

17<sup>th</sup> Crucifer Genetics Workshop

# BRASSICA 2+10

September 5th – 9th

Saskatoon, SK



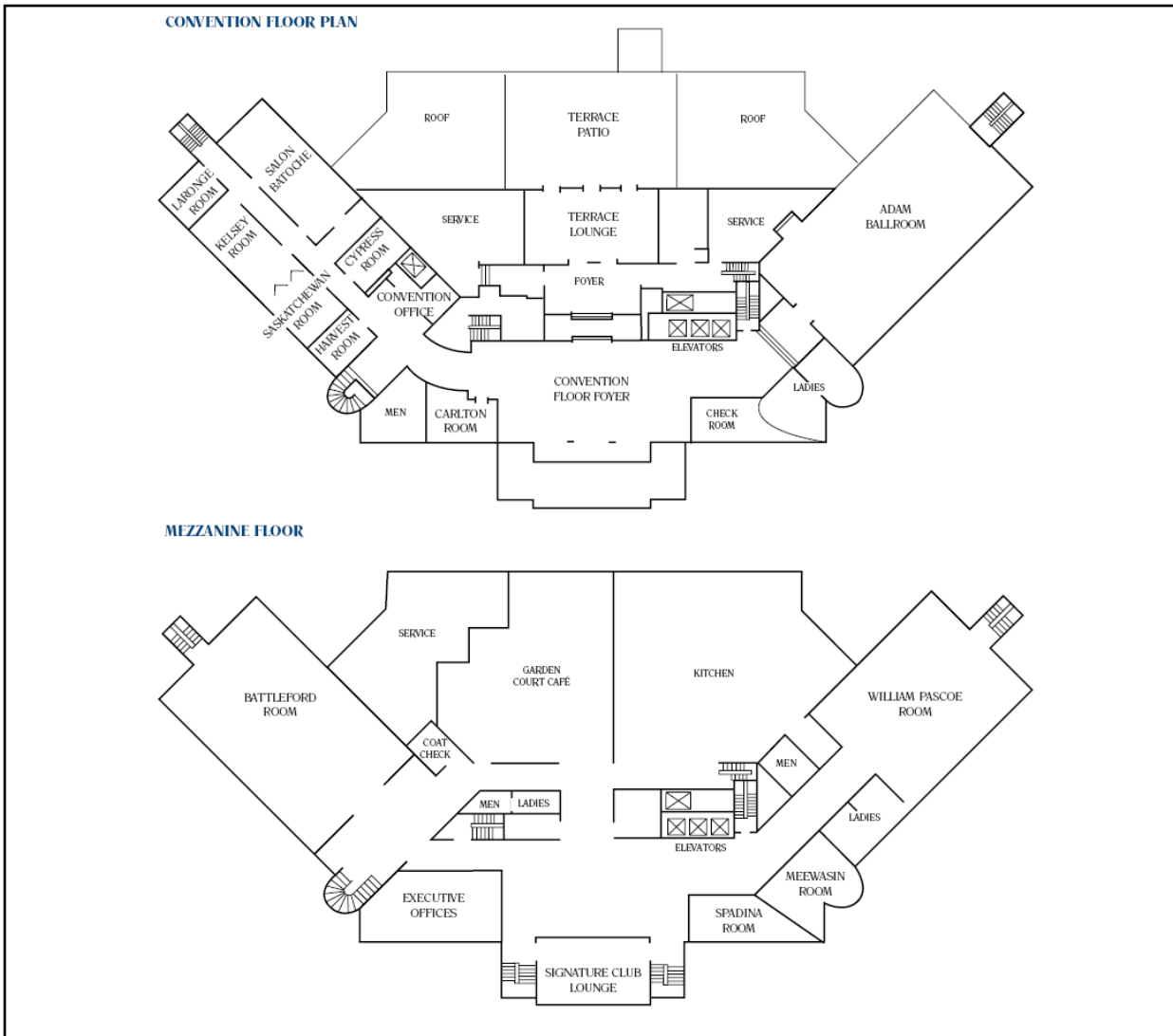
PROGRAM & ABSTRACTS BOOK



# Floor Plan

Delta Bessborough Hotel

Saskatoon, SK, Canada



## PROGRAM AT A GLANCE

### Brassica2010: 17<sup>th</sup> Crucifer Genetics Workshop

Note: All talks in the ADAM BALLROOM, the Delta Bessborough Hotel, 601 Spadina Crescent East, Saskatoon

