



**WA STATE AGRICULTURAL
BIOTECHNOLOGY CENTRE
(SABC)**

**ANNUAL REPORT
2001**

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1. Summary of staffing and resources

2. Aims of the SABC for 2002

3. Highlights of research in the SABC

4. SABC research links and collaborations

5. Overview of research undertaken in the SABC

Rumen Biotech

Rumen Biotech has experienced delays in the major project, to protect livestock against fluoroacetate poisoning, because of regulatory requirements. A licence has been obtained, to allow a contained toxicity trial on cattle, and funding for this research is being discussed among the funding agencies. In the meantime, research continues on the structure and function of gene promoters in the prominent ruminal bacterium *Butyrivibrio fibrisolvens*. The use of selected promoters to express novel genes in *B. fibrisolvens* is an important future aim. A second project on the characterisation of chromosomal integration mechanisms in the important gastrointestinal bacterium *Bacteroides uniformis* is currently examining the expression of the relevant genes in both *E. coli* and *B. uniformis*. Manipulation of the integration genes and integration attachment sites is the next stage in this work.

Animal Virology

- A major grant has been obtained for the development of diagnostics and a vaccine for the control of the bovine lentivirus causing Jembrana disease in cattle in Indonesia. Partners in the investigation are the Disease Investigation Centre in Bali, LIPI Biotechnology in Bogor, and Balitvet in Bogor.
- Genetic characterisation of Australian strains of porcine circoviruses detected in the Australian pig herd, the development of diagnostic reagents, and investigation of their role in disease, is continuing. While these viruses are associated with disease in North America and Europe, including post-weaning mortalities and skin diseases, no definitive evidence has been obtained that these viruses are associated with similar diseases in Australia. An explanation for the apparent differences between the Australian situation and elsewhere is being investigated. These studies funded by the Pig Research and Development Corporation.
- A project investigating the role of equine herpesviruses in respiratory disease in horses is continuing, and funded by the Rural Industries Research and Development Corporation. The objectives are to develop methods of differentiating latent and active herpesvirus infection, thus enabling interpretation of the significance of herpesviruses detected in horses with respiratory disease, and whether activation of latent virus infections contributes to the development of respiratory disease or decreased performance.

Publications (2001)

1. HARTANINGSIH, N., DHARMA, D.M.N., SOEHARSONO, S. and WILCOX, G.E. (2001) The induction of a protective immunity against Jembrana disease in cattle by vaccination with inactivated tissue-derived virus antigens. *Veterinary Immunology and Immunopathology* 78: 163-176.
2. BASSAMI, M. R., YPELAAR, I., BERRYMAN, D., WILCOX, G. E. and RAIDAL, S. R. (2001) Genetic diversity of beak and feather disease virus detected in psittacine species in Australia. *Virology* 279: 392-400.

3. PHENIX, .K.V., WESTON, J.H, YPELAAR, I, LAVAZZA, A., TODD⁴ D., WILCOX, G.E. and RAIDAL, S. R. (2001).Nucleotide sequence analysis of a novel circovirus of canaries and its relationship to other members of the genus Circovirus of the family Circoviridae. *Journal of General Virology* 82:2805-2809.
4. METHAROM, P., TAKYAR, S., XIA, H. Q., ELLEM, K. A., WILCOX, G. E. and WEI, M. Q. (2001) Development of disabled, replication-defective gene transfer vectors from the Jembrana disease virus, a new infectious agent of cattle. *Veterinary Microbiology* 80: 9-22.

Human Oncology

- **Childhood leukaemia research**

Work continues to identify and characterise genes regulated by the T-cell oncoprotein HOX11 which is involved in acute lymphoblastic leukaemia (T-ALL) in humans. Our previous studies have identified two target genes of HOX11, *aldehyde dehydrogenase 1a1* and *Slim1*. The former is intriguing because of its demonstrated role in synthesizing retinoic acid, a key modulator of several cellular process including differentiation. We have formally demonstrated that *ALDH1A1* is a HOX11 target gene, as the *ALDH1A1* promoter can be transactivated approximately 12-fold in human erythroleukaemia cells in a HOX11-dependent manner. In a search for further HOX11 target genes, guanine nucleotide dissociation inhibitor beta (*GDIβ*) was found to be upregulated in erythroleukaemia cells as a result of enforced HOX11 expression. Significantly, *GDIβ* was aberrantly expressed in three of three HOX11 positive T-ALL cell lines, but was not detectable in seven HOX11 negative T-ALL cell lines tested, nor in normal human thymocytes. To produce large amounts of purified HOX11 protein for further studies, we purified human HOX11 expressed in *E. coli* as a soluble and functional glutathione S-transferase (GST) fusion protein. The biological activity of the recombinant protein was verified by the specific binding of GST-HOX11 to DNA containing consensus HOX11 recognition sites.

- **Mesothelioma research**

A sophisticated study was undertaken to identify tumour suppressor genes downregulated in cancerous mesothelioma cells compared to normal mesothelial cells using Affymetrix GeneChip technology. A number of genes were identified by this analysis, many of which are involved in cell adhesion processes, and these are being followed up in further investigations.

Veterinary Immunology

- **Flea vaccine research**

A number of genes encoding potential protective flea gut antigens have been identified using a molecular approach. Cloning of the corresponding full-length genes will be followed by experimental vaccine trials to test their efficacy.

