

A POSSIBLE HERBICIDE OPTION FOR CHICKPEA: METRIBUZIN AS POST-EMERGENT



PHOTO 1: (L to R) one tolerant accession and three susceptible chickpea cultivars to metribuzin post-emergent at 200 g/ha.

by Ping Si

Recent research conducted at CLIMA has found that two chickpea elite accessions were tolerant to metribuzin post-emergent at a rate similar to that used in narrow-leaved lupin (Photo 1). All chickpea cultivars were susceptible to the same rate of metribuzin applied post-emergent. This finding provides a promise that metribuzin can be used as a post-emergent herbicide to control wild radish and other broad-leaf weeds in chickpea.

Chickpea has been a poor cousin of narrow-leaved lupin in terms of herbicide options for weed management. There are very limited options for post-emergent herbicides in chickpea, making weed control very difficult. Broad-strike® can be used in chickpea for post-emergent application, but quite often it causes crop damage and weed control is inadequate. There are pre-emergent herbicides available to chickpea. They do their jobs at sowing and at very early stage of seedling growth. As chickpea has slow early growth, weeds germinated after the crop emergence would become dominant in the

paddock. This lack of weed control during the post-emergent results in chickpea crops infected with wild radish (Photo 2). Having a good post-emergent herbicide and chickpea cultivars with improved herbicide tolerance would ensure its seed yield and its place as a valuable rotational crop in the Australian dryland farming system.

Metribuzin is a versatile herbicide and has been used either pre-emergent or post-emergent in various crops for many years. It controls wild radish and other broad-leaf weeds when applied post-emergent. When applied pre-emergent, it retards growth of grass weeds. Metribuzin is registered in chickpea to use only as pre-emergent, but safe as post-emergent in narrow-leaved lupin. It can also control the weeds resistant to Group B herbicides which are widespread in the wheat-belt. Among the weeds which developed resistance to common herbicides, fewer weeds were found to be resistant to metribuzin. Metribuzin is still effective in controlling broad-leaf weeds.

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The two tolerant accessions of chickpea were identified after screening 100 germplasm accessions with a diverse genetic and geographic background from across the world. Nature has evolved some herbicide tolerance in chickpea. Luckily, these two tolerant accessions also have good agronomic characteristics. They provide the foundation for developing chickpea cultivars tolerant to metribuzin post-emergent so that this herbicide can be used safely in chickpea. We are collaborating with the national chickpea breeding program to make good use of the metribuzin tolerant chickpeas.

Germplasm screening is part of the continued endeavour, funded by GRDC, to improve herbicide tolerance in pulse crops of chickpea, field pea and lupin.

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DIRECTOR'S REPORT



Prof. William Erskine

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During the period I attended the International Conference on Grain Legumes - Quality Improvement, Value Addition and Trade at Kanpur, India from February 14 to 16, 2009 to present a plenary lecture on Approaches to Base Broadening in Grain Legumes co-authored by Drs Byrne, Clarke, Clements and Si. At the same Conference the former CLIMA Director Professor Kadambot Siddique was presented with a Gold Medal from the Indian Society of Pulse Research and Development by Dr APJ Abdul Kalam, former President of India. I also visited the National Botanical Research Institute in Lucknow, where I gave a seminar on CLIMA's research.

Pulse breeding in Australia is now dominated by Pulse Breeding Australia (PBA). To increase the integration of pre-breeding at CLIMA with mainstream pulse breeding, CLIMA has sought closer links with PBA. Following my visit to the PBA meetings in Adelaide in March 2009, CLIMA has been invited to be an Observer to the PBA Board and a participant to PBA Coordination Group meetings for a period of 12 months. This arrangement will allow time to identify future possible synergies between CLIMA and PBA. At the same meetings in Adelaide CLIMA scientists Drs Heather Clarke, Ping Si and Janine Croser were invited to present their on-going pre-breeding research to link with PBA breeders.

During April I visited East Timor, where CLIMA's largest project the Seeds of Life II funded by ACIAR/AusAID, is active to become familiar with in-country activities. The project targets food security in East Timor and is embedded within the Ministry of Agriculture and Fisheries. To date the project has made dramatic research

progress by introducing and testing a range of germplasm for key staple crops. Extensive evaluation by farmers has revealed dramatic yield advantages over the local cultivar in maize of 53%, in peanut of 31%, in rice of 23% and in sweet potato of 80%, accompanied by improvements in size and eating quality averaged over on farm trials (> 170 on-farm trials/crop). The project is set to profoundly affect food security in East Timor.

