Enhancing Canola Through Genomics

CANOLA GENOMICS NEWSLETTER

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GenomePrairie

Introduction

Welcome to the third issue of "Canola Genomics Newsletter". We are presenting the progress of the "Enhancing Canola through Genomics" project after 15 months since the start of the project. As in the previous issues, some of the topics studied in this project are reviewed. Lester Young and Martin Reaney describe the importance of metals in plant seeds and some implications in human and animal diet. The authors also describe how the synchrotron can be used to analyze elements of importance in the canola seed composition. Prakash Venglat, Daoquan Xiang and Raju Datla working on the seed composition activity describe the embryo development in *Arabidopsis* seeds, the model species closely related to canola. A significant effort within this project deals with bioinformatics such as the analysis and annotation of ESTs. Jacek Nowak describes some of the bioinformatics resources that are used within the project. Other highlights of the project presented in this issue include the creation of a project scientific and management advisory committee and an overview of the DNA microarray training organized by the project. Finally, as with the first issue, we are presenting a brief biography of four scientists involved in the project.

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Project Update

Now in its second year, the "Enhancing Canola through Genomics" Genome Canada funded project has made a significant progress on most of the milestones proposed initially in research activities related to seed development and composition. Scientists from Agriculture and Agri-Food Canada (AAFC) and from the National Research Council (NRC) involved in the project were able to accomplish the following:

- Construction of 27 embryo and seed specific cDNA libraries
- Identification of more than 10,000 Brassica unigenes from more than 60,000 ESTs related to seed development
- A technique for plant hormone profiling using HPLC / MS has been developed
- Fingerprinting and contiging for 20,475 *Brassica napus* BAC clones
- Mutants identified in each step of sinapine and phytate biosynthesis

In addition, several scientists involved in the project presented posters and oral presentations at the NRC Genome Health Initiative meeting held in Montreal on May 16-19, 2004 and at the AAFC Genomics meeting held in Ottawa on June 2-4, 2004.

On May 5th the project held its second scientific meeting to review the project progress. Participants included PDFs, technicians, students and guests. The scientific advisory committee (see p. 5) attended the meeting and provided feedback and advice following 18 short presentations covering various research activities of the project.

Analysis of seed metals using the Synchrotron

Lester Young and Martin Reaney**, AAFC-Saskatoon Research Centre*

Seeds store fairly high concentrations of calcium, iron, zinc and other metals. These metals reserves are mobilised during germination as a ready supply of the enzyme cofactors necessary for rapid growth. The supply of metals in the seed allows rapid growth of the seedling until the roots are large enough to draw the metals from the soil. Many genes control the capture, transport, storage and metabolism of metals in plants, so an alteration in the concentration or location of metals in the seed may indicate a mutation in a metal handling gene.

Understanding metal physiology in plants may have implications in human and animal diets. Many people are thought to have iron or zinc deficient diets, so understanding metal storage in seeds may lead to more nutritious crops. Plants are also being studied currently for their ability to clean up heavy-metal pollution (phytoremediation). Understanding metal transport, metabolism and storage will help this area of research.

We are using synchrotron-based X-ray fluorescence spectra (XFS) to determine both the amount and location of metals in *Arabidopsis thaliana* seeds. We are using *A. thaliana* as it is closely related to canola, so genetic changes in metal handling are likely to be similar in either plant. *A. thaliana* seeds are much smaller than canola seeds and lend themselves to easier analysis in a synchrotron.

Our initial results suggest that tissues with higher levels of metabolic activity during germination have higher concentrations of manganese (Fig 1). The localisation of manganese in rapidly growing tissues is necessary as the turnover of enzyme cofactors is higher in these tissues. Manganese is also known to accumulate in mitochondria, organelles which are in higher concentrations in rapidly growing tissues. Our results provide spatial information about the distribution of metals which have been extremely difficult to obtain in the past using other techniques.

We will be looking at metal storage in different varieties of *A. thaliana* seeds plants to determine how metal accumulation varies between different populations. We will also be screening a library of *A. thaliana* seeds created at AAFC that have individual genes disabled to identify genes which affect metal storage. Identification of these genes in *A. thaliana* will enable better understanding of metal metabolism in both *Arabidopsis* and canola.

