collaboration, with a visit by one of the Peruvian collaborators planned for August to October in 2007.

**This research is supported by CLIMA.**

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Genetic variation in narrow-leaved lupin (NLL) accessions and breeding programs

**PRINCIPAL INVESTIGATORS:** Dr Susan J. Barker (UWA), Prof. Mike Jones (Murdoch), Dr Bevan Buirchell (DAFWA), Mr James Ponds (UWA)

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The overall aim of this project is to provide information about the distribution of genetic variation in narrow-leaved lupin (*Lupinus angustifolius*, NLL) accessions from across the Mediterranean region, and to determine the extent to which this variation is reflected in current breeding programs in Australia and internationally. The other aim is to proceed with postgraduate student training in the area of plant molecular genetics (molecular plant breeding).

Statistical (biplot) analysis of many of the 2000 accessions of wild lupins from 31 countries available in the national NLL collection was performed, to select average and outlier accessions across the range of regions represented in the collection. Characters considered were time to flowering; seed weight; % protein; height; soil, altitude, and rainfall; and ‘comments of interest’. Approximately 100 accessions were selected and three or four representatives of each accession were grown for molecular genetic analysis. Some seed diversity within accessions has been noted and final numbers to be assessed for each accession will be determined empirically.

DNA isolation techniques were established. Amplified fragment length polymorphism (AFLP) and limited simple sequence repeat analysis were performed, with various methods of visualisation tested to determine the optimum method for future work. AFLP analysis of all accessions in the
selection has now been performed. Some additional samples are still required to be processed for highly variable accessions.

Alongside the genotype and accession relationship analysis, a second project has been initiated that investigates variation in lupin allergenicity. In Western Australia, most people do not normally contact lupin so are unlikely to be directly sensitised to lupin proteins. However, overseas examples of individuals known to be sensitive to peanuts and having a severe reaction following consumption of white lupin seed proteins, led to a collaboration with Professor Peter Sly at the Telethon Institute for Child Health. We are examining cross-reaction of NLL seed proteins to plasma of adults who are allergic to peanut. We aim to establish the range of proteins in lupin seed that are cross-reactive, using plasma from 10 to 20 individuals. Variation between lupin genotypes in the expression of the allergenic proteins will be established, along with variation between individual sera in which proteins are recognised. Data from these studies will inform the lupin breeding program of any potential to reduce the allergenic component of lupin seeds.

This research is supported by the Australian Research Council Linkage program (LP0348023), the Department of Agriculture and Food WA (DAFWA), the School of Plant Biology (UWA), the Telethon Institute for Child Health Research and the National Health and Medical Research Council.

Lupin germplasm characterisation

**PRINCIPAL INVESTIGATORS:** Dr Fucheng Shan (UWA), Dr Bevan Buirchell (DAFWA)

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Breeders recognise the importance of genetic resources to their breeding programs and have been actively involved in accumulating and using wild accessions since lupin breeding began in earnest in Australian in the early 1970s. Through collection missions and importing germplasm from overseas breeding programs, Australia now holds the largest collection in the world for narrow-leafed lupin and has a substantial collection of nearly all the other species from the Mediterranean region and North Africa. The Australian Lupin Collection held in the Department of Agriculture and Food Western Australia (DAFWA) contains 1301 wild/landraces of *L. angustifolius*, 180 lines of *L. luteus*, 763 lines of *Lupinus albus* and 36 lines of *L. mutabilis*. This collection has been tapped for sources of anthracnose resistance, phomopsis resistance, pleiochaeta root rot resistance and high protein over the last 30 years. While there has not been a concerted effort to evaluate the collection for a number of other traits it has, nonetheless, served as a useful resource for novel traits.

To use this germplasm efficiently, characterisation of it is a high priority. Since characterisation of such a large collection in a short period is almost infeasible, identification of a core collection to represent the genetic diversity of the whole collection is a practical starting point. Once the core subset is established, the germplasm can then be evaluated and characterised for yield and agronomic components, quality traits, biotic and abiotic stresses. If a trait of interest is identified in a subset of the core collection, it is possible to go back to the whole collection and further evaluate the accessions represented by that subset of the core collection. The information is useful to breeders in identifying germplasm with novel or improved traits that can be incorporated into breeding programs.

The aims of this research are:

1. to develop a core collection representing the genetic diversity of the Australian Lupin Collection based on DNA markers but also incorporating other available geographical and evaluation data
2. to screen the core collection for the characters or traits identified as priorities covering yield components, agronomic, quality traits, and biotic and abiotic stresses
3. to provide elite germplasm to lupin breeders.

This research commenced in July 2005.

Agriculture Research Western Australia has undertaken a survey of its leading lupin researchers to gather a list of traits and their priority (total 18 biotic and 21 quality traits) for screening. This research alliance has highly experienced researchers in the area of lupin grain quality, legume pathology and agronomic/breeding traits. These researchers have access to world-class facilities at the University of Western Australia, Department of Agriculture and Food WA (DAFWA), Chemistry Centre (W.A.), State Agricultural Biotechnology Centre WA at Murdoch University and CSIRO to carry out most of the experiments and analysis proposed.
Core establishment and evaluation

A core collection of 120 accessions in *L. angustifolius* has been established based on the DArT markers and their habitat parameters using Maximim software. This core covers the distribution area of *L. angustifolius* with most core accessions from Spain and Greece. A comparison of habitats between the whole and core collections of *L. angustifolius* showed that their habitats are quite similar, indicating that the core can represent habitats of the whole collection. Analysis of diversity of 10 available evaluation traits showed that there was no significant difference of diversity between the whole collection and the core. This indicated that the core has well reserved the diversity of the original collection.

This core has been grown out in a screenhouse at the UWA Shenton Park Field Station for evaluation on their yield components.

DNA extraction

All the wild/landraces in the species *L. angustifolius* (1301 accessions), *L. albus* (763 accessions), *L. luteus* (180 accessions) and *L. mutabilis* (36 accessions) were grown out for young leaf collection. DNA extraction has been completed for the above species except 36 accessions in *L. mutabilis*.

DArT array development

A diverse number of accessions covering each lupin species were used to set up the arrays for the diversity analysis. After genotyping over 100 *L. angustifolius* samples across 6000 random clones in three separate experiments, over 500 polymorphic markers were identified. The highest quality markers (350) were selected for final genotyping array development together with a set of control clones. The array has been used for routine genotyping of the *L. angustifolius* collection. A total of 191 markers have been generated across 1300 accessions in *L. angustifolius*.

“Through collection missions and importing germplasm from overseas breeding programs, Australia now holds the largest collection in the world for narrow-leafed lupin and has a substantial collection of nearly all the other species from the Mediterranean region and North Africa.”

*Dr Fucheng Shan (right) and Dr Bevan Buirchell (left) evaluating narrow-leafed lupin representative accessions in the screenhouse at the UWA Shenton Park Field Station*
Next steps

DArT markers will be generated for the other three species *L. luteus*, *L. albus* and *L. mutabilis*. A core for these three species will be established and grown out for yield and agronomic evaluation. Evaluation on quality traits and biotic stresses will be carried out for the core collection of *L. angustifolius*.

This research is supported by the Grains Research and Development Corporation (GRDC) – (UWA00092).

Characterisation and evaluation of wild *Cicer* genetic resources to accelerate chickpea improvement in Australia

**PRINCIPAL INVESTIGATORS:** Dr Fucheng Shan (UWA), Dr Heather Clarke (UWA), Dr Guijun Yan (UWA), Assoc. Prof. Julie Plummer (UWA), Prof. Kadambot Siddique (UWA)

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Improved chickpea varieties with resistance to pests and diseases and tolerance to environmental stresses are increasingly required for the chickpea industry, but it is difficult to make rapid improvement within chickpea due to its narrow genetic diversity. In contrast, its wild relatives in the genus *Cicer* possess a wide range of valuable traits which potentially could be introgressed through hybridisation into chickpea cultivars. Since the project commenced on 1 July 2002, a total of 218 accessions and selections belonging to eight wild annual *Cicer* and one perennial species have been assembled at CLIMA from various world gene banks. They have been characterised using DNA fingerprinting based on AFLP analysis. The major findings are discussed below.

1. Accession identity established and duplication and misidentification clarified

DNA profiles of the world collections of 108 original accessions and 110 selections have been generated. Identification keys for each accession have been established. Duplicate and misidentifications have been clarified in the germplasm, which provided a standard protocol to check the identity of selected parental accessions in breeding and related research programs. The results have been communicated to chickpea breeders and researchers.

Geographical locations of maximum genetic variation of the wild annual *Cicer* species
2. Phylogenetic relationships between and within annual *Cicer* species established to help chickpea improvement

Phylogenetic analysis of wild annual *Cicer* accessions and six chickpea cultivars, based on AFLP data, revealed four distinct groups corresponding well to the cultivar and primary, secondary and tertiary gene pools of chickpea. The phylogenetic relationships incorporated with other published evaluation data will be useful for parental selection in chickpea improvement programs.

3. Range of diversity reveals that cultivated chickpea can be improved by introgression of genes from its wild relatives

Genetic analysis based on AFLP data revealed that the extent of diversity varied considerably and was unbalanced between species with greatest genetic diversity found in *Cicer judaicum*. *C. arietinum* (chickpea) has the least genetic variation. This factor could explain why yield improvement of chickpea on a global basis has been slow when compared to cereal crops, which reinforces the necessity to introduce valuable genes from its wild relatives.

4. Hot spots identified for further wild *Cicer* collections

For the first time geographic patterns of genetic variation in *C. reticulatum*, *C. echinospermum*, *C. bijugum*, *C. judaicum* and *C. pinnatifidum* were established using AFLPs. Based on the current collections, the maximum genetic diversity of *C. reticulatum*, *C. echinospermum*, *C. bijugum* and *C. pinnatifidum* was found in south-eastern Turkey, while Palestine was the centre of maximum genetic variation for *C. judaicum*. This information provides a solid basis for the design of future collections and *in situ* conservation programs for wild annual *Cicer* (Shan et al 2005).

In addition, the techniques developed in this study led to a new project at CLIMA funded by GRDC, ‘Lupin Germplasm Characterisation’ (UWA 00092).

This research is supported as a Post Doctoral Fellowship to Dr Fucheng Shan by the Grains Research and Development Corporation (GRDC – PDF 38).

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**Exploring genetic variation in *Cicer* leaf and root exudates for better resistance to pests and improved nutrition**

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Chickpea plants secrete acidic exudates through glandular trichomes (hairs) on the surface of their leaves, stems, pods and roots. The aim of this pilot project is to explore the genetic variation among cultivated chickpea (*Cicer arietinum*) and several annual members of the genus *Cicer*.

Such visible exudates from chickpea leaves and pods have attracted interest for many years and the carboxylates malate and oxalate were long reported to play a role in plant–insect interaction in chickpea. In comparison to other crops which lack the leaf exudate, chickpea is relatively unaffected by colonisation of most insects including aphids; most insects are either deterred by the acid or are killed by its low pH of 1.3. Budworm (*Helicoverpa sp./ pod borer*) is one exception, and there is strong evidence that chickpea exudates actually play a role in attracting the moth to the chickpea crop.

Research at UWA has also highlighted the role of carboxylates in the root exudates of chickpea with regard to the mobilisation of nutrients from P-poor soils in Australia. We therefore need to know if there is a link between above ground and below ground exudation, such that selection for above ground exudation (to deter insects) might have consequences for below ground function (such as P uptake).

Wild relatives of chickpea among the genus *Cicer* are a valuable genetic resource for chickpea improvement, and it is important to know if the wild species also produce exudates on their surfaces. Resistance to insects and fungal pathogens have been identified in some of the annual species. On the other hand, our experiences growing wild *Cicer* species in the UWA glasshouse tell us that some plants are particularly susceptible to aphid and thrip infestations. For example,
C. bijugum requires regular chemical sprays to control the pests during the summer months. Given the limited genetic diversity among chickpea cultivars, it is also of great interest to identify and quantify rhizosphere carboxylates for a genetically and ecologically diverse group of annual Cicer species.

Our results so far show that there is genetic variability in the density of leaf trichomes between Cicer species. There are also some differences between species for the length of these trichomes. Trichomes are being examined on leaves, petiole/rachis and stipules. The response of cowpea aphid and blue green aphid to several Cicer species grown in cages in a controlled temperature environment will be assessed in coming weeks. We will also analyse the leaf exudates by high pressure liquid chromatography, examining which organic acids are present, as well as their quantity, in each species. Similarly, the carboxylate concentration in the rhizosphere of experimental plants will be analysed. We will then look for correlations between the insect response, the morphology of the leaves and the chemistry of the exudates. Exudates from above ground and below ground will be compared.

This research is supported by CLIMA.

### Interspecific hybridisation in lupins

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Lupin breeding in Australia could benefit from the additional genetic diversity of interspecific hybrids. Currently the value of lupins is constrained by several seed quality factors which contribute to their position as a relatively low value feed grain. Improving seed quality of *Lupinus angustifolius* (narrow-leafed lupin), the most widely adapted species, by increasing protein and oil content or other specific components (e.g. sulphur amino acids) would allow the grain to be sold in a wider range of feed and food markets. Combining desirable attributes among the key crop species (*L. angustifolius*, *L. albus*, *L. luteus* and *L. mutabilis*) would help to improve an individual species more rapidly. For example, a priority is to transfer the seed quality, the brown spot and pleiochaeta root rot tolerance traits from *L. luteus* to *L. angustifolius*.

Transferring anthracnose resistance, metribuzin tolerance, wide adaptation or aphid tolerance from *L. angustifolius* to *L. luteus* would be enormously beneficial in the breeding of yellow lupins. Similarly, transferring high oil and protein characters from pearl lupin (*L. mutabilis*) to *L. angustifolius* would be valuable if this were possible. Some preliminary results in Europe with the crop lupin species have
shown that hybridisation is possible, for example, between *L. angustifolius* and *L. luteus*. Interspecific hybridisation was successful among the rough-seeded lupin species in work done in Western Australia in the past 15 years by breeders.

A range of microscopy techniques are being applied at the Centre for Microscopy and Microanalysis at UWA to selfed, immature seeds of *L. angustifolius* to characterise the development of normal embryos. This is being compared to the growth of hybrid embryos to examine possible barriers to the formation of interspecific hybrid seed and therefore postulate the best stage to rescue these embryos through *in vitro* culture. Methods include hand and microtome sectioning, SPURRs resin embedding, several staining techniques and the use of light, multiphoton confocal or electron microscopy.

Examination of pollen growth down the stigma will help to assess if there are pre-zygotic barriers in particular cross combinations. Microscopy studies will provide valuable information to increase the focus and improve efficiency with fewer cross-species on which to concentrate project resources.

In this first year of the project, a hand-crossing program of approximately 1200 plants to identify best species/breeding line cross combinations has been conducted with all combinations of four cultivars or breeding lines of *L. angustifolius*, *L. luteus*, *L. albus* and *L. mutabilis* during the winter season of 2006 and conclusions are still being drawn. So far, production of seeds at low rates has been achieved in most combinations among the four species.

However, data suggests that *L. angustifolius* is the best species to use as the maternal parent for crossing with the other three species. The most successful combination was *L. angustifolius* × *L. luteus*, followed by *L. albus* × *L. mutabilis*, *L. angustifolius* × *L. albus* and *L. angustifolius* × *L. mutabilis*. These putative hybrids produced seed, which will be tested using the MFLP molecular marker method at DAFWA. Tissue culture techniques are currently being developed to rescue putative hybrids that do not reach maturity on the mother plant.

The intended outcomes for the project are as follows:

- Produce true F1 and backcrossed hybrid lines by focusing on crossing *L. angustifolius* or *L. luteus* with *L. luteus*, *L. albus* and *L. mutabilis* and additionally *L. albus* × *L. mutabilis*.
- Develop best methods for achieving true hybrids among the crop lupin species including direct hand-crossing and seed production on the plant and through embryo rescue.
- Develop a routine validation of the hybrid nature of the resulting lines by molecular marker methods already in use at DAFWA for lupin breeding programs within species.

**This research is supported by the Grains Research and Development Corporation (GRDC) – (UWA00094).**
Improved herbicide tolerance for pulses in the Western Region

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Weed control is more difficult in narrow-leafed lupin and pulses than in cereals. It has become a critical issue not only for the profitability of lupin and pulse production but for weed management in cropping rotations. The widespread emergence of herbicide resistance in wild radish populations has reduced the ability to control this weed in crops. Lupin and pulse cultivars with increased tolerance to herbicides will assist in clawing back the dramatic decline in area sown to lupin and pulse since 1999.

This project screens a diverse source of lupin and pulse genetic materials and produces mutant populations of narrow-leafed lupin and desi chickpea for the development of cultivars with increased tolerance to herbicides. Major progress has been made and is discussed below.

Two lupin mutants originated from cultivar Tanjil were identified in 2005 as highly tolerant to metribuzin (Group C). A field trial at Shenton Park in 2006 confirmed their superior tolerance to metribuzin at high rates with great yielding capacity. They also have good resistance to anthracnose in the disease nursery in 2006.

- Screening for increased tolerance to carfentrazone-ethyl (Group G) was carried out at a very high rate among mutants of lupin and chickpea in Merredin in 2006. About 250 individual lupin plants of Mandelup origin were tagged as highly tolerant and they will be harvested by hand. Chickpea M2 populations had high survival rate after carfentrazone-ethyl application and they will be harvested in bulk and re-screened again at a higher rate next year.

- Germplasm of lupin and chickpea up to 100 accessions each were screened against herbicides of isoxaben (Group K) and mesotrione (Group F) in 2005. All lupin and chickpea germplasm were highly tolerant to isoxaben post-emergent. Some accessions of lupin and chickpea showed good level of tolerance to mesotrione.

- Germplasm of field pea, faba bean and lentil of 200 accessions in total were screened against four herbicides (isoxaben, isoxaflutole, mesotrione and carfentrazone-ethyl) in 2006. Large variation exists among the germplasm of pea, lentil and faba bean in tolerance to isoxaben, isoxaflutole and carfentrazone-ethyl. Mesotrione killed most pea, lentil and faba bean germplasm.

- More mutant populations of lupin Mandelup and chickpea Sonali and Genesis 509 were induced and grown to produce seed in 2006. These mutation populations are the source materials for screening for increased tolerance of target herbicides.

- A broad suite of herbicide chemistries that are not used in current dryland cropping system in Australia were examined with lupin cultivars and wild radish under glasshouse conditions.

