

CANOLA GENOMICS NEWSLETTER

Issue 2, January 2004



GenomePrairie

Introduction

Welcome to the second issue of “Canola Genomics Newsletter”. In this issue we are presenting the progress of the “Enhancing Canola through Genomics” project since the official start of the project. As in the first issue, we would like to introduce you to some of the topics studied in this project. Joan Krochko describes the microspore derived embryos phenomena in *Brassica* and how genomics tools are used to study this phenomenon. Ali Hannoufa working on the seed composition activity describes the anti-nutritional factors remaining in canola seed. Scientists in this project are using several genomic tools and resources. Two articles in this issue are presenting a brief overview of the genomic methodologies. First, Jeff Pylatuik presents the microarray technology. Then Chris Lewis is describing an internet resource used by the researchers involved in the project but also available publicly to other researchers interested in *Arabidopsis* and canola genomics. Public outreach to provide information about new scientific areas such as genomics is done by several means including museum exhibits. In this issue Donna Coad discusses the “Geee! in the Genome” exhibit that is coming to Saskatchewan in January 2004. Finally, as with the first issue, we are presenting a brief update on some of the scientists involved in the project.

Project Update

The three year co-funded “Enhancing Canola through Genomics” project by Genome Canada, the National Research Council and Agriculture and Agri-Food Canada started in April 2003. Significant research and recruitment progress was achieved during the first nine months of the project. The additional hiring of staff allowed the project to progress on both seed development and seed composition activities. Currently there are 40 scientists working full time on the project. As an indication of this progress, eight presentations related directly to this project were given at recent genomics meetings and four new peer-reviewed papers were published or are in press.

On the seed development activity, several cDNA libraries representing different stages of seed development have been constructed. Approximately 15,000 ESTs were sequenced and characterized; thus achieving the targeted number in year 1. Current experiments are focusing on the characterization of selected genes that may have an important role in seed development. Microarray experiments looking at differential gene expression during dormancy and seed germination have started and the data are being analyzed. A signalling chip containing 600 genes involved in plant hormones, signaling pathways and seed development was prepared. Among the 600 genes, 35 are specifically related to

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seed maturation and germination. With regards to the seed composition activity, research is underway on all aspects. In particular, *Arabidopsis* activation-tagged and T-DNA knockout lines are used to have better understanding of lipid synthesis and regulation pathways. In order to gain insights on the regulation of Seed Storage Protein (SSP) genes, approximately 23,000 M1 plants transformed with GUS genes have been screened and 69 putative mutant plants were selected. Among these, 12 lines with phenotype of reduced reporter (GUS) activity have been confirmed. The Tandem Affinity Purification cassette has been re-synthesized to optimize for codon usage in Crucifers. This last construct will be used to identify potential SSP-specific transcription factors protein interactions.

To identify “rate limiting” genes involved in secondary metabolites biosynthesis, 70 *Arabidopsis* mutant lines with knockouts in genes affecting the biosynthesis of phytate, sinapine and lignin have been obtained either by acquisition from TAIR, i.e. SALK lines, or by screening AAFC’s knockout population. Finally, fingerprinting of 11,000 *B. napus* BAC clones has been completed and approximately 10,000 *A. thaliana* activation tagged lines have been generated.

(continued on page 3)

Plants from Pollen: Microspore Embryogenesis in *Brassica*

Joan Krochko* and Alison Ferrie**, NRC-Plant Biotechnology Institute

The current literature documents only one natural occurrence of cells from pollen grains developing into seed embryos in the land plants (5). However, isolated immature pollen grains (microspores) of many plant species can be induced to switch from a gametophytic developmental program to an embryonic developmental program by the application of a stress treatment (heat, cold, osmotica, colchicine). Efficient protocols have been developed for *Brassica napus* and this species is now a model system for the study of early events in plant embryogenesis as well as embryo maturation. The resultant doubled haploids are a useful resource for gene mapping as well as for breeding purposes due to the rapidity and ease in generating homozygous lines. In addition, the availability of large numbers of developmentally synchronous microspore-derived embryos provides a convenient platform for experiments involving large-scale biochemical, molecular and physiological analyses.