Australian Centre for Necrotrophic Fungal Pathogens (ACNFP)

Septoria pathogenicity

Aim; to identify and characterize the genes and proteins that contribute to pathogenicity in necrotrophic fungal pathogen. We have concentrated on the major wheat pathogen *Stagonospora nodorum*. Our approach is to identify fungal genes by EST sequencing and investigating their role by gene disruption. Physiological studies have been initiated to examine aspects of pathogenicity and to investigate methods to inhibit fungal growth.

In order to organize and analyze the data emerging from our own and other public fungal pathogen genomics projects, we have designed and implemented an initial version of FOGREG, the fungal orthologue gene registry. A bioinformatics package, written to our design by Soren Rasmussen is used throughout.

- **Physiology of infection**

We have initiated a series of physiological studies of *S. nodorum* pathogenesis. The aims of these studies are to develop quantitative assays of pathogenicity, acquire experimental material for cDNA libraries, acquire physiological evidence for mechanism of pathogenicity and develop means to inhibit fungal growth in planta.

A number of *S. nodorum* strains expressing one of two GFP (green fluorescent protein) constructs (obtained from Barbara Howlett) were made. Using the confocal, these strains are being used to examine the mode of infection. They would also be useful in experiments to test quantitatively the growth of the fungus, such as assessments of wheat accessions and breeding lines.

A key technique for our strategy to dissect pathogenicity is the ability to analyse the effect of disrupting the expression of genes on the properties of *S. nodorum*. Progress in all stages of the procedure has been very rapid.

We are now expanding our list of target genes to include a focus on genes involved in peroxisome structure and function.

Medicago Resistance

Our aim is to characterize the genes and protein required for disease resistance to necrotrophic pathogens. The aim of this project is to genetically characterize disease resistance in *Medicago truncatula* to one or more necrotrophic pathogens. The first step is to find necrotrophic pathogenic isolates. The second stage is to find differential resistance in ecotypes of *M. truncatula*.

We have acquired core collection of ecotypes. These include key ecotypes used to create genetic maps in France and the USA, recombinant inbred line (RIL) ecotypes and their progeny, and in addition some 200 SARDI ecotypes are being single seed descended to create homozygous inbred lines. Ecotypes maintained by SARDI tend to be seed bulked from a number of individuals collected at a given location. The level of heterozygosity is unknown, but should be excluded as it can cause problems in assessing phenotypes. We have also

established a set of skeleton genetic markers for the Medicago genome to aid initial gene mapping studies.

We have screened ca. 30 necrotrophic fungal isolates for pathogenicity to Medicago. The isolates were from infected Medicago found locally at Murdoch University, from fields at Ballards Seeds at Narringin, and from a collection at AgWA. The most virulent group of pathogens was of the genus Phoma. However, Ascochyta, Alternaria, Botrytis, Colletotrichum and Stemphylium were found to be pathogenic to varying degrees.

To find differential resistance in ecotypes of Medicago, we have investigated the response of in excess of 100 ecotypes from SARDI to infection with several key pathogenic necrotrophs (including Ascochyta rabiei, Botrytis fabae and P pinodella). In each plant-pathogen interaction, differential temporal resistance was evident, whereby the cotyledons and the first true leaf showed enhanced susceptibility. A range of partial resistance phenotypes was also observed with phenotypically different resistance and disease symptoms being apparent. In individual, fully resistant ecotypes, resistance can be associated with and without localised cell death and cellular changes, while susceptible ecotypes display different patterns of chlorosis and necrosis.

Six of the most resistant ecotypes are currently being taken through a crossing program to produce F2 segregating populations. These populations will be available as a resource for other gene mapping and cloning experiments.

2001	GRDC	Virulence and genetics variability in the spot-type net blotch pathogen of barley in Western Australia; with Rob Loughman, Sangiv Gupta	AUS\$113,500	36
	GRDC	Development of a Legume-specific Bioinformatics Resource for legume researchers; with Matthew Bellgard et al. researchers	AUS\$206,060	36
	ARC-LIEF	Title: Joint controlled environment facility for research and development in plant biotechnology in Western Australia. With Lambers et al	AUS\$600,000	
	ARC-LIEF	Proteomics facility for biotechnology research in WA. With Wilcox et al	AUS\$340,000	
Murdoch totals	5		AUS\$4,460,000	552

National and International Expert Committees

2001 Member Australian Research Council EAC for Biology
 2002 Member Review Panel CRC for Tropical Plant Protection (12)

Invited speaker at conferences

2001 Asilomar Fungal Genetics, California

CRCTPP, Brisbane
Discovery Meeting, CSIRO PI, Canberra
Australasian Plant Pathology Society, Cairns (43)

Invited Speaker in Universities and Research Institutes

2000 Birmingham University
University of Wales Aberystwyth
Aventis Crop Science, Gent
Aventis Crop Science, Lyon
University of Western Australia
 TMRI, San Diego
Du Pont, Delaware
 Syngenta Jeallots Hill
Monsanto Cambridge

Conference Organiser

2001 Fungal Genetics Conference, Asilomar, session organiser

Editorial Boards

1993-date Microbiological Research
1999-date Physiological & Molecular Plant Pathology
1999-date Molecular Plant Pathology, Senior Editor