I attended a workshop of The Global Partnership Initiative for Plant Breeding Capacity Building (GIPB) hosted at Bioversity International in Rome 7-8 May 2009 to develop the curriculum for an e-learning course in pre-breeding. The GIPB was formed at the Food and Agriculture Organization (FAO) of the United Nations, Rome, because of the worldwide shortage of plant breeders in both the developing and developed world. I was invited to present the overview of 'Issues in Pre-Breeding' at the workshop. This e-learning course is targeted at developing-country breeders, gene bank managers, university professors, and associated fields, and will be also useful to students at CLIMA and UWA.

Dr Hulya Sipahi (Central Research Institute for Field Crops, Ministry of Agriculture and Rural Affairs, Ankara, Turkey) visited CLIMA for training in legume molecular genetics from January to March 2009 (see page 7).

A major milestone in the period was the movement of Dr Oonagh Byrne from CLIMA to DAFWA in April. Oonagh was with CLIMA in various capacities since 1997. She was a full-time researcher on two consecutive GRDC funded grants (1997 – 2004) on the topic of pea weevil resistance in field pea and did her PhD on the "Incorporation of pea weevil resistance from wild pea (*Pisum fulvum*) into cultivated field pea (*Pisum sativum*)" completing in 2005. She worked as a research associate in a joint CLIMA/Plant Biology ARC-Linkage project entitled "Fast tracking pea weevil resistance into field pea cultivars through interspecific hybridisation" which started in June 2007. In order to facilitate the continued input of Oonagh into the project she has been appointed an Adjunct Research Fellow to CLIMA. As the ARC project continues until June 2011 her place has been taken by Dr Nader Aryamanesh. Dr Nader Aryamanesh is no stranger to CLIMA having done his PhD on Chickpea *Ascochyta* blight with Dr Heather Clarke. Welcome back on board – Nader! (see page 8)

Dr Shyama Weerakoon joined CLIMA to work in the GRDC-funded Break Crop Herbicide Tolerance project from March 2009 (see page 8). Welcome - Shyama.

A NEW HERBICIDE OPTION FOR CHICKPEA

continued from page 1

This breakthrough has been a team effort involving Dr Ping Si (CLIMA), Drs Yinglong Chen and Shyama Weerakoon (CLIMA), Profs William Erskine (CLIMA) and Steve Powles (WAHRI) together with the national chickpea breeding program, who supplied the germplasm. We are currently conducting detailed experiments to characterise the tolerant accessions.

PHOTO 2: Chickpea crops infested with wild radish



RESEARCH REPORTS

HYBRID BREAKTHROUGH COULD BOOST PROSPECTS FOR LUPINS

by Jon Clements, Leah Leong, John Quealy, Larissa Prilyuk, Hua'an Yang, Bevan Buirchell & Gordon Francis