Research on *Brassica* microspore-derived embryos (MDE) was first initiated in Agriculture Canada and the University of Guelph in the early 80's and has been a continuing part of the core research program at PBI since 1990. In addition to *Brassica napus*, successful microspore embryogenesis and doubled haploid production have been achieved with several other *Brassica* species (e.g. *B. rapa*, *B. juncea*, *B. oleracea*, *B. carinata*, *B. nigra*) (2). There is also ongoing research at PBI on the development of embryogenesis protocols for other taxa within the *Brassicaceae*, as well as specialty crops, i.e. herbs, spices, medicinals, and legumes. One species that remains recalcitrant in this respect is *Arabidopsis thaliana*, and to date there are no published reports of a reliable microspore embryogenesis system for *Arabidopsis*. A more complete understanding of the cellular events occurring during microspore embryogenesis would aid in the development of protocols for genotype-independent generation of doubled haploid plants for breeding programs.

The identification and isolation of the factors and genes necessary and sufficient to induce embryogenesis in the absence of fertilization (i.e. microspore embryogenesis, somatic embryogenesis, apomixis) is an area of great interest to many labs and yet there are still very few definitive answers. Some gene and protein changes involved in early microspore or somatic embryogenesis have been reported in the literature (1, 6). SERK, a somatic embryogenesis receptor kinase (6), LEAFY COTYLEDON2, a B3 domain transcription factor (7), LEAFY COTYLEDON1, a HAP3-like transcription factor (4), BABY BOOM, an AP2 domain transcription factor (1), and AGL15, AGAMOUS-Like 15 (3), have all been shown to induce embryogenesis when over-expressed in plants. Clearly these proteins are sufficient to induce the rest of the embryogenic program and therefore are involved very early in the cascade of embryogenic-specific gene changes. In contrast, the stress-related factors and events involved in the initial

induction process in microspore-derived embryos are still unknown. In *Brassica* sp. embryogenic competence is dependent upon the developmental stage of the microspores and the transition is most effectively achieved if the stress is applied at the mid- to late-uninucleate or early-binucleate stages. The cellular changes occurring during this stress period, marking the transition to an embryogenesis program, include alterations in microspore polarity and loss of the asymmetric first pollen mitosis. Heat shock proteins, MAP kinase signaling pathways and cell cycle factors have been implicated in the induction of microspore embryogenesis. In related studies, others have suggested that the microspore-embryogenic ability in *Brassica* crops is controlled by two loci with additive effects (8).

Our research program has been initiated to isolate and identify genes involved in the induction of embryogenesis and early embryo development utilizing the *Brassica* microspore system. This will involve sequencing of ESTs from tissue-specific cDNA libraries and subtracted cDNA libraries, protein phosphorylation studies and metabolite analyses. Such data will be used to elucidate the early events involved in the transition from pollen to developing embryo and ultimately provide insights into improving embryogenesis in recalcitrant plant species.



- 1- Boutilier K et al. (2002) Plant Cell 14: 1737-1749;
- 2- Ferrie AMR 2003 In: Doubled Haploid Production in Crop Plants. Maluszynski M, Kasha KJ, Forster BP, Szarejko I (eds) Kluwer Academic Publishers;
- 3- Harding EW et al. (2003) Plant Physiol 133: 653-663;
- 4- Lotan T et al. (1998) Cell 93: 1195-1205;
- 5- Pichot C et al. (2001) Nature 412: 39;
- 6- Schmidt EDL et al. (1997) Develop. 124: 2049-2062;
- 7- Stone SL et al. (2001) Proc Natl Acad Sci USA 98: 11806-11811;
- 8- Zhang FL, Takahata Y (2001) Theor Appl Genet 103: 254-258.

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Anti-Nutritional Factors Remaining in Canola Seed

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Brassica crops in Western Canada are grown primarily as a source of seed oil (40%) although they are also significant sources of high quality protein (~37% on an oil-free basis), which at present, is used primarily as a low grade animal feed.