Ph D Students

Lene Bindslev Jensen 1997-2001 (Carlsberg and Copenhagen University)

Teaching

Plant Biotechnology 3 lectures

Administration

Murdoch – Divisional Board, Institutional Biosafety Committee, Greenhouse Committee,

Publications

Refereed Articles

1. KINANE J, DALVIN ST, BINDSLEV L, HALL A, GURR SJ, OLIVER RP (2000)
Evidence that the cAMP Pathway Controls Emergence of both Primary and Appressorial

- Germ-Tubes of Barley Powdery Mildew *Molecular Plant Microbe Interactions* **13** 494-502
2. SOLOMON PS, OLIVER RP (2001) The nitrogen content of the tomato leaf apoplast increases during infection by *Cladosporium fulvum*. *Planta* **213** 241-249
 3. SEGERS G, BRADSHAW N, ARCHER D, BLISSETT K, OLIVER RP (2001) Alcohol Oxidase is a Novel Pathogenicity Factor for *Cladosporium fulvum* but Aldehyde Dehydrogenase is Dispensable *Molecular Plant Microbe Interactions* **14** 367-377
 4. BUSSINK H-J, OLIVER R (2001) Identification of two highly divergent catalase genes in the fungal tomato pathogen, *Cladosporium fulvum*. *European Journal of Biochemistry* **268** 15-24
 5. NIELSEN PS, CLARK AJ, OLIVER RP, HUBER M, SPANU PD (2001) Hcf-6, a novel class II hydrophobin from *Cladosporium fulvum* *Microbiological Research* **156** 59-63
 6. SOLOMON PS, NIELSEN PS, CLARK AJ, OLIVER RP (2000) Methionine synthase, a gene required for methionine synthesis, is expressed *in planta* by *Cladosporium fulvum*. *Molecular Plant Pathology* **1** 315-323
 7. THOMAS SW, RASMUSSEN SW, GLARING MA, ROUSTER JA, CHRISTIANSEN SA, OLIVER RP. (2001) Gene identification in the obligate fungal pathogen *Blumeria graminis* by expressed sequence tag analysis. *in press Fungal Genetics and Biology* **33** 195-211
 8. BUSSINK H-J, CLARK AJ, OLIVER RP (2001) The *Cladosporium fulvum* Bap1 gene: evidence for a novel class of Yap-related transcription factors with ankyrin repeats in phytopathogenic fungi *European Journal of Plant Pathology* **107** 655-659
 9. SOLOMON PS, OLIVER RP (2002) Evidence that *Cladosporium fulvum* metabolises γ -aminobutyric acid during infection of tomato. *Planta* **214** 414-420
 10. BINDSLEV L, KERSHAW MJ, TALBOT NJ, AND OLIVER RP (2001) Complementation of the *Magnaporthe grisea* Δ cpkA mutation by the *Blumeria graminis* PKA-c gene: functional genetic analysis of an obligate plant pathogen. *Molecular Plant Microbe Interactions* **14** 1368-1375
 11. WIRSEL SGR, RUNGE-FROBOSE C, AHREN DG, KEMEN E, OLIVER RP & MENDGEN KW (2002) At least four species of *Cladosporium* sympatrically colonise *Phragmites australis*. *In press Fungal Genetics and Biology*
 12. LENE BINDSLEV, RICHARD P OLIVER & BO JOHANSEN (2002) *In situ* PCR for detection and identification of fungal species *In press Mycological Research*

Other Publications

1. RICHARD OLIVER LENE BINDSLEV, JULIA KINANE STEVE THOMAS, PETER SOLOMON, GREER WILSON, HENK-JAN BUSSINK, MARILYN PIKE Using genetics and genomics to dissect fungal pathogenicity. APPS Cairns 2001 pp 57-60
2. OLIVER RP, GRANDBASTIEN MA (2001) An Experimental approach to investigate horizontal gene transfer between organisms. In EC-sponsored research on Safety of genetically modified organisms. Ed Kessler C, Economidis I. EC pp 20-21

Veterinary Microbiology group (David Hampson)

Work in 2001 continued to focus on development of improved PCR diagnostics for detecting and identifying a number of different species of intestinal spirochaetes in human and animal faeces.

A recombinant DNA-based serological assay was developed for detection of serum antibodies against *Brachyspira hyodysenteriae* (the agent of swine dysentery). Work commenced on the

development of recombinant vaccines for the control of intestinal spirochaete infections in pigs and poultry.

Publications

1. Brooke CJ, Clair AN, Mikosza ASJ, Riley TV and Hampson DJ (2001) Carriage of intestinal spirochaetes by humans: epidemiological data from Western Australia. *Epidemiology and Infection* 127:369-374.
2. Brooke CJ, Hampson DJ, Riley TV, and Lum G (2001) Failure to detect *Brachyspira pilosicoli* in the bloodstream of Australian patients. *Journal of Clinical Microbiology* 39:4219.
3. La T and Hampson DJ (2001) Serologic diagnosis of *Brachyspira (Serpulina) hyodysenteriae* infections. *Animal Health Research Reviews* 2:45-52.
4. Mikosza ASJ and Hampson DJ (2001) Human intestinal spirochetosis: *Brachyspira aalborgi* and/or *Brachyspira pilosicoli*? *Animal Health Research Reviews* 2:83-91.
5. Mikosza ASJ, La T, de Boer WB and Hampson DJ (2001) The comparative prevalence of *Brachyspira (Serpulina) pilosicoli* and *Brachyspira aalborgi* as the etiologic agents of histologically-identified intestinal spirochetosis in Australia. *Journal of Clinical Microbiology* 39:347-350.
6. Mikosza ASJ, La T, Margawani KR, Brooke CJ and Hampson DJ (2001) PCR detection of *Brachyspira aalborgi* and *Brachyspira pilosicoli* in human faeces. *FEMS Microbiology Letters* 197:167-170.
7. Stephens CP and Hampson DJ (2001) Intestinal spirochaete infections in chickens: a review of disease associations, epidemiology and control. *Animal Health Research Reviews* 2:101-110.