Figure 1: Insert Mn fluorescence map of a single *A. thaliana* seed. Higher concentrations of manganese are indicated by blue contour lines.

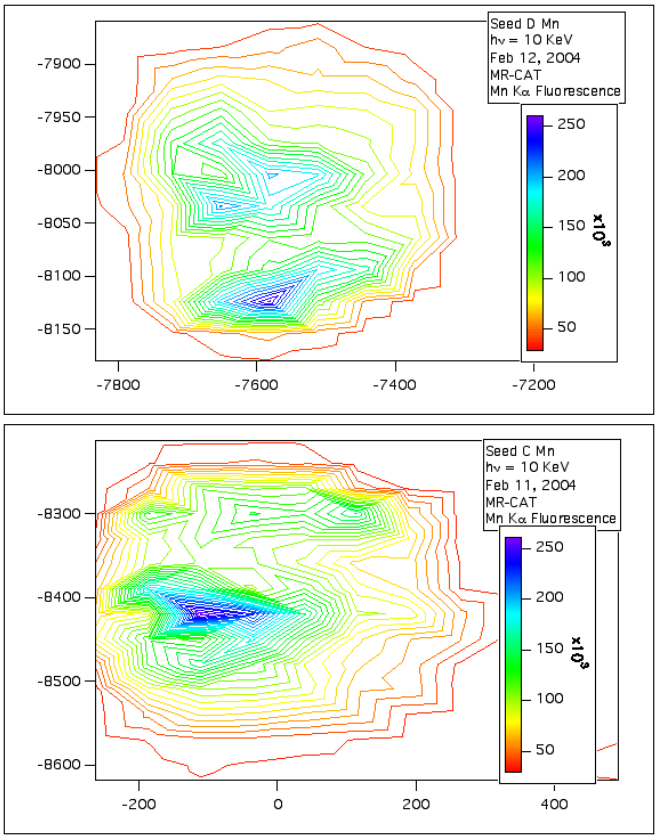

Table 1: ICP-MS determination of metal concentrations (~ppm) in 0.1 g of *Arabidopsis thaliana* seeds

Mg	Ca	Mn	Fe	Co	Ni	Cu	Zn
3580	5160	60	235	0.2	2.4	12	45

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Brassica zygotic embryogenesis,

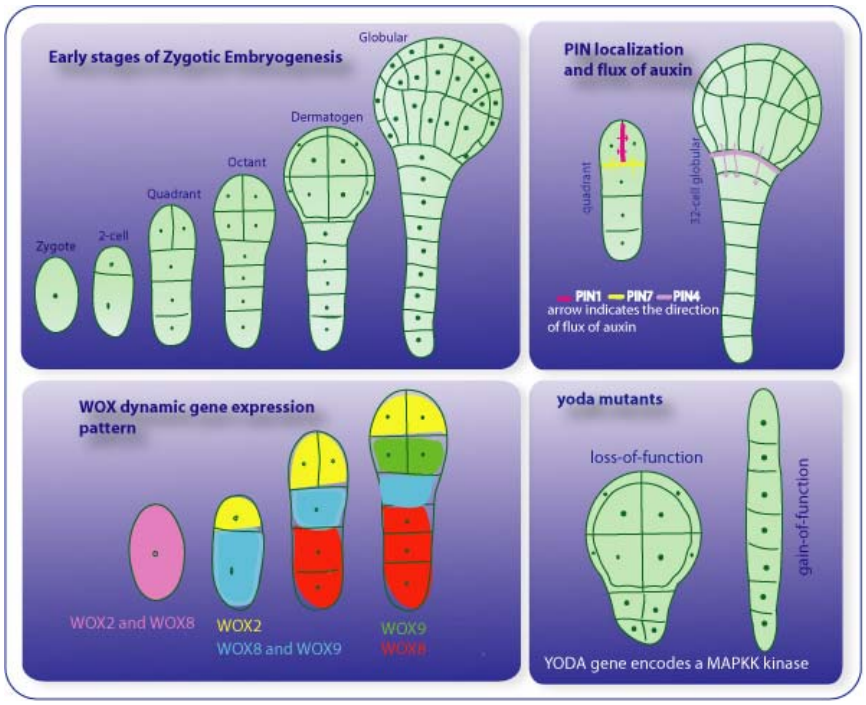
Prakash Venglat[*]*, Daoquan Xiang*[†]* & Raju Datla*[§]*, NRC-Plant Biotechnology Institute*

The main research focus of our group under the Genome Prairie project is zygotic embryogenesis, with the goal to identify genetic factors that are involved in patterning and pattern elaboration which contribute to diverse seed characteristics. Specifically, we are using different *Brassica* species that display noticeable variation in seed size and number. This article highlights the roles of three key players in zygotic embryogenesis that appeared in the recent scientific literature.