<i>Sunday Sept 5</i>	<i>Monday Sept 6</i>	<i>Tuesday Sept 7</i>	<i>Wednesday Sept 8</i>	<i>Thursday Sept 9</i>
	7:30 Continental Breakfast & Registration (Convention Floor Foyer)	7:30 Continental Breakfast & Registration (Convention Floor Foyer)	7:30 Continental Breakfast (Convention Floor Foyer)	
	<b>Session 1A: Seed Quality</b> 8:30 Keynote: Jitao Zou (CANADA) 9:10 Larry Sernyk (USA) 9:30 Kun Lu (CHINA) 9:50 Fred Peng (CANADA)	<b>Session 3A: Comparative &amp; Functional Genomics</b> 8:30 Keynote: Thomas Altmann (GERMANY) 9:10 Wang Jun (UK) 9:30 Nirala Ramchiary (KOREA) 9:50 Mark Belmonte (CANADA)	<b>Session 5A: Breeding and Trait Genetics</b> 8:30 Keynote: Wallace Cowling (AUSTRALIA) 9:10 Keynote: You-Rong Chai (CHINA) 9:50 Christian Obermeier (GERMANY)	<b>Excursion to Wanuskewin Heritage Park</b> 9:30: Departure from Hotel
	10:10 Coffee Break	10:10 Coffee Break Sponsored by Cargill Inc	10:10 Coffee Break sponsored by Pioneer Hi-Bred Ltd	
	<b>Session 1B: Seed Quality</b> 10:40 Keynote: Peter Eastmond (UK) 11:20 Wentao Zhang (CANADA) 11:40 Youlian Pan (CANADA) 12:00 Abha Agnihortri (INDIA)	<b>Session 3B: Comparative &amp; Functional Genomics</b> 10:40 Keynote: Rod Wing (USA) 11:20 Keith Adams (CANADA) 11:40 Xiaowu Wang (CHINA) 12:00 Sateesh Kagale (CANADA)	<b>Session 5B: Breeding and Trait Genetics</b> 10:40 Keynote: Anne-Marie Chèvre (FRANCE) 11:20 Zhengying Cao (CHINA) 11:40 Marilyn Cruz-Alvarez(USA) 12:00 Jo-Anne Relf-Eckstein (CANADA)	
	12:20 Lunch	12:20 Lunch	12:20 Lunch	12:00 Lunch
13:30 – 17:00 (Adam Ballroom) <b>Brassica Genome Sequencing Workshop</b> The <i>Brassica rapa</i> reference sequence On-going <i>B. oleracea</i> reference sequencing	<b>Session 2A: Development</b> 13:30 Keynote: Tim Sharbel (GERMANY) 14:10 Robertson McClung (USA) 14:30 Ming-Jun Gao (CANADA) 14:50 Fangqin Zeng (CHINA)	<b>Session 4A: Response to the Environment</b> 13:30 Keynote: Rick Amasino (USA) 14:10 Steve Robinson (CANADA) 14:30 Richard Fletcher (USA) 14:50 Allan Feurtado (CANADA)	<b>Session 6A: Genetic Diversity</b> 13:30 Keynote: Mark Beilstein (USA) 14:10 Guy Barker (UK) 14:30 Renate Schmidt (GERMANY) 14:50 Jinling Meng (CHINA)	14:30: Departure from Heritage Park
15:00 Coffee Break Sponsored by Illumina Inc	15:20 Coffee Break Sponsored by Beckman Coulter Inc	15:20 Coffee Break Sponsored by Life Technologies Inc	15:20 Coffee Break Sponsored by Genome Prairie	15:00: Arrival at Delta Bessborough
<i>Brassica napus</i> reference sequencing Re-sequencing and SNP discovery General discussion/future priorities	<b>Session 2B: Plant Products</b> 15:50 Keynote: Daniel Kliebenstein (USA) 16:30 Diana Zuluaga (NETHERLANDS) 16:50 Maurice Moloney (UK) 17:10 Guusje Bonnema (NETHERLANDS)	<b>Session 4B: Response to the Environment: Biotic Stress</b> 15:50 Keynote: Barbara Howlett (AUSTRALIA) 16:30 Régine Delourme (FRANCE) 16:50 Sandra Konietzki (GERMANY) 17:10 Ben Vosman (NETHERLANDS)	<b>Session 6B: Genetic Diversity</b> 15:50 Keynote: Angela Hay (UK) 16:30 Stephen Ridge (AUSTRALIA) 16:50 Anja Bus (GERMANY) 17:10 Feng Li (JAPAN)	
17:00 – 21:00 (Convention Floor Foyer) Registration				
19:00 – 22:00 (Terrace Patio & Lounge) Opening Reception	17:30 – 19:30 Poster Session (Terrace Lounge & Convention Floor Foyer)	19:00 Cocktails (Convention Floor Foyer) 20:00 BANQUET (Adam Ballroom)	17:30 SCIENTIFIC PORTION ENDS	



Delta Bessborough Hotel  
Web site: <http://www.brassica2010.ca>

## TABLE OF CONTENTS

Program at a Glance.....	1
Detailed Program.....	3
Organizing Committee.....	10
Supporters.....	11
Abstracts:	
Session 1: Seed Quality.....	19
Session 2: Development & Plant Products.....	29
Session 3: Comparative and Functional Genomics.....	39
Session 4: Response to the Environment.....	49
Session 5: Breeding and Trait Genetics.....	59
Session 6: Genetic Diversity.....	69
Posters.....	79
Participant List.....	143

# PROGRAM BRASSICA2010

SUNDAY SEPTEMBER 5<sup>TH</sup> – THURSDAY SEPTEMBER 9<sup>TH</sup>

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## SUNDAY SEPTEMBER 5<sup>TH</sup> 2010 (AFTERNOON / EVENING)