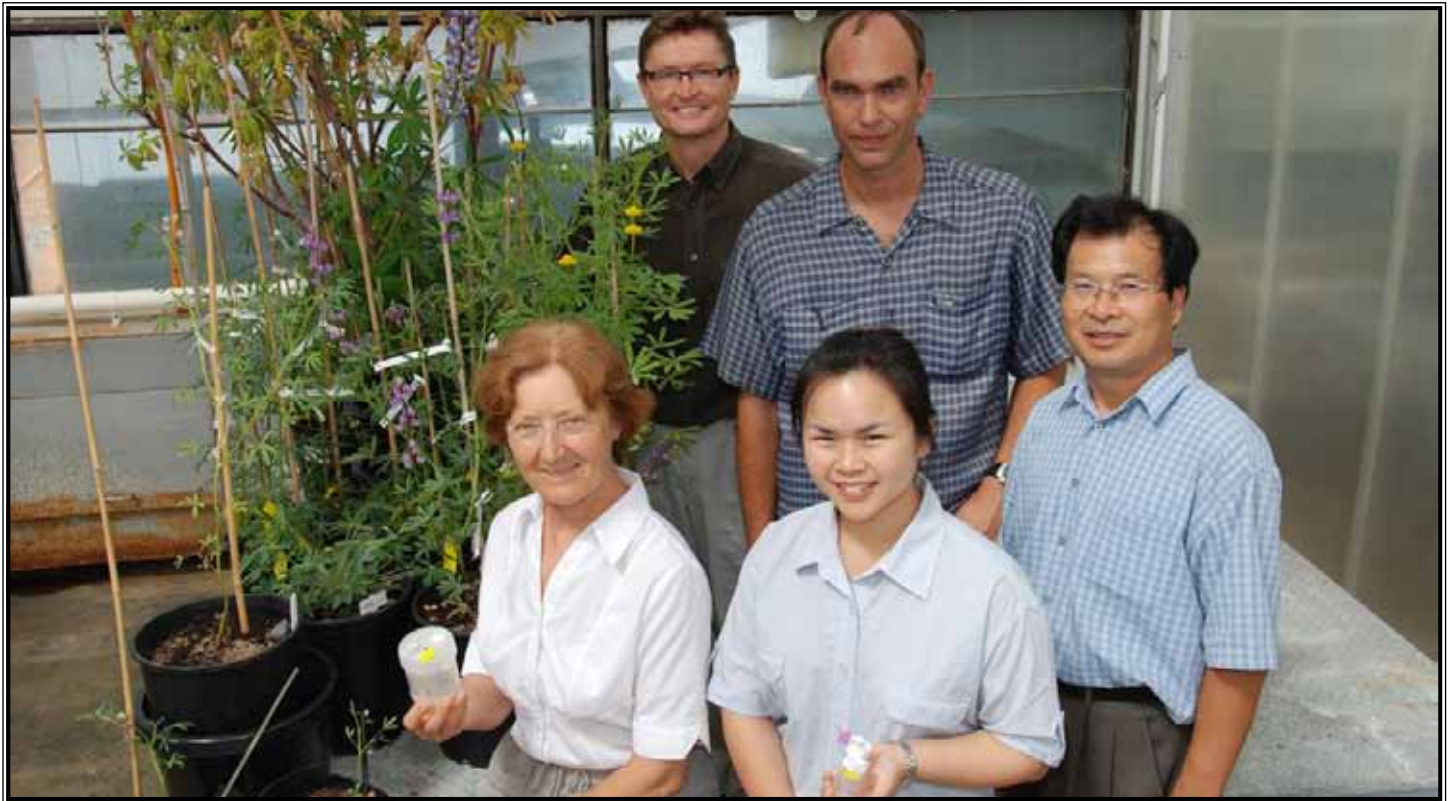


Photo by Brendon Cant & Associates

Pictured are (l to r): Dr Larissa Prilyuk, Ms Leah Leong; Rear (l to r): Dr Jon Clements, Mr John Quealy, Dr Hua'an Yang with a flowering hybrid *L. angustifolius* x *L. luteus* plant and tissue culture plantlets which will be bred further to generate breeding lines with what it is hoped will possess superior traits in lupins.

A research group at CLIMA, The University of Western Australia, have produced hybrid plants that can provide a pathway to transfer best plant characteristics between narrow-leaved lupin and yellow lupin. The GRDC-funded project has attempted a number of cross combinations among the four crop lupin species with an emphasis on narrow-leaved lupin (*Lupinus angustifolius*), the most important grain legume in Australia due to its adaptation to infertile sandy soils, reasonable tolerances to diseases and pests and its use as a break crop. Yellow lupin (*L. luteus*) has a superior seed quality to narrow-leaved lupin but it has lower yield and suffers from susceptibility to anthracnose and aphid damage. The team made approximately 1600 crosses during 2008 and it was not until December 2008 that we finally had confirmation that some of those crosses had produced embryos which were true hybrids. Through the crossing of specific genotype combinations and refining the embryo rescue methods in tissue culture, a few flowering F₁ hybrids between *L. angustifolius* x *L. luteus*, have been produced. It had been a difficult process because these two species have quite different chromosome numbers.

This world-first breakthrough in producing flowering hybrid plants between these two species opens up the opportunity to allow the transfer of traits from *L. luteus* to *L. angustifolius* or vice-versa and this would make the crop much more attractive to growers through potentially increased market value of grain in the case of narrow-leaved lupin or improved plant traits and yield for yellow lupin. Project leader, Dr Jon Clements, said that "the breakthrough had been a team effort involving research assistants Ms Leah Chong, John Quealy, Dr Larissa Prilyuk, Gordon Francis and molecular breeder Dr Hua'an Yang". Confirmation of the hybrids was by visually intermediate plant characteristics and also through molecular marker analysis. Plants have a pale yellow and pink appearance which is intermediate between the pink-flowered *L. angustifolius* parent that was used and the bright yellow flower colour of *L. luteus*.