This research is supported by the Grains Research and Development Corporation (GRDC) – (UWA 97).
Effective weed control is critical to profitable lupin production. The emergence of herbicide resistant weeds and the poor metribuzin tolerance of the anthracnose resistant cultivars (Tanjil and Wonga) have become major obstacles for many growers. The project addressed the need to breed lupins with 1) improved tolerance to metribuzin by understanding the genetic basis of triazine tolerance, and 2) novel tolerance by mutation to isoxaflutole and carfentrazone-ethyl, with potential to control herbicide-resistant weeds. Tolerant breeding lines were developed and provided to the National Lupin Breeding Program, along with efficient screening procedures to facilitate future breeding.

This project developed a reliable glasshouse bioassay to differentiate lupin genotypes with tolerance or susceptibility to metribuzin in the seedling stage. The lupin breeding program at DAFWA adopted this method in 2003 and screened more than one thousand advanced breeding lines to select elite lines having a high level of metribuzin tolerance. Genetic studies show that metribuzin tolerance in lupin is under single gene control. This knowledge enabled the DAFWA program to develop appropriate breeding and selection strategies for rapidly improving and maintaining tolerance to metribuzin. Further, this knowledge was used by GRDC Project DAW 00102 to develop an MFLP marker which should further improve the efficiency of breeding. Notably, our screening identified that advanced breeding line WALAN 2173 (high protein, anthracnose resistant and good yield) was not a pure line and contained about 25% metribuzin susceptible individuals. With this information the DAFWA breeding program made a metribuzin-tolerant reselection (WALAN 2173M) which was released as Coromup in August 2006.

A large amount of new germplasm was developed from this project, through crossing between tolerant and susceptible genotypes or from induced mutation. Tolerant progenies (F4) from the study of inheritance for metribuzin tolerance are available for breeding program to take up. Germplasm with tolerance to the newer herbicides isoxaflutole and carfentrazone-ethyl was developed through induced mutation with chemical mutagens EMS and Azide. These mutant lines (30 tolerant to isoxaflutole and 16 to carfentrazone-ethyl) have been selected for tolerance in both M2 and M3. They were seed-increased in 2005 and will be characterised for tolerance in a new GRDC project. We also identified Tanjil mutants with very high tolerance to metribuzin (better than Mandelup). These mutants are being fast-tracked as potential high anthracnose resistant and metribuzin tolerant varieties.

This research is supported by the Grains Research and Development Corporation (GRDC) – (UWA 0042).
Developing interspecific hybrids between chickpea and its wild relatives

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Cultivated chickpea belongs to the species *Cicer arietinum*. The species has no known wild populations, so chickpea's closest wild relatives are among other species within the genus *Cicer*. In order to create improved chickpea cultivars which express multiple disease and abiotic stress resistance, it is necessary to access a large gene pool. Many attempts have been made worldwide to introgress genes from wild *Cicer* into the cultivar with limited success. Only two annual *Cicer* species, *C. reticulatum* and *C. echinospermum*, are easily hybridised with chickpea using conventional crossing methods. The other six annual species and a large number of perennial species remain an inaccessible source of genes for chickpea improvement. To tackle this problem, an international collaboration was established between CLIMA, the Crop Development Centre at the University of Saskatchewan, Canada (CDC) and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India.

The collaborative project has generated hybrids between cultivated chickpea and accessions of *C. bijugum*, *C. pinnatifidum* and *C. judaicum*. This achievement was made possible using in vitro technology to rescue immature seeds to tissue culture in the laboratory before they abort from the mother plant in the glasshouse. Without rescue, most hybrids of *C. pinnatifidum* and *C. judaicum* abort from the mother plant 14 to 21 days after pollination, whereas *C. bijugum* abort as early as seven days. Transfer of techniques developed at ICRISAT has enabled the successful rescue in vitro of 14-day-old hybrids between several Australian cultivars and *C. pinnatifidum* at CLIMA. The international collaboration has further developed techniques and complex media for the rescue of very immature hybrids between chickpea and *C. bijugum*. Hybridity of *in vitro* plantlets has been confirmed using DNA technology.

Detailed microscopic studies of embryo development in immature seeds, comparing the normal development of selfed chickpea seeds with hybrids, identified barriers to chickpea x *C. bijugum* hybrid seed development for the first time. This
research helped to identify the best timing to rescue hybrids from the mother plant, and provided useful information about embryo development. We also applied plant growth regulators to the flower buds at pollination to promote pod set and to maintain the hybrid pod on the mother plant as long as possible before rescue.

A robust system is required to enable breeders to incorporate wide crosses in their chickpea improvement programmes. This project continues to develop reliable protocols which can be applied to any chickpea cultivars for wide crosses. Albinism and recalcitrance to rooting in hybrid plantlets in vitro are two major factors limiting our success in transferring large numbers of plantlets from controlled conditions in the laboratory to pots in the glasshouse. We are currently examining the causes. In the future, we will also quantify pollen viability and fertility of the flowers of hybrid plants and investigate the chromosome arrangements of the new hybrids.

This research is supported by the Grains Research and Development Corporation (GRDC) – (UWA00091).

Genetics of Ascochyta blight resistance in chickpea and wild Cicer species

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Ascochyta blight, caused by fungal pathogen Ascochyta rabiei (Pass) Lab, is a major biotic constraint limiting chickpea production world-wide. Very little information is available on diallel crosses involving wild Cicer species in relation to the genetics of Ascochyta blight resistance. The major objective of this study is to determine the genetic components of ascochyta resistance in chickpea and wild Cicer accessions from the primary genepool of chickpea.

The genetics of Ascochyta blight resistance was studied in five different 5 x 5 half-diallel cross sets involving seven genotypes of chickpea (ICC 3996, Almaz, Lasseter, Kaniva, 248-Isoline, IG 9337 and Kimberley Large), three accessions of C. reticulatum (ILWC 118, ILWC 139 and ILWC 184) and one accession of C. echinospermum (ILWC 181). Infested chickpea debris from the previous year was spread between rows as inoculum in the field. Both F1 and F2 diallel sets were screened visually using a 1-9 scale where 1 is unaffected and 9 is dead.

Estimates of genetic parameters showed significant additive and dominant gene actions. The variance (Vr) and the parent–offspring covariance (Wr) were calculated from the family means. The slope of the regression line in Wr/Vr graph differed significantly from zero but not from unit, indicating that the frequency of dominant alleles was different among parents and the model was adequate. The frequency of dominance alleles was higher than recessive alleles. Susceptibility was dominant over Ascochyta blight. The study also suggests the involvement of both major and minor genes. The recessive alleles were concentrated in the two resistant parents ICC 3996 and Almaz. Results suggest that the wild Cicer accessions may have different major or minor resistant genes. Overall, narrow-sense heritability for Ascochyta blight resistance was about 85% and 60% for F1 and F2 diallel sets, respectively. Broad-sense heritability was greater than 94% for all diallel sets.

The results show that Ascochyta blight resistance is controlled by recessive genes. High narrow-sense heritability indicates that additive gene effects were more important in the inheritance of the trait and high genetic gain can be achieved in breeding resistant chickpea cultivars. The knowledge from this research will help plant breeders to pyramid all available resistance genes effectively in the Cicer germplasm into the cultivated chickpea.

This research is supported by the Ministry of Science, Research and Technology of Iran, and CLIMA.
Transformation of narrow-leafed lupin (NLL) with the anti-apoptosis gene, p35, and impact on necrotrophic fungal pathogen disease symptoms

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Apoptosis or programmed cell death is involved in the normal growth and development of many organisms and is a process that recently has been identified during necrotrophic fungal attack of susceptible plant tissues. As necrotrophic fungi require plant cell death to feed and replicate, blocking cell death initiated by these fungi might be a strategy to explore for improvement of crop species where genetic resistance is not found.

To this end, we have obtained the p35 gene from Professor D Gilchrist, UC Davis, and inserted the gene into a susceptible cultivar of narrow-leafed lupin (NLL). The gene was stably integrated into the lupin genome, expressed in leaves, roots and stems, and did not detectably alter normal plant development or fecundity in 12 transgenic lines obtained.

These transgenic lines were evaluated for disease symptoms following inoculation with three necrotrophic fungal diseases: anthracnose (Colletotrichum lupini); brown leaf spot (Pleiochaeta setosa) and root rot (Pleiochaeta setosa). In each case, a significant reduction of disease symptoms was observed for some lines that generally correlated with the abundance of p35 gene transcripts.

Results of this research support the hypothesis that fungal necrotrophs induce programmed cell death in lupin plants as part of the disease syndrome. The materials we have generated will be useful in further investigation of the importance of limiting programmed cell death in necrotrophic fungal disease. Localised expression of anti-apoptosis genes specifically in infected tissues may prove a durable form of resistance in species where resistant germplasm does not exist.

This research was supported by the School of Plant Biology (UWA), the Grains Research and Development Corporation (GRDC) – (UWA309) and ARC Linkage International Program (LX0346900).

Genetic mapping of the narrow-leafed lupin genome and molecular cloning of a key domestication gene

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Narrow-leafed lupin (NLL; Lupinus angustifolius L.) is the most important grain legume crop in southern Australia. Little is known about the genetic structure of the NLL genome and so in this study, we set out to improve our understanding by making a genetic linkage map.

Two things are required to make a genetic map: a segregating mapping population and plentiful genetic markers. A mapping population based on a cross between wild (P27255) and domesticated (83A:476) NLL was developed at the Department of Agriculture and Food Western Australia. These parents were chosen to ensure maximum genetic variation in the mapping population and to allow the mapping of key domestication traits. For genetic markers, we used gene-based molecular markers because such markers are transferable between related species and hence allow analysis of comparative genome structure between species.

Together with national and international collaborators, we developed the first gene-based map of NLL and conducted a comparative analysis of the genome structure of NLL with the model legume species, Medicago truncatula. We found that, despite the wide evolutionary distance between NLL and M. truncatula, there remained several conserved regions between the two genomes. We also found strong evidence of widespread genome duplication in the NLL genome, possibly arising from an ancient polyploidisation event.
On the map, we identified the locations of five key domestication genes. One of these domestication genes (the early flowering time gene, \( K_u \)) mapped to genomic region which is conserved between \( N L L \) and \( M. \) truncatula. On the basis of the shared genome structure in this region, it should be possible to use the wealth of genomic resources available in the model \( M. \) truncatula to assist in the molecular cloning of \( K_u \), which would be of significance for basic and applied research. We are currently attempting the molecular cloning of \( K_u \) with the support of an internal CLIMA grant.

This research was supported by the Grains Research and Development Corporation – (GRDC) – (UWA372). An internal CLIMA grant is supporting the cloning of \( K_u \).

**Development of molecular markers for anthracnose resistance in *Lupinus albus***

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*Lupinus albus* is an important lupin species which was successfully grown on the red loam soils in the northern agricultural region of Western Australia before the outbreak of anthracnose in 1996. The only albus cultivar, Kiev Mutant, proved to be highly susceptible to lupin anthracnose and consequently the albus industry was wiped out in Western Australia. Selection for anthracnose resistance is now one of the top priorities in albus lupin breeding in Australia. The development of molecular markers that tag the anthracnose resistance gene in *Lupinus albus* would significantly facilitate the breeding and selection of new albus cultivars for anthracnose resistance.

While molecular breeding has been very successful in narrow-leaved lupin, no funding has previously been available for marker work for albus lupin. In 2006, a mini-project funded by CLIMA was undertaken to search for molecular markers for anthracnose resistance in albus lupin.

The MFLP, a DNA fingerprinting technique developed at CLIMA in 2001, was applied for this work. The parents and representative F8 derived recombinant inbred lines (RILs) from a cross of P27174 (resistant) x Kiev Mutant (susceptible) were used in obtaining the fingerprints in 41 MFLP gels. Three candidate molecular markers were identified as linked to the anthracnose resistance gene. One of the markers (marker ‘Lin B’) is a co-dominant marker. The other two markers (markers ‘Lin A’ and ‘Lin C’) are dominant markers. All of these markers showed correct DNA banding patterns on the eight resistant plants tested.

Among the eight susceptible plants, seven plants showed the marker banding pattern corresponding to anthracnose disease phenotype. The one plant showing inconsistence for each marker would be due to genetic recombination between the marker site and the R gene on the chromosome. However, if we make selection based on a combination of markers ‘Lin A’ and ‘Lin B’, we would be able to select only those eight resistant plants and discard the eight susceptible plants. The results indicated that a combination of both markers ‘Lin A’ and ‘Lin B’ increased the accuracy in marker-assisted selection for anthracnose resistance in albus lupin. The marker ‘Lin C’ showed the same banding pattern as the R-allele band of marker ‘Lin B’ on the 16 plants, indicating that markers ‘Lin C’ and ‘Lin B’ are closely located on the chromosome in relation to the R gene. In the financial year 2006/07, GRDC has granted additional funding which will enable us to complete the sequencing and conversion of the above markers into simple polymerase chain reaction (PCR)-based, implementable markers for albus lupin breeding.

This research is supported by CLIMA.
Development and implementation of molecular markers in Australian lupin breeding program

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In the molecular world it is easy to get large numbers of molecular markers, but it is very difficult to get a marker that is implementable in practical plant breeding. The difficulty lies in the fact that a molecular marker must meet the following requirements before it can be used for marker-assisted selection to provide tangible benefit in a plant breeding program:

- The marker is closely linked to an agronomic trait of industry significance.
- The marker is co-dominant, which enables breeders to differentiation homozygous individual (e.g. RR) from heterozygous individuals (e.g. Rr).
- The marker is more cost-effective than the traditional field or glasshouse based selection to warrant the use of marker-assisted selection.
- The marker is high-throughput and amenable to large numbers of samples.

Apart from the above well-known challenges, another major factor limiting the use of molecular markers in practical breeding is that many cultivars in a plant species not containing the target gene may show the target marker band (false positive), which is the reason why many marker breeding programs engage in the time-consuming process of ‘marker validation’ to sort out which crosses are suitable for which markers.

To address this challenge, the lupin marker team at DAFWA has developed a new strategy of applying the marker validation concept during the marker development stage so that only those markers with a wide application potential are selected and developed. During the last two year, several excellent new markers were developed or identified in lupin, including:

- development of a co-dominant, simple PCR-based marker linked to anthracnose resistance in cultivar Mandelup. Initially, nine candidate markers were discovered within three months by using MFLP, a new DNA fingerprinting technology developed at CLIMA. The nine candidate markers were ‘validated’ by testing on key lupin cultivars, and the number of false positives ranged from one to 10. Only the candidate marker with the least false positives was selected and converted into a simple PCR-based, cost-effective marker for routine marker implementation.

Because Mandelup is the highest yielding lupin cultivar in Australia, it has been extensively used as a parental line in crossing in the lupin breeding program. This newly developed marker, termed ‘AnMan’, enables the breeder to select for anthracnose resistance from progenies with pedigrees relating to Mandelup. Together with the markers ‘AntjM1’ and ‘AntjM2’ linked to anthracnose resistance in Tanjil previously developed at DAFWA, the Australian breeders are now able to fix the anthracnose resistance at the early stage (F2) in the breeding cycle for most of the breeding lines with molecular markers.

- development of a co-dominant, simple PCR-based marker tagging the soft-seediness gene **moll**. The marker is so close to the gene that no recombination was detected among all the 115 RILs resulted from a domesticated x wild cross. The marker banding pattern correlated perfectly with all the domesticated cultivars and all the wild collection accessions tested. This marker is applicable in wide range of domesticated x wild crosses.

- discovery of a co-dominant marker tagging the phomopsis resistance gene in Tanjil. By using MFLP, four candidate markers were identified. After validation tests on the commercial lupin cultivars, one marker with widest application potential was selected. This marker provides new tool in selection for phomopsis resistance in the lupin breeding program.

Following the initiation of application of molecular markers in lupin breeding in 2003/04, marker implementation in the Australian lupin breeding program has experienced a ‘taking off’ stage during 2005/06.
International collaboration to develop robust protocols for doubled haploid production in field pea and chickpea

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Doubled haploid (DH) populations derived from F1 hybrid material enable the selection of elite homozygous progeny with improved combinations of the more difficult polygenic traits such as yield, biotic and abiotic stress resistance and quality requirements. Routine development and use of haploid plants in breeding programs has yet to be achieved for any Fabaceae species. To redress this lack of information in the pulse crops, the GRDC funded a project UWA00035 (2002–2005) to establish a national and international collaborative effort to research DH technology for chickpea (Cicer arietinum L.) and field pea (Pisum sativum L.). This collaboration has resulted in the first report of in vitro grown pollen-derived proembryos in chickpea and field pea.

Routine protocols for the induction of haploid embryogenesis from isolated microspores have been developed for a range of chickpea genotypes and one field pea genotype. Within the project we have improved the percentage of microspores responding to embryo induction, optimised a wide range of protocol parameters for responsive genotypes and made significant inroads towards the development of haploid plants. As part of our multi-faceted approach, we have also undertaken research into intact anther culture, with success in the development of plantlets (ploidy level yet to be confirmed) from anther-derived callus in chickpea.

A further important achievement of this project has been the building of linkages with Canadian pulse researchers. This collaboration has led to cooperation in other cell biology projects (UWA00036) and the collaborative research is now being extended to include a group at INRA, France who have expertise in legume embryology and an interest in field pea doubled haploidy. In addition, national linkages between SARDI and CLIMA researchers were also strengthened by collaborative efforts on aspects of this project.

GRDC funding has enabled us to make important progress towards our goal of DH production in chickpea and field pea. Further funding has been secured from ARC (see project report for LP0562111) in order to finalise protocol development for doubled haploid plant development in these target species. Once developed, these protocols will be freely available to Australian pulse breeders and researchers.

This research was supported by the Grains Research and Development Corporation (GRDC) – (UWA00035).
Accelerating the genetic improvement of grain legumes for Australia by developing doubled haploid technology for field pea and chickpea

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GRDC-funded research undertaken at The University of Western Australia in collaboration with Canadian researchers at The University of Saskatchewan resulted in the world-first production of pollen-derived proembryos in chickpea and field pea (see above report on UWA00035). This breakthrough offered an excellent opportunity to fill the gap in fundamental haploid embryology information in these species and to apply this new knowledge to develop protocols for routine doubled haploid (DH) plant production.

The Council of Grain Grower Organisations Ltd (COGGO) has generously provided funding to enable the establishment of an Australian Research Council (ARC) Linkage Project to build upon the results of UWA00035. The overall aim of the project is to understand the process of haploid embryogenesis in chickpea (*Cicer arietinum* L.) and field pea (*Pisum sativum* L.) on a cellular level. We will use this improved understanding of haploid embryogenesis pathways to develop world-first *in vitro* protocols for the production of doubled haploid plant material in these species.

The forecasted outcomes of the project will be:

- an improved knowledge of fundamental embryology in Fabaceae
- novel protocols for *in vitro* doubled haploid plant production in chickpea and field pea
- doubled haploid germplasm for the Australian pulse improvement programs.