Phil O'Brien

- **Engineering Resistance to Fungal Diseases**

We have been investigating the use of recombinant antibody genes to engineer resistance to the fungal pathogen *Rhizoctonia solani*. Genes for anti-pectinase antibodies have been cloned and expressed in bacteria. The antibodies are potent inhibitors of polygalacturonases from a variety of fungal species. The genes have been modified for expression in plants and transformed into tobacco. Initial experiments confirm that the genes are being expressed in the transgenic tobacco.

- **Virulence of *R. solani***

Mechanisms of virulence of *R. solani*: *R. solani* is an example of a root infecting necrotrophic fungus and is unusual in that the vegetative cells contain 6-12 nuclei. To study virulence factors we have investigated transformation of this pathogen. Transformation of protoplasts with a hygromycin resistance gene driven by either an ascomycete or basidiomycete promoter was unsuccessful. Transformation of has been achieved using a modified gene introduced by *Agrobacterium tumefaciens*.

- **Eradu Patch**

This is a disease of lupin 1st discovered in YEAR. The pathogen characterized as a Thin Binucleate Rhizoctonia (TBR) is very difficult to grow making surveys of the disease impractical. Using a DNA detection test developed by us we have surveyed the distribution of the pathogen in wheat and lupin crops in WA. The results show that the disease is more widespread than previously considered.

WADA-Crop Improvement Institute/SABC laboratory

R. Appels, Leader of Western Node of Molecular Plant Breeding CRC

D. Hodgson, Laboratory manager

The WADA/SABC laboratory covers a wide range of activity with a focus on molecular markers that can be applied to breeding, in order to trace agronomically significant traits including biotic and abiotic stresses, and end-product quality attributes. A key aim is to establish high throughput analyses based on markers, and where possible target candidate genes, that are carefully validated for the respective trait. Collaborating organizations include Molecular Plant Breeding CRC University of Adelaide, University of Western Australia, Curtin University, CSIRO-Plant Industry, University of Southern Queensland (USQ).

WHEAT QUALITY AND DISEASE RESISTANCE MARKER DISCOVERY AND VALIDATION

Michael Francki

The last quarter (November 2001-January 2002) has seen the development of research projects in molecular marker discovery, validation and implementation across WA adapted germplasm. The specific research projects have been compiled in collaboration with the wheat breeders and cereal chemists at the Department of Agriculture, South Perth. The specific projects include:

- **Marker discovery and validation for grain quality**

Alternative loci controlling flour swelling volume using a doubled haploid population (132 individuals) derived from the cross Cadoux x Reeves. Both parents are null-4A for GBSS and vary in FSV. Validation of markers developed from the NWMMP across WA DH populations for flour colour and stability (Westonia x Janz; Cadoux x Reeves); milling yield (WAWHT2046 x Carnamah; Cadoux x Reeves), water absorption (WAWHT2127 x WAWHT2074) and grain size (Westonia x Janz).

Parents of populations are being grown in glasshouse and preliminary SSR analyses for developing structural framework maps and validating existing markers for specific traits will commence in February 2002. DH populations have been sorted in preparation for planting in glasshouse and field trials. Field trials of DH populations will commence in May 2002 but exact locations are yet to be determined. Additional funding (operating costs and travel, technical assistance) to support validation and implementation activities has been sought from GRDC through the AWCMMMP.

- **Disease resistance**

The on-going project and funds transferred from UWA to Department of Agriculture and VAW CRC to develop WA wheat with high level Septoria resistance has identified in excess of 110 polymorphic SSR markers suitable for mapping spring and winter wheat populations. A further 70 SSR markers will be analysed of polymorphisms between parents before screening mapping populations. Recent published reports have shown that high level of pre-harvest tolerance is linked within 15-30 cM from loci controlling red grain colour in winter wheat. Winter wheat parents with Septoria resistance used in this study will be phenotyped in 2002 to validate alternative sources of pre-harvest sprouting useful for introgressing into spring wheat varieties.

Potential markers recovered from *Aegilops tauschii* septoria resistant lines by various earlier researchers in a variety of projects are being reassessed and used to assay polymorphisms in lines from a *A. tauschii* cross (R xS) to uncover any primers that could be applied to hexaploid wheat. Where possible NWMMP populations will be used to map probe positions as an initial guide to their value as markers.