GRDC has provided funding to continue this research for another 3 years. The hybrids will be backcrossed to Western Australian lupin cultivars to incorporate them into the breeding program run by

Senior Plant Breeder at the Department of Agriculture and Food Western Australia (DAFWA), Dr Bevan Buirchell. The tracking of introgressed genes from either species will be aided by molecular marker assisted breeding in molecular geneticist Dr Hua'an Yang's laboratory at DAFWA and further cytogenetic work at UWA. CLIMA Director, Professor William Erskine, expects lupin growers in Australia to be keen to see grain values increase for lupins. The research outcomes provided here enhance the prospects of increasing protein and sulfur amino acids in narrow-leaved lupin and therefore grain value.

Dr Clements acknowledged the financial support of GRDC during the 3 year project. "We also want to thank past project team member Dr Julia Wilson for her input in the first two years of the project, Mr Damber Shrestha who assisted with the crossing effort, and UWA project collaborators Dr Bevan Buirchell, Dr Heather Clarke, Professor Craig Atkins, Professor John Kuo, Dr Mark Sweetingham and Professor Kadambot Siddique for their input to make this outcome possible" said Jon Clements.

RESEARCH REPORTS

BIOTECHNOLOGY BOOST FOR PROMISING ALTERNATIVE OILSEED CAMELINA

by Janine Croser, Kioumars Ghamkhar, Nader Aryamanesh, Margaret Campbell, Clive Francis

Camelina sativa (False Flax, Gold of Pleasure) is an ancient oilseed crop recently identified by UWA researchers as a promising, high omega-3 alternative to canola (*Brassica napus*) for cool temperate environments in Southern Australia.

Historically, camelina has been used as food oil in Europe and is currently registered as commercial food oil in many European countries and Canada. Camelina contains an exceptional amount of the omega-3 fatty acids, up to 45% of total seed oil. The total seed oil content is around 40% and the remainder of the seed can be used for meal for human consumption and stock feed. The oil also contains a unique antioxidant complex of tocopherols, carotenoids, and phosphatides conferring resistance to heat and rancidity.

Camelina also has desirable agronomic traits, which make it compatible with reduced tillage systems. It is non-shattering, has a fast early growth rate and is very hardy, enduring cold as well as heat and drought. Importantly, camelina has high resistance to blackleg under Australian conditions, yields comparable to canola, requires a low seeding rate and competes well with weeds due to early vigour. These factors indicate camelina would be compatible with environmental goals of reducing energy, herbicide/ pesticide use and protecting soils from erosion.

There are early indications camelina can also be used to make biodiesel, an environmentally friendly alternative to diesel fuel. The lower production inputs mean it may be produced for significantly less than other biodiesel crops, making the fuel competitive in price with its petroleum counterpart.

CLIMA researchers have access to a substantial germplasm collection of this species brought to CLIMA from the Vavilov Institute, St Petersburg, Russia (as part of successive RIRDC projects NPP02-30 and NPP04-37). Breeders have identified a critical need for access to biotechnology tools in order to fully exploit the potential available within the camelina germplasm.



Camelina (Camelina sativa) flowering in the field in WA

A UWA small grant has enabled us to i) undertake a fluorescent AFLP screening and analysis of genetic diversity available within the accessions of *C. sativa* held at UWA and ii) ascertain the potential for applying *Cruciferae*-based doubled haploid development protocols to camelina.

We have found a very high degree of dimensionality in the data of band frequencies. Two groups of accessions were identifiable on a scatter diagram of the first two dimensions of the Principal Coordinate Analysis (PCoA). The molecular diversity also showed some relationship with geographical distribution but strong relationship with climatic conditions. Three accessions also were identified as outliers by the PCoA.

We have used the AFLP inferred genetic diversity results to select three diverse

parents for experiments aimed at adapting doubled haploid protocols from related species (*Brassica napus* and *Brassica rapa*) to *C. sativa*. We have successfully linked morphological size to microspore developmental stage and have undertaken a series of experiments to identify the necessary triggers and culture conditions to switch from gametic (normal) to sporophytic (haploid) developmental pathways. Induction of haploid development has been achieved and efforts are now focused on the development of haploid embryos and plants from these induced cells.