To achieve these outcomes we will:

I. Develop novel methods for the routine induction of sporophytic development from the male gametes of chickpea and field pea. This will be accomplished through continued optimisation of parameters known to be critical to haploid embryology including donor plant genotype and growing conditions, inductive stress, isolation methodology, tissue culture medium composition and culture regime.

II. Use multiple microscopy techniques such as fluorescence, differential interference contrast, multiphoton confocal and transmission scanning electron to elucidate the haploid division pathways and the cellular processes involved in the switch from gametophytic to sporophytic development in chickpea and field pea.

III. Use the fundamental information from microscopy investigations coupled with optimised parameters from (I) to develop protocols for haploid embryo maturation, plant regeneration and effective chromosome doubling, culminating in doubled haploid plant production.

To date, our research has focussed on detailed observation of microspore developmental stages and linking these stages with bud morphology to enable more synchronous populations of microspores for establishing cultures. Electroporation of anthers and microspores has also been undertaken following reports of success (Ochatt pers. comm.) in field pea using this technique by INRA researchers. The next phase of the project will use scanning electron microscopy and transmission electron microscopy techniques to observe pollen growth in culture. Fluorescent microscopy will be used to identify and quantify the modes of haploid proembryo development.

This research is supported by Council of Grain Grower Organisations Ltd (COGGO) and the Australian Research Council (ARC) – (LP0562111).
Enhancing double haploid research in cool season legumes by investigating haploidy in the tropical legumes cowpea and winged bean

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According to isolated reports, haploid plant production has been achieved in the phaseoloid species soybean (*Glycine max* L. Merr.), pigeonpea (*Cajanus cajan* L.), cowpea (*Vigna unguiculata* L. Walp.) and winged bean (*Psophocarpus tetragonolobus* L.D.C.). As yet, there has been no haploid plant production in any of the galegod legumes (chickpea, field pea, lentil, lathyrus or lupin).

A pilot project has been established with CLIMA internal funding support to replicate the published protocols for the phaseoloid species, specifically those for winged bean and cowpea. The aim of this project is to collect information of value to our chickpea and field pea doubled haploid (DH) research program. This will be achieved through the development of techniques that may be directly or indirectly applicable to the chickpea and field pea DH research. We surmise there is likely to be significant areas of commonality in the *in vitro* culture requirements of the tropical and temperate leguminous species that cannot be identified without undertaking experiments with both.

Winged bean germplasm has been collected and plants of six genotypes grown under short day conditions in the controlled environment facility at The University of Western Australia (UWA). Brightfield microscopy has been used to track microspore development *in situ* and to identify morphological markers to assist in choosing buds for culture. Experiments have begun to test a variety of stress pretreatments and to culture intact anthers on a range of embryo induction media. Planned experiments include the application of isolated microspore culture protocols and the electroproportion of intact anthers and isolated microspores. Microspore development will be tracked using fluorescence microscopy after fixing and staining with DAPI. Initial results from anther and isolated microspore culture are expected by the end of 2006.

If successful in winged bean, we will undertake similar research using cowpea as the target species. This study is planned to run initially for six months.

This research is supported by CLIMA.

New ascochyta resistant, high yielding and quality kabuli chickpea varieties released for Australia

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Ascochyta blight, caused by *Ascochyta rabiei*, is the most damaging disease of chickpea in most parts of the world. The disease has caused widespread yield losses in Australia and a significant decline in the area of production. To rejuvenate the industry, new varieties with high levels of Ascochyta blight resistance and integrated management packages are necessary. In this project we developed an international collaboration to fast-track the release of new improved kabuli chickpea varieties with Ascochyta blight resistance for Australia. The project commenced in 1998 when Ascochyta blight was not well established in Australia and relied on off-shore screening in Turkey to identify improved germplasm from the world’s major kabuli chickpea improvement programs. The major objectives were to select Ascochyta blight resistant chickpea crossbred lines with high quality likely to be well adapted to Australian conditions, introduce promising lines to Australia and fast-track new varieties for commercial production.

Kabuli chickpea crossbred lines and commercial varieties from the International Centre for Agricultural Research in Dry Areas (ICARDA) in Syria, the Aegean Agricultural Research Institute (AARI) in Turkey, and Australia were screened for resistance to Ascochyta blight and agronomic traits in Turkey during 1998 to 2001 (GRDC-funded CLIMA project UWA248NR). More than 2000...
breeding lines and varieties were screened and approximately 300 superior lines were selected and introduced to Australia.

The initial quantity of seed introduced to Australia was less than 50 seeds of each crossbred line (initially grown in a quarantine glasshouse) which limited evaluation to agronomic adaptation in small field plots in Western Australia. Further evaluation (yield, agronomy, disease management and seed quality) was more extensive, expanding from one field scale replicated yield trial in 2002 to multiple sites across southern Australia from 2003 to 2005 (COGGO funded project CGO/2 2002). Disease screening was undertaken in South Australia, Victoria and New South Wales where the disease had established by 1998 and continued at interstate locations in 1999 and 2000, and in Western Australia between 2002 and 2005 after the disease had spread through the western region. Seed production of the most promising crossbred lines commenced by selecting single plants from plots at Bindoon between 1999 and 2001. Seed bulk-up continued in Western Australia at irrigated and dryland sites between 2001 and 2005, and under commercial conditions in Western Australia and Victoria in 2005.

The international collaboration with ICARDA and AARI enabled us to screen a large number of chickpea lines prior to import to Australia. Selection of lines and seed production carried out concurrently with development of agronomic and disease management practices allowed the release in 2005 of the first kabuli chickpea varieties with moderate resistance to Ascochyta blight and large, high quality seed. The release of Almaz and Nafice to growers was achieved in less than seven years after introduction into Australia and will improve the confidence in the Australian chickpea industry.

This research was supported by the Council of Grain Grower Organisations Ltd (COGGO) and Grains Research and Development Corporation (GRDC).
The project conducts a great part of the early breeding cycles at ICRISAT where specialised facilities and manpower resources help to enrich germplasm for agronomic characteristics necessary for adaptation to Western Australia together with resistance to Ascochyta blight and other diseases. Lines are then fixed and late generation selection occurs in Western Australia. Pollen selection techniques and specialised facilities at CLIMA are contributing to develop cold tolerant and ascochyta resistant germplasm which is shared between Western Australia and ICRISAT. Collaboration with PAU allows screening of Western Australian-targeted lines against a wider range of ascochyta pathotypes and other characteristics in an environment that has some similarities with the Western Australian grain belt.

In less than two years the project now has a high degree of Ascochyta blight resistance in a large number of breeding lines that are being tested at various stages in Western Australia. Some of this material is already in the regional Crop Variety Testing trials. Six lines will be fast-tracked with a view to release an ascochyta resistant, high yielding and high quality desi chickpea variety by 2009/2010.

This research is supported by the Council of Grain Grower Organisations Ltd (COGGO) – (CGO 3-2004).
Faba bean breeding and selection for the Western Region

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The total area cropped to faba bean in Western Australia expanded rapidly in the early 1990s reaching a peak of about 40,000 ha in 1997. Disease (chocolate spot) epidemics and a series of dry seasons subsequently caused the area to fall to less than 10,000 ha. The release of Fiesta, a variety with improved chocolate spot resistance, and better disease management strategies have since seen a modest recovery in the faba bean industry. Nevertheless, at its current level the industry is well below its medium term potential of over 100,000 ha in Western Australia.

There are two centres for breeding faba bean in Australia: Adelaide, South Australia and Narrabri, New South Wales. Germplasm from these centres is evaluated in Western Australia. The aim is to develop varieties with resistance to chocolate spot, ascochyta and rust, the three main faba bean diseases, as well as increased yield and yield stability. Fiesta is now the most widely grown faba variety in Western Australia and has become the standard against which improvements in disease resistance and yield are measured. However, Fiord will produce higher yields than Fiesta in Western Australia in disease free situations.

Germplasm was evaluated in Stage 2 (S2), Stage 3 (S3) and Stage 4 (S4) trials at several locations. The S2 trial was situated at Bindoon and involved 185 lines, mainly crossbreds obtained directly from the breeding centres in Adelaide and Narrabri. Stage 2 trials are mainly used for seed multiplication. Stage 3 trials were conducted at Dongara, Merredin, Katanning and Scaddan. About 120 lines were evaluated at each site. Inclusion of lines in trials was dependant on the availability of seed. More lines originating from New South Wales were included at Dongara and Merredin, while more lines from South Australia were included at Katanning and Scaddan. About 75% of the lines were common across sites.

Stage 4 trials were located at Dongara, Mingenew, Bolgart, Kojonup (analysed as two trials as two replicates ended up 50 m away from the remainder of trial on a different soil type) and Esperance. Five current varieties plus four lines that produced high yields in S2 and S3 trials in 2004 were included.

Key messages from Stage 3 trials
- SP98002 and 735*683/15 were the two top ranked lines, performing particularly well at Merredin and Katanning. SP98002 is slightly earlier flowering than most released varieties and performed creditably at Mingenew in the Stage 4 trial.
- 1270*278/10, 974*(611*974)/42 and 483/5 are three lines being considered for multiplication by National Faba Bean Improvement Program due to improved ascochyta resistance and good seed characteristics. 1270*278/10 did well at most sites, and was ranked equal third overall, and did particularly well at Scaddan. 974*(611*974)/42 did well at the southern sites and was ranked 7 and 5 at Scaddan and Katanning. Both lines performed well in 2004 trials.
- 483/5 tended to do better at northern sites in both the Stage 3 and 4 trials.

Key messages from Stage 4 trials
- FiestaAR*(IC*AS)/3 did well at southern sites but not in the north. It has good ratings for ascochyta resistance.
- At the majority of sites, Fiord was amongst the lowest yielding varieties, with the notable exception of Mingenew which had very low disease pressure.
- Nura (a new release with improved disease resistance, short in height, late flowering variety) was the highest yielding variety at Esperance Downs Research Station (a high rainfall southern site) and in two replicates of the Kojonup trial, but was disappointing elsewhere.

This research is supported by the Grains Research and Development Corporation (GRDC).
The potential of the pearl lupin (Lupinus mutabilis) for southern Australia

Pearl lupin: development of the first Australian cultivar for commercial evaluation

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Pearl lupin (Lupinus mutabilis) originates from South America where high alkaloid types have been cultivated for centuries and eaten as a traditional food after de-bittering.

Its grain protein and oil levels rival and in some cases exceed soybean. Oil quality is very good being high in unsaturated fatty acids and low in erucic acid. Pearl lupin has a thin seed coat (13%) similar to soybean, making it highly suitable for de-hulling. The protein is also of higher quality than narrow-leaved lupin in terms of lysine and sulphur amino acid levels.

After some preliminary crossing and field evaluation in 2000/01, GRDC project UWA00043, ‘The potential of the pearl lupin (Lupinus mutabilis) for southern Australia’ began in July 2002. The project demonstrated that pearl lupin had clear potential as a high value legume crop for production under winter–spring cropping systems in southern Australia and that pearl lupin should receive in excess of $100/tonne premium over narrow-leaved lupin in high specification aquaculture and monogastric feed markets. The first pearl lupin varieties would be most profitable in more reliable rainfall environments (greater than 350 mm growing season rainfall) and relatively fertile, well drained soils.

The level of future breeding investment should become evident based on the performance of the first varieties developed. A potential area of production at least as big as the peak L. albus area across Australia (250,000 ha) is ultimately envisaged. These estimates could be quite conservative given an outlook for higher fossil fuel and nitrogenous fertiliser prices. Like other lupin crops, pearl lupin can fix substantial quantities of nitrogen as well as provide a disease break to cereal and oilseed crops.

There is increasing interest and investment in lupin processing in Australia. Plans to de-hull large quantities of L. angustifolius are advancing rapidly in Western Australia with both regionally based, small-to-medium scale operations and a large-scale plant under construction in Perth by Australasian Lupin Processing, a joint venture between Cooperative Bulk Handling Ltd and George Weston Foods. There are also lupin de-hulling facilities in regional New South Wales and lupin kernel-flour milling operations in Western Australia and Victoria. Wet processing of lupin kernel meal into protein and fibre fractions are also planned. The upswing of interest has been sparked by new opportunities in the aquafeed and food ingredient markets. Pearl lupin would add the new dimension of up to 20% edible quality oil (or potentially higher with a trade-off in protein). Discussions with commercial companies have confirmed that pearl lupin would be highly sought, for oil extraction and as a protein-rich source material. The amino acid profile and functionality of pearl lupin is similar to other lupin species and compares favourably with soy protein.

In the second GRDC-funded project, ‘Pearl lupin: development of the first Australian cultivar for...’
commercial evaluation’ (UWA00093), the agronomic suitability and yield of 31 fully domesticated *L. mutabilis* breeding lines with early flowering, low alkaloid, non-shattering and basically sound plant structure were evaluated at Shenton Park field plots in 2005. The best line produced 72% and 74% the seed yield of Tanjil and Andromeda respectively, while yielding 22% higher than Wodjil. *L. mutabilis* biomass was similar to Wodjil but lower than Tanjil and harvest index values were generally lower than the other crop species. Aphid colonisation of these low alkaloid lines indicates that they were all less susceptible than Wodjil. Several lines are less susceptible to anthracnose than *L. albus* cv. Kiev Mutant but well short of the resistance of *L. angustifolius* cv. Tanjil. Variation exists in brown spot resistance but all lines are relatively susceptible. The total alkaloid levels in these varieties ranged from 0.001% to 0.018%. Seed oil ranged from 12.8% to 17.1% and protein ranged from 38.3% to 46.3%.

The 10 best lines were selected and sown in yield trials at Mount Barker, York and Geraldton in 2006. The Geraldton and York trials have been badly drought affected. There is still the need to evaluate lines with extra early flowering times, using material which is available in subsequent streams of crossing in the project.

More South American landraces have been obtained from a Portuguese collaborator (80 lines) and from a Chilean collaborator (20 lines). There is also the potential to use many of the approximately 80 other closely related lupin species to *L. mutabilis* from the Andean region, most of which are known or assumed to have the same chromosome number as *L. mutabilis* and may be crossable with it. We hope to access some representatives of these species in future years. By the end of this current project the level of future breeding investment should become evident based on the performance of the germplasm developed. Interest has been expressed by pork researchers to collaborate in agronomic evaluation of lines in eastern Australia and feeding trials.

This research is supported by the Grains Research and Development Corporation (GRDC) – (UWA00043 and UWA00093).

Improvement of yellow lupins (*Lupinus luteus*) in Western Australia

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Yellow lupin is a highly suitable feed for poultry, pigs and the aquaculture industry because the grain has higher protein content (38% to 40%) and better amino acid balance than narrow-leafed lupins. Its protein rich grain and potential for production in many parts of southern Australia have attracted researchers. Low grain yield and susceptibility to aphid feeding damage are the main obstacles for the expansion of this industry. Initially, yellow lupin was targeted in highly acidic and aluminium toxic soils of the eastern wheatbelt in Western Australia. But aphids are more of a problem in this region and also yellow lupins seem to require more moisture than narrow-leafed lupin. Currently, its adaptation in cooler southern regions is being investigated.

There are indications that gramine can provide tolerance to aphids in yellow lupins. Gramine, a tryptophan derived indole alkaloid, is a relatively safe compound compared to the quinolizidine group present in albus and narrow-leafed lupins.

Drs Jon Clements and Kedar Adhikari collect yellow lupins in Portugal.
The current advanced breeding lines have little or no gramine. Presently gramine is being incorporated into the breeding lines with the anticipation of improving aphid tolerance. With collaboration from CSIRO, new techniques of screening plants against aphids have been developed in the glasshouse and screenhouse conditions.

Yellow lupin was primarily bred for the northern European environment and the ‘ideotype’ is not suited to Western Australian conditions. However, the ideotype in Australia is yet to be defined. It was hypothesised that reduction in flowering time would result in higher grain yield as experienced in narrow-leafed lupin. However, the early flowering types also had lower biomass production, possibly associated with the lower plant stature and failed to increase the yield potential. Recently a new source of early flowering in a relatively tall background has been identified and this will be evaluated further.

Yellow lupin has high apical dominance and produces pods primarily on the main stem. The pod development in the first order and subsequent branches starts only when the pods are fully formed on the main stem. By this time, there is already initiation of moisture stress and the branches fail to develop pods. This is considered the major reason for its lower yield. A genotype with indeterminate growth habit that produces pods not only on the main stem, but also on branches is required to increase the yield level.

To increase the genetic diversity, new germplasm are being introduced from overseas through collection trips and international collaborators. From these germplasm, three sources of resistance to anthracnose have been identified and they are being incorporated into the breeding lines. A recent trip by Drs K. Adhikari and J. Clements in Portugal with the help of the Portuguese Curator, Dr Eliseu Bettencourt, has enriched the genebank with interesting accessions, such as *Lupinus hispanicus* from a soil pH of 8.0 with a calcareous component (unusual since the species is found mainly on acid soils), and *L. luteus* from altitudes as high as 830 m whereas previous collections have come from 500 m or lower.

This research is supported by the Grains Research and Development Corporation (GRDC).
New oilseed options for Australian farmers and industry

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The aim of this project was to deliver to farmers and industry new oilseed options for export and local production, including:

- oilseeds for industrial use and production of biodiesel
- oilseeds for the production of quality mustard oils for export
- oilseeds that contain high contents of essential fatty acids: Omega-3 or Gamma linolenic acid.

A single oilseed system based on *Brassica napus* is unlikely to be the most suitable for all environments and disease exposure. There is a need for alternative oilseeds, which have both high yield and oil content and which may have low production costs compared to canola. *B. juncea* and *B. carinata* both have potential in this area, for export or biodiesel. The high erucic acid Crambe (*Crambe abyssinica*) had potential in southern Australia and had an expanding market in Europe and USA as industrial oil. Quality mustard seed is in demand for export to India with a market there of 200,000 tonnes, mainly for *B. juncea*.

The market for health food supplements is rapidly growing and there is an increasing demand for oils containing essential fatty acids such as Omega-3 and Omega-6.

Promising material was sought for the three project objectives:

1. an early line of *B. juncea* (mustard) with high yield suitable for biodiesel
2. single plant selections of mustards with a desirable mustard flavour but with better oil quality in terms of reduced erucic acid
3. lines of Golden Flax and Camelina very high in oil and Omega-3 fatty acids and Camelina.

The project has supplied seed of the high yielding mustards to the Department of Agriculture and Food WA for biodiesel studies. DAFWA produced 70 tonnes of mixed seed for production of biodiesel in government vehicles.

Crambe is undergoing world-wide demand on account of its qualities as a species with high erucic acid. Harvested yields of the best lines 94053 at 1.2 tonnes per hectare were equal to that of canola but must be discounted by the characteristic of pod wall retention accounting for up to 50% of the harvested yield.