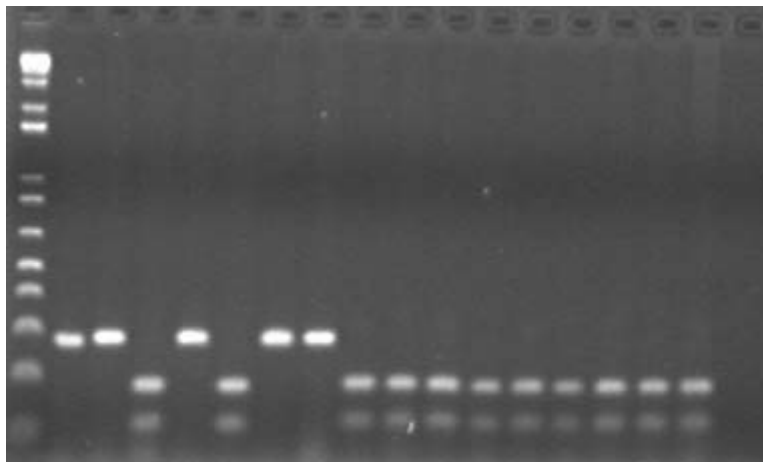
- **Personnel**

The Department of Agriculture was successful in attracting a PhD student, Ms Karon Ryan, and commenced her studies on 29 January 2002. Ms Ryan has a background in molecular genetics and is enrolled through Murdoch University with Dr Michael Francki, Prof Mike Jones and Prof Rudi Appels as supervisors. Ms Ryan will focus her studies on marker discovery and validation on at least one mapping population for grain quality attributes and exploit publicly available EST databases and their application for genome mapping. The studies on the *A. tauschii* sequences are being carried out by Modika Perera and Meredith Carter. The Research Officer positions funded by the VAW CRC and GRDC will be appointed March 2002.

IMPLEMENTATION OF MARKERS FOR *MLO9* AND THERMOSTABLE BETA AMYLASE IN BARLEY

Max Paris and Chengdao Li

Several thousand DNA samples have been assayed using SNPs identified in the *mlo9* and the thermostable beta-amylase genes. Primer extension using dideoxy nucleotides and MALDI-TOF were used initially for both of the genes and double haploid lines for progression into the breeding program at WADA were assayed using this system. On a large scale backcross lines were assessed for the presence or absence of the gene. For the thermostable beta-amylase gene, the characteristic SNP assayed was located in a restriction enzyme site (CCGG) and this observation provided the basis for a simplified



gel based assay where the diagnostic fragment was PCR amplified and then digested. As shown below a 271 bp fragment was indicative of the normal alleles and the presence of two digestion products was the result of the presence of the diagnostic T to C change.

MARKERS FOR NET FORM NET BLOTCH IN BARLEY

Mehmet Cakir, Sanjiv Gupta, Chengdao Li

The barley cross Tallon x Kaputar was mapped using microsatellite and AFLP sequences and the resulting genetic linkage map used to analyse the association of resistance to net form net-blotch (NNB) to particular quantitative trait loci. A major (80% of variation accounted for) and highly significant ($P < 0.001$) QTL was discovered on chromosome 6H.

The NNB-6H QTL was defined by both AFLP and microsatellite markers. In a parallel study 5 barley crosses were studied using bulk segregant analyses (BSA), to search for markers associated with NNB resistance. The AFLP and microsatellite sequences used to study the BSA samples were the same as those that were mapped in the Tallon x Kaputar. In 2 of the crosses used for BSA (Pompadour x Stirling; WPG8412 x Stirling), a number of AFLP and microsatellite sequences were found to differentiate between the resistant and sensitive “bulks”. In three cases, for the AFLP sequences, those that differentiated between the resistant and sensitive “bulks” also mapped to the NNB-6H QTL defined in the Tallon x Kaputar cross.

The study has provided the basis for defining, and validating, several DNA markers developed for high throughput analyses for tracing this trait in a breeding program. These include SSR markers Bmag0173, EBmac0874 and AFLP M9 green, a 311 bp band associated with NNB resistance and M7 yellow, a 116 band associated with NNB sensitive type.

COLD TOLERANCE AT FLOWERING AND BUDWORM RESISTANCE IN CHICKPEA.

Heather Clarke (UWA), Tanvir Khan (WADA)

Improvement in chickpea yield has been achieved through a novel breeding schedule based on pollen selection during hybridisation. New introductions showing some resistance to budworm are also being introgressed. In the current study, screening methods in the laboratory (pollen tube assay; molecular markers), controlled environment growth rooms (low temperature; Petri-dish assay for budworm resistance) and in the field are being compared and optimised. The project is funded by GRDC (UWA354).

MARKERS LINKED TO PEA WEEVIL RESISTANCE IN FIELD PEA

Oonagh Byrne and Darryl Hardie

Twenty families derived from a cross between resistant x sensitive field pea F3 selections were analysed using a bulked segregant analysis approach. Although the population size was small, the use of DNA fingerprint techniques provided 10 DNA sequences that were investigated further. Follow-up studies indicated that 6 of the DNA sequences were potentially linked to pea weevil resistance and confirmation studies have been planned.

ABIOTIC STRESS MARKERS FOR WHEAT

Meredith Carter and Rudi Appels

The milestones for this project are defined in the GRDC project DAW 724. Early targets in this project are to validate markers for A1 and B tolerance in WA wheat germplasm. For the *B-1* locus the psr680 probe has been sequenced in preparation for designing primers for PCR. For the *B-3* locus psr160 is the probe of interest. Crosses of interest for the A1 tolerance include Sunco x Tasman, Cranbrook x Halberd, Cascades x RAC875, Embropa 16/2*/Cascades, Cascades2*/Cascades2*.Maringa and Cascades2*/Cascades2*.Br25 (Brazilian sources A1 tolerance on group 4), Carnamah/Karlgarin and the many Delhaize ET8 (A1 tolerant) and ES8 (A1 sensitive).