The information and methodology developed in this project will be used in follow-up breeding projects to select optimal parents and rapidly produce adapted varieties with desired quality characteristics.

RESEARCH REPORTS

PARTNERSHIP BETWEEN NARC, CLIMA, ICARDA AND ACIAR
HAS DELIVERED SUPERIOR LENTIL VARIETIES TO NEPAL

by Clive Francis



Dr Renuka Shrestha (kneeling) with Nepali women farmers in a lentil field. Dr Shrestha completed her PhD in 2005 on lentil adaption to drought at The University of Western Australia with a John Allright Fellowship from ACIAR

Nepal Agricultural Research Council (NARC) has released ILL 7982 (FLIP96-50L) and ILL 6829 (FLIP89-71L) with the names *Maheshwar Bharati* (named after the first coordinator of the national grain legumes research program) and *Sagun* (gift/good luck), respectively in 2008. During an ACIAR supported project supervised by Professor Clive Francis (CLIMA) these varieties were bred at the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria largely through the efforts of Dr Ashutosh Sarkar who is now based in India. Dr Sarkar succeeded current CLIMA Director, Professor William Erskine, as lentil breeder in the highly successful ICARDA program. They were introduced to NARC, Nepal through International Nurseries as LISN-SL 1996 and LIYT 1996. These varieties are released for Kathmandu Valleys and similar environments, and the mid hills and river basin of Nepal where crop duration is about 30 days longer as compared to terai/ inner terai, the traditional growing area. As

Stemphyllium blight and wilt root rot are becoming severe in terai/ inner terai, popularization of these varieties plays a significant role in lentil production in the mid hills where the incidence of these diseases is negligible. Maheshwar Bharati and Sagun matured in about 160 days with average yields of 1450 kg/ha and 1350 kg/ha, respectively. These varieties are also performing well in the lower altitudes of the terai which borders India and is the source of major lentil production.

Farmers of Lalitpur, Bhaktapur, Palpa, Dhankuta and Ramechhap districts are very much encouraged to include lentil in their cropping system. Farmers preferred these varieties due to high yield (25-140% higher), large seed size (10-35% larger) and low wilt and root rot diseases as compared with check variety Simal (LG 7). Moreover, the short plant stature of Maheshwar Bharati, makes it resistant to lodging in excessive moisture and high fertility conditions.

RESEARCH REPORTS

GENERATION ACCELERATION PROTOCOL (GAP) – A NOVEL WAY TO ACCELERATE LUPIN BREEDING

By Janine Croser, Christine Munday, Bevan Buirchell
and William Erskine

In plant breeding, it is of great value to accelerate generations by shortening each cycle to enable faster fixation of new genetic traits. As yet there is no doubled haploid technique for lupin, which would be the fastest route to a true breeding plant. In lupin breeding Dr Bevan Buirchell (DAFWA) uses single pod descent under field conditions to achieve two generations per year.

We are currently developing an *in vitro* based generation acceleration protocol (GAP) to double the current rate of generation turnover for lupin.

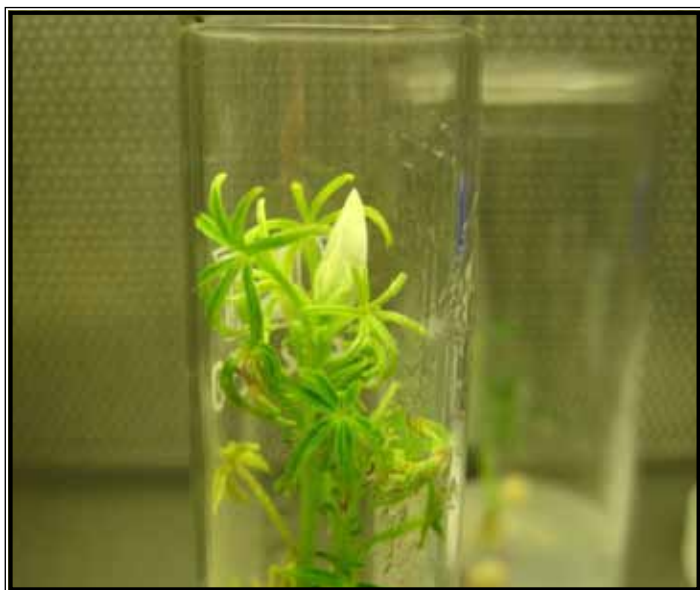
The GAP is applied as follows: seeds are sterilized, placed into culture and then subjected to appropriate light, temperature and medium composition regimes to induce flowering in the shortest possible time frame.