While high in oil and protein and rich in essential amino acids and fatty acids, *Brassica* seed also contains anti-nutritional components, notably sinapine, fibre and phytate, which hinder use of the meal as an animal feed. Among edible oilseeds, sinapine is unique to *Brassica* seed. Levels range from 0.7% to 3%, with about 90% of it present in the embryo (non-hull) fraction. When consumed, sinapine can cause unpleasant flavours in the meat, milk and eggs. Upon consumption of larger amounts it can cause serious growth and reproduction problems (1). Dietary fibre represents about a third of the canola meal remaining after oil extraction. High fibre content can have adverse effects on diet digestibility and feed efficiency, and reduce oil and protein yields. Phytate, which ranges from 2.0-4.0% in the seed, has both nutritional and environmental impacts. As a result of chelation, phytate lowers the bioavailability of mineral nutrients. Phytate also reduces phosphorus digestibility thereby increasing phosphate in animal waste, which can pollute water systems (2). Therefore, sinapine, fibre and phytate have been identified as targets for elimination from *B. napus* seeds in order to increase the value of the canola meal.

Efforts to identify low sinapine *Brassica* germplasm to generate low sinapine varieties by classical plant breeding have met with little success. Approximately 40% reduction in sinapine has been achieved by metabolic engineering, e.g. by down

-regulating the expression of the ferulic acid 5-hydroxylase (F5H) gene. Development of yellow-seeded cultivars of canola at the Agriculture and Agri-Food Canada's Research Centre in Saskatoon has lowered fibre content due to the thinner hull of the yellow-seeded types. Metabolic mutant selection also holds promise for reducing fibre. Several mutants deficient in the fibre component lignin have been identified in many plant species, including maize and *Arabidopsis*. In addition, several genes in the phenylpropanoid pathway, including caffeic acid *O*-methyltransferase (COMT), hydroxycinnamyl alcohol dehydrogenase (CAD) and phenylalanine ammonia lyase (PAL), have been targets for down-regulation to reduce lignin content in plants (3). Phytate levels in the feed may be reduced by treatment with microbial phytases; however this is an expensive process which is cost-effective only in parts of the world where there are high penalties for disposing manure with high phosphorus content. Mutant selection has yielded low phytate crop varieties (2), and up to 40% reduction in phytate was achieved by genetic engineering (Keller, personal communication).

Developing a canola variety having significantly reduced levels of phytate, sinapine and fibre would represent a major contribution to improving the canola meal not only for livestock feed, but also potentially for direct human consumption.

1- Pearson et al., 1980, J. Sci. Food Agric. 31, 898

2- Raboy, 2001, Trends Plant Sci. 6, 458

3- Anterola and Lewis, 2002, Phytochemistry, 61, 221

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Project Update (continued from page 1)

Two networking meetings were set-up to enhance the interaction among the plant genomics projects. The first meeting involved the principal investigators (PIs) for Genome Prairie's Functional Genomic Abiotic Stress and the Enhancing Canola projects in Saskatoon on 27 Aug. 2003. The second meeting involved the project leaders, managers and some PIs of all Genome Canada plant projects during the Genome Canada annual meeting on 26 Sep. 2003. Scientists exchanged information and ideas on their respective project and discussed possibilities of collaboration.

Three national genomics meetings were held in Saskatoon in August 2003. These three meetings saw the participation of all the PIs involved with the Enhancing Canola project. The PIs participated at different levels including organizations of the meetings, chairing of sessions, oral and poster presentations. In addition, the Enhancing Canola project progress was presented at the Genome Canada meeting held in Toronto on Sep. 25-26, 2003.

Finally, the Enhancing Canola project held recently its first scientific meeting on Nov. 19, 2003 in Saskatoon to review the project progress. Sixty participants including PDFs, technicians and students attended the meeting where 24 short presentations were given. The scientific presentations were followed by a networking meeting.



Raju Datla (NRC) and Kevin Rozwadowski (AAFC) at the Nov. 19 project meeting.