The product of fertilization, zygote, divides asymmetrically resulting in a small apical cell that gives rise to the embryo proper and a large basal cell that gives rise to the extra-embryonic suspensor. Lukowitz et al. (2004) have identified a MAPKK kinase that specifies through an as yet unknown signaling pathway the suspensor fate of the basal cell. They screened for mutants that show defects in the early stages of embryogenesis and identified several candidates one of which was called *yoda*. In the recessive mutant *yoda*, the basal cell failed to produce a suspensor and divided aberrantly resulting in cells with an embryonic fate. Positional cloning of *YODA* revealed that it encodes a MAPKK kinase. The dominant-negative lines of *YODA*, which harbor a deletion in the non-catalytic domain of the MAPKK kinase and exhibit constitutive kinase activity, produced embryos with long suspensors or in extreme cases the whole embryo was represented by a long suspensor. These findings suggest that some key developmental switches are regulated by MAPKK kinases in the specification of cell fate during embryogenesis of plants and animals.

The plant hormone auxin has been shown to play a critical role in the specification of polarity and axis establishment during embryogenesis. However, it has not been clear how a small diffusible molecule, IAA (auxin), can be localized to different parts of the embryo to trigger changes during development. *PIN* (derived from *pin-formed* mutant) family of proteins function as auxin efflux carriers and allow translocation and localization of auxin in different tissues. Friml et al. (2003) have identified mutants of the different members of the *PIN* gene family and studied the localization dynamics of the key PIN proteins during early stages of embryo patterning. They used an auxin-inducible promoter tagged to GFP to track the presence of auxin in the zygote and later embryo stages. Auxin response signal was initially observed in the apical proembryo cells up to 32-cell stage after which the signal was absent in the proembryo and present in the uppermost cell of the suspensor (hypophysis). This switch in the auxin gradient from the proembryo (up to 32-cell stage) to the upper suspensor cell later is coordinated by the localization of PIN7, PIN1 and PIN4. Embryos of *pin* mutants exhibit defects in the early cell division patterns that are attributed to polarity establishment and later in the patterning of the hypocotyl and root meristem.

Homeobox genes such as the *HOX* gene family members are transcription factors involved in regulation of cell fate during animal embryogenesis. In a recent study, Haecker et al. (2004) have identified several members of the *WOX* gene family that are specifically expressed in different embryo stages. *WOX* stands for *WUSHCEL*-related homeobox genes. *WUSCHEL*, the first member of this family is a marker for stem cell fate in the shoot apex. The dynamics of the expression pattern of the different WOX members suggest that they could play a significant regulatory role in cell fate specification during plant embryogenesis.



References:

- Lukowitz et al. 2004. Cell, **116**: 109-19.
- Friml et al. 2003. Nature, **426**: 147-53.
- Haecker et al. 2004. Development. **131**: 657-68.

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Enhancing Canola through Genomics project bioinformatics resources

Jacek Nowak, NRC-Plant Biotechnology Institute*

Genome sequencing and microarray experiments generate a significant amount of data that is used to determine the structure and function of genes. These data are analyzed using bioinformatics approaches, which include the development and application of computer programs and the development of databases (Baxevanis & Ouellette 2001; Ouzounis & Valencia 2003). With the assistance of five bioinformaticians, the ECTG project uses the necessary infrastructure including high-performance computers and specialized algorithms to conduct the bioinformatic work. Bioinformaticians are involved at different levels and have expertise in many areas e.g. ESTs warehousing and analysis, microarray experimental data processing and statistical analysis, genome mapping.