Following *in vitro* seed set, the immature embryos are dissected from the seed coat and then returned to *in vitro* conditions for germination, growth and seed set.

The process is repeated as many times as is required to obtain the desired generation. At the final generation, seed is allowed to fully mature *in vitro* and is then returned to the conventional breeding programme.

Recent advances in this technology overseas have shown it can be used to advance as many as eight generations per year in the early flowering field pea cv. Frisson and more than ten in *Arabidopsis* (Ochatt *et al.*, 2009).

CLIMA researchers have successfully induced *in vitro* flowering and seed set in *Lupinus angustifolius* L. and are currently optimising conditions to reduce generation time and widen the protocol to a range of genotypes.



Narrow leafed lupin cv. Tanjil flowering *in vitro*

COMPARING THE OLD AND THE NEW ... A 'P'PhD

by Federico Ribalta



Generation of homozygosity and genome fixation in field pea
(*Pisum sativum* L.)

Federico Ribalta joined the Centre for Legumes in Mediterranean Agriculture (CLIMA), University of Western Australia, in April 2008. He became a research officer on the ARC Linkage Project (0562111) "Accelerating the genetic improvement of grain legumes for Australia by developing doubled haploid technology for field pea and chickpea".

Federico was awarded a Master of Agricultural Sciences at the National University of La Plata, Argentina (March 2008). During his Masters studies, he carried out a research project on the "Characterisation of different regenerants and parental genotypes of symbiotic mutants of pea (*Pisum sativum* L) and *Medicago truncatula Gaertn*" in collaboration with the National Agricultural Research Institute in France (INRA) and supervised by Dr Sergio Ochatt (INRA, France).

Federico has recently been granted a scholarship from the Grains Research & Development Corporation (GRDC) to pursue his PhD studies jointly with CLIMA and the School of Plant Biology, University of Western Australia. The subject of the PhD research is "Generation of homozygosity and genome fixation in field pea (*Pisum sativum* L.)"

During his PhD research Federico will test the hypothesis "legume doubled haploids (DH) have a great potential value in plant breeding" by comparing DH-derived material with recombinant inbred lines (RILs) produced using the conventional methodology of single seed descent (SSD). DH- and SSD- derived populations will also be compared with RILs obtained using novel *in vitro* flowering and seed set technology for shortening of generation time. The strategies will be compared on the basis of economic (time and cost) and genetic (integrity of regenerants) outcomes.

His PhD research will be co-supervised by Dr Sergio Ochatt (INRA, France), Dr Janine Croser (CLIMA), Prof. William Erskine (CLIMA) and Assoc. Prof. Patrick Finnegan (Plant Biology, UWA).