The Application of Microarrays in Advancing Functional Genomics Page 4

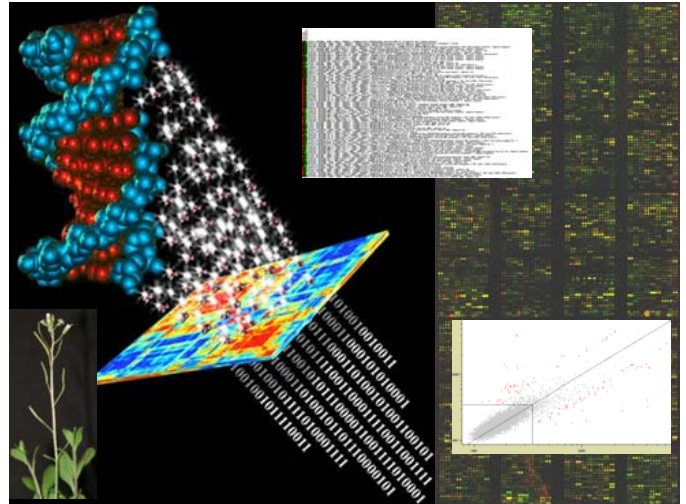
Jeff Pylatuik*, *NRC-Plant Biotechnology Institute*

Scientists have high expectations that genomic approaches in biology will revolutionize our understanding of the molecular mechanisms controlling the development and physiology of plants. The logical progression from the well-established field of structural genomics (i.e. the mapping and sequencing of genomes) is functional genomics, which aims to assess the function of all genes in a systematic fashion. Essential to the success of this goal has been the emergence of microarray (or gene chip) technology to study gene expression. The underlying assumption of microarray analysis is that information on where and when a gene is expressed provides information about its function.

Microarrays commonly consist of gene-specific nucleotides (cDNA, genomic, or synthetic oligonucleotides) spotted on a glass surface. Alternatively, short nucleotides can be synthesized in situ on a quartz wafer using photolithography and oligonucleotide chemistry (i.e. Affymetrix). Hybridizing labeled mRNA from plants displaying different developmental, physiological, or genotypic states permits the simultaneous monitoring of tens of thousands of transcripts in a single step, providing an "expression profile" for that condition.

The explosion of microarray data, combined with sequence data and proteomic data has led to the development of computational genomics, which aims to model functional principles of biological systems. Thus, the applications of microarray data is being expanded beyond the simple identification of up- or down-regulated genes in response to a particular condition. Data generated from microarray analysis can be applied to identify *cis*-regulatory elements in a genome, by comparing sequence and expression data. Modified microarray techniques, in which promoter sequences are spotted on arrays, have been used successfully with immunoprecipitation techniques to identify target genes of transcription factors. Lastly, microarray, sequence, and protein-protein interaction data have been successfully combined to identify new and complex regulatory modules. These regulatory modules involve groups of co-regulated genes, their transcriptional regulators, and how the module functions in response to different conditions.

Accompanying this incredible informative power are several challenging issues facing microarray technology. Many studies have found that results obtained from different platforms are incomparable. mRNA splice variants can't be distinguished on most microarray platforms.



"Full genome" synthetic oligonucleotide arrays are designed based on a large number of loci whose annotation is only tentative. Lastly, there is an unprecedented informatics and analytical challenge in dealing with large amounts of data that is often inherently noisy, instable, and biased. Efforts toward resolving these issues, in particular the data analysis, have progressed considerably and continue to evolve rapidly. As a result, researchers face a daunting challenge in keeping current with improved methods of statistical analysis and interpretation of microarray data. Statistical rigor and validation by standard methods such as RT-PCR or Northern hybridization is increasingly demanded by journals and peer reviewers. As such the quality of the data continues to improve.

Microarray technology is an exciting and promising new development in the field of functional genomics. Several challenges lie ahead but current and future applications of the technology and data it generates has, and will continue to, contribute considerably to our understanding of plant development and physiology.