Mustards *B. juncea* and *B. carinata* again yielded as well or better than canola in regional yield trials. A selection (21) made during the course of the project will be evaluated for world markets as part of the related marketing study, UWA87A.

High linolenic lines of *Camelina sativa* and golden linseed yielded some 40% less than canola in trials but command a substantial price premium in the oilseed market, nationally and internationally. They are major species in the related marketing study.

Test marketing of the most promising material in this project is undertaken as project UWA87A. Good markets for Camelina oil and mustard have been located and the best mustard (Sel 21) is incorporated in biodiesel trials and marketed in Bangladesh. The linseed market is strong in the health industry and will be fast-tracked. A Crambe initiative commenced independently in Victoria has not been up to expectations.

This research is supported by the Rural Industries Research and Development Corporation (RIRDC) – (UWA74A, NPP02-30).
Mustards: Promising biodiesel crops for the wheatbelt – management strategies, feasibility and herbicide tolerance development

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The project has focused on developing a high yielding agronomic package for Indian and Ethiopian mustards as biodiesel crops. The project has built on research that was conducted by the Department of Agriculture and Food WA, CLIMA and Muresk Institute. The project consists of several field experiments to be conducted at Muresk Institute, Northam and Dryland Research Institute, Merredin, to determine the best varieties, optimum rates and timing of fertiliser application, seeding rate, time of sowing, and weed, pest and disease management.

Planning and conducting of these trials was done in collaboration with Margaret Campbell, Research Officer (Oilseeds), CLIMA, UWA. Standard field experimental methods with adequate replication of treatment combinations were used. Measurements on crop growth, grain yield, oil yield and quality are being made. Demonstration trials to showcase the high yielding agronomic package will be conducted in collaboration with grower groups such as Ninghan Farming Group in Mukinbudin, Liebe Group in Buntine, Mingenew–Irwin Group in Mingenew and Western Australian No-Till Farming Association in Meckering, in a second year of the project.

The difficulty of controlling cruciferous weeds, for example, wild radish, is a major constraint to growing mustard as the lines currently available are tolerant of few herbicides. They are susceptible to any herbicides that could be used for the control of cruciferous weeds. This will severely limit the adoption of these crops by farmers. Lines tolerant of particular herbicides useful in the control of cruciferous weeds are being developed using conventional breeding methods. The three herbicides chosen for the development of herbicide tolerance in the mustards were the pre-emergent herbicide, Balance™ and two post-emergent herbicides, metribuzin and MCPA amine.

Concurrently with the research described above, the project has prepared, summarised, communicated and discussed currently available information on the research topics, as well as reported on the research progress to wheatbelt people. The task was carried out as a series of workshops, which also covered other relevant topics, related to the best available advice/guidance for those who are considering investing in biodiesel. The workshops were delivered by specialists on particular aspects in selected geographically spread centres in the wheatbelt, including Merredin, Esperance, Lake Grace and Kondinin. Discussions raised awareness and addressed issues associated with the biodiesel opportunity for the wheatbelt.

A feasibility summary paper was produced which considered the competitive advantages and the constraints to developing partial or total manufacturing of biodiesel in and outside the wheatbelt. Considerations included canola, other oilseeds crops, crushing / oil extraction, marketability of canola meal, manufacturing of biodiesel on-site or off-site and transport costs.

The potential benefits from this project are applicable to all the shires of the wheatbelt of Western Australia.

This research is supported by the Wheatbelt Regional Development Scheme.
SUB-PROGRAM GL2 – Disease and Pest Management

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Sub-program GL2 tackles the most persistent scourge of legume crops and pastures worldwide – the damaging losses to diseases and pests. The program is highly regarded and has achieved widespread international recognition. Several team members participated in and helped to organise the key international symposia on legume pathology and genetics. The program uses the most up-to-date techniques, such as metabolomics, marker-assisted breeding and transformation in combination with the tried and tested methods such as accession collection, breeding and integrated pest management. The model species, Medicago truncatula, is a centre of much of our activity but we also use a wide range of crop and pasture species. The program’s funding is growing and is sourced from a range of national and international agencies including the Grains Research and Development Corporation (GRDC), Australian Research Council (ARC), Department of Education, Science and Training (DEST), Australian Centre for International Agricultural Research (ACIAR), Council of Grain Grower Organisations Ltd. (COGGO), the European Union (EU) and National Science Foundation (NSF).

Seeds of Life – East Timor

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The Seeds of Life program in East Timor commenced on 1 September 2005 with the goal of improved food security in East Timor. The purpose of Seeds of Life is the use of improved crop varieties and associated technologies which result in increased food production. The five-year project, jointly funded by the Governments of East Timor and Australia, is embedded as a program within the Ministry of Agriculture, Forestry and Fisheries (MAFF). The program has four components with specific objectives:

1. seed production and storage and distribution
2. evaluation of new germplasm and associated technologies
3. on-farm demonstrations and trials (OFDTs), and
4. program management and coordination and institutionalisation of crop research and extension in MAFF.

Capacity building is a priority for the program and is embedded within the four components. The program develops the participatory crop research capacity of MAFF and East Timor and raises the level of skills in germplasm collection and conservation, seed production and storage and farmer participatory research skills.

Twenty-three replicated trials to evaluate maize, peanut, cassava and pigeonpea germplasm were installed in the first main growing season. At least two local varieties were within each trial. In addition, five varieties of locally available velvet beans were compared. Select maize varieties demonstrated yield advantages in excess of 100% over locals. LYDMR (Late Yellow Downey Mildew resistant) and Suwan5, which proved to be superior yielding selections in previous years, will continue to be included in on-farm trials. Introduced sweet potato clones demonstrated yield advantages in excess of 130% over the local varieties. Two cassava clones outyielded local varieties by 40% to 60% and outyielded one peanut variety. New peanut varieties also performed well.

Six hundred and fifty-four OFDTs were installed during the main growing season from October 2005 to April 2006. Included were 196 maize, 146 sweet potato, 41 cassava, 187 peanut, 4 velvet bean and 80 rice trials. In addition, 23 maize and 23 peanut OFDTs were established during the second season.

Gender disaggregated views on the new varieties were collected. The yield advantage for introduced composites identified in replicated, on-station trials were also illustrated under farmers conditions for maize. LYDMR and Suwan5 outyielded local varieties by approximately 50%. Test peanut varieties of PT5 and GN11 yielded more than locally grown peanuts in seven of the eight sub-districts.
Genetic dissection of fungal disease resistance in legumes using *Medicago truncatula*

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Fungal necrotrophic diseases are the greatest constraint to the long-term viability of grain legume production in Australia. We are applying powerful molecular genetic approaches to analyze resistance to necrotrophic fungal pathogens using *Medicago truncatula*. *M. truncatula* has many of the features of a model plant and is well suited for these studies since Australia has a very large collection of *M. truncatula* accessions. *M. truncatula* is also phylogenetically related to the most important legume crops (pea, faba bean, chickpea, lentil, lucerne and clover). The identification of genes from the *M. truncatula* genome involved in the pathogen recognition (classical R-genes) or in the defence reaction should aid in the identification of homologous genes in other legume crops.

Alternatively, the genes could be transferred to legume crops.

Progress in this GRDC project has been very good and the project has gained a significant international profile. For example, the CSIRO and Murdoch groups are full partners in a 6th European Framework Project entitled ‘New strategies to improve grain legumes for food and feed’ and have obtained a DEST Innovation Access grant that has facilitated the linkage of this CLIMA/GRDC project with the large EU project and uptake of cutting edge resources.

A major part of the project has been the screening of the Australian *M. truncatula* collection.
At present, 75 isolates representing 29 species have been screened against eight or nine *M. truncatula* accessions with appropriate host legumes (lucerne, chickpea, lentil, faba bean, field pea, and lupin) being included in the tests. We have now completed the screen of 100 *M. truncatula* accessions with 11 fungal isolates which include eight foliar pathogens (*Colletotrichum trifoli*, *C. coccodes*, *Stagonospora meliloti*, Phoma sp, *P. medicaginis*, Ascochyta pinodes, *A. lentis*, *A. rabiei*) and three root pathogens (*Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*) and have identified accessions with large differences in susceptibility versus resistance.

Excellent progress has been made in developing mapping populations with over 20 successful crosses covering resistance/susceptible accessions for seven fungal species and in a number of cases large F2 populations.

In the case of the foliar pathogens there has been particularly good progress in the genetic analysis of genes for resistance to *Phoma medicaginis* and *Ascochyta lentis*. Two major genes are involved: recessive in the case of Phoma and dominant in the case of Ascochyta. In Ascochyta, the genes segregate with Mendelian ratios consistent with major effects. The genes for resistance to Phoma are quantitative. Mapping of these genes is well advanced.

Further work on elucidating the mechanism of defence against key foliar pathogens has also been initiated. We have focused on an isolate of *Phoma medicaginis* called OMT5 and a pair of resistant and susceptible ecotypes. Gene expression experiments by quantitative PCR using defence marker genes have shown several differences in the response to Phoma between these ecotypes and a comprehensive gene expression screen using a 16,000 oligo microarray was carried out with colleagues in Germany and has helped identify genes/pathways linked to resistance, including the phenylpropanoid pathway.

In the case of root pathogens good progress has also been made. For example, *F. oxysporum* accessions with high resistance or susceptibility have been isolated. Moreover, the use of mapping populations has already led to the identification and low resolution mapping of three unlinked loci involved in the resistance response, with one of the quantitative trait loci being responsible for over 50% of the total resistance. While accessions with complete resistance to *R. solani* have not been found in spite of extensive screening, accessions with moderate degrees of resistance have been identified as well as some that are extremely susceptible. Progress has been made using genetic approaches to begin to dissect out pathways important for moderate resistance to this important root pathogen, which causes losses for a wide range of crops.
We have also been developing molecular and transformation tools for the analysis of differential gene expression in *M. truncatula*, including sensitive RT-PCR assays for analysing control and defence marker gene expression and transcriptomic approaches involving oligo microarrays and transcription factor profiling. Substantial efforts to transform Mt have been undertaken with the development of both transient and stable transformation assays involving Agrobacterium mediated, particle bombardment and hairy root transformation. These transgenic resources are providing valuable insight into the defence pathways used by legumes for resistance to fungal pathogens and potentially valuable genes/regulatory motifs.

We have also taken a directed approach to study potential *M. truncatula* closely related genes known to regulate defence responses in other plants. We have identified over 50 potential members of this family of transcription factors in *M. truncatula*. Time course expression studies revealed early induction of several of these *M. truncatula* Ethylene Response Factors by foliar and root pathogen inoculation. Treatment of plants with the defence regulators ethylene and/or methyl jasmonate suggest these genes are likely to be involved in defence against necrotrophic pathogens. Two of these genes have been introduced into *M. truncatula* using *Agrobacterium rhizogenes* or *A. tumefaciens*. This is allowing the study of activation of individual defence signalling pathways by pathogen challenge or over-expression or silencing of candidate genes.

This research is supported by the Grains Research and Development Corporation (GRDC) – (UWA00038).

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**Integrated management of Botrytis grey mould of chickpea in Bangladesh and Australia**

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Chickpea production in Australia increased rapidly during the 1990s but subsequently declined due to the impact of severe disease outbreak, principally Ascochyta blight. Botrytis grey mould (BGM) has also caused considerable yield and quality losses to Australian chickpea industry over the years. This crop history parallels the decline of chickpea in Bangladesh where BGM alone was principally responsible for the decline of crop area. This project aimed to benefit chickpea production in both countries through improving the BGM resistance of chickpea varieties and developing integrated crop management (ICM) packages to increase yield stability. The specific objectives of the project were to:

- assemble and screen a wide range of chickpea germplasm for resistance to BGM
- produce and distribute seed of varieties less susceptible to BGM to farmers in Bangladesh
- fine-tune and demonstrate integrated disease management packages in Bangladesh and Australia
- provide training to Bangladeshi scientists in Australia and Bangladeshi extension workers and farmers in Bangladesh.
Field screening to identify chickpea lines with useful levels of resistance to BGM has been conducted in Bangladesh over four seasons. Nearly 500 genotypes were screened in 2003/04, 208 in 2004/05 and 254 in 2005/06. From the second season onwards, entries comprised promising selections from the previous season and new entries.

Screening was conducted each year at two locations in Bangladesh, at Jessore and Ishurdi, where BGM is endemic, and for one year only at Tarahara, a high BGM risk site in Nepal. There were clear differences in reaction to BGM at each location in all seasons and some lines with moderate levels of resistance were identified, with a consistent reaction across sites and seasons.

In parallel, controlled environment screening was conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India and Horsham in Victoria. Resistance ratings were generally not consistent between field screening in Bangladesh and growth room at ICRISAT and Horsham. Nevertheless, it was possible to identify lines with relatively greater resistance in both growth room and field assessments.

Selected lines with superior resistance are currently being used as parental genotypes in chickpea breeding programs in Australia, Bangladesh and ICRISAT. A set of genotypes assessed as either relatively resistant or susceptible were grown at Ishurdi and Jessore in 2005/06 to determine whether the BGM rating values based on plant symptoms correspond with yield loss as determined in plots with high or low BGM severity. However, disease control with fungicide spraying was poor and relative yield loss between genotypes could not be clearly differentiated.

Isolates of *Botrytis cinerea* have been collected in Bangladesh, India, Nepal and Australia since 2003 and subjected to PCR-based microsatellite DNA analysis. This analysis showed a high degree of variation within sub-populations and across the population in general, which suggests that multiple resistance genes and mechanisms will be required if durable resistance is to be achieved in chickpea varieties.

A series of on-farm trials were conducted in Bangladesh in order to evaluate various components of ICM packages under farmers’ conditions. On-farm variety evaluations were conducted in Bangladesh to determine farmers’ preferences for varietal characteristics in this BGM-prone environment. From the first season, ICM packages incorporating best-bet technologies for BGM management were tested in farmer-managed operational scale plots. The recommended ICM practice is to use a variety less susceptible to BGM, reduced seed rate, canopy management (delayed sowing and thinning) to prevent excessive vegetative growth, and need-based foliar application of fungicide. Other important components of the ICM package are application of superphosphate, fungicide seed treatment and management of pod borer.
In the 2002/03 season, 100 such demonstrations were conducted across five districts of Bangladesh and yield increases of 20% to 50% were observed. Similar on-farm activities have been conducted each year until 2005/06 when 641 demonstrations were conducted across the eight districts. District mean yields in ICM plots generally exceeded 1 t/ha\(^{-1}\), making chickpea very competitive with other cropping options for the winter season.

In parallel with ICM demonstrations, seed villages were established in Bangladesh in 2004/05 and 2005/06 to encourage and train farmers in chickpea seed production and to ensure future dissemination of quality seed of improved chickpea varieties.

This research is supported by the Australian Centre for International Agricultural Research (ACIAR) – (CS1-2001-039).

### Plant health management for faba bean, chickpea and lentil – WA component

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This project builds on the achievements of a previous project (CS1-2000-066) and continues the focus on screening and exchange of germplasm to improve disease resistance, management and yield of the pulse crops (faba bean, chickpea and lentil). These improvements will benefit Australia as well as Central and West Asian countries. Additionally, threats to pulse crops in Australia are being minimised by pre-emptive screening and breeding for resistance to exotic diseases and strains of endemic pathogens should there be an incursion of one of these diseases into Australia. This project also promotes collaboration among food legume researchers within Australia, ICARDA and in partner countries and thus strengthens research capacity in National Agricultural Research Systems.

#### Faba bean

Faba bean germplasm from ICARDA's gene bank was evaluated for resistance to chocolate spot and Ascochyta blight in screening nurseries at Lattakia (Syria), under artificial inoculation. Lines resistant to both diseases were identified and six were introduced to Australia. Using wider sources of resistance reduces the risk of breakdown in disease resistance which is more likely when a single source of resistance is used.

#### Chickpea

Screening of chickpea in greenhouses at ICARDA was undertaken for resistance to Pathotype III of Ascochyta blight. The screening identified 14 lines with acceptable levels of resistance (disease score of less than 5) to this pathotype. These 14 selections were also tested under field conditions against a mixed infection of Pathotypes I and II and were found resistant. In addition, during 2005/06, approximately 21,000 chickpea genotypes were screened in the field at ICARDA for resistance to Ascochyta blight Pathotypes I and II and high levels of resistance were observed in 24% of the material (rating \(\leq 3\)).

Around 8500 chickpea genotypes were screened at ICARDA for resistance to Fusarium wilt, in the sick plot at Tel Hadya during 2005/06 cropping season. Among this material were 92 Australian chickpea genotypes re-tested for their performance under high wilt disease: 40 lines were confirmed as resistant to the disease. Twenty-five chickpea lines from CLIMA and DAFWA were also evaluated. However, only three of these demonstrated satisfactory levels of resistance.

#### Lentil

ICARDA evaluated 210 lentil genotypes from Australia for resistance to Fusarium wilt during the 2006 season. Among the Australian varieties, only Cumra was highly resistant and all others were susceptible to highly susceptible. Within the breeding material tested, 52 lines were found to be highly resistant. None of the lines tested showed combined resistance to both Fusarium wilt and Ascochyta blight. Since Fusarium wilt is seed-transmitted in chickpea, even at a very low level, continued vigilance is needed to avoid introduction of this disease to Australian farming systems.

#### Integrated crop management

Field trials were conducted in the 2004 and 2005 growing season to determine an economic and robust fungicide management package for
improved chickpea varieties. In parallel with fungicide management research, epidemiology research has been progressing to ensure that recommended fungicide timings will protect the crop from wind-borne spores as well as seed-borne sources. Trials were established at two high rainfall sites (Northam and Dongara) with the new kabuli varieties, Nafice and Almaz, which were released by CLIMA in 2005 in collaboration with ICARDA. Both varieties are moderately resistant for Ascochyta blight and produce large and high quality seed. The resistance to Ascochyta blight displayed by these varieties indicates that no more than two fungicide sprays of chlorothalonil will be required to achieve effective ascochyta control.

**Collaboration**

During 2005 and 2006, Australian pulse pathologists met to discuss and maintain collaboration in different aspects of resistance screening and development of disease control packages for field pea, chickpea, faba bean and lentil. Also during the past two years, pulse pathologists from ICARDA visited CLIMA and other Australian project partners for scientific exchange and strengthening of the alliance.

This research is supported by the Australian Centre for International Agricultural Research (ACIAR) – (CIM-2004-003).