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- 13:30    **BRASSICA SEQUENCING WORKSHOP (PART I)**                      *Adam Ballroom*  
*Chair: Ian Bancroft, John Innes Centre, Norwich, UK*
1. *The Brassica rapa reference sequence*  
Xiaowu Wang, Institute of Vegetables and Flowers, Beijing, China
  2. *On-going B. oleracea reference sequencing*  
Andrew Sharpe, NRC-Plant Biotechnology Institute, Saskatoon, Canada  
Shengyi Liu, Oil Crops Research Institute, Wuhan, China
  3. *Brassica napus reference sequencing around the world*  
Boulos Chaloub, INRA, Evry, France  
Jinling Meng, Huazhong Agricultural University, Wuhan, China
- 15:00    *Break SPONSORED BY ILLUMINA INC*
- 15:30    **BRASSICA SEQUENCING WORKSHOP (PART II)**                      *Adam Ballroom*
4. *Other Brassicas, Re-sequencing , SNP discovery and Bioinformatics Resources*  
Jacqui Batley, University of Queensland, Brisbane, Australia  
Graham King, Rothamsted Research, Harpenden, UK  
Ian Bancroft, John Innes Centre, Norwich, UK  
Isobel Parkin, Agriculture and Agri-Food Canada, Saskatoon, Canada
  5. *General discussion and future priorities*
- 17:00–    *Registration*                                                                              *Convention Floor Foyer*  
21:00
- 19:00–    *Opening Reception*                                                                              *Terrace Lounge & Patio*  
22:00

# PROGRAM BRASSICA2010

SUNDAY SEPTEMBER 5<sup>TH</sup> – THURSDAY SEPTEMBER 9<sup>TH</sup>

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MONDAY SEPTEMBER 6<sup>TH</sup> 2010 (MORNING)

(ALL SCIENTIFIC TALKS IN ADAM BALLROOM, CONVENTION FLOOR)

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- 7:30 - 13:00     *Registration*     *Convention Floor Foyer*
- 7:30     *Continental Breakfast*     *Convention Floor Foyer*
- 8:30     **SESSION 1A: SEED QUALITY**  
*Chair: Kevin Rozwadowski, Agriculture and Agri-Food Canada, Saskatoon, CANADA*  
**Keynote: Jitao Zou**, NRC-Plant Biotechnology Institute, Saskatoon, CANADA  
"Functional Significance of Lysophosphatidylcholine Acyltransferase in Acyl Group Editing and Triacylglycerol Biosynthesis in Seeds"
- 9:10     **Larry Sernyk**, Dow AgroSciences, Indianapolis, USA  
"Developments in High Oleic Acid, Low Linolenic Acid Canola Breeding at Dow Agrosciences"
- 9:30     **Kun Lu**, Southwest University, Beibei, P.R. CHINA  
"Expression Quantitative Trait Loci Analysis of *BAN*, *F3H* and *TT19* Genes in *Brassica napus*"
- 9:50     **Fred Peng**, University of Alberta, Edmonton, CANADA  
"Genome-Wide Comparative Analysis of the B3 Superfamily of Transcription Factors in *Arabidopsis*, *Brassica rapa*, Castor Bean, and Rice"
- 10:10     *Break*
- 10:40     **SESSION 1B: SEED QUALITY**  
*Chair: Jitao Zou, NRC-Plant Biotechnology Institute, Saskatoon, CANADA*  
**Keynote: Peter Eastmond**, Warwick HRI, University of Warwick, Wellesbourne, UK  
"Triacylglycerol Hydrolysis in Crucifers"
- 11:20     **Wentao Zhang**, Agriculture and Agri-Food Canada, Saskatoon, CANADA  
"Analysis of the Seed Quality Traits of *Brassica napus* by Genome-Wide Gene Expression Quantitative Trait Locus Mapping"
- 11:40     **Youlian Pan**, NRC-Institute for Information Technology, Ottawa, CANADA  
"Discovery of Transcriptional Regulatory Machineries in Seed Development of *Brassica napus*"
- 12:00     **Abha Agnihotri**, Amity University, Noida, INDIA  
"Fatty Acids Composition and Fungal Disease Resistance in Advance Progenies of Indian Mustard (*B. juncea*) Derived from Interspecific Cross (*B. juncea*/*B. carinata*) and Microspore Mutagenesis"
- 12:20     *Lunch*

# PROGRAM BRASSICA2010

SUNDAY SEPTEMBER 5<sup>TH</sup> – THURSDAY SEPTEMBER 9<sup>TH</sup>

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## MONDAY SEPTEMBER 6<sup>TH</sup> 2010 (AFTERNOON / EVENING)