- 1- Li H, Wang, W. (2003) *Curr. Opin. Gen. Dev.* 13: 611-616.
- 2- Ge H et al (2003) *Trends Genet.* 19: 551-560.
- 3- King HC and Sinha MD (2001) *JAMA* 286: 2280-2288.

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The Brassica / Arabidopsis Genomics Initiative (BAGI) is a project at the Saskatoon Research Centre of Agriculture and Agri-food Canada. The goal is to develop information on the molecular and genetic structure, and function of *Brassica* genomes. To this end the initiative has developed a number of resources, including 3' and 5' sequences for *B. napus* cDNA clones, a *B. napus* BAC library, *B. napus* genetic mapping populations, *Brassica* microsatellite markers, and an *Arabidopsis* activation tagged population. More information about the initiatives resources can be found online at <http://brassica.agr.gc.ca> or <http://www.brassica.ca>.

The present emphasis of the BAGI website is on the Brassica / Arabidopsis Comparative Genome Browser (BioViz), which displays *Brassica* DNA relative to homologous regions of the *Arabidopsis* genome. This imparts contextual information on the *Brassica* sequence through access to the *Arabidopsis* annotation, as well as providing a loose clustering of *Brassica* multi-gene families relative to their *Arabidopsis* homologue. Currently there are more than 70,000 3' and 5' *Brassica napus* EST sequences available in the browser, and in future we plan to include expression information from Microarray and SAGE studies. Details regarding EST library construction will be available online in the new year.

Sequences are aligned to the *Arabidopsis* genomic (BAC) sequences using NCBI BLAST, and the hits identified by BLAST are stored in a MySQL database. The sequence and annotation for the *Arabidopsis* genome was obtained from The Institute for Genomic Research (TIGR).

Publicly available *Brassica* EST sequences from NCBI and the *Brassica oleracea* genomic sequence from TIGR will be added to the browser in the near future. If you have a col

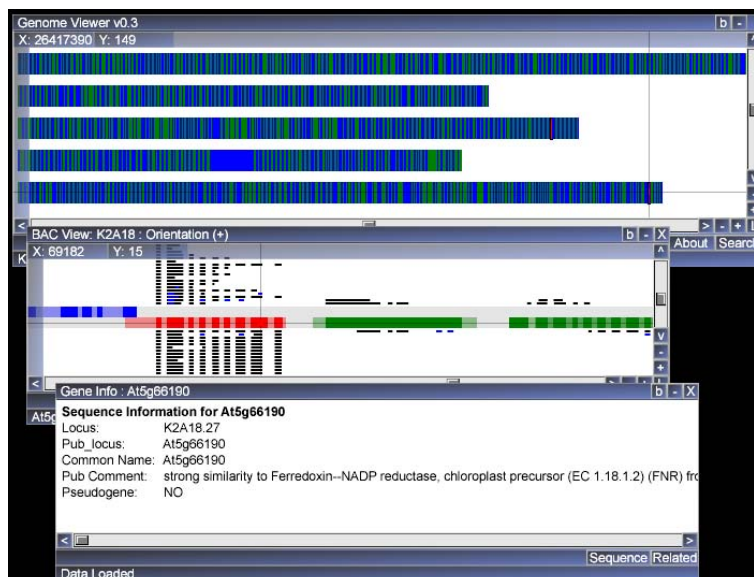
lection of Brassica sequences that you'd like displayed in the browser please contact the author.

The client side of the browser is based on a Graphical User Interface (GUI) object library built using Scalable Vector Graphics; a relatively new graphics standard based on XML. There are a number of advantages to an XML+SVG based genome browser rather than the standard bitmap based browser. 1) Because SVG is a vector graphics format you can zoom and pan an SVG image without losing fidelity (in other words, no grainy images). This also means you don't need to wait for the server to generate a new image every time you would like to examine your data from a different perspective. 2) Because SVG is dynamic and interactive, new data can be added to the existing image and the image can be changed in response to user events (i.e. clicking on a gene).