Later in the project water logging will become a target and current work in WADA/UWA is defining the phenotype for assessing the *Dracula4/2*Brookton* cross. A candidate gene is pyruvate decarboxylase (*pdc*, step before alcohol dehydrogenase in detoxifying alcohol) in terms of allowing survival under anaerobic conditions.

Personnel: Fiona Drake-Brockman is providing technical support

REGENERATING AND TRANSFORMING MANGO

Vaughan Agrez, Soon-Chye Tan, Zora Singh

The project has succeeded in developing a partial *in vitro* plant regeneration system for mango cv's 'Kensington Pride' (KP) and 'R2E2'. It has been established that the nucellus surrounding the developing embryo is the tissue that provides the most responsive explant for the initiation of *in vitro* cultures and media formulations have been optimised accordingly. The project is currently in the process of optimising the maintenance and maturation media for embryonic mango cultures. Initial work has also commenced on developing selection media for future transformation studies.

TEMPERATURE SHOCK TREATMENTS FOR KILLING MEDFLY FLY LARVAE IN FRUIT

Louise Williams, Francis Delima (WADA)

Temperature shock treatments on fruit destined for export markets are key steps in minimising problems with Medfly infestation. This project is undertaking a detailed calibration of Medfly larvae lethality and the accumulation of heat shock (HS70) protein in specific tissues, as cold and hot temperature shocks are applied. Assays for HS70 include gel electrophoresis and antibody-based techniques.

NEW MARKERS FOR LATE MATURING ALFA AMYLASE (LMA) IN WHEAT

Meredith Carter, Mike Jones

Cleo-Inia x Janz derived doubled haploid lines were studied using AFLP and bulk segregant analysis (BSA) to identify markers linked to late maturity alpha amylase (LMA). The two parental lines and bulks (10 high LMA segregants and 10 low segregants) were screened with 52 Pst/Mse primer combinations and 5 AFLP fragments were identified as being potentially linked to LMA. A 450 bp fragment was chosen for further characterisation and converted to a dominant PCR assay. In checking the bulks used for the BSA experiment 9/10 of the individuals showed the expected band.

Polymorphism within the parents of the NWMMP, for the 450 bp fragment are currently being searched for in order to map the DNA sequence to a chromosome region. To date the Cranbrook x Halberd parents are not suitable for mapping – the parents show no sequence differences within the entire 450 bp fragment that is amplified in the PCR assay, as judged from direct sequence studies. The aim of the genetic mapping is to confirm whether the sequence maps to the chromosome region established to carry the major QTL for LMA, namely, the distal region of 7BL.

A NEW COMPOSITE GENETIC MAP FOR WHEAT

Rudi Appels

A large international collaboration (regular contact with Dr P Gustafson, USDA Missouri, in particular) is being carried out to compile a composite genetic map for wheat. The data so far includes the maps for Fukuho x Oligo (CIMMYT), Synthetic x Opata (Canada and France), Clark's Cream x NY line (Cornell), data from the NWMMP (Australia). Approximately 3,000 markers are in the composite map and is intended to provide a resource for finding new markers in a region of interest. The genetic map, aligned to the physical map using data from deletion lines), is complemented by EST mapping to deletion lines and thus candidate genes can be identified to regions where QTLs have been mapped.

MAPPING NEW QTLs IN WHEAT AND BARLEY

Mehmet Cakir, Chengdao Li, Wujun Ma, Peter Langridge, Rudi Appels

This project includes the molecular genetic mapping of the barley crosses, Tallon x Kaputar, Patty x Tallon, 9104 x Dash and WABAR2080xTR232, as well as the curation of the NWMMP wheat crosses Cranbrook x Halberd, CD87 x Katepwa, Sunco x Tasman and Egret x Sunstar. For the barley cross Tallon x Kaputar the data characterising 65 doubled haploid lines, taken together with information from maps constructed overseas, has provided a map of adequate resolution (241 markers genotyped and 160 assigned to chromosomes) to study a number of agronomic and quality based traits to define QTL. The Map Manager software has provided the basis for analysing the data and, using the permutation test option, defined highly significant associations of chromosome regions and trait variation. Although the relatively small size of the population limits the detailed interpretation, it is evident from the available data that the results from the Tallon x Kaputar cross are consistent with observations coming from other crosses. In the wheat area, groups around Australia have used the NWMMP crosses to assign their gene or sequence of interest and in terms of additional markers for these crosses the acquisition of this additional information is continuing to enhance the value of the NWMMP maps. A high level of activity in characterizing the flour from the doubled haploid lines in the populations (by groups around Australia) also means a large body of information is in place to carry out analyses for QTL controlling the respective traits. In a collaboration with CSIRO-PI a detailed analysis of Mixograph variables is being carried out and results are suggesting the QTL associated with the HMW glutenin on 1B are particularly sensitive to interactions with other QTLs (such as protein). This work is in collaboration with colleagues at INRA (France) and will provide valuable confirmation of information in a completely independent cross, in a European environment and independently generated Mixograph data.