Bioinformaticians from AAFC and NRC collaborate on sharing bioinformatics resources, in particular, the implementation of secure web base tools for data integration and exchanges between the two institutions. Assistance to other scientists is provided in microarray image processing using QuantArray and ScanArray software's, data storage and analysis using BASE and GeneSpring software's and developing software to manage clones sequences that will be part of a cDNA Brassica microarray currently in development.

Bioinformatics team is also providing support for a large scale Analysis of ESTs data resulting from the project. Over 60 thousand Brassica ESTs sequences were acquired, catalogued and analyzed using automated processing pipelines. High Performance Computing (HPC) with Beowulf cluster technology, a custom made supercomputer with 64 microprocessors are used to automatically and rapidly analyze EST sequences. Continuing optimization and application of sophisticated tools and algorithms for mass analysis of sequence data in a distributed and parallel computing environment resulted in an automated system for functional classification and gene annotation. This effort is still in progress and will be implemented in later months.

References:
- Baxevanis AD & Ouellette BF. 2001. Bioinformatics: a Practical Guide to the Analysis of Genes and Proteins. J Wiley & Sons, Inc.
- Ouzounis CA & Valencia A. 2003. *Bioinformatics.* **19**: 2176-2190.

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Future conferences and training courses

- 15th International Conference on *Arabidopsis*, Berlin, Germany, 11-14 Jul. 2004, www.arabidopsis2004.de/
- Gordon Research Conference, Plant Molecular Biology, 18-23 Jul. 2004, Holderness School, Plymouth, NH, www.grc.uri.edu/programs/2004/plantmol.htm
- International Arabidopsis Vascular Development Conference, Canmore, Canada, 13-15 Aug. 2004, www2.biology.ualberta.ca/iavd2004/
- 2nd Canadian Plant Genomics Workshop, Quebec, 29 Aug.- 1st Sep, 2004, www.cpgw-acgp.ca/quebec2004/en/
- The 5th Genomic Arabidopsis Research Network Meeting (GARNet), Leicester, UK, 1-2 Sep. 2004, www.york.ac.uk/res/garnet/meetinginfo2004.htm
- Agricultural Biotechnology International Conference, (ABIC 2004), Cologne, Germany, 12-15 Sep. 2004 http://www.abic2004.org/
- TIGR's XVI Genome Sequencing and Analysis Conference (GSAC). 27 - 30 Sep. 2004; Washington, D.C., www.tigr.org/conf/gsac/
- 7th Annual Conference on Computational Genomics. 21-24 Oct. 2004; Reston, Virginia, www.tigr.org/conf/cg/
- The Applied Computational Genomics Course, 27 Nov. - 5 Dec., 2004, Halifax, NS, Canada, www.gcbioinformatics.ca/training.htm

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The enhancing canola genomic project appoints an advisory committee

The "Enhancing Canola" project has formed a Scientific and Management Advisory Committee (SMAC) to provide scientific advice and strategic guidance to the project. The SMAC is composed of three members expert in management, seed development and composition. The members are Dr. John Thompson (Chair), Dr. Steve Barnes and Dr. John Harada. Dr. John Thomson is professor at the University of Waterloo. His research interests include: plant development in particular comparative aspects of senescence and stress and the role of hormones and the involvement of free radicals; molecular cloning of genes involved in senescence and stress. Dr. Thompson is also Vice President of Research and Development with Senesco Technologies**.** Dr. Thompson has a PhD in Biology from the University of Alberta, Edmonton and is a Fellow of the Royal Society of Canada. Dr. John Harada is Professor of Plant Biology at the University of California Davis; he is chair of the graduate program. His research interests include molecular, genetic, biochemical, and genomic dissection of embryogenesis and seed development in plants. He teaches Plant Growth and Development and Molecular and Cellular Biology of Plants. Dr. Harada has a PhD in Biochemistry from the University of Washington. Dr. Steve Barnes is Group Leader at Advanta (SES Europe, Belgium). He is responsible for the development and implementation of molecular methods in the beet and canola breeding programs. Dr. Barnes research interests include plant genome structures, BAC and ESTs resources and marker assisted selection in canola and sugar beet. Dr. Barnes obtained his PhD in Genetics at the University of Cambridge, UK. He taught genetics at the University of Nottingham and serves in several international committees dealing with plant breeding and genomics. The SMAC members receive copies of the progress reports and other relevant documentation. They will evaluate the project progress regularly and will offer recommendations to the project Steering Committee how to achieve maximum impact from this Genome Canada investment. The SMAC participated recently to the 2nd project scientific meeting (see p. 1) and visited the various AAFC and NRC laboratories involved in the project.