The server side of the browser uses a collection of CGI's to access data stored in the database or XML and plain text files. As such it should be possible to adapt the browser to work with alternate databases.

More information on selected topics can be found at:
Brassica / Arabidopsis Comparative Genome Browser:
http://www.svgopen.org/2002/papers/lewis_et_al_bioviz_genome_viewer/
SVG Information: <http://www.svgx.org/>
SVG Discussion: <http://groups.yahoo.com/group/svg-developers/>
SVG GUI: <http://homepage.usask.ca/~ctl271/cgui/>
SVG Specification: <http://www.w3.org/TR/SVG/>

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Genome Prairie invites you...

Donna Coad, Genome Prairie*

Do you remember how much fun it was when you were a kid and you learned by doing? This is exactly what the Gee in Genome is all about. Imagine watching your children or your parents as they experience a number of activities such as extracting DNA or creating a 3D protein. These are just a few of the hands on activities you will experience on your visit to the exhibit.

The Gee in Genome is an innovative, multi-dimensional public education project geared for children grade 5 to adult. The exhibit was developed by the Canadian Museum of Nature, presented nationally by Genome Canada in partnership with the Canadian Institutes of Health Research, hosted by the Saskatchewan Science Centre in Regina and presented locally by Genome Prairie. The project is a bilingual, hands on traveling exhibition which includes: a suitcase exhibit, a series of interactive public programs, curriculum-based school programs, youth forums, a national forum series and a dynamic Web component. The web site supports the objectives of the physical exhibition and programming with detailed information for the public, teachers and student alike.

The 2500 sq. ft. exhibition distills contemporary biomedical research into an entertaining and educational experience for its visitors. A musical analogy woven throughout the exhibit helps explain genomics and addresses the interest of teenagers and young adults. The exhibit serves as the center piece for connecting the public to the scientific community and developing interest for careers in science and technology. The approach builds on Dr. Michael Smith's commitment to youth and his role as a teacher who guides our young people as they work to solve the great mysteries of life. There are five zones, each with a different focus but all with hands on interaction.

The museum will visit approximately ten cities across the



country over a three period and will then return to Ottawa to be housed permanently at the Museum of Nature.

On behalf of Genome Prairie and the Saskatchewan Science Centre, we invite you, your family and friends to enjoy the first ever traveling exhibit on the study of genomics. We would also ask you to mark your calendars for the Public Forum which will be held February 19th at the Kramer Imax Theatre, Saskatchewan Science Centre. The topic is Genomes: Wonders and Worries. Our guest panelists, to name a few are: Dr. Lorne Babiuk and Dr. Timothy Caulfield.

For detailed information on the Gee in Genome, we invite you to visit our web site at www.genomeprairie.ca

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Future conferences

- Plant and Animal Genome XII Conference, San Diego, California, Jan. 10-14, 2004, <http://www.intl-pag.org/>
- Genomics in an open society, GE³LS Symposium, Vancouver, Feb. 5-7, 2004, <http://www.genomecanada.ca/ge3ls2004/>
- Plant Responses to Abiotic Stress, Santa Fe, New Mexico, Feb. 19-24, 2004, <http://www.keystonesymposia.org/>
- Comparative Genomics of Plants, Taos, New Mexico, Mar. 4-9, 2004, <http://www.keystonesymposia.org/>
- Crop Functional Genomics, Jeju, South Korea, Apr. 7-10 2004, <http://www.cfg2004.org/>
- The Biology of Genomes, Cold Spring Harbor, May 12-16, 2004, <http://meetings.cshl.org/2004/2004genome.htm>
- 1st Symposium on Enabling Technologies for Proteomics, May 13, 2004, Montreal, www.conciergeconnection.com/etp2004
- 15th International Conference on Arabidopsis, Berlin, Germany, Jul. 11-14 2004, <http://www.arabidopsis2004.de/>
- 2nd Canadian Plant Genomics Workshop, Quebec, Aug. 29-Sep. 1st, 2004
- Plant Genomes, Cold Spring Harbor, Dec. 9-12, 2004, <http://meetings.cshl.org/2004/2004meetings.htm>