Personnel: Julie Uhlmann is providing technical support.

HIGH THROUGHPUT IMPLEMENTATION OF MARKERS FOR BREEDING

Meredith Carter, Grant Daggard, Kevin Gale, Dave Hodgson, Mike Jones, Rudi Appels

Using standard analytical procedures, markers for Wx-B1, BYDV, Sr2, VPM segment, and flour colour have been applied to a total of over 2000 DNA samples from the WADA wheat breeding program. The material analysed included doubled haploid lines as well as breeding lines to confirm the presence of traits of interest. The project is also establishing solid phase PCR as a means for both stabilising the DNA recovered from the matrix mill (established in an earlier SABC project) and simplifying DNA-based assays for plant breeding. The technology uses DNA chemically linked through a 5'-P group and ethylene

glycol *bis*[succinimidylsuccinate] to Nucleolink plates (Nunc) in order to immobilise the DNA template for PCR (using fluorescein labelled primer). Based on collaborative work with CSIRO-PI, PCR products from a multiplexed reaction are then assayed using a hybridization approach analogous to the DIAPOPS systems (Nunc) and the required product assayed using anti-fluorescein-AP, Fab fragments in an ELISA-type assay. In parallel with the lab-based tests, the transfer of electronically acquired ELISA-reader data into breeders data systems is being established together with the appropriate bar-coding systems to track DNA samples to plants in the field.

Plant Virus Group

Participating members: Dr Steve Wylie, Mrs Belinda Welsh, Ms Kanokwan Ratanosanobon, Mr John Blinco, Ms Dora Li.

2001 was an eventful year for the Plant Virus Group. Steve and Belinda continued to grow and analyse transgenic lupins and faba beans from the Transgenic pulse Development Project. Three faba bean lines homozygous for a cucumber mosaic virus (CMV) resistance gene were identified from the 13 analysed. These will be analysed for CMV resistance in 2002. They also completed a complicated new construct for resistance to CMV in pulses. Kanokwan began to look for differences in the interactions between *Agrobacterium* and two cultivars of narrow-leafed lupin, a transformation-competent one and a recalcitrant one. She immediately found differences in bacterial attachment between the two cultivars. Dora used degenerate primers to isolate part of a resistance gene analogue (RGA) from yellow lupin, the first RGA isolated from that species. She will attempt to isolate the full gene in 2002 and try to ascertain its function. John finished a full-length infectious clone of CMV as part of his efforts to identify avirulence components of the virus. He began work making a clone of a virulent strain of the same virus.

In July, Steve and John visited Dr Rob Potter at Cornell University in upstate New York and whilst there spoke with a number of prominent scientists involved in plant pathogen interactions. They then met up with Dora in New York before travelling to Madison for the 10th International Congress of Molecular Plant Microbe Interactions where they each presented posters. They then visited UC at Davis and Berkley to present their work and speak with other researchers. They were particularly fortunate to have a talk with Professor George Bruening, Director of the Center for Engineering Plants for Resistance Against Pathogens at UC Davis. In August, all members of the group attended the 12th Annual Combined Biological Sciences Conference in Perth. In November Steve, along with Prof Considine, presented a one day workshop on grapevine viruses to growers. In late November, most members of the group introduced their work to GRDC members John Cullen, Julianne Lloyd-Smith and Darrel Dent in a review of progress of the Transgenic Pulse Development Project.

- **Grants**

Steve Wylie was granted \$6000 by the GRDC to attend the 10th International Congress of Molecular Plant Microbe Interactions.

- **Publications/Presentations**

1. Ratanasanobon K, S. Wylie, M. Jones (2001) Investigations into improving the transformation efficiency of *Lupinus angustifolius*

2. Proceedings of the 14th CBSM, Perth,
3. Ratanasanobon K (2001) Towards Unraveling Why Gene Transfer to Lupins is So Difficult: Progress to Date and Experiments Planned for Year 2002. Lupin Mini Symposium, Perth
4. Wylie S.(2001) Clever constructs for GM lupins to resist diseases. Lupin Mini Symposium, Perth
5. Wylie S., J. Kueh¹, K. Ryan¹, B. Welsh¹, Y. Hu³, MGK Jones (2001) A comparison of bean yellow mosaic virus pathotypes in *Lupinus angustifolius*. Proceedings of the 10th International Congress of Molecular Plant Microbe Interactions, Madison.
6. Blinco JH, Wylie SJ, Jones MGK (2001) Generating a full-length infectious clone of cucumber mosaic virus to determine virulence regions. Molecular Plant Microbe Interactions, Madison.
7. Li DA, Buirchell B, Jones RAC, Jones MGK (2001) Towards the identification of molecular markers linked to and the isolation of a novel resistance gene to cucumber mosaic virus in *Lupinus luteus*. Proceedings of the 10th International Congress of Molecular Plant Microbe Interactions, Madison

Towards Insect Resistance in Transgenic Plants.