**Predictive models and decision systems for virus diseases and aphid vectors of lupin and canola**

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Aphids and the viruses they transmit cause major economic losses in legume and canola crops in Australia. This project is developing innovative predictive models and decision support systems for Beet western yellow virus (BWYV) in canola, Bean yellow mosaic virus (BYMV) in lupin, and aphid feeding damage in both canola and lupin. These models will greatly improve our understanding of the factors driving virus epidemics and aphid outbreaks. Following extensive validation with data previously collected in the Western Australian wheatbelt, the predictive models and decision support systems will be extended to end-users, resulting in considerable productivity gains, reduced costs and environmental benefits.

Forecasting models for epidemics of disease-causing pathogens that depend on a ‘green-bridge’ of susceptible herbaceous plants for their survival over the dry summer period in the Western Australian wheatbelt rely mainly on accurate data for daily temperature, rainfall and evaporation to function effectively. Climatic factors, especially rainfall, determine the size of the ‘green bridge’ which in turn determines the likelihood that a damaging epidemic will develop. Previously, the input of climatic data into such models has been done manually and has been a very time consuming exercise. As a consequence, forecasts based on such models focused on large areas (at the shire level), and were not usually updated on a
regular basis as the growing season progressed. However, research within this project on automation of data retrieval enabled forecasts for 475 points across the wheatbelt to be done with considerably less labour. This permits regular updates of predictions with a minimum of effort and also allows forecasts to focus on small areas.

Thus far models for greenness and BYMV incidence have been completed, and are being calibrated and validated. The BYMV model should be ready to forecast the 2007 season, with the BWYV and feeding damage models to follow later in the year.

This research is supported by the Australian Research Council (ARC) – (LP0669515) and DAFWA.

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**Imagination of lupin through virus resistance and better grain quality**

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Twenty plants from each of seven lines of transgenic lupin derived from three transformation events were tested for stability of resistance to Bean yellow mosaic virus (BYMV). All plants were progeny derived from BYMV-resistant parents. The plants were randomly distributed on benches in a temperature-controlled compartment of the physical containment glasshouse at Murdoch University.

Prior to challenge with virus all plants were tested for the presence of the resistance transgene. There were some problems with the use of a new 96 well DNA extraction protocol that should have speeded up the analysis. Ball bearings were placed in each tube, together with the sample and extraction buffer, then shaken to disrupt the tissue. However, this method gave inconsistent results over three separate DNA extractions. A subset of the plants was independently tested. In parallel a well-tried DNA extraction of the plants was undertaken which generated repeatable PCR results. Results were virtually the same so virus challenge was done when the plants were 23 days old. All lines were homozygous for the virus-resistance gene.

The plants were challenged with BYMV by sap inoculation three times every eight to 11 days. Of the seven lines tested, all but one showed symptoms of BYMV infection on at least one plant. Infected plants were shown to contain the virus in newly emerged leaves by RT-PCR specific for BYMV. The one highly resistant line was tested for presence of BYMV using the same method and none was detected.

The result was surprising because these lines had been chosen from two previous generations of resistant parents. All non-transgenic control plants were infected with BYMV. Some Quillinock control plants showed unusual symptoms and these were tested by ELISA which showed that they were infected with Cucumber mosaic virus (CMV) through the seed. CMV is seed-borne in lupin.

This research is supported by Murdoch University, Department of Agriculture and Food Western Australia (DAFWA) and CLIMA.
All of the important grain legume crops in Australian cool-season agriculture have offshore origins, largely around the Mediterranean basin, and many have comparatively short histories within Australia. These are compelling arguments to study the adaptation of these crops to their target environments, both in Australia and internationally, to understand their potential based on their responses to key stresses faced within the growing season and beyond. This knowledge improves plant breeders’ understanding of their crops of interest, and leads to the production of better-adapted varieties.

This is the research ethos which unites the work undertaken at UWA, DAFWA, CSIRO and our international partners within the GL3 Sub-program, spanning all the major grain legume crops including narrow-leafed lupin, chickpea, lentil, faba bean and lathyrus. The scope of the work is similarly broad, ranging from measuring the effects of modifying enzyme activity in the seed to assessing adaptation in a genotype by environment framework, optimising farm practice with participatory research, and investigating storage effects on seed quality.

**Traits for yield improvement of chickpea for drought-prone environments of India and Australia**

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The project aimed to bring together chickpea breeders and physiologists to study the adaptation of chickpea to water-limited environments in India and Australia. A large genotype by environment study was conducted at seven sites in India and five sites in Australia with over 70 putatively drought-resistant chickpea lines.

The study showed that phenology was central to chickpea adaptation in both countries. Chickpeas that flowered and podded early escaped drought and were the highest yielding in Australia and in southern and central India. In northern India, the highest yielding chickpeas were those that were intermediate in flowering and podding. The project identified genotypes that were regionally adapted to the north or south of India and those that were widely adapted in both countries. Some of the widely adapted genotypes introduced from India are now being used in the Australian chickpea breeding program. None of the Australian genotypes evaluated in India performed well because they were susceptible to Fusarium wilt and/or were too late to flower and pod.
Physiological studies were conducted on a limited number of genotypes under rainout shelter facilities at Merredin in Western Australia, the Indian Institute of Pulses Research (IIPR) in Kanpur, the Indian Agricultural Research Institute (IARI) in New Delhi and at CCS Haryana Agricultural University in Hisar. Methodologies were developed to evaluate a large number of lines for the accumulation of solutes (osmotic adjustment) in breeding programs. The results show that osmotic adjustment in chickpea is poorly inherited and does not correlate with higher yields when the plants are exposed to terminal drought. The project fostered close interaction between Indian and Australian scientists and forged firm scientific and social ties that have led to new collaborative projects between the two countries, and ongoing interest in new work. Moreover, the project has provided guidance to the chickpea breeding programs in India and Australia, and helped shape the nature of CLIMA’s current chickpea research.

This research is supported by the Australian Centre for International Agricultural Research (ACIAR) – (CS1/1996/07).

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**Improvement of salinity and boron toxicity tolerance in chickpea**

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This joint project between CLIMA and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is screening chickpea lines for salinity and boron toxicity tolerance. Salinity is a problem in Australia and southern Asia, while boron toxicity is a problem in southern Australian soils otherwise suited to chickpea.

When grown in a Vertisol soil, screening of 263 accessions of chickpea, including 211 accessions from ICRISAT’s mini-core collection (comprised of 10% of the core collection and 1% of the entire collection), showed a six-fold range of variation for seed yield under salinity (application of 80 mM NaCl at seeding), with several genotypes yielding 20% more than a previously-released salinity tolerant cultivar. While desi genotypes had higher salinity tolerance than kabuli genotypes, the range of variation was similar in both, indicating that breeding for salinity tolerance can be undertaken in both groups.

Biomass in the vegetative stage (50 days after sowing) was not correlated with seed yield under...
Can final seed size in chickpea (*Cicer arietinum* L.) be predicted and easy to maintain under terminal drought?

**PRINCIPAL INVESTIGATORS:** Dr Patrizia Gremigni (CSIRO), Adjunct Prof. Neil C. Turner (UWA/CSIRO)

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Seed size is an important quality trait in chickpea, particularly in kabuli where it is strongly correlated to market value. This project examines the role of sucrose synthase (SuSy) in determining seed size using transgenic lines with different levels of SuSy between tolerant and sensitive genotypes with similar phenology, to produce balanced RILs for future evaluation. The crosses are as follows:

1. Early types: JG 11 (tolerant) * ICCV 2 (sensitive)

The appropriate level of boron to add to the Alfisol soil to induce a 50% reduction in biomass and a 50% reduction in pod number was determined in a glasshouse experiment in 2005/06. Currently, 108 lines are being screened in three replicates in a special salinity/boron toxicity screening facility at ICRISAT for boron toxicity tolerance (15 ppm boron) and for both salinity and boron toxicity tolerance (80 mM NaCl + 10 ppm boron).

This research is supported by the Council of Grain Grower Organisations (COGGO), CLIMA and ICRISAT.
activity. Seed coat and cotyledon dry weights were measured throughout the seed filling phase in three T2 sucrose synthase (SuSy) over-expressing lines obtained from the desi chickpea Semsen. The developmental pattern was similar in Semsen and the transgenic lines and SuSy over-expression had no detrimental effect on seed yield.

There was a positive linear correlation ($r^2 = 0.50$) between seed size at maturity and SuSy activity during rapid seed filling in all three T2 SuSy over-expressing lines, but the over-expression of SuSy had a little beneficial effect on seed size. This confirms previous observations that SuSy measured during rapid seed filling is associated with seed size at maturity. These observations were based on a population of RILs (F7 progeny) derived from 1) two crosses between the large-seeded kabuli type Kaniva and the two small-seeded desi type Tyson and CTS 60543, and 2) with nine chickpea cultivars grown in the field under either irrigated or rain-fed conditions.

Considering the high level of homology among the SuSy sequences of other plant species and the finding of a quantitative trait loci for seed size that maps to the SuSy2 gene in the pea genome, the development of a SuSy gene-based marker for seed size in chickpea breeding appears possible. In preliminary work, primers for areas of high homology of SuSy sequences from available database entries (Arabidopsis, pea, common bean, Medicago) were designed and used to amplify by PCR the genomic DNA of the cultivars Kaniva, Tyson and CTS 60543, parents of the RILs mentioned above. Two distinct DNA bands of about 1000 and 820 bp were amplified from the desi cultivars Tyson and CTS 60543, as well as from the kabuli cultivar Hera (used as the positive control). However, there was evidence for a polymorphism between Tyson and CTS 60543 in the primer binding region of the larger fragment, which was virtually absent from the CTS 60543 amplification products.

This research is supported by the Grain Pool of Western Australia Pty Ltd, CSIRO and CLIMA.

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**Lathyrus and lentil in the cropping systems of Nepal**

**PRINCIPAL INVESTIGATORS:** Prof. Clive Francis (UWA), Dr Renuka Shrestha (NARC), Mr Ram Neupane (NARC), Mr N.K. Yadav (NARC), Dr Ashutosh Sarker (ICARDA)

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Despite a difficult year with the unsettled political situation consequent upon Maoist activity in Nepal, significant progress in most of the six defined objectives has been made. This is thanks largely to the combined efforts of Nepal Agricultural Research Council (NARC) staff. The completion of the project objectives can be achieved in 2006/07.

Disease screening for wilt and Stemphylium blight was largely successful in 2005/06 and comprised a major part of the program. Particularly encouraging was the finding that a number of selections had tolerance of both diseases. These included ILL 6811, ILL6256, ILL7164, ILL8093 and ILL6408. Lines like ILL7982 which were selected for farmer participation on the basis of wilt and Stemphylium did not perform as well in 2005/06 trials which strongly indicates the need for several years of data before final conclusions can be drawn on disease resistance. Nevertheless, the results and associated yield and seed quality data will continue to be the main criteria for introduction to farmer participatory research.

In the different regions of Nepal, farmer participation has been a feature of NARC activities and one strongly encouraged by ICARDA. More than 250 farmers in the various regions received seed samples or participated in NARC trials. Lines like ILL 7723, ILL7164, ILL7982, ILL7537 ILL4402 and ILL7979 have all been distributed to farmers in the Terai (Central, East and West) and the Mid-hills regions. Cases for formal registration of new lines are to be considered prior to next planting season. Already one new cultivar, Shital, has resulted directly from the program.

Lathyrus lines were grown in Perth as an F4 population. Growth vigour and maturity were assessed. After testing for the neurotoxin ODAP, only low ODAP lines are forwarded to Nepal for field evaluation. Pale flower colour which can be used as a marker has also been selected in Perth and 28 F4 lines will be forwarded to Nepal for row evaluation in 2006/07. Seed increase as F5 lines of all lines will be conducted in Perth during 2006 to ensure adequate seed supplies for further bulking by NARC. The F4 population in Nepal failed in 2005 – a combination of drought and herbicide drift.
Better crop germplasm and management for improved production of wheat and barley and pulse and forage legumes in Iraq

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An ACIAR-funded project (CIM/2004/024) commenced in May 2005 with the major objective to identify, promote and widely disseminate among farmers in the rain-fed cropping regions of northern Iraq 'best-bet' improved varieties and crop management systems for wheat and barley and pulse and forage legumes. The key activities include the introduction, evaluation and selection of improved germplasm of wheat, barley and pulse and forage legumes for northern Iraq rain-fed farming systems; development of improved cropping system management options suited to the region; and enhanced capacity of Iraqi research and extension programs to identify and evaluate potentially valuable germplasm and better crop/soil management technologies and promote their adoption by farmers.

To provide background and a base for future impact assessment, a baseline survey was developed and conducted with 260 farmers in July/August 2005 by the Ministry of Agriculture.
Physicochemical changes in faba bean (*Vicia faba* L.) after long-term storage at high temperature

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Legume seeds are mostly preserved in dry storage at ambient temperature. High temperature during storage can reduce legume seed quality. This research investigated physicochemical changes after long-term storage in faba bean (variety Fiesta) at a range of temperatures (5, 15, 25, 37, 45 and 50°C).

**Effect of storage temperature on some physical properties**

High storage temperature caused substantial changes in hydration and swelling coefficients of beans that reflect the capacity to imbibe water in a reasonable length of soaking time. After 18 h soaking at 25°C, hydration and swelling coefficients were significantly lower (p = 0.05) in samples stored at higher temperatures, especially those stored > 37°C, as compared to samples stored at lower temperatures (≤ 25°C).

Solute and electrolyte leakage also increased with increased storage temperature. Faba bean stored at > 37°C exhibited 18 to 36 mg/g solute leakage whereas seeds stored at ≤ 25°C had only 4 to 7 mg/g solute leakage. Bean hardness tested by the hard-to-cook test was substantially affected by storage temperature (r = –0.99). After 8 hours soaking followed by 2 hours cooking, the puncture force required for seeds stored at 5°C was 3.3 Newton/seed whereas seeds stored at 50°C required the much higher puncture force of 15.2 Newton/seed.

**Effect of storage temperature on some chemical properties**

Acid detergent fibre and lignin content increased substantially with storage temperature and suggest that storage of faba bean at higher temperatures (> 37°C) for 12 months causes thickening of cotyledon cell walls, which may be a key cause of deterioration in cooking quality. In contrast, the concentration of total free phenolic constituents (mostly tannins) in the testa substantially decreased with higher storage temperature and time.

**This research is supported by an Australian Research Council (ARC) – Linkage grant, DAFWA, Chemistry Centre (W.A.), CLIMA and UWA.**
Maximising the potential of the L Hist G*E trials to describe lupin adaptation to Mediterranean Australia

PRINCIPAL INVESTIGATORS: Dr Jens Berger (CSIRO), Dr Jairo A. Palta (CSIRO), Dr Bevan Buirchell (DAFWA)

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Over the past decade, lupin yields in Western Australia have been stagnant at 1.1 t/ha and the sown area has declined from a high of 1.3 million ha to 0.5 million ha in 2005. Grower confidence in lupin has decreased because of low profitability associated with low and unstable seed yield and declining prices. We suggest that a focussed approach to documenting lupin adaptation to real world stresses faced throughout their growing region can help guide breeders in producing cultivars for specific environments.

The L Hist trials are a series of 51 site and year combinations of the complete set of lupin cultivars released since 1967 grown in Mediterranean Australia (largely in Western Australia but also in eastern States). Most trial datasets only comprise information on plot yields, but since 2005 CSIRO scientists working under the CLIMA umbrella have been augmenting this with data on: 1) phenology, 2) biomass development throughout the growing season, 3) seed and biological yield distribution across branch orders, 4) harvest index, and 5) yield components collected from each site.

This information will be used to characterise genotypes to investigate how different cultivars are adapted to different types of environment, and demonstrate how varieties have changed over time since the initial release of Uniwhite in 1967. This is important because there is little published work describing adaptation in target environments, and consequently little guidance for breeding programs to produce material targeted at specific abiotic stresses. This is reflected in the prevailing view that there is no specific adaptation in modern lupin cultivars, and that the latest releases will be better everywhere in the lupin growing region.

This research is supported by CLIMA, CSIRO and DAFWA.

"We suggest that a focussed approach to documenting lupin adaptation to real world stresses faced throughout their growing region can help guide breeders in producing cultivars for specific environments."
The objective of the alternate oilseeds project was to establish current and potential markets and strategies for production and handling of the range of alternative oilseeds, which can be brought into commercial production over the next two years.
Development of value-added plant protein products for the aquaculture feeds sector

PRINCIPAL INVESTIGATOR: Assoc. Prof. Brett Glencross (Dept of Fisheries, WA)
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Expansion of the market base for lupin is one of the key objectives for increasing demand for the grain and potentially increasing the price received by the farmer. Lupin is still regarded primarily as a feed grain and therefore market development within the highest paying feed sector will deliver better long-term grain prices. The aquaculture industry is not only the highest priced feed sector but also has been the fastest growing animal production sector, domestically and internationally, for over ten years. To position itself to capitalise on opportunities in this growth, the grains industry has sought to explore the potential for feed grain use in this market sector. The aquaculture feed sector is keen to engage to reduce their risk exposure of heavy dependence on the use of fishmeals, which are an expensive and highly volatile commodity.

To explore the potential of feed grains and lupin, in particular, a range of value-added grain products (kernel meals, protein concentrates and protein isolates) from several grain varieties (narrow-leafed lupin, yellow lupin, pearl lupin) have been developed and evaluated in three aquaculture species (trout, Atlantic salmon, prawn).

Annual workshops have been held since 2003 to promote the technology uptake and seek industry input into the R&D process. The technology has been transferred to grain processors (CBH Group and Weston Technologies) and end-users (Skretting Australia, Ridley Aquafeeds), with significant domestic uptake of the use of lupin kernel meals in aquaculture feed formulations. This has had flow-on effects of stimulating international use of lupin kernel meals in aquaculture feed formulations. Protein concentrate production has seen significant interest in the potential use in human food market sectors.

Following on from industry adoption, key initiatives have focussed on developing quality assessment mechanisms to determine key quality criteria for continued market development and to provide grain processors and end-users with objective means to gauge product value among suppliers and shipments. This has seen the intensive assessment of a small sample-set (75 samples) of lupin kernel meals for the development of near infra-red spectroscopy calibrations for digestible protein and energy from these meals when fed to fish. Sampling has been completed, and calibration is still to be finalised.

This research is supported by the Grains Research and Development Corporation (UWA00062), Fisheries Research and Development Corporation (2004/236), Skretting Australia, Weston Technologies and CBH Group.

Brett Glencross with Barramundi
Improved lupin grain quality and yield through genetic manipulation of key physiological traits

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Despite lupin being a highly useful crop for Australian agriculture, there are several inherently negative characteristics of lupin relative to other legume species. Narrow-leaved lupin has a very thick seed coat, expressed as a percentage of whole seed weight, typically 24% to 25% compared to only 7% in soybean and 9% in field pea. Lupin seed coat is mainly comprised of cellulose associated with hemicellulosic and pectic polymers and reduces metabolisable energy of whole seed for monogastrics. De-hulling is relatively expensive based on current prices received for lupin. Reductions in hull proportion has the potential to channel extra assimilate into kernel or additional seed numbers per pod.