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- 13:30 **SESSION 2A: DEVELOPMENT**  
*Chair: Raju Datla, NRC-Plant Biotechnology Institute, Saskatoon, CANADA*  
Keynote: Timothy F. Sharbel, Leibniz Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Gatersleben, GERMANY  
“Heterochrony and Hybridization: The Evolution of Apomixis in the Genus *Boechera*”
- 14:10 C. Robertson McClung, Dartmouth College, Hanover, USA  
“Genetic Architecture of Circadian Clock Function in *Brassica rapa*”
- 14:30 Ming-Jun Gao, Agriculture and Agri-Food Canada, Saskatoon, CANADA  
“A Scarecrow-Like Protein Interacts with HDA19 and Negatively Regulates Seed Maturation Program and Anthocyanin Biosynthesis in *Arabidopsis* Seedlings”
- 14:50 Fanggin Zeng, Huazhong Agricultural University, Wuhan, P.R. CHINA  
“Two Duplicate *CYP450X* Homologous Genes *BNMS1* And *BNMS2* Are Required for Pollen Exine Formation and Tapetal Development in *Brassica napus*”
- 15:20 *Break* SPONSORED BY BECKMAN COULTER INC
- 15:50 **SESSION 2B: PLANT PRODUCTS**  
*Chair: Maurice Moloney, Rothamsted Research, Harpenden, UK*  
Keynote: Daniel Kliebenstein, University of California, Davis, USA  
“Identifying Causal Genotype-Phenotype Linkages: How Plant-Breeding Helped *Arabidopsis*/Glucosinolate Research and Vice Versa”
- 16:30 Diana Zuluaga, Wageningen University, Wageningen, The NETHERLANDS  
“Risk Assessment of *Brassica oleracea* Glucosinolate Biosynthesis on the Soil Detritus Food Web”
- 16:50 Maurice Moloney, Rothamsted Research, Harpenden, UK  
“Production of Human Apolipoprotein A1 in Transgenic Safflower Seeds”
- 17:10 Guusje Bonnema, Wageningen University, Wageningen, The NETHERLANDS  
“Genetic Dissection of the *Brassica rapa* Metabolome Using a Genetical Genomics Approach: A Case Study of Six Biosynthetic Pathways”
- 17:30 *Poster Session* *Terrace Lounge & Conventional Floor Foyer*
- 19:30 *Dinner on your own*

# PROGRAM BRASSICA2010

SUNDAY SEPTEMBER 5<sup>TH</sup> – THURSDAY SEPTEMBER 9<sup>TH</sup>

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TUESDAY SEPTEMBER 7<sup>TH</sup> 2010 (MORNING)

(ALL SCIENTIFIC TALKS IN ADAM BALLROOM, CONVENTION FLOOR)

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- 7:30 - 11:00     *Registration*     *Convention Floor Foyer*
- 7:30     *Continental Breakfast*     *Convention Floor Foyer*
- 8:30     **SESSION 3A: COMPARATIVE & FUNCTIONAL GENOMICS**  
*Chair: Graham King, Rothamsted Research, Harpenden, UK*  
Keynote: Thomas Altmann, Leibniz Institut für Pflanzengenetik und  
Kulturpflanzenforschung (IPK), Gatersleben, GERMANY  
"Molecular and Genetic Analysis of Biomass- and Metabolite-Heterosis in  
*Arabidopsis*"
- 9:10     Wang Jun, Rothamsted Research, Harpenden, UK  
"Comparison and Refinement of Methods to Generate Integrated Genetic Maps  
for *Brassica napus*"
- 9:30     Nirala Ramchiary, Chungnam National University, Daejeon, REPUBLIC OF  
KOREA  
"Identification of Potential MicroRNAs and their Targets in *Brassica rapa* L."
- 9:50     Mark Belmonte, University of Manitoba, Winnipeg, CANADA  
"Laser Capture Microdissection and RNA Profiling of *Arabidopsis* Seed Reveals  
Dynamic Changes in Gene Activity within and between Seed Compartments  
over Time"
- 10:10     *Break SPONSORED BY CARGILL INC*
- 10:40     **SESSION 3B: COMPARATIVE & FUNCTIONAL GENOMICS**  
*Chair: Isobel Parkin, Agriculture and Agri-Food Canada, Saskatoon, CANADA*  
Keynote: Rod Wing, University of Arizona, Tucson, USA  
"Development and Analysis of Comparative Genomics Platforms from Closely  
Related Species: *Oryza* and Brassicaceae as Case Studies"
- 11:20     Keith Adams, University of British Columbia, Vancouver, CANADA  
"Divergence in Alternative Splicing Patterns between Duplicated Gene Pairs in  
Polyploid *Brassica napus*"
- 11:40     Xiaowu Wang, Institute of Vegetables and Flowers, Beijing, P.R. CHINA  
"Genome Structure Comparison between *B. rapa* and *A. thaliana*"
- 12:00     Sateesh Kagale, Agriculture and Agri-Food Canada, Saskatoon, CANADA  
"Comparative Analysis of the *Arabidopsis* and Brassica EAR Repressomes"
- 12:20     *Lunch*

# PROGRAM BRASSICA2010

SUNDAY SEPTEMBER 5<sup>TH</sup> – THURSDAY SEPTEMBER 9<sup>TH</sup>

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## TUESDAY SEPTEMBER 7<sup>TH</sup> 2010 (AFTERNOON / EVENING)

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### 13:30 **SESSION 4A: RESPONSE TO THE ENVIRONMENT**

*Chair: Guusje Bonnema, Wageningen University, Wageningen, The NETHERLANDS*

Keynote: Richard Amasino, University of Wisconsin, Madison, USA  
“Vernalization: Remembering Winter with an Environmentally Induced Epigenetic Switch”

14:10 Steve Robinson, Agriculture and Agri-Food Canada, Saskatoon, CANADA  
“Treasure from the Desolation Islands and Beyond”

14:30 Richard S. Fletcher, Colorado State University, Fort Collins, USA  
“Translational Genomics in the Brassiceae: Predicting Candidate Genomic Regions Affecting Drought Avoidance in *Brassica napus*”