Presentation of the project Scientists

Adrian Cutler. Dr. Adrian Cutler's objective within the "Enhancing Canola through Genomics" project is to study genes involved in late seed maturation and germination. Dr. Cutler works at the National Research Council, Plant Biotechnology Institute as a Senior Research officer in the area of molecular and biochemical characterisation of genes involved in stress, ABA metabolism and hormone signaling. Dr. Cutler also worked on plant cold Tolerance and plant cell biology. Dr. Adrian Cutler obtained his PhD in 1978 at Florida State University. He then completed a postdoctoral position at the University of California. He joined PBI-NRC in 1983 after a Research Associate position at the University of Saskatchewan. He is a coauthor of 62 plant biotechnology-related publications in refereed journals. He is also a co-inventor on three patents applications related to plant biotechnology. Dr. Cutler is also a principal investigator with the Genome Prairie Functional Genomics of Abiotic Stress project.



Selected publications from Dr. Cutler's group.

- Zhou R., S. J. Ambrose, M. M. Galka, A. J. Cutler, T. M. Squires, M. K. Loewen, K. Nelson and S. R. Abrams. 2003. A new abscisic acid catabolic pathway. *Plant Physiol.* In Press.
- Zhou R., T. M. Squires, S. J. Ambrose, S. R. Abrams, A. R. S. Ross and A. J. Cutler. 2003. Rapid extraction of abscisic acid and its metabolites for liquid chromatography-tandem mass spectrometry. *Journal Chromatography A.* 1010: 75-85
- Chiwocha S., S. Abrams, A. J. Cutler, A. Ross, A. Kermode, S. Ambrose and M. Loewen. 2003. Metabolic profiling of four classes of plant hormones and their major metabolites by liquid chromatography electrospray tandem mass spectrometry: an analysis of hormone turnover associated with thermodormancy and germination of lettuce (*Lactuca sativa* L.) seeds. *Plant Journal* 35: 405-417
- Qi. Q., Y. Huang, A.J. Cutler, S.R. Abrams and D.C. Taylor. 2003. Molecular and biochemical characterisation of an aminoalcoholphosphotransferase (AAPT1) from *Brassica napus*: effects of low temperature and abscisic acid treatments on AAPT expression in *Arabidopsis* plants and effects of over-expression of BnAAPT1 in transgenic *Arabidopsis*. *Planta.* 217: 547-558.

Jitao Zou. Dr. Jitao Zou's objective within the "Enhancing Canola through Genomics" project is to study genes involved in carbon partitioning and lipid metabolism. Dr. Zou works at the National Research Council, Plant Biotechnology Institute as a Senior Research Officer in the area of plant lipid metabolism. Other areas of interest include physiology and biochemistry of plant stress response and seed and anther development. Dr. Jitao Zou obtained his PhD in 1990 at the Institute of Botany, Chinese Academy of Sciences, Beijing. Then he completed a postdoctoral position at Yale University before joining NRC-PBI in 1992. He is a coauthor of 27 plant biotechnology-related publications in refereed journals. He is co-inventor on six patents and patent applications related to plant lipid metabolism



Selected publications From Dr. Zou's group.

- Wei Y, W Shen, M Dauk, F Wang, G Selvaraj and J Zou (2003) Targeted-gene disruption of glycerol-3-phosphate dehydrogenase in *Colletotrichum gloeosporioides* reveals evidence that glycerol is a significant transferred nutrient from host plant to fungal pathogen. *J. Biol. Chem.* In press
- Zheng Z, Q Xia, M Dauk, W Shen, G Selvaraj and J Zou (2003) The *Arabidopsis AtGPAT1*, a member of a glycerol-3-phosphate acyltransferase gene family, is essential for tapetum differentiation and male fertility. *Plant Cell* 15: 1872-1887.
- Shen W, Y Wei, M Dauk, Z Zheng and J Zou (2003) Identification of a mitochondrial glycerol-3-phosphate dehydrogenase from *Arabidopsis thaliana*: evidence for a mitochondrial glycerol-3-phosphate shuttle in plants. *FEBS Letters* 536: 92-96.
- Marillia E, BJ Micallef, M Micallef, A Weninger, KK Pedersen, J Zou and DC Taylor (2003) Biochemical and physiological studies of *Arabidopsis thaliana* transgenic lines with repressed expression of the mitochondrial pyruvate dehydrogenase kinase. *J. Exp. Bot.* 54: 259-270.