Modika Perera and Michael G.K. Jones

The Australian pulse industry is worth about \$400 million per annum. Plant sucking insects such as aphids are serious pests of pulses that reduce crop yield and transmit phytopathogenic viruses. The yellow lupin (*Lupinus luteus*) variety Wodjil is particularly susceptible to aphid damage and this may limit its potential use. In Western Australia aphids are also vectors of two serious lupin viruses: cucumber mosaic virus (CMV) and bean yellow mosaic virus (BYMV). Aphid species, which frequently attack pulse crops in Western Australia such as cowpea aphid (*Aphis craccivora*), green peach aphid (*Myzus persicae*), bluegreen aphid (*Acyrtosiphon kondoi*) and potato aphid (*Macrosiphum euphorbiae*) are the main focus of this project. The control of these pests currently relies heavily on cultural methods and applications of chemical pesticides. Some aphid populations have developed resistance to insecticides. The overall aim of this project was to investigate potential novel sources of aphid resistance genes that may be used for lupin crop improvement, and to study gene expression using different promoters when a plant is attacked by chewing and sucking insects. This project has several parts and there are as follows:

- ***Development of different plant expression cassettes using a wound-inducible promoter**

In order to study the expression of a wound-inducible promoter we have generated a recombinant plasmid pMP4 using a β -glucuronidase (*gus*)/ green fluorescent (*gfp*) fusion construct driven by the *Shpx6b* gene promoter (wound-inducible promoter) from the tropical legume *Stylosanthes humilis* (Dr J.M. Manners-CRC for Tropical Plant Pathology, University of Queensland. This construct was introduced into aphid/insect susceptible tobacco (*Nicotiana tabacum*) cv.'Dynes' using *A. tumefaciens* strain AGLO and produced 48 T₀ transgenic tobacco plants. The transgenic status of these plants was confirmed using Polymerase Chain Reaction (PCR) primers complementary to the *shpx6b* gene promoter to amplify the 600bp fragment. From 48 positive *in vitro* transformants in selection, 20 were randomly selected and transferred into the PC2 glasshouse and T₁ seeds were collected. These T₁ seeds are being analysed further to confirm the expression pattern conferred by the *shpx6b* promoter during insect attack and to check the inheritance of the transgene.

- ***Analysis of gene expression pattern of this wound-inducible promoter during insect attack**

Transgenic (T₁) tobacco leaves with the pMP4 construct were infected with aphids (sucking insect), potato tuber moth (PTM)(chewing insect), were wounded with forceps and were treated with 25 μm plant defense regulator Methyl Jasmonate (MeJa). Non-transgenic tobacco leaves were treated/infected as negative control. The control and experimental leaves were examined for *gus* expression 48-72 hours after infection/treatment by histochemical staining with 5-bromo-4-chloro-3-indole β-D-glucuronide (X-gluc) and *gfp* gene expression using fluorescence microscopy. The results of histochemical staining with X-gluc during the infection of aphids, strong discrete few blue spot were obtained at aphid feeding sites. For chewing insects, there was a strong expression of respective gene but the expression was widely distributed along the edges of damaged parts of the leaves. A similar strong induction of *gus* activity were observed in the positive controls treated with MeJa as well as the leaves mechanically wounded with forceps. The *gfp* expression pattern was quite similar to *gus* expression and, was observed in the feeding sites of both sucking and chewing insects in different level. The obtained results indicated that, the Shpx6b wound-inducible promoter was rapidly and strongly induced during the plant wound response, suggesting that the Shpx6b gene promoter will be potentially useful for genetic engineering of insect and /or pathogen resistance in transgenic plants.

- **Lupin transformation with the construct (pKGMi-11) carrying the complete plant pathogen resistance gene *Mi***

The plant resistance gene *Mi*, from tomato, confers resistance to three species of root-knot nematodes and to aphid species. Work to transform aphid susceptible narrow-leaved (*Lupinus angustifolius*) cultivar Myallie and yellow lupin (*Lupinus luteus*) variety Wodjil has been completed using the cosmid vector pKGMi-11 carrying the complete plant pathogen resistance gene *Mi*. To date we have inoculated 2,590 meristems of *L. Luteus* and 1,580 meristems of *L. angustifolius*. A series of narrow-leaved and yellow lupin shoots were selected and were transferred to aerated hydroponics solution in a PC2 glasshouse. Genomic DNA was extracted from putative transgenic lupin leaf tissues for analysis of the transgenic status of these plants. Specific primers were used to amplify the *Mi* gene sequence and other *Mi* components to verify that there had been an insertion of the complete sequence into selected plants. So far we have not been able to detect any of the *Mi* gene components in these selected plants. In addition, to the *Mi* specific primers we also used a specific primer pair designed to amplify the kanamycin resistance (*npt11*) selectable marker gene. PCR amplification of genomic DNA from three positive *in vitro* transformants (Myallie) and two positive Wodjil transformants using primers for *npt11* were amplified the expected fragment of 644 bp of the *npt 11* gene. The results obtained so far suggest that there may be incomplete introduction of the large *Agrobacterium Mi* insert into the lupin regenerants.

- ***Research on novel sources of aphid resistance**

Performed choice and no-choice bioassays with different aphid species following microscopic technique using different legume crops to investigate potential sources of insect resistance that may be use for lupin crop improvement. Results so far obtained indicate that *Lupinus albus* cultivar Kiev mutant is a potential source for aphid resistance that can be investigated further to isolate, identify and charecterise potentially useful genes of aphid resistance that can be transferred for other legume species via genetic engineering.

The major findings of this research is planned to present at the Australian Entomological Society, 33rd AGM and Scientific Conference, Esplanade Hotel, Frementle, WA 22-27 September 2002.

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