14:50 J. Allan Feurtado, NRC-Plant Biotechnology Institute, Saskatoon, CANADA  
“The *Arabidopsis* C2H2-Type Zinc Finger Enhydrous Promotes the Transition to Germination by Regulating Light and Hormonal Signaling during Seed Maturation and Early Imbibition”

15:20 *Break* SPONSORED BY LIFE TECHNOLOGIES INC

### 15:50 **SESSION 4B: RESPONSE TO THE ENVIRONMENT: BIOTIC STRESS**

*Chair: Régine Delourme, INRA, Le Rheu, FRANCE*

Keynote: Barbara Howlett, the University of Melbourne, Melbourne, AUSTRALIA

“Mechanisms of Mutations in the Blackleg Fungus, *Leptosphaeria maculans*, Leading to Breakdown of Disease Resistance in Canola”

16:30 Régine Delourme, INRA, Le Rheu, FRANCE  
“A Connected Multicross Design to Explore the Diversity of Stem Canker Quantitative Resistance Factors in Winter Oilseed Rape (*Brassica napus* L.)”

16:50 Sandra Konietzki, Freie Universität Berlin, GERMANY  
“Identification of *Verticillium* Resistance Loci in *B. alboglabra*”

17:10 Ben Vosman, Wageningen University Research, Wageningen, The NETHERLANDS  
“Whitefly Resistance in Cabbage”

19:00 *Cocktails*

*Convention Floor Foyer*

20:00 *Banquet*

*Adam Ballroom*

21:00 *Entertainment followed by dancing*

# PROGRAM BRASSICA2010

SUNDAY SEPTEMBER 5<sup>TH</sup> – THURSDAY SEPTEMBER 9<sup>TH</sup>

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WEDNESDAY SEPTEMBER 8<sup>TH</sup> 2010 (MORNING)

(ALL SCIENTIFIC TALKS IN ADAM BALLROOM, CONVENTION FLOOR)

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- 7:30     *Continental Breakfast*     *Convention Floor Foyer*
- 8:30     **SESSION 5A: BREEDING AND TRAIT GENETICS**  
*Chair: Rod Snowdon, Justus Liebig University, Giessen, GERMANY*  
Keynote: Wallace Cowling, Canola Breeders Western Australia Pty Ltd, West Perth, AUSTRALIA  
“Exploiting Genetic and Genomic Information - New Challenges and New Opportunities for *Brassica* Breeding”
- 9:10     Keynote: You-Rong Chai, Southwest University, Beibei, P.R. CHINA  
“Molecular Dissection of the Phenylpropanoid-Flavonoid-Proanthocyanidin Pathway and its Association with Yellow Seed Traits in *Brassica*”
- 9:50     Christian Obermeier, Justus Liebig University, Giessen, GERMANY  
“Mapping for *Verticillium longisporum* Resistance and Marker Development for Marker-Assisted Selection in Winter Oilseed Rape”
- 10:10    *Break SPONSORED BY PIONEER HI-BRED LTD*
- 10:40    **SESSION 5B: BREEDING AND TRAIT GENETICS**  
*Chair: Jinling Meng, Huazhong Agricultural University, Wuhan, P.R. CHINA*  
Keynote: Anne-Marie Chèvre, INRA, Le Rheu, FRANCE  
“Dynamic of *Brassica* Genomes through Homologous and Homeologous Recombinations”
- 11:20    Zhengying Cao, Huazhong Agricultural University, Wuhan, P.R. CHINA  
“Characterizing Differentiated DNA Methylation Loci in the Genome of *Brassica napus* and their Impact on Trait Variation”
- 11:40    Marilyn Cruz Alvarez, Florida Gulf Coast University, Fort Myers, USA  
“Differences in Expression of the *CAULIFLOWER (CAL)* Gene do Not Correlate with Phenotypic Differences in the F2 Progeny of Cauliflower and Rapid Cycling *Brassica oleracea* (Rbo) Hybrids”
- 12:00    Jo-Anne Relf-Eckstein, Agriculture and Agri-Food Canada, Saskatoon, CANADA  
“Genetic Investigations into the Yellow Seed Phenotype in *Brassica napus* Canola”
- 12:20    *Lunch*

# PROGRAM BRASSICA2010

SUNDAY SEPTEMBER 5<sup>TH</sup> – THURSDAY SEPTEMBER 9<sup>TH</sup>

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## WEDNESDAY SEPTEMBER 8<sup>TH</sup> 2010 (AFTERNOON / EVENING)