Another characteristic of lupin is the high proportion of dry matter in pod walls. In current varieties of *L. angustifolius*, about 32% to 35% of the pod dry matter is in the pod walls. Corresponding values for other species are 29% in *L. albus* and about 47% in *L. mutabilis*. By comparison, this proportion is as low as 13% in *Pisum sativum* and 24% for *Phaseolus vulgaris*. The project therefore aimed to provide breeders with new *L. angustifolius* breeding lines having lower seed coat or pod wall proportion and altered seed quality (particularly higher protein).

Further aims of the project were to provide alternative lower alkaloid *L. angustifolius* genotypes and reduced shattering *L. luteus* germplasm. There are some indications in the literature and through general breeding experience that the current low alkaloid gene *iucundus* is associated with lower yield potential and with split-seed disorder where manganese is at low levels on infertile sands.

Yellow lupin cultivars and breeding lines currently do not have optimal non-shattering genes for our hot, dry summer finishes to the season. The project aimed to identify better reduced shattering germplasm for use in the yellow lupin breeding program.

The project produced several sources of lower seed coat or pod wall *L. angustifolius* lines that can be used by the breeding program in Western Australia. Lines consisted of lower seed coat (low SC) proportion, single plant derived mutants (originating from a mutation population of a DAFWA breeding line (83A473), crosses (cv. Tanjil and low seed coat wild type x M11257 mutant and wild type x low SC breeding line diallel crosses) and selected reduced seed coat DAFWA breeding lines (e.g. WALUP2049). These lines had seed coat proportions down to 18.0%. Several wild *L. angustifolius* had SC proportions of 19% to 20% and these accessions were from Turkey, Greece and Cyprus. Heritability of mutant and wild type lower seed coat was moderate. Sources of lower pod wall in *L. angustifolius* were entirely from wild type sources in the Australian Lupin Collection.

Pod wall proportions of Greek wild accession P26576 was 25.8% compared to Tanjil at 32.2%. The lower pod wall proportion appears to be primarily due to lighter pod walls. Inheritance of the low pod wall trait in the wild genotype showed broad and narrow-sense heritabilities of 0.51 and 0.44, respectively. Lines resulting from these crosses have lower pod wall proportion as well as fully non-shattering pods. Reducing seed coat proportion of whole seed and pod wall proportion in *L. angustifolius* breeding programs is possible given the existing variation available in wild and mutant germplasm. Crosses with larger seeded breeding lines or cultivars can provide further reductions in seed coat proportion.

The project also identified mutant higher protein (up to 37%) *L. angustifolius* lines in late flowering backgrounds. Introggression of the higher protein will be attempted through crossing to current breeding lines such as Mandelup and Coromup. Mutant and foreign breeding line selections were identified and shown to have different low alkaloid alleles using test crosses.

The project identified the lowest available seed coat and pod wall germplasm for *L. luteus*, a species with a high seed coat (25%) and extremely high pod wall proportion (42%). The variation, however, was not great and lowest value for seed coat was 22.1% in a Portuguese wild type. Screening for lower pod shattering among imported breeding lines and cultivars of *L. luteus* highlighted several lines from Europe and one from USA collections that will be useful for the development of this trait in breeding programs.

This research was supported by the Grains Research and Development Corporation (GRDC) – (UWA0009).
Marketing of alternative oil seeds

**PRINCIPAL INVESTIGATORS:** Ms M.C. Campbell (UWA), Prof. C.M. Francis (UWA)

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The objective of this project was to establish current and potential markets, and strategies for production and handling of the range of alternative oilseeds which can be bought into commercial production over the next two years if prices and demand are established.

New oilseeds, if profitable, are a valuable addition to the farming system in providing alternatives to canola in the rotations. This would greatly aid control of diseases such as black leg to which most of the alternatives are not susceptible. There is the potential to increase national oilseed production where the frequency of canola cropping is limited by the disease. New lines would also provide the potential to grow profitable crops in drier areas (the mustards) and sandy soils (Camelina), environments where current canola cultivars are not well adapted.

The major outcome sought was to integrate commercial control and marketing in a marketing plan developed in conjunction with consultants arranged by the commercial partner Grain Pool of Western Australia. This organisation is currently the largest marketer of oilseeds in Australia. The Grain Pool’s experience in handling of oilseeds in conjunction with partner CBH Group (Australia’s largest bulk handling organisation) was the key contribution to the project.

A market of yellow oriental mustard has been established in south-eastern Asia and its future will depend of the alignment with canola prices. The high Omega-3 oil of Camelina is keenly sought in Europe and an oil shipment of 6 tonnes is planned to UK or Finland which in the future will be seeking larger quantities of this quality oil. There is a clear market for golden linseed in the boutique organic market and non organic forms. Because of much slower seed increase rates than the Brassicas, large scale production will not take place until 2007.

Although a strong demand for industrial high erucic acid oil exists in USA and Europe, difficulties and handling costs of the seed in husks greatly diminishes farmer interest in Australia.

The market for Camelina oil, mustard and golden linseed has been established. Further development will be in the hands of specialist growers in league with marketing companies.

This research is supported by the Rural Industries Research and Development Corporation (RIRDC) – (NPP04-37).

Assessment of molecular responses by fish to inclusion of grain meals as dietary protein sources

**PRINCIPAL INVESTIGATOR:** Assoc. Prof. Brett Glencross (Dept of Fisheries, WA)

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With the adoption of feed grain use by the aquaculture feed sector there has been an increasing level of interest in the overall physiology of the animal, particularly in gut health in response to increased use of these resources. Typically, most fish are not evolved to deal with dietary carbohydrates which are largely treated as xenocompounds within the digestive system. One way to examine the impact of such molecules and raw materials on the animal is to examine changes in protein expression when fed different raw materials.

To examine this, rainbow trout were fed diets containing 30% of two different lupin varieties with soybean meal or solely fishmeal as the protein sources. After a 28-day period on the test diets, samples of liver, intestine and blood were collected from multiple fish (n=3) within each tank and multiple (n=4) tanks for each treatment, processed and sent to France for analysis. In France at the INRA Station d’Hydrobiologie, samples were extracted and electrophoresed using the INRA and Department of Fisheries equipment and expertise.

Analysis was not conclusive due to poor resolution of the electrophoresis gels. Several weeks were
spent trying to resolve the electrophoresis problem and in discussion with French colleagues of outputs achieved. The time in France was inadequate to resolve the electrophoresis problems and to fully complete to work. Subsequent discussion with other researchers in this area revealed that BioRad precast-minigels were not reliable during this period and that this was not an isolated case.

Fortunately, reference samples were kept in Perth in case of problems (loss of samples, electrophoresis problems) in France. These samples are yet to be re-run and are waiting on purchase of new electrophoresis equipment following the breakage of Department of Fisheries equipment during freighting back to Australia.

This research is supported by CLIMA.

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<tr>
<th>Bioactive phytochemical and phytonutrient profiling of lupin, soybean and chickpea</th>
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<tr>
<td><strong>PRINCIPAL INVESTIGATORS:</strong> Dr E. Swinny (CCWA), Dr D. Harris (CCWA)</td>
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<tr>
<td><strong>EMAIL:</strong> <a href="mailto:ESwinny@ccwa.wa.gov.au">ESwinny@ccwa.wa.gov.au</a></td>
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The aim of this project is to establish a comparative phytochemical profile of lupin, soybean and chickpea. Such a profile would provide a valuable tool for legume quality assessment and monitoring of holistic responses of the biological system to external stimuli.

The first groups of phytochemicals under investigation in this project are tocopherols, tocotrienols and folate. These groups of phytochemicals are very topical regarding nutritional aspects and very little is known about them as associated with the above legumes. Therefore, the first stage of this project involved the development of analytical methods for the analysis of tocopherols, tocotrienols and folate. The next group of phytochemicals being examined are the xanthophylls and carotenoids.

There are a number of methods described in the literature for the determination of tocopherols and tocotrienols in foods. These include using gas chromatography and high performance liquid chromatography (HPLC). Gas chromatography sample preparation and pre-treatment prior to analysis is tedious and time consuming. This project has focussed on HPLC methodology. Thus far a method has been developed for alpha, gamma and delta tocopherol using reverse phase HPLC coupled with photodiode array detection. However, UV detection has been unsuccessful at detecting low concentrations of tocopherols in the various extracts of *L. angustifolius*, *L. albus* and *L. luteus* lupin and Kimberley Large chickpea that were analysed. A normal phase HPLC method with fluorescence detection is currently being developed and good progress has been made.
Current analytical methodology for folates involves microbiological, protein binding or chromatographic assays. HPLC allows for quantification of the different vitamer forms of folate. However, current methods require rigorous sample preparation and few vitamer standards are available. Currently, this project is focussing on the development of a method for folates using fluorescence detection.

Regarding the analysis of xanthophylls and carotenoids, selected samples of lupin and chickpea have been extracted with various solvents and analysed by HPLC. Carotenoids have been detected but have not yet been identified. The project is ongoing.

This research is supported by CLIMA.

### MicroRNAs in the phloem of white lupin

**PRINCIPAL INVESTIGATORS:** Ms Caren Rodriguez (UWA), Dr Penny Smith (University of Sydney), Prof. Craig Atkins (UWA).

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The phloem long-distance translocation system of plants functions as both a nutrient delivery system and an information pathway by which signal molecules are disseminated throughout the plant. These signals could include not only traditional signalling factors, such as growth-regulators but also proteins and ribonucleic acid (RNA). A role for RNA as a signalling molecule is emerging.

Different RNA molecules including small RNAs like microRNAs are present in phloem. MicroRNAs are a recently discovered class of small noncoding RNAs that play a significant regulatory role in both plants and animals by targeting messenger RNAs for cleavage or translational repression. In plants, many of the targets of microRNAs are messenger RNAs encoding transcription factors that play a role in developmental processes and may also have a role in regulating plant responses to stress.

We are investigating the transport of microRNAs in *Lupinus albus*, a plant from which phloem exudate can be easily isolated. Phloem sap has been collected by making shallow incisions in different organs of the plants. The presence of microRNAs in phloem of *L. albus* had already been confirmed through cloning and hybridisation approaches in a previous study, suggesting that they could be transported.

In the current study, grafting experiments are being employed to investigate microRNAs translocation through a graft junction in the model plant *Arabidopsis thaliana* for which mutants in microRNA biogenesis and action are available.

According to the preliminary results, movement of microRNAs through a graft junction does not seem to be evident. Gene expression in phloem vascular tissue in *L. albus* is also been studied using laser capture microdissection technique. We are also investigating small RNA binding proteins in lupin phloem exudate. These proteins could be involved in microRNAs trafficking within the plant.

This research is supported by the Australian Research Council (ARC), Scholarship for International Research Fees and University Postgraduate Award UWA.

Phloem sap exudate collected from *Lupinus albus* after making shallow incisions in pods and inflorescences.
Background

The harshly Mediterranean climate of south-western Australia with hot dry summers has favoured the development of ley-farming systems based on annual pasture species. Despite the unequivocal success of subterranean clover and annual medics, several contemporary factors have challenged their sustainability. These include excessive dependence on herbicides, incomplete use of water and nutrients, pest and diseases, lack of persistence under intensive cropping and the high cost of seed production.

\[\text{Trifolium spumosum L.}\]

These challenges have been a focus of the scientific research effort by CLIMA and its funding partners over the last two decades. A second generation of new annual legume species has now been developed with a suite of desirable characteristics to help overcome these challenges. Five new species are now successfully in commerce over 1.5 million ha in Western Australia: yellow serradella (*Ornithopus compressus*), French serradella (*O. sativus*), gland clover (*Trifolium glanduliferum*), biserrula (*Biserrula pelecinus*) and balansa clover (*T. michelianum*). CLIMA partners are now seeking to maintain their past outstanding successes in commercialising new annual pastures and several new species are at an advanced stage of testing.
The adoption of these new pasture species has steadily increased in recent years, helped by rising prices for meat and wool, the increased demand for legume nitrogen in cropping systems and the need to control herbicide resistant crop weeds in a pasture phase. Pasture based farming systems have also continued to evolve in tandem with new opportunities created by species and cultivar development. One such development is the concept of phase farming where short periods of pasture are used tactically to break up extended cropping sequences. The development of aerial seeding legumes that can be cheaply harvested by conventional header harvesters has been critical for this development. The success of the Pasture Legume Program is testament to the ability of the research team to recognise constraints to production in the field and link them to particular plant attributes that can be incorporated into new cultivars effective on a commercial scale.

Activities

Genetic resource enhancement of key annual pasture legumes continues to be a major component of CLIMA activities through the Department of Agriculture and Food Western Australia (DAFWA). In this, the network of international linkages established by CLIMA plays a major role. Collection tours of Cyprus, the Cyclades Islands (Greece), Eritrea, Israel and Armenia have been completed with an emphasis on forage legumes. These collections currently under assessment by Mr R Snowball, Curator of the National Trifolium collection, have resulted in an influx of new and novel germplasm – more than 800 new accessions, to accompany the priority species from the older germplasm collection. Work is continuing on the development of core collections for key species that encompass more than 70% of the variability of all accessions. Molecular techniques such as amplified fragment length polymorphism (AFLP) are now being used to complement eco-geographical data using bladder clover as a model species.

The Western Australian component of the National Annual Pasture Legume Improvement Program (NAPLIP) led by DAFWA was concluded in June 2006 after several cycles of funding (Grains Research and Development Corporation and Australian Wool Innovation) over nine years. Nationally, over 20 new cultivars were released, many of which were initially developed as a key part of the first phase of CLIMA. A number of novel species are also at an advanced stage of testing including Eastern star clover (T. dasyurum), bladder clover (T. spumosum) and Lotus ornithopodoides, all with potential roles in cropping systems on fine textured soils. In conjunction with Victorian collaborators, DAFWA is also developing selections from another CLIMA import, Moroccan clover (T. isthmocarpum) which has tolerance of waterlogging and mildly saline soils.

The movement toward new and novel legumes is challenging, particularly as there is a need to demonstrate that sufficient ‘duty of care’ has been undertaken prior to commercialisation. This is expensive in terms of funds and manpower. There is also a need to research problems as they arise post commercialisation. In this context CLIMA, through DAFWA and the Chemistry Centre (W.A.), is currently researching the issue of occasional photosensitivity in sheep grazing biserrula pasture. An analysis of the secondary plant compounds in biserrula is a major component of this study.

Two pasture cultivars were released jointly with DAFWA in 2006 following a project supported by the Rural Industry Research and Development Corporation (RIRDC) to overcome constraints to seed production in purple clover (T. purpureum) and sulla (Hedysarum coronarium). Purple clover cv. Electra™ should be highly productive in medium and high rainfall areas for grazing as well as hay production over a range of soil types. Sulla cv. Flamenco® is a highly productive, short-lived perennial species for neutral to acid fertile soils in medium to high rainfall areas and may have useful anthelmintic properties. With the establishment of the CRC for Plant Based Management of Dryland Salinity, species tolerant of saline and/or waterlogged soils are now being developed. While perennial species are favoured for high water use, in the central and northern areas of the Western Australian grain belt, the hot dry summers reduce their impact and vigorous annuals able to survive into the early summer may well have a role, either alone or in mixtures.

Pasture or forages for pharmaceutical use, especially those which have high contents of phytoestrogens or other flavonoids developed by CLIMA, are in use in commercial red clover health products. They and other species are demonstrating significant anti-cancer cell activity in a RIRDC-supported project. Other research includes the quest for pasture species with special nutritive...
features for health, meat, wool, milk, or aquaculture production. (e.g. anthelmintic activity, essential fatty acids). Assessments of the nutritive value of new pasture species are being simplified through the development of near infra-red spectroscopy (NIRS) technology by CLIMA through CSIRO Livestock Industries.

### Pasture Legume Sub-programs

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<tr>
<th>Sub-program</th>
<th>Leader</th>
<th>Major research components</th>
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<tr>
<td>PL1</td>
<td>Germplasm Development for Pasture Legumes</td>
<td>Mr Richard Snowball DAFWA</td>
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<tr>
<td></td>
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<td>- Collections in new locations</td>
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<td></td>
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<td>- Eco-geography, characterisation and evaluation of new germplasm</td>
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<td>- Core collection technology</td>
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<td>PL2</td>
<td>Pasture Biotic Interactions</td>
<td>Dr Hayley Norman CSIRO</td>
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<td></td>
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<td>- Defined NIR technology for pasture quality</td>
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<td></td>
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<td>- Definition of specific nutritive or biotic advantages</td>
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<td>- Relative feeding value of new pasture species for meat and wool production (duty of care)</td>
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<td>PL3</td>
<td>Annual Pasture Legume Improvement</td>
<td>Dr Clinton Revell DAFWA</td>
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<td></td>
<td></td>
<td>- Agronomic evaluation of species adapted to acid and neutral soils</td>
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<td>- Research into variation in key adaptive characters of the test species</td>
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<tr>
<td>PL4</td>
<td>Novel Uses of Forage Legumes</td>
<td>Prof. Clive Francis UWA</td>
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<tr>
<td></td>
<td></td>
<td>- Novel pasture/forage legume alternatives</td>
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<td>- Legumes with pharmaceutical or aquaculture advantages</td>
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“Pasture or forages for pharmaceutical use, especially those which have high contents of phytoestrogens or other flavonoids developed by CLIMA, are in use in commercial red clover health products.”
The Australian Trifolium Genetic Resource Centre (ATGRC) hosted by the Department of Agriculture and Food Western Australia (DAFWA) is part of the Australian National Genetic Resource Centre or Network. It has the national mandate for Trifolium, Ornithopus and other species generally adapted to acid soils. Other genera including annual and perennial, exotic and native pasture legume species are also held. Details of holdings can be found at [www.agric.wa.gov.au](http://www.agric.wa.gov.au) (search on ‘genetic resource centre’). Germplasm of tropical forages and pasture species adapted to alkaline soils are held in other genebanks in the national centre. The Australian Grains Research and Development Corporation (GRDC) provides funding for the operation of the National Genetic Resource Centre.

Pasture germplasm development in CLIMA is currently supported by activities connected with the ATGRC. Core activities include germplasm acquisition, characterisation, conservation and distribution. These support research and development by any bone fide researchers from recognised research organisations anywhere in the world. However, much of the germplasm and its associated information are used for local plant improvement programs. Germplasm acquisition through collecting seeds from the wild has been a continuing activity with recent missions focussing on short season environments in the Mediterranean region. One such mission undertaken in 2005 (not reported here) was successful in collecting early flowering accessions of *Trifolium* species from Cyprus and the Greek island of Folegandros. The collecting of native forage species from rangeland areas in Western Australia has also contributed to holdings in the ATGRC.