Project scientists participate to DNA microarrays training

DNA microarrays technology is an important tool used in the Enhancing Canola project by several scientists for the study of seed development and composition. The project organized a hands-on DNA microarrays introductory course on Feb 10-13, 2004 and a DNA microarray workshop on Feb. 26-27, 2004 to train new scientists and to provide up-to-date information on this technology. The hands-on course saw the participation of ten scientists from NRC and AAFC involved directly in the project. At the DNA microarray workshop, scientists involved in DNA microarrays research provided 10 presentations. Four experts in DNA microarray design and data analysis were invited to give a lecture in this workshop. The guest speakers were Sorin Draghici (Wayne State University, Detroit), Nicolas Tinker (AAFC, Ottawa), Neil Winegarden (University Health Network, Toronto) and Chris Bowman (NRC-Institute for Biodiagnostics, Winnipeg). Approximately 110 scientists primarily from three Genome Prairie projects (FGAS, Pathogenomics, ECTG) attended the workshop. The program and talks presented at the workshop are available on the internet in the project website. (www.genomeprairie.ca/canola/presentations.htm).

The DNA microarray workshop was possible thanks to the sponsors: NRC-PBI, AAFC-Saskatoon, Genome Prairie, Ag-West Biotech Inc., FGAS project at Genome Prairie, VIDO and Amersham.

Presentation of the project Scientists

Kevin Rozwadowski

Dr. Kevin Rozwadowski's objective within the "Enhancing Canola through Genomics" project is to investigate the regulation of seed storage protein gene expression by identifying members of transcription factor complexes using epitope-tagged proteins. Other current research interests of Kevin centre around DNA recombination and repair in plants including: development of technologies for modulating DNA recombination frequency during meiosis and in vegetative cells, development of gene targeting technologies, identification and characterization of members of protein complexes facilitating repair of double-strand breaks, and investigation of signal transduction pathways and regulation of the cell cycle in response to DNA damage. Kevin is a research scientist at the Agriculture and Agri-Food Canada laboratory in Saskatoon. He received his PhD in Molecular Biology and Genetics at the University of Guelph in 1996 followed by postdoctoral research experience at NRC and AAFC in Saskatoon before joining AAFC as a Research Scientist. Kevin is a coauthor of eight plant related publications in refereed journals. He is also a co-inventor on five patent applications related to plant biotechnology.

Selected papers/patents of Kevin Rozwadowski

- Venglat SP, Dumonceaux T, Rozwadowski K, Parnell L, Babic V, Keller W, Martienssen R, Selvaraj G, Datla R. 2002. The homeobox gene BREVIPEDICELLUS is a key regulator of inflorescence architecture in Arabidopsis. Proc Natl Acad Sci U S A. 99:4730-5.
- Qi Q, Rajala RV, Anderson W, Jiang C, Rozwadowski K, Selvaraj G, Sharma R, Datla R. 2000. Molecular cloning, genomic organization, and biochemical characterization of myristoyl-CoA:protein N-myristoyltransferase from Arabidopsis thaliana. Journal of Biological Chemistry. 275:9673-83.
- Huang J, Hirji R, Adam L, Rozwadowski KL, Hammerlindl JK, Keller WA, Selvaraj G. 2000. Genetic engineering of glycinebetaine production toward enhancing stress tolerance in plants: metabolic limitations. Plant Physiology. 122:747-56.
- Rozwadowski K; Lydiate D. Modulation of Meiotic Recombination (PCT/WO /02/22811 A2, 2002).

Yuhai Cui

Dr. Yuhai Cui's objective within the "Enhancing Canola through Genomics" project is to study the regulation of seed storage proteins. Yuhai's lab is using a transgenic screening approach to identify genes controlling two conglycinin subunit gene promoters. *Arabidopsis thaliana* is being employed as model system for this study. Yuhai completed his B.Sc. in Microbiology at Shandong University, M.Sc. and PhD in Plant Microbiology at the Chinese Academy of Sciences. Shortly after completing his PhD Yuhai did a postdoctoral at Cornell University. In 1999, Yuhai earned a PhD in Plant Molecular Genetics at the University of Guelph, where he also did his postdoctoral research. In 2000, Yuhai joined AAFC as a Research Scientist in London. He is a coauthor of 14 plant related publications in refereed journals.