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- 13:30 **SESSION 6A: GENETIC DIVERSITY**  
*Chair: J. Chris Pires, University Missouri-Columbia, Columbia, USA*  
Keynote: Mark Beilstein, Texas A&M University, College Station, USA  
“Hidden Histories in Brassicaceae: Phylogeny, Molecular Clocks, and Hybridization”
- 14:10 Guy Barker, Warwick HRI, University of Warwick, Wellesbourne, UK  
“Analysis of Allelic Variation within *Brassica oleracea*”
- 14:30 Renate Schmidt, Leibniz Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Gatersleben, GERMANY  
“Analysis of Allelic Diversity in *Brassica napus*”
- 14:50 Jinling Meng, Huazhong Agricultural University, Wuhan, P.R. CHINA  
“Development of a Population for Substantial New Type *Brassica napus* Diversified at Both A/C Genomes”
- 15:20 *Break* SPONSORED BY GENOME PRAIRIE
- 15:50 **SESSION 6B: GENETIC DIVERSITY**  
*Chair: Lars Østergaard, John Innes Centre, Norwich, UK*  
Keynote: Angela Hay, University of Oxford, Oxford, UK  
“Evolution of Petal Loss in *Cardamine hirsute*”
- 16:30 Stephen Ridge, University of Tasmania, Hobart, AUSTRALIA  
“Molecular Tools for Understanding Flowering Time in Cauliflower Hybrid Seed Crops”
- 16:50 Anja Bus, Max Planck Institute for Plant Breeding Research, Cologne, GERMANY  
“Population Structure, Genetic Diversity, and Linkage Disequilibrium in a *Brassica napus* Diversity Set”
- 17:10 Feng Li, Tohoku University, Sendai, JAPAN  
“Identification and Characterization of a *Brassica* Orthologue of *Arabidopsis* *GLABRA1* Responsible for Leaf Hairiness”
- 17:30 Farewell comments; scientific portion of workshop ends

# PROGRAM BRASSICA2010

SUNDAY SEPTEMBER 5<sup>TH</sup> – THURSDAY SEPTEMBER 9<sup>TH</sup>

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## THURSDAY SEPTEMBER 9<sup>TH</sup> 2010

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9:30 – *Excursion to Wanuskewin Heritage Park*  
15:00

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9:30 Departure from Bessborough  
10:00 DVD Presentation  
10:30 Kimaskikinawaw Medicine Walk  
11:15 Gallery & Exhibits self directed  
12:00 Lunch in South Room  
13:00 Mānokawēwin Tipi Raising  
13:45 Unstructured time  
14:00 Traditional Dance Performance  
14:30 Departure from Wanuskewin  
15:00 Arrival at Delta Bessborough

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## ORGANIZING COMMITTEE

**Raju Datla** NRC - Plant Biotechnology Institute, Saskatoon

**Régine Delourme** INRA, Le Rheu, France

**Pierre Fobert (Co-Chair)** NRC - Plant Biotechnology Institute, Saskatoon

**Wilf Keller** Genome Prairie, Saskatoon

**Jinling Meng** Huazhong Agricultural University, Wuhan, P.R. China

**Lars Østergaard** John Innes Centre, Norwich, UK

**Isobel Parkin (Co-Chair)** Agriculture and Agri-Food Canada, Saskatoon

**J. Chris Pires** University Missouri-Columbia, Columbia

**Andrew Sharpe** NRC - Plant Biotechnology Institute, Saskatoon

**Rod Snowdon** Justus Liebig University, Giessen, Germany

**Jitao Zou** NRC - Plant Biotechnology Institute, Saskatoon

**Yarnel Bender** NRC - Plant Biotechnology Institute, Saskatoon

**Lana Culley** NRC - Plant Biotechnology Institute, Saskatoon

## ACKNOWLEDGEMENTS

Selina Beaudin, Guusje Bonnema, Steve Karcz, Pam Marfleet, Maurice Moloney, Carrie Ogden, Kevin Rozwadowski



## **NATIONAL RESEARCH COUNCIL, PLANT BIOTECHNOLOGY INSTITUTE**

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## **NRC GENOMICS AND HEALTH INITIATIVE "BIORENEWABLE OIL FOR FOOD AND FUEL" PROJECT**

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## **CANADIAN CANOLA SEQUENCING INITIATIVE (CAN-SEQ)**

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**SESSION 1:**  
**SEED QUALITY**  
**ORAL PRESENTATION ABSTRACTS**







**EXPRESSION QUANTITATIVE TRAIT LOCI ANALYSIS OF *BAN*, *F3H* AND *TT19* GENES IN *BRASSICA NAPUS*****Kun Lu, Cunmin Qu, Kai Zhang, Junxin Lu, Yourong Chai, and Jiana Li**

Chongqing Rapeseed Engineering Research Center; Key Laboratory of Biotechnology and Crop Quality Improvement of Ministry of Agriculture; College of Agronomy and Biotechnology, Southwest University, Tiansheng Road 216#, Beibei, Chongqing 400716, P. R. CHINA