VISITORS

MOLECULAR BIOLOGY TRAINING FOR TURKISH VISITOR

By Kioumars Ghamkhar

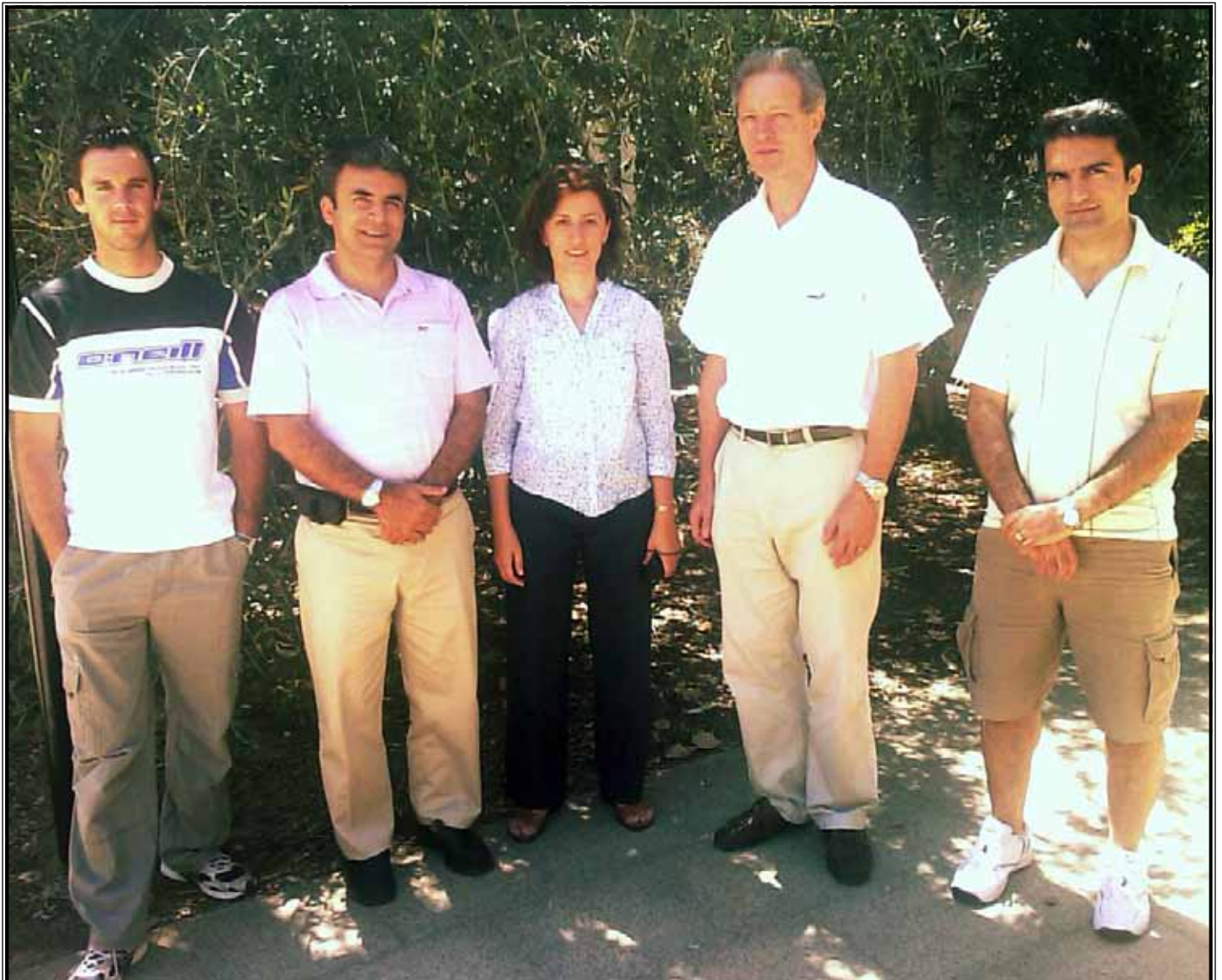
Dr Hulya Sipahi (Central Research Institute for Field Crops, Ministry of Agriculture and Rural Affairs, Yenimahalle, Ankara) is a biotechnologist working on doubled haploidy and molecular markers in barley who recently visited CLIMA for three months.

In the past, Hulya has been interested in grasses (particularly barley) in Turkey. Recently, she has been asked to start research on molecular markers in legumes, many of them originating from Turkey or its neighbouring countries. Hulya had a good background in molecular biology and it helped her going through the intense training, smoothly. Her passion for molecular biology and legumes indicated Hulya would be an excellent contact and collaborator for future joint projects between UWA and Turkey.

Dr Ghamkhar spent substantial time working closely with Dr Sipahi during her visit. Other CLIMA researchers particularly Dr

Aryamanesh, helped considerably during her training. Dr Croser and her technical staff also contributed to the doubled haploidy part of the visit for a week. Dr Sipahi's visit was fully funded by the Turkish Ministry of Agriculture and has enabled her to i) learn cutting edge fluorescent DNA fingerprinting techniques and ii) meet a range of scientists in the field of biotechnology across universities and research organizations in WA; e.g. University of Western Australia, Murdoch University, and Department of Agriculture and Food WA.

The information and methodologies acquired by Dr Sipahi during her visit will be used in her follow-up projects in Turkey to improve legume germplasm. Hulya and I are also very thankful to Dr Heather Clark and Professor William Erskine for their roles in accommodating her in Perth during this visit.