Selected papers of Yuhai Cui.

- Brugiere N, Cui Y, Bi Y-M and Rothstein SJ. 2001. The AtPP gene of the Brassica napus S locus region is specifically expressed in the stigma and encodes a protein similar to a methyltransferase involved in plant defense. Sex. Plant Reprod. 13:309-314.
- Brugiere N, Cui Y and Rothstein SJ. 2000. Molecular mechanisms of self-recognition in Brassica self-incompatibility. Trends Plant Sci. 5:432-8.
- Bi Y-M, Brugiere N, Cui Y, Goring DR and Rothstein SJ. 2000. Transformation of *Arabidopsis* with a *Brassica* SLG/SRK region and ARC1 gene is not sufficient to transfer the self-incompatibility phenotype. Mol. Gen. Genet. 263: 648-54.
- Cui Y, Bi Y-M, Brugiere N, Arnoldo M and Rothstein SJ. 2000. The S locus glycoprotein and the S receptor kinase are sufficient for self-pollen rejection in Brassica. Proc. Natl. Acad. Sci. U. S. A. 97: 3713-3717.

Presentation of the project Scientists *(continued from p. 6)*

Suzanne Abrams

Dr. Sue Abrams's objective within the "Enhancing Canola through Genomics" project is to develop a general mass spectrometric method to profile hormones and their metabolites in a plant tissue. The method is being applied to a variety of studies including seed development and dormancy, high temperature and osmotic stress in seeds. Other research interests of Sue's include natural products chemistry, organic synthesis, development of ABA analogs as plant growth regulators and the application of NMR and MS in plant biotechnology research. Sue is a Principal Research Officer at the National Research Council in Saskatoon. She is an adjunct professor at the University of Saskatchewan, Department of Chemistry and a Principal Investigator with the Genome Prairie Functional Genomics of Abiotic Stress project. She received her PhD in organic chemistry from Dalhousie University in 1977 and joined NRC in 1978. Sue is an author on 114 publications in refereed journals and is an inventor on four patents/patent applications related to plant growth regulators.

Selected recent papers of Sue Abrams

- Ross ARS, Ambrose SJ, Cutler AJ, Feurtado JA, Kermode AR, Nelson K, Zhou R, Abrams SR. 2004. Determination of endogenous and supplied deuterated abscisic acid in plant tissues by HPLC-ESI MS/MS with MRM. Analytical Biochemistry. 329:324-33.
- Zhou R, Ambrose SJ, Galka MM, Cutler AJ, Squires TM, Loewen MK, Nelson K, Jadhav A, Taylor DC, Abrams SR. 2004. A new abscisic acid catabolic pathway. Plant Physiology. 134:361-369.
- Chiwocha SDS, Abrams SR, Ambrose SJ, Cutler AJ, Loewen M, Ross ARS, Kermode AR. 2003. A Method for Profiling Classes of Plant Hormones and Their Metabolites using Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry: An Analysis of Hormone Regulation of Thermodormancy of Lettuce (*Lactuca sativa* L.) Seeds. The Plant Journal. 35:405-417.
- Razem, F.A., M. Luo, J-H. Liu, S.R. Abrams, and R.D. Hill 2004 Purification and characterization of a barley aleurone abscisic acid-binding protein Journal of Biological Chemistry. 279: 9922-9929.

Fawzy Georges

Dr. Fawzy Georges' objective within the "Enhancing Canola through Genomics" project is to study functional genomics of early stages seed development with particular emphasis on sugar metabolism and signaling. Fawzy is a Senior Research Scientist at the National Research Council in Saskatoon. His other research interests include: molecular genetics and genetic engineering of plants, antinutritional plant carbohydrates, protein engineering and phospholipid signaling for superior and improved traits. Fawzy is an adjunct professor at the University of Saskatchewan, department of Biochemistry. He received his PhD in Bio-organic chemistry at the University of Ottawa in 1977 followed by postdoctoral research experience at McGill University and the University of Alberta. He is a coauthor of 40 plant related publications in refereed journals. He is also a co-inventor on six patents/patent applications related to plant biotechnology.