Systematic and in-depth morphological characterisation of species collections has for the most part taken second stage to the preliminary evaluation and conservation of newly collected germplasm. However, recent activities have focussed on establishing more complete character data sets. New germplasm of *Biserrula pelecinus* collected from Morocco, Spain, the Canary Islands and Eritrea was characterised in 2005 by Nadia Bazihizina, a visiting undergraduate student from the University of Florence (not reported here). A core collection can now be developed using eco-geographic and morphological data to aid in future germplasm selection. The entire collection of *Trifolium spumosum* was characterised in 2004 and will be used to authenticate a core collection based on eco-geographic and molecular data. A new project to commence in 2007 managed by Kioumars Ghamkhar will develop a core collection of *Trifolium subterraneum* using the most up-to-date technologies. Germplasm conservation and distribution are continuing activities of the ATGRC and provide the necessary support for all germplasm users, now and well into the future.

An emerging challenge to the introduction of pasture species not present in Australia is undertaking a weed risk assessment to enable their introduction. This has been made all the more difficult due to Biosecurity Australia adopting a fully species-based system of plant introduction and their conservative approach to weed risk assessment. Patrizia Gremigni has undertaken many weed risk assessments of pasture species over the past 12 months with financial support from Australian Wool Innovation. The list of species that researchers discover during overseas visits which show considerable potential continues to grow.

The core activities of the ATGRC continue to be undertaken by Kris Gajda with financial support from the GRDC. Ben Cohen and Brad Wintle completed their work with the Centre in January and June 2006, respectively.
Germplasm collection of *Trifolium* and other pasture legumes species from short season, low latitude regions in the Mediterranean

**PRINCIPAL INVESTIGATORS:** Mr Richard Snowball (DAFWA), Assoc. Prof. Mike Ewing (DAFWA), Mr Kris Gajda (DAFWA), Mr Brad Wintle (UWA), Dr Sarita Bennett (UWA), Mr Graeme Sandral (UWA), Mr Brad Nutt (DAFWA), Prof. Clive Francis (UWA), Mr Mehreteab Aberra (DAFWA).

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Collecting missions were undertaken in Israel and Morocco in 2006, the last year of this project. Earlier missions to Lebanon, Spain, the Canary Islands, Morocco and Eritrea have been reported in previous CLIMA biennial reports.

Phillip Nichols and Richard Snowball spent two weeks collecting pasture legumes in Israel in May 2006. They were accompanied by Rivka Hadas, Curator, Volcani Genebank; Shmuel Galili, Volcani Center; and Jaime Kigel, Hebrew University. Local botanist Yair Ur with assistance from fellow botanist Dvora Schitzer led the mission. Some seeds were also provided by Mimi Ron, Curator, Mt Scopus Botanic Garden in Jerusalem.

According to *Flora Palaestina* many pasture legume species with potential can be found in Israel. Existing seed collections of *Trifolium* species originating from Israel have shown great promise in recent plant improvement programs in Western Australia. Israel also offers a great diversity in climate and landform including very dry, semi-desert environments in the south, a humid Mediterranean climate in coastal areas, warmer climates inland at low altitudes on the Jordan Valley, and a colder montane climate in the northern highlands.

Much of the country was covered during the collecting mission. However, there was a focus on the coastal strips between Tel Aviv and the Gaza and north of Netanya to Haifa, the Golan Heights, adjacent Galilee, and the dry area south of the West Bank between the Gaza and the Dead Sea. Despite the high level of industrialisation and urbanisation, natural environments with a high species diversity protected from overgrazing were found. A total of 562 accessions from 104 species and 25 genera were collected from 55 collection sites. Of the 562 accessions, 483 were annual pasture legumes, 29 were perennial pasture legumes, 39 were annual grain legumes, 10 were unidentified legumes and one was a non-legume herb.

The most significant discoveries include *Biserrula pelecinus* collected from 15 sites from the Golan Heights to the Central Negev Desert; *Trifolium spumosum* collected from 25 sites in 10 districts nearly doubling the existing collection; *T. glanduliferum* collected from 10 sites in Upper Galilee and the Golan Heights; *T. dasyurum* collected from 16 sites from the Golan Heights to the Northern Negev, and *T. purpureum* the most common species collected from 31 of the 55 sites visited.

Daniel Schafferman from Israeli Gene Bank, is acknowledged for his assistance in cleaning and preparation of seed prior to sending to Australia. Yoram Kapulnik and Avi Perevolotsky, Volcani Center, were excellent hosts during our visit.

Nezha Saidi, Curator of the forage genebank in Rabat, organised a follow-up collecting mission to areas of interest identified in the previous mission to Morocco in 2004. She was accompanied by Chaouki Al Faiz, Imane Thami Alami, E. El Mnouar, A. Dam, K. Bakhy, H. Ouabbou and H. Hilali. Provinces visited included Khemisset east of Rabat in the Middle Atlas, Kenitra just north-east of Rabat, Tanger and Larache in the north, and Agadir near the Anti Atlas in the south. Over seven days, 134 accessions of pasture species were collected from 29 sites. Six new accessions of *Biserrula pelecinus* were collected including one from the Anti Atlas site at a latitude of 30 degrees and 3 minutes north and an altitude of 740 m. Other species of interest collected include *Lotus ornithopodioides*, *Ornithopus compressus*, *Trifolium cherleri*, *T. isthmocarpum*, *T. resupinatum* and *Anthyllis tetraphylla*.

High priority species from both collections in Israel and Morocco will be grown and characterised in field plots at the Medina Research Station, Perth in 2007.

"High priority species from both collections in Israel and Morocco will be grown and characterised in field plots at the Medina Research Station, Perth in 2007."
Improving the utilisation of germplasm of *Trifolium spumosum* L. by the development of a core collection using eco-geographical and molecular techniques

**PRINCIPAL INVESTIGATORS:** Dr Kioumars Ghamkhar (UWA), Mr Richard Snowball (DAFWA)

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A core collection is a limited number of accessions derived from an existing germplasm collection and represents the genetic spectrum in the whole collection. There is no question of the need for core collections from large germplasm collections. However, only a few core collections in the world have been developed using molecular markers only and have resulted in some disappointing outcomes. This reflects the need for better exploitation of these techniques together with morphological and eco-geographical data to increase the level of confidence in the reliability of core collections. The future of large germplasm collections, therefore, will depend on how well these data are exploited.

Although techniques such as microsatellites, inter simple sequence repeats, random amplified polymorphic DNA, restriction fragment length polymorphism, and a dozen other markers can be used, amplified fragment length polymorphism (AFLP) is overall one of the fastest and most informative techniques for high throughput molecular characterisation of germplasm.

The aims of this work are:

1. to develop a core collection of *Trifolium spumosum* to provide to the breeders the smallest number of accessions with the maximum diversity as a model for other pasture legume species, and

2. to set up a methodology for core collection development in this pilot study to be adopted in larger germplasm collections of other pasture and grain legume species.

A total of 398 accessions from the Australian ex situ collection of *T. spumosum* were investigated for complete or near complete passport/eco-geographical information. The near complete information was completed using ArcGIS, Geography Network, Encarta World Atlas and Global Gazetteer, and were double-checked in Google Earth for their location. The collection of 317 accessions with complete eco-geographical information was completed using ArcGIS, Geography Network, Encarta World Atlas and Global Gazetteer, and were double-checked in Google Earth for their location. The collection of 317 accessions with complete eco-geographical

Kioumars Ghamkhar, Richard Snowball and Bradley Wintle in the field deciding on Agromorphological traits
and passport data was then stratified into five eco-regions and two sub-eco-regions. MStrat Software was used to select a preliminary core of about 30% of the collection (95 accessions). To include all potential diversity in this subset, breeders were asked to nominate accessions with possible agronomic value and these accessions were added to the 95 accessions.

Fluorescent AFLP markers were used and processed in an ABI 3730 DNA analyser followed by further processing by GeneMapper software. Mstrat, and NTSys-pc software are being used to develop a final core collection containing 30% of the preliminary subset. This core collection of 32 accessions will be selected using AFLP and eco-geographical data. The final subset (core collection) should cover the highest diversity with lowest repetition within the smallest collection size. At the final stage of the project, this core will be validated using morphological data in MStrat. This research will also identify deficiencies in genetic diversity resulting in better targeted collecting.

This research is supported by the Grain Research and Development Corporation (GRDC) – (UWA00005).

Methods for molecular characterisation of *Trifolium spumosum* L. germplasm

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There are several methods for screening genetic diversity within a germplasm collection using molecular markers. Methodologies vary from bulking all individual samples of each accession to choosing a number of individual plants without bulking. Only a few studies have adopted the latter, due to financial constraints and even those studies that have adopted this methodology have mostly failed to justify the logic behind the chosen number of the individual plants. *Trifolium spumosum* shows a broad morphological diversity within accessions. Thus it is important to investigate the need for individual sampling and the optimum number of individuals for molecular characterisation of this species. Establishing the best methodology is crucial to the success of the main GRDC-funded project (UWA00005) aimed at developing a core collection.

A subset of the original germplasm selected on the basis of eco-geographical data was characterised by fluorescent AFLP markers using both a number of individual plants and their bulk. The selected plants represented a range of morphological variation within and between the accessions of the species. Result showed that using five, six or seven plants gave the highest level of discrepancy in DNA banding patterns with minimum cost. As there was only a slight difference in the level of discrepancy between these numbers, it was decided to adopt the most economic sampling strategy of five individual plant leaf tissues accompanied by a bulk of them. This bulk of the five individual plants was also used to compare the combined data of the individuals with data from the bulk.
This result has been adopted for the whole eco-geographical subset in the GRDC project (UWA00005). This will enable the capture of maximum molecular diversity in *T. spumosum* with minimum costs.

**This research is supported by CLIMA.**

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**Morphological characterisation of *Trifolium spumosum* L. germplasm collection**

**PRINCIPAL INVESTIGATORS:** Mr Richard Snowball (DAFWA), Mr Bradley Wintle (UWA), Dr Kioumars Ghamkhar (UWA)

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The Australian Trifolium Genetic Resource Centre holds approximately 16,000 accessions, mostly of species of *Trifolium* and *Ornithopus* collected from wild environments of countries with a Mediterranean climate. Over the past 10 to 15 years efforts have been directed to collecting, growing and conserving new germplasm to support the research and development of alternative pasture species with potential. Consequently, little comprehensive morphological screening of entire species collections at the one time has been undertaken. *Trifolium spumosum* L. is one of a few alternative species that has been the focus of recent collecting and field evaluation. A project aimed at improving the utilisation of the collection of this species through the development of a core collection is near completion at UWA. A thorough morphological analysis of the germplasm was undertaken simultaneously to add significant value to the final core collection.

A total of 11 morphological characters and their states were developed and are described below.

<table>
<thead>
<tr>
<th>Character</th>
<th>Character states (CTS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf marks</td>
<td>4 CTs subdivided to 8 sub-CTs (discrete)</td>
</tr>
<tr>
<td>Leaf size</td>
<td>10 – visual rating</td>
</tr>
<tr>
<td>Flowering time</td>
<td>Quantitative (discrete)</td>
</tr>
<tr>
<td>Stem habit</td>
<td>5 – visual rating</td>
</tr>
<tr>
<td>Stem thickness</td>
<td>Quantitative (continuous)</td>
</tr>
<tr>
<td>Stem number</td>
<td>5 – visual rating</td>
</tr>
<tr>
<td>Corolla colour</td>
<td>3 – qualitative (discrete)</td>
</tr>
<tr>
<td>Calyx pigmentation</td>
<td>6 – visual rating</td>
</tr>
<tr>
<td>Seed number</td>
<td>Quantitative (discrete) per pod</td>
</tr>
<tr>
<td>Seed yield per flower head</td>
<td>Quantitative (continuous)</td>
</tr>
<tr>
<td>Individual seed weight</td>
<td>Quantitative (continuous)</td>
</tr>
</tbody>
</table>

Six plants from each of 320 accessions from the germplasm collection were grown and characterised at the Medina Research Station south of Perth. The collected data will be used:

- to validate the first subset developed using eco-geographical data
- to combine with molecular and eco-geographical data to form the final core collection for the species.

This will later be compared with a core collection based on molecular and eco-geographical characterisation. In this way the use of molecular methods can be authenticated by checking that morphological diversity is captured. This work is directly linked to project UWA00005 (above).

Most of the data collection was undertaken by Brad Wintle while Kris Gajda contributed in the later stages of the project. Valuable advice on the development of character states was provided by Brad Nutt and Angelo Loi.

**This research is supported by CLIMA.**
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The Pasture Biotic Interactions Sub-program PL2 conducts research into the relationships between pasture legumes and other living organisms in their environment. Research areas include the plant–livestock interface, diet selection, nutritional and anti-nutritional properties of forage, insect resistance, rhizobial interactions and viral diseases. Other research includes the quest for pasture species with special nutritive features for health, meat, wool, milk or aquaculture production. (e.g. anthelmintic activity, essential fatty acids). We continue to develop near infra-red spectroscopy technology as a means of rapid and inexpensive screening of large numbers of plant genotypes.

SUB-PROGRAM PL2 – Pasture Biotic Interactions

Using stable carbon isotopes to measure short-term diet selection in sheep grazing annual legumes with either saltbush or subtropical perennial grasses

PRINCIPAL INVESTIGATORS: Dr Hayley Norman, Mr Matt Wilmot, Dr Dean Thomas, Mr Allan Rintoul, Dr Dean Revell (all CSIRO Livestock Industries) with support from Mr Tim Wiley (DAFWA), Ms Lidia Bednarek (UWA), Mr Neil Ballard (Ballard’s Seeds)

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There is increasing impetus to incorporate perennial plants such as shrubs and temperate and subtropical grasses into farming systems in southern Australia. Unfortunately, many of these perennial plants have poor nutritive value so they tend to be grown in combination with high value annual legumes. These systems require careful management to prevent over-grazing of the annual legume component and to maximise livestock utilisation of the perennial component.

The performance of livestock grazing forage mixtures largely depends on the relative intake – abundance and timing – of the components in the mixture. A better understanding of diet selection and utilisation will allow targeted improvement in animal performance and profitability.

Unfortunately, techniques to measure diet selection in grazing ruminants are often inaccurate, time consuming and expensive. This small project was initiated to explore the stable carbon isotope technique as a means of predicting short-term diet selection by sheep grazing mixed pastures.

Hayley Norman with sheep used in a CLIMA-funded carbon isotope study
The stable carbon isotope technique is based on differences in carbon isotope accumulation in plants with different photosynthetic pathways. About 99% of carbon in nature is the $^{12}$C isotope. The C3 pathway of photosynthesis discriminates against $^{13}$C in favor of $^{12}$C much more than the C4 pathway. This leads to different isotope ratios in the plant biomass and influences isotope ratios throughout the food chain. Most of the annual legumes that are used in farming systems in southern Australia have C3 photosynthetic pathways while a number of the perennials grasses and shrubs that are planted by farmers have a C4 photosynthetic pathway.

The aim of this study was to test proof-of-concept of the stable carbon isotope technique as a practical tool for estimating the relative proportion of an individual ruminant’s diet that is legume hay, subtropical perennial grass or saltbush. We were particularly interested in which animal tissues or byproducts provided the most accurate prediction of intake and the time it takes for tissues to reflect a change in diet. This will provide a basis to design grazing systems with appropriate combinations of forages to provide a suitable diet for the animals while achieving the goals of profitability and sustainability. It also provides a valuable research tool to researchers.

In summary we found that $\delta^{13}$C of faeces, wool, plasma, urine, rumen solids, rumen liquor and urine can all be used to predict the relative consumption of plants with either C3 or C4 photosynthetic pathways in the diet of sheep fed known mixtures for a period of 18 days. While all tissues allowed differentiation between diets, only faecal and rumen samples had reached isotopic equilibration within the experimental period (equilibration of 7 days). When rumen or faecal samples were used, the technique was very accurate, predicting the diet with less than 5% error. Plasma and wool samples did not reach isotopic equilibrium within 18 days but may be used to rank animals within flocks for longer-term dietary preferences.

This research is supported by CLIMA.
The Annual Pasture Legume Improvement Program draws together research activities that focus on the breeding and selection of new pasture legumes for Western Australian farming systems. Emphasis is placed on overcoming the constraints in current cultivars as well as considering the needs of future farming systems. This has required the innovative use of species not previously used in managed agriculture.

Research in the sub-program is also concerned with the impact of pasture legumes on key sustainability parameters such as water use and the integration of legumes, including lucerne, into cropping systems of Western Australia.

Twenty-one new cultivars were commercialised nationally during this project:

- Urana<sup>a</sup> (SE003), Napier<sup>a</sup> (YL012), Coolamon<sup>a</sup> (SM012), Izmir<sup>a</sup> (SE008) and Mintaro<sup>a</sup> (BM031) subterranean clovers
- Mauro<sup>b</sup> (LCP7/16) biserrula
- Yelbin<sup>a</sup> (GEH72.2a) and King (87GEH74A) yellow serradellas
- Erica<sup>a</sup> (FHS3) and Margurita<sup>a</sup> (FHS7) French serradellas
- Prima<sup>a</sup> (CPI 87182) gland clover
- SARDI Persian (SA33798) and Lusa (H14172) Persian clovers
- Wilpena<sup>a</sup> (E4) and Moonbi<sup>a</sup> (G7) sullas
- Bindaroo (SA 8460) button medic
- Cavalier<sup>a</sup> (Z1186) and Scimitar<sup>a</sup> (Z497) burr medics
- Toreador<sup>a</sup> (Z1065) Medicago tornata x M. littoralis hybrid
- Jester<sup>a</sup> (Z194) barrel medic
- Jota<sup>a</sup> sweet clover (Melilotus albus).

The Western Australian project team made a major contribution to development of the majority of these cultivars. A number of other lines have been selected for release as new cultivars in 2007, pending the outcomes of duty of care trials. These include GCN39 eastern star clover (Trifolium dasyurum), CFD27 bladder clover (T. spumosum), SA5045 Trigonella balansae and KRC-2 balansa clover (Trifolium michelianum).
Six new cohorts have been field tested since 2004. These cohorts consist of *Lotus ornithopodioides*, purple clover, midseason Moroccan clover, late flowering French serradella, midseason and late flowering RLEM seedling tolerant subterranean clovers. Glasshouse screening to measure seedling resistance to red-legged earth mite (RLEM) has been conducted on 840 pasture legumes in this project. Screening for clover scorch resistance has also been conducted on 506 lines. Targeted characterisation to date has been conducted on 852 lines in the following species: purple clover, *Trifolium cherleri*, *T. spumosum*, *Hymenocarpus circinatus* and French and Moroccan serradellas.