*CLIMA visitor Hulya Sipahi was updated on the latest biotech activities and projects at CLIMA.
(L to R) Mr Federico Ribalta, Dr Kioumars Ghamkhar, Dr Hulya Sipahi, Prof. William Erskine, and Dr Nader Aryamanesh*

NEW FACES

FAST TRACKING PEA WEEVIL RESISTANCE INTO FIELD PEA CULTIVARS

By Nader Aryamanesh, Guijun Yan, Tanveer Khan, Darryl Hardie and Willie Erskine



Pea weevil is a major insect pest in cultivated peas in Australia and worldwide. The only form of control of this insect is regular chemical pesticide application and seed fumigation. The

objectives of this project are to study the genetic basis of resistance to pea weevil and introgression of pea weevil resistance from wild pea into cultivated pea. Introgressed pea populations derived from a resistant *P. fulvum* accession and a cultivar field pea have been developed and crossed into several elite Australian pea cultivars which have been bred for improvement in standing ability and blackspot resistance.

This project is funded by an ARC Linkage project and initially conducted by Dr Oonagh Byrne. Dr Nader Aryamanesh took over the project in April 2009.

Nader graduated with MSc degree from Tabriz University, Iran in 2002. Nader

was awarded a scholarship to do a PhD in the School of Plant Biology and CLIMA, UWA and graduated from his PhD in 2008 working on QTL mapping of ascochyta blight resistance in chickpea. Nader worked as a Research Officer in the school of Plant Biology on molecular approach in detecting chromosomal rearrangements in wide hybridization in *Brassica* species and also selection of purslane as a vegetable source of omega 3 fatty acid through chemical analysis and AFLP markers in 2008. Nader has taken a new position as a Research Associate to work on pea weevil resistance through identification and introgression of resistance genes to cultivated field pea from its wild accession.

IMPROVING HERBICIDE TOLERANCE IN GRAIN AND PASTURE LEGUMES

By Shyama Weerakoon

Shyama joined CLIMA in March 2009 to work in a project, funded by GRDC, on improving herbicide tolerance in grain and pasture legumes, together with Dr Ping Si, and Profs Willie Erskine and Steve Powles. The project conducts experiments to identify best plants/lines with herbicide tolerance in lupin, chickpea, field pea and French serradella from mutated populations or diverse germplasm and improves knowledge of herbicide tolerance in these crop/pasture species. The selected lines and improved knowledge will strengthen future breeding for herbicide tolerance in legumes.

Shyama graduated with a special degree in Botany from the University of Colombo, Sri Lanka. She was awarded a scholarship to do a PhD at the School of Plant Biology, UWA. She graduated with her PhD in the areas of Brassica plant physiology and genetics in 1999. Shyama worked at the Open University of Sri Lanka as a senior lecturer in Botany until 2007. She carried out research on genetic variation among mustard germplasm in Sri Lanka, impact of seasonal variations on mustard seed yield and producing interspecific hybrids to improve fatty acid profile of mustard germplasm in Sri Lanka.



CLIMA seminar series

The CLIMA seminar series continues on a monthly basis.

Please contact Janine Croser
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to suggest topics and speakers.

In particular, we value the earliest possible advice of seminars offered by visitors.

CLIMA PUBLICATIONS January–June 2009

Bramley, H., Turner, N.C., Turner, D.W. and Tyerman, S.D. 2009. Roles of morphology, anatomy, and aquaporins in determining contrasting hydraulic behavior of roots. *Plant Physiology* 150, 348-364

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THE LENTIL BOOK

Success in the literary world - *The Lentil Book* (The Lentil: Botany, Production and Uses) has now been published by CABI International, edited by W. Erskine (UWA), F. Muehlbauer, A. Sarker and B. Sharma. There are five CLIMA publications included as detailed in the Publications List in this newsletter.

Readership

This book will be of interest to those working in horticulture, agronomy and botany with a particular focus on lentils.

Description

The lentil with its ancient origin is today very much a crop of the modern world confronted with issues of food security, poverty, water scarcity and the need to find sustainable agricultural systems in a changing climate. In the last three decades the global production of lentil had almost tripled due not only to larger harvest areas but also and more importantly to an increase in productivity. The Lentil provides a timely synopsis of the current knowledge associated with this highly digestible and protein-packed grain with its ability to use water efficiently and to grow in marginal environments.

NEWSLETTER CREDITS**Volume 10, Number 1 July 2009****EDITOR**

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LAYOUT

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