Selected papers/patents of Fawzy Georges

- Dong J, Keller W, Yan W and Georges F. 2004. Gene expression at early stages of *Brassica napus* seed development as revealed by transcript profiling of seed abundant cDNAs. Planta. 218: 483-491.
- Athmer L, Kindrachuk J, Georges F, Napper S, 2002. The influence of protein structure on the products emerging from Succinimide hydrolysis". Journal of Biological Chemistry. 277: 30502-30507.
- Shaterian J, Georges F, Hussain A, Waterer D, De Jong H and Tanino KK. 2004. Salt Stress Resistance Associated with Abscisic Acid and Differential Expression of the Calreticulin (CR) Gene in Diploid Potato Clones. Environmental & Experimental Botany, in Press.
- Keller WA, R. Datla, J.-Z. Dong, F. Georges, A.A.K. Hussain and G. Selvaraj, Methods and Compositions for Modifying Levels of Secondary Metabolic Compounds in Plants. (PCT/CA99/00056, 1999).

Future conferences and training courses *(continued from p. 4)*

- 2nd Biological Language Conference, 18-19 Nov. 2004, Carnegie Mellon University, Pittsburgh, http://flan.blm.cs.cmu.edu/blc2004/
- Plant Genomes, Cold Spring Harbor, NY. 9-12 Dec. 2004, http://meetings.cshl.org/2004/2004meetings.htm
- Plant and Animal Genome XIII Conference, San Diego, California, *15-19 Jan. 2005*, www.intl-pag.org/
- Keystone Symposia: Plant Cell Signalling - In vivo and omics Approaches 1 - 6 Feb. 2005; Santa Fe, New Mexico, www.keystonesymposia.org
- Keystone Symposia: Proteomics and Bioinformatics, 8 - 13 Apr. 2005; Keystone, Colorado, www.keystonesymposia.org

Website of interest…

www.brassica.info.

This website is managed by Dr. Graham King from the Horticulture Research Institute in the UK. The website contain information related to *Brassica* genomics and genetics. Information on national and international research program, a wide range of *Brassica* experimental resources, including plants, reference populations, clone libraries, genetic markers is available on the website.

More information…

More information on the institutions involved in the "Enhancing Canola through Genomics" project is available using the following Internet links:

AAFC-Saskatoon: www.agr.gc.ca/science/saskatoon
AAFC-London: www.agr.gc.ca/science/london
AAFC-Ottawa: www.agr.gc.ca/science/ecorc
Genome Prairie-Calgary: www.genomeprairie.ca
NRC/PBI-Saskatoon: pbi-ibp.nrc-cnrc.gc.ca/en/pbi.htm

An electronic form of this newsletter and other information about the project including the contact information of the scientists involved in the project are available at www.genomeprairie.ca/canola/.

If you have comments about this newsletter or would like to contribute with a relevant article, please send your comments and contributions to: Faouzi.Bekkaoui@nrc-cnrc.gc.ca

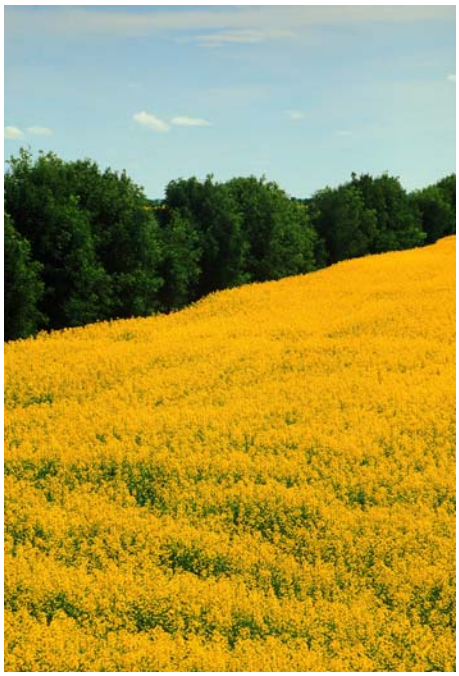

Photo courtesy the Canola Council of Canada

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