Martin Reaney. Dr. Martin Reaney's objective within the "Enhancing Canola through Genomics" project is to use analytical methods for the study of genes involved in seed composition. Dr. Reaney works at Agriculture and Agri-Food Canada in Saskatoon as a Research Scientist in the area of lipid biochemistry. He is involved in developing new technology for processing oilseeds and producing commercial products. He has assisted Canadian manufacturers to develop and implement new technology for adding value to crops and crop products.

Dr. Martin Reaney obtained his PhD in 1989 at the University of Saskatchewan in the area of Biochemistry and Plant Physiology. Dr. Reaney was a director of research at Feed Energy Inc. in Des Moines, IA for two years before joining Agriculture and Agri-Food Canada in 1990. He is a co-author of 32 plant biotechnology-related publications in refereed journals. He is also a co-inventor on nine patents related to plant biotechnology



Selected publications and patents of Dr. Martin Reaney.

- Reaney MJ, Liu Y-D, Westcott ND. 2003. Method for commercial preparation of conjugated linoleic acid. United States Patent # 6,420,577
- Lang X, Dalai AK, Bakhshi NN, Reaney MJ, Hertz PB. 2001. Preparation and characterization of bio-diesels from various bio-oils. *Bioresour Technol.* 80:53-62.
- Reaney MJ, Liu YD, Taylor WG. 2001. Gas chromatographic analysis of Diels-Alder adducts of geometrical and positional isomers of conjugated linoleic acid. *J. Am. Oil Chem. Soc.* 78:1083-1086
- Reaney MJ, Tyler NJ, Brown K. 1999. Practical nuclear magnetic resonance analysis of liquid oil in oilseeds: I. Factors affecting peak width. *J. Am. Oil Chem. Soc.* 76:859-862

Jas Singh. Dr. Jas Singh's objective in Genome Prairie's "Enhancing Canola through Genomics" project is to elucidate the role of LEA (late embryogenesis abundant) proteins in seed development and their interactions with other seed components. Jas is a senior research scientist at the Eastern Cereal and Oilseed Research Centre of Agriculture and Agri-Food Canada in Ottawa and leads the AAFC/CCGI genomics group there. His research interests are in the development of frost tolerance and cold acclimation in crop plants especially in *Brassica*.

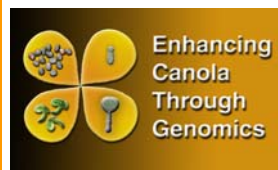
Jas received his Ph.D. in Biochemistry at McGill University in 1971 followed by postdoctoral research in biochemistry at Cornell University in the areas of isolation and characterization of electron transport proteins in chloroplast and in bacteria. Subsequently, he carried out biophysical work on membrane behavior in frost injury before adopting a molecular genetics approach.



He has authored/co-authored over 50 scientific papers and was the co-inventor of several patents related to the subject. Jas is well known for his work on cold acclimation in crop plants and was a member of the Editorial Board of *Plant Physiology* for nine years.

Selected publications and patents of Dr. Jas Singh

- Gao M-J, Allard G, Byass L, Flanagan AM, Singh J (2002) Regulation and characterization of four CBF transcription factors from *Brassica napus*. *Plant Mol Biol* 49: 459-471
- Singh J, White TC and Jiang C (inventors) Canadian Patent 2146712. Issued 2002/05/26. Cold Induced promoter from winter *Brassica napus*.
- Ouellet T, Singh J, Tao T and Simmonds J (inventors) US patent 6515204B1. Issued 2003/02/04. Corn Silk Gene and Regulatory Region.



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