Flavonone 3-hydroxylase (F3H), anthocyanidin reductase (ANR) encoded by *BANYULS* (*BAN*) gene and glutathione S-transferase encoded by *TRANSPARENT TESTA 19* (*TT19*) gene are three key enzymes in flavonoid pathway, playing important roles in the synthesis and transport of anthocyanin and proanthocyanidin. However, little is known about the expression variations and the regulatory network in *Brassica*. In this study, we determined their expression levels in seeds of 30 days after flowering (DAF) in F<sub>7</sub> recombinant inbred lines (RILs) derived from a cross between *Brassica napus* cultivars Youyan2 (black-seeded) and GH06 (yellow-seeded), and characterized the locus-level regulatory network related to *B. napus BAN*, *F3H* and *TT19* genes. Expression quantitative trait loci (eQTL) mapping revealed five, seven and eight eQTLs for *BAN*, *F3H* and *TT19* genes, respectively. Marker E4M8 was associated with *qBAN-8-2* and *qTT19-8-2*, while marker H022L18-2 was linked with *qBAN-16-4* and *qF3H-16-6*, implying that there might be upstream regulatory genes in E4M8 and H022L18-2 marker flanking regions. In further analysis, we identified four *trans*-eQTLs (*qBAN-16-5*, *qF3H-16-5*, *qBAN-16-4* and *qF3H-11-2*) close to the location of the major QTL controlling yellow-seeded trait of *B. napus*. The 200-kb flanking sequences of the four eQTL on *B. rapa* chromosome A09 showed well synteny to partial continuous fragment of chromosome 1 of *Arabidopsis* genome, suitable for candidate gene predication of eQTLs. Based on gene finding results, seven transcription factors were suggested to be the potential upstream candidate(s) controlling expression variations of *BAN*, *F3H* and *TT19* genes. These results could provide a new approach for constructing regulatory pathways that contribute to complex traits, such as yellow-seeded trait.

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**ANALYSIS OF THE SEED QUALITY TRAITS OF *BRASSICA NAPUS* BY GENOME-WIDE GENE EXPRESSION QUANTITATIVE TRAIT LOCUS MAPPING**

**Wentao Zhang<sup>1</sup>, Erin Higgins<sup>1</sup>, Larissa Ramsay<sup>2</sup>, Kerry Boyle<sup>2</sup>, Yarnel Bender<sup>2</sup>, Nirmala Sharma<sup>2</sup>, Andrew Sharpe<sup>2</sup>, Pierre Fobert<sup>2</sup>, and Isobel Parkin<sup>1</sup>**

1. Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK, S7N 0X2, Canada
2. NRC-Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, SK, S7N 0W9, Canada

Oilseed rape (canola), *Brassica napus*, is an economically important crop as its seed products are invaluable sources for both human nutrition and renewable energy. The overall value of the *B. napus* seed is determined mainly by the seed quality traits: oil and protein content and composition, along with some anti-nutritive components including glucosinolates, fibres, erucic acid, phenolics etc. These quality traits exhibit a quantitative inheritance and are controlled by a complex genetic system. To dissect this genetic architecture, in the current research, we performed gene expression quantitative locus (eQTL) mapping analysis on the developing seeds from two large double-haploid populations of spring type *B. napus*. The first was generated from a cross between a cultivated *B. napus* line and a newly re-synthesized line, the second from a cross between a traditional black seeded canola line and a yellow seeded line. Global eQTLs were identified and mapped using transcriptome data from 44K feature Agilent *B. napus* arrays in concert with genotype data from genetic markers across the *B. napus* genome. Results from this research in combination with classical QTL mapping provided important information for identifying candidate genes that control seed quality traits. Also, mapping such eQTLs allows us to construct genetic regulatory networks to help elucidate the mechanisms underlying the variation observed for seed quality traits. Furthermore, high density mapping of gene expression markers generated from the transcriptome profiling data of the mapping population will provide resources for other such studies in *B. napus*.

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# SESSION 1B: SEED QUALITY

MONDAY SEPTEMBER 6<sup>TH</sup>

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## FATTY ACIDS COMPOSITION AND FUNGAL DISEASE RESISTANCE IN ADVANCE PROGENIES OF INDIAN MUSTARD (*B. JUNCEA*) DERIVED FROM INTERSPECIFIC CROSS (*B. JUNCEA*/*B. CARINATA*) AND MICROSPORE MUTAGENESIS

**Abha Agnihotri<sup>1</sup>, Deepak Prem<sup>2</sup>, and Kadambari Gupta<sup>3</sup>**

1. Professor & Head, Centre for Agricultural Biotechnology, Aimt, Block E-3, Amity University Uttar Pradesh, Sector 125, Noida-201303 (NCR) India
2. Centro de Investigaciones Biológicas, Biological Research Centre (CIB), C.S.I.C. Ramiro de Maeztu, 9, 28040 MADRID, SPAIN
3. Topworth Group of Companies, A- 21, II<sup>nd</sup> Floor, Green Park Main, NEW DELHI-110016, INDIA

The presently cultivated Indian mustard varieties do not conform to the international nutritional standards of low erucic/ high oleic acid, and are susceptible to fungal diseases resulting in huge economic losses. To meet these objectives three genotypes of *B. Juncea* [resbj-837 and resbj-830 from Rothamsted research, UK having tolerance to *Peronospora parasitica*, and teri(oe)m21-1 a low erucic acid line] were used for interspecific hybridization with *B. carinata* var. Kiran. The hybrids were obtained through sequential embryo rescue. An efficient doubled haploid (DH) production protocol was established in *B. juncea* var. Varuna and pusa and used for microspore mutagenesis by using ENU and EMS. The DH mutant progenies and those derived from interspecific crosses were screened for fa profile and disease resistance.