Considerable efforts have been made to promote NAPLIP products to industry over the last two years with presentations to major industry field days and demonstration sites established in conjunction with key growers groups. In a survey of Western Australian wheatbelt farmers in 2005, only 31% of all new pasture sowings were the traditional subterranean clover and annual medics. The balance was sown to new species developed by NAPLIP including French serradella 25%, biserrula 17% and yellow serradella 10%.

This research was supported by the Grains Research and Development Corporation (GRDC) – (UWA 360) and Australian Wool Innovation (AWI).

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### Seed production limits sulla and purple clover as fodders

**PRINCIPAL INVESTIGATORS:** Assoc. Prof. Mike Ewing (UWA), Mr Peter Skinner (DAFWA), Dr Phil Nichols (DAFWA), Mr Richard Snowball (DAFWA)

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This four-year project sought to identifying sources of genetic variation in sulla (*Hedysarum coronarium*) and purple clover (*Trifolium purpureum*) that overcome seed production problems that limit the current commercial use of these species. The project was concluded in March 2006 and culminated in the successful release of two improved cultivars, Flamenco\(^A\) (HRN83-A) sulla and Electra\(^TM\) (139465NM) purple clover.

Sulla is a biennial species adapted to fertile neutral and alkaline soils. Flamenco\(^A\) sulla was selected out of an accession collected in Tunisia by Dr Walter Graves (USDA) and was chosen on the basis of high forage and seed production, confirmed over three years of field testing. This variety is moderately hard-seeded and can be de-hulled with serradella processing technology.

The project also identified a selection of genotypes with low levels of hard seed at harvest time and is the subject of a new research program.

Purple clover is a long season annual legume. Electra\(^TM\) purple clover was selected from an accession collected in Turkey and was chosen on the basis of high seed yield and greater ease of seed extraction from the calyx (threshability). This variety also combines greater tolerance to clover scorch disease (*Kabatiella caulivora*). Prior to this project, tolerance to clover scorch was not known in purple clover and the disease was regarded as a key constraint to its wide-scale use. A second round of plant selection in purple clover has been undertaken and a cohort of lines was tested nationally through NAPLIP.

The research was supported by the Rural Industries Research and Development Corporation (RIRDC) – (UWA 65A) and CLIMA.
**e-Variety Profiler for annual legume pastures**

**PRINCIPAL INVESTIGATORS:** Dr Perry Dolling (DAFWA), Dr James Fisher (DAFWA)

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The e-Variety concept has been successfully developed for cereals and grain legumes to package information in a user-friendly electronic format. The e-Variety Profiler for Cereals is based on an Excel spreadsheet and captures variety specific information on all agronomic characteristics including flowering time, herbicide tolerance and pest and disease tolerance. This becomes a useful decision support tool for variety selection.

This project sought to extend the e-Variety concept to annual legume pastures in order to provide an easy electronic reference to help growers make decisions about pasture species and cultivar selection. Subterranean clover has been used as a model species and agronomic information packaged in a stand-alone HTML format. A prototype has now been produced and awaits further development for inclusion of other pasture species. The concept version allows a user to enter the database using different entry points including annual legume species or variety, rainfall region or location, farming system and soil type.

*This research was supported by CLIMA.*

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**Enhanced capacity for plant chemistry analysis associated with duty of care conducted on new pasture plants**

**PRINCIPAL INVESTIGATORS:** Dr Clinton Revell (DAFWA), Dr Ewald Swinny (CCWA), Dr Shao-Fang Wang (CCWA)

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An appropriate duty of care process needs to be demonstrated as a central element of the development and commercialisation of new species and cultivars of annual pasture legumes. This requires an assessment of the positive and negative impacts on the environment and the grains and livestock industries. There is also a need to respond to problems that may emerge after cultivars are commercialised and adopted by industry.

Many of the risks for animal health are usually related to ‘secondary plant compounds’, which are defined as compounds that are not directly part of the plants’ normal biochemical pathways, but are involved in adaptation of plants to their environments. This project examined the plant chemistry of two annual legumes, biserrula (*Biserrula pelecinus*) and bladder clover (*Trifolium spumosum*).

Biserrula has been in commerce for over six years but in recent years a number of outbreaks of photosensitisation have been reported. Investigations to date suggest it is likely to be a primary photosensitivity arising from secondary plant compounds. Solvent extracts from biserrula were analysed by high performance liquid...
chromatography (HPLC) and liquid chromatographic mass spectrometry for phenolics (flavonoids, coumarins, flavonols, flavones), chlorophylls, carotenoids, pyrrolizidine alkaloids and riboflavin. The flavones were identified as apigenin and luteolin (and their glycosides) but there are no reports in the literature relating these compounds to photosensitivity in grazing animals. No difference in phenolic, carotenoid and chlorophyll profiles was evident in extracts from high and low risk plant samples. The analysis also suggests there are no coumarins, furanocoumarins, pyrrolizidine alkaloids or riboflavin present in biserrula. Further work is required to establish the identity of the compound(s) responsible for photosensitivity in biserrula.

Bladder clover is at an advanced stage of testing but routine testing for isoflavones revealed the presence of some unidentified compounds. Solvent extracts (including dichloromethane, methanol, 70% methanol and 30% methanol) from bladder clover were prepared. The chemical profile studies by HPLC indicate that bladder clover contains coumarins, dicoumarins, flavonoids, isoflavonoids and tannins, with dicoumarin as the major constituent in the plant. The impact of this compound on animal production needs to be investigated.

This research was supported by CLIMA.

**Comparative soil water use and water extraction of recently developed annual pasture legume species and cultivars**

**PRINCIPAL INVESTIGATORS:** Mr Darryl McClements (DAFWA), Dr Perry Dolling (DAFWA), Dr Clinton Revell (DAFWA)

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Annual pasture species with the capacity for extended spring growth are likely to use more water and to slow groundwater recharge. They may also produce more biomass and maintain quality for longer into the summer. Late maturity and deep roots are plant characters likely to enhance extended spring growth. The work described has been undertaken to measure water use of recently developed annual pasture legume species and cultivars. These combine extended flowering with deep rooting capabilities (yellow serradella, biserrula) and are compared to traditional species such as subterranean clover, volunteer annual pasture and lucerne perennial pasture.

A mid-slope site with a deep duplex sandy loam soil profile was selected south-east of Wickepin, Western Australia. Neutron probe access tubes were installed to a depth of 2 m in the centre of 20 m x 3 m plots.

Pasture treatments were established in autumn 2005 and included *Biserrula pelecinus* cv. Casbah, *Ornithopus sativus* cv. Margurita/Erica mix, *Trifolium subterraneum* cv. Dalkeith, *Trifolium vesiculosum* cv. Cefalu, *Medicago sativa* cv. Scepter and volunteer pasture. Water use by the long season annuals at the end of the first growing season was similar to lucerne and greater than subterranean clover and volunteer pasture. Summer rainfall in 2006 was uncharacteristically high and contributed to increasing soil water under all treatments except lucerne.

Clearly, perennial pastures have much better capabilities to maintain soil water deficits than annual pastures (particularly after summer rainfall) but long season annuals do dry the soil to greater depth than unimproved pasture and subterranean clover.

This research was supported by CLIMA.
Pasture and forage legumes have traditionally been used in Australia as a source of feed for grazing animals. Other countries, notably China, have long exploited a range of legumes for their pharmaceutical and nutraceutical benefits. Following the trends in Europe and the United States, there is now a rapidly increasing demand for legumes in Australia. CLIMA has an advanced program in the production of red clover with enhanced phytoestrogens which are now in commerce. It is exploring the considerable genetic resource available for wider opportunities in the field. Progress in selection of legume extracts with anti-cancer activity has produced promising results and will be extended to include an extensive range of forage legumes. Success in selection of novel legumes will add to options for farmers to exploit high value products from environmentally friendly plants.

Pasture or forages for pharmaceutical use, especially those which have high contents of phytoestrogens or other flavonoids developed by CLIMA, are in use in commercial red clover health products. They and other species are demonstrating significant anti-cancer cell activity in a RIRDC-supported project. Other research includes the quest for pasture species with special nutritive features for health, meat, wool, milk, or aquaculture production. (e.g. anti-helminthic activity, essential fatty acids would be assessed in a wide range of species). CLIMA is also investing in the development of rapid analytical techniques as well as finetuning existing techniques used to determine the nutritive value of pastures.

### Investigation into legumes with pharmaceutical and aquaculture potential

**PRINCIPAL INVESTIGATORS:** Dr Shao Fang Wang (CCWA), Prof. Peter Leedman (UWA), Dr Kevin Foster (DAFWA), Ms Viki Russell, Assoc. Prof. John Howieson (Murdoch), Prof. Clive Francis (UWA)

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Legumes have been used as traditional medicines in China, India, Egypt, and other Asian countries. Recently, there is a large untapped potential for the utilisation of forage legumes in industries other than mainstream agriculture as a source of protein, dietary phytohormones and new drugs. Two outstanding market opportunities present themselves:

- the extraction of phytohormones and the development of new drugs
- aquaculture feeds for the developing intensive fish and prawn feed industries.

Both activities require a fundamental understanding of the chemical make-up of the target legume and the utilisation of functional bioassays.

**Research aims**

1. Develop a capacity for chemical analysis of legumes such that species from our unique genebank with valuable pharmaceutical or aquaculture benefit can be developed.
2. Undertake chemical identification of isoflavonoids and bio-active compounds from legumes and assess their efficacy in mammalian breast/prostate cancer proliferation assays.
3. Generate a basic knowledge of the chemistry of annual pasture legume germplasms such that cultivars can be developed to suit specific industries.
4. Develop cultivars for specific industries.
5. Develop a preliminary understanding of the agronomy of the new species.

Based on our legume expert’s experiences on phytoestrogens and background knowledge, and following interrogation of the literature and seed availability, legume seeds were selected from genetic research centres within Australia and grown in the DAFWA field station. The plants

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**SUB-PROGRAM PL4 – Novel Uses of Forage Legumes**

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**CLIMA BIENNIAL RESEARCH REPORT 2005-2006**
covered over 160 legume accessions from 29 genera and 47 species. 220 crude alcoholic and aqueous extracts were prepared at the Chemistry Centre (W.A.) for screening with an anti-proliferative activity test against human breast and prostate cancer cell lines at the Western Australia Institute for Medical Research. Some extracts were prepared from different parts (green tissue, seed, root). One-third of crude extracts demonstrated significant anti-proliferative activity against breast and prostate cancer cell lines. Some extracts have IC50 value of less than 20 ug/ml, and a few extracts with IC50 value lower than 2 ug/ml. The anti-cancer activity varied with the plant genus, species and variety.

Several crude extracts with strong anti-cancer activity were selected for further fractionation to identify active compounds. By using the bioassay-guided fractionation approach, several compounds were isolated and their structures were determined using spectroscopic methods. These compounds have shown strong anti breast and prostate cancer activity in vitro, some up to 100 times stronger than biochanin A and genistein. The potential use of these compounds and their derivatives as a drug should be further investigated. In vivo tests with these compounds for anti-cancer activity is strongly recommended as a next step to develop lead compounds for the pharmaceutical industry. A toxicity study and structural and activity relationships need to be followed up.

This project also studied flavonoids and isoflavonoids for anti-proliferative activity against breast and prostate cancer cell lines. The activity of these compounds depends on the structural type, substitute groups and positions. The relationship between structures and activities was suggested.

Pasture legume seeds are an under-evaluated resource. Our chemical analysis of the nutritional and anti-nutritional values of seed of more than 40 pasture legumes showed that some legume seeds have high protein content, good fatty acid profiles, good nutritional value and low anti-nutrition characteristics. These legume seeds have great potential in aquaculture feed. A fish feeding trial with a legume seed is currently being developed by a Masters project at Curtin University of Technology. A further grant is needed to study a fish feeding trial with a few legumes, fish biology, digestion, and properties of protein and seed powder.

This research is supported by the Rural Industries Research and Development Corporation (RIRDC) – (UWA 73A) and CLIMA.
The provocative research into human therapeutics suggest isoflavones may offer a number of health benefits related to heart disease, osteoporosis, menopause symptom relief and possibly cancer. Soya bean is the best-known form of dietary isoflavone and raw products contain up to 0.2% isoflavone. Other legumes (notably some of the Trifolium species) have much higher levels in their leaves (2% or more). The presentation of the principal types of phytoestrogens (isoflavones) in a variety of pharmaceutical formulations such as tablets and capsules is being undertaken in Australia and the United States by the company Novogen Ltd. These are based on red clover and utilise lines with enhanced isoflavones selected by the CLIMA/DAFWA program.

We appreciate the support Novogen has given over the past 11 years with which we have achieved the aims of modifying and enhancing the isoflavone content by some 200% of key red clovers such as Quinqueli and Redquin. This very successful research partnership will in the future be seen as an excellent working model of how our university and industry can work efficiently together in new research ventures. While we believe there is an opportunity to improve Pawera isoflavones considerably, we realise that the economic priority for Novogen is to more sharply increase free isoflavones in the hay. Technology needs to be explored for maximising the percentage of leaf blade and preconditioning the freshly harvested leaf to reach Novogen’s 1.5%-free aim. We suggest that a visit to Switzerland and Sweden which currently market pure isoflavones would be useful and are exploring our contacts in these countries.

▲ Suite of legumes growing at Medina Research Station

In the future we should discuss the value to the wider industry of a strain like Genstar Null which is quite unique and likely to be sought by farmers. It has extremely low formononetin and is easily distinguished by its lack of leaf mark. Arrangements to share royalties may be explored under PBR. In addition, the promising anti-cancer activity in some of our plant extracts are under study as part of the RIRDC-funded project ‘Investigation into legumes with pharmaceutical and aquaculture potential’.

**This research is supported by Novogen Ltd.**
Scientific Journal Articles


Agricultural Research


**Review Articles, Books and Book Chapters**


**Conference Publications**


**Technical Publications**


### VISITORS TO CLIMA – 2005-2006

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<tr>
<th>Name</th>
<th>Institution</th>
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<tbody>
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<td>Dr Heather Clarke Dr Jon Clements</td>
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<td>Department of Agriculture and Food WA and Pulse Association of the South East</td>
<td>Dr Debbie Thackray</td>
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<td>Minister for Agriculture and Food, Government of Western Australia</td>
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<td>Protein Research Foundation, South Africa</td>
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<td>Seeds of Life project, East Timor</td>
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## THESES PASSED – Postgraduate Students 2005-2006

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<tr>
<th>Name</th>
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<tr>
<td>Dr Oonagh Byrne</td>
<td>Incorporation of pea weevil resistance from wild pea (<em>Pisum fulvum</em>) into cultivated field pea (<em>Pisum sativum</em>)</td>
<td>Dr Penny Smith Dr Guijun Yan Dr Darryl Hardie</td>
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<tr>
<td>Dr Renuka Shrestha</td>
<td>Adaptation of lentil (<em>Lens culinaris medikus</em>) to rainfed environment – response to water deficits</td>
<td>Prof. Kadambot Siddique Prof. Neil Turner Assoc. Prof. David Turner</td>
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CLIMA's mandate

“To apply leading edge science to the problems and priorities identified by the Western Australian grain and pasture legume industries with the objective of creating value for the industries and the wider community.”

CLIMA’s vision

“Innovation in Legume Science and Technology”

A Centre of Excellence in grain and annual pasture legume research and development that leverages the strengths of its partners to address the problems and priorities of the Western Australian grain and pasture legume industries – to be achieved through strategic scientific research and development, linked to an applied base.

The Centre intends to be a world leader in problem-focused legume research and will achieve this by drawing on the expertise within its four partner organisations.

CLIMA’s objective is to add value to the activities of its clients, core partners and staff and in doing so, maximise the benefits of co-operation and co-ordination of research.

2005-2006 BIENNIAL RESEARCH REPORT

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Acronyms

AAAC Australian Association of Agricultural Consultants
AARI Aegean Agricultural Research Institute
ACIAR Australian Centre for International Agricultural Research
ARC Australian Research Council
ATFCC Australian Temperate Field Crops Collection
ATGRC Australian Trifolium Genetic Resource Centre
AusAAD Australian Agency for International Aid Development
AWA Australian Women in Agriculture
AWCC Australian Winter Cereals Collection
AWI Australian Wool Innovation
BARI Bangladesh Agricultural Research Institute
CCWA Chemistry Centre (WA)
CDCCrop Development Centre, University of Saskatchewan
CLIMA Centre for Legumes in Mediterranean Agriculture
CGGGO Council of Grain Grower Organisations Ltd
CSIRO Commonwealth Scientific and Industrial Research Organisation
DAFWA Department of Agriculture and Food Western Australia
DEST Department of Education, Science and Training
DNRF Department of Natural Resources and Environment, Victoria
GRDC Grains Research and Development Corporation
HNU Haryana Agricultural University, Hissar, India
IARI Indian Agricultural Research Institute, New Delhi
ICARDA International Centre for Agricultural Research in Dry Areas
ICRISAT International Crops Research Institute for the Semi-Arid Tropics
ICPR Indian Institute of Pulses Research
INRA Institute of National Institute for Agricultural Research
IOPR Institute of Oil and Pulse Research, Gulbarga, Karnataka, India
JNKVV Jawaharlal Nehru Krishi Agricultural University
LWRC Land and Water Research and Development Corporation
MAFF Ministry of Agriculture, Forestry and Fisheries, East Timor
NARIP National Annual Pasture Legume Improvement Program
NARC Nepal Agricultural Research Council
NFIP National Faba Bean Improvement Program
NSF National Science Foundation, USA
PAU Punjab Agricultural University, India
QDPI Queensland Department of Primary Industries
RARS Regional Agriculture Research Station, Ishurdi
RIRDC Rural Industries Research and Development Corporation
SARDI South Australian Research and Development Institute
USDA United States Department of Agriculture
UWA The University of Western Australia
VIDA Victorian Institute for Dryland Agriculture

Abbreviations

AFUP amplified fragment length polymorphism
BCMV Bean cucumber mosaic virus
BGM Botrytis grey mould
BWYV Beet western yellow virus
BYMV Bean yellow mosaic virus
CMV Cucumber mosaic virus
DH doubled haploid
HPLC high performance liquid chromatography
ICM integrated crop management
MFLP Microsatellite-anchored Fragment Length Polymorphism
NIR near infra-red reflectance
NIRS near infra-red spectroscopy
NLL narrow-leaved lupin
OFDTs on-farm demonstrations and trials
PCR polymerase chain reaction
RLEM red-legged earth mite
RDA ribonucleic acid