Six elite genotypes, derived from *B. juncea*/*B. carinata* [ $BC_3F_2/BC_2F_3$  progenies] were selected for low erucic/high oleic acid and tolerance to white rust and *alternaria* blight ( $di < 2$ ). The DH mutant progenies, screened up to the dh-m<sub>3</sub> showed high variability in FA profile; palmitic 3.2-16 %, oleic 18.4-44 %, linoleic 8.0-37 %, linolenic 4.0-16 % and erucic acid < 2.0 to 40%. The DI for white rust ranged from 0.6 to 2.6 and for *alternaria* blight 0.03 to 1.0 under epiphytotic field conditions, and 1.3 to 3.3 when assessed by detached leaf method. A total of 15 lines developed by the two strategies form a valuable gene pool for developing indian mustard with improved oil quality and high tolerance to fungal diseases for oilseed sustainability in an environment friendly manner.

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**SESSION 2:**  
**DEVELOPMENT**  
**&**  
**PLANT PRODUCTS**  
**ORAL PRESENTATION ABSTRACTS**



**HETEROCHRONY AND HYBRIDIZATION: THE EVOLUTION OF APOMIXIS IN THE GENUS BOECHERA**

**Timothy F. Sharbel**

Apomixis research group, Leibniz Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Corrensstrasse 3, D-06466 Gatersleben, Germany

Apomixis, a natural form of reproduction in plants whereby seeds are produced asexually, is hypothesized to result from the deregulation of developmental pathways leading to sexual seed development. Hypotheses seeking to explain the mechanisms which trigger deregulation employ the global genomic effects of hybridization and polyploidy. In particular, we are interested in the first step of apomixis, the production of meiotically unreduced gametes (apomeiosis).

Using a SuperSAGE analysis of microdissected live ovules at 4 developmental stages, from 3 sexual and 3 apomictic accessions, we have sequenced over 2.2 million mRNAs to show over 6000 differentially-expressed mRNAs. Approximately 400 of these mRNAs demonstrated heterochrony, whereby gene expression was shifted between sexual and apomictic ovules. The genes to which the mRNA tags belong were determined by homology searches to sexual and apomictic flower-specific transcriptome libraries which we sequenced using 454 technology.

Most heterochronic and stage-specific tags were significantly downregulated during early apomictic ovule development, and 110 were associated with reproduction. Finally, we show that apomixis-specific gene expression is characterized by a significant overrepresentation of transcription factor activity. We hypothesize that apomeiosis is associated with global downregulation at the megaspore mother cell stage, as influenced by the relative titer of *trans*-acting regulatory factors, which is effectively halved in the hybrid (apomictic) genome due to divergent transcriptional regulator and promoter sequence evolution in the parental genomes. As diploid apomicts ancient hybrids, these data support the postulated link between hybridization and asexuality.

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**IDENTIFYING CAUSAL GENOTYPE-PHENOTYPE LINKAGES: HOW PLANT-BREEDING HELPED *ARABIDOPSIS*/GLUCOSINOLATE RESEARCH AND VICE VERSA****Daniel J. Kliebenstein**

Department of Plant Sciences, University of California, Davis, USA

Natural genetic variation and the resulting phenotypic variation between individuals within a species have been of longstanding interest in wide-ranging fields. In humans, natural genetic variation is frequently at the core of an individual's susceptibility to cancer and other debilitating disorders. In plants, natural genetic variation is the basis of plant breeding and an important foundation of ecology and evolution. However, the molecular underpinnings of this phenotypic variation are relatively uncharted.

The topic of natural genetic variation is also of critical importance to plants and their ability to respond to biotic stresses because a plants metabolic defense repertoire is highly variable across natural genotypes. This diversity provides both a complex impediment to designing the optimum genotype as well as a dramatic opportunity to utilize this diversity to address several questions. The first question of most direct interest is what are the genes underlying differences in how plants produce secondary defense metabolites? This same question and the answers obtained however also allows us to use this applied/ecological field of research to begin developing fundamental tools and quantitative genetic theory that can be applied to almost any phenotype. We have been using genomics methodologies such as transcriptomics, targeted metabolite profiling and metabolomics to better understand how genetic diversity in glucosinolates is controlled and what the genetic basis of this may be. In this presentation I will focus on how the combination of quantitative genetics and glucosinolate secondary metabolism is changing our understanding of the term "secondary" as well as how this novel metabolite class is illuminating what may be fundamental theories of quantitative genetics. In each case, the fundamental illumination as direct application consequences and the two cannot be separated. In the seminar I will focus on the following main areas.

- 1) Combining phylogenetics, expression QTLs and phenotypic QTLs to identify unknown genes.
  - 2) Combining metabolomics and network expression analysis to identify the molecular basis of epistatic networks controlling plant secondary metabolism and how "secondary" is integrated into the whole plant.
  - 3) A novel approach to identify causal candidate genes within genome wide association mapping at a greater than 75% success rate and further implications for secondary metabolism pleiotropy.
  - 4) How glucosinolates identified a new but previously ignored fundamental phenotype.
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# SESSION 2B: PLANT PRODUCTS

MONDAY SEPTEMBER 6<sup>TH</sup>

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## GENETIC DISSECTION OF THE *BRASSICA RAPA* METABOLOME USING A GENETICAL GENOMICS APPROACH: A CASE STUDY OF SIX BIOSYNTHETIC PATHWAYS

Dunia Pino del Carpi<sup>1,2</sup>, Ram Kumar Basnet<sup>1</sup>, Danny Arends<sup>3</sup>, Ric de Vos<sup>4</sup>, Kim Boutilier<sup>4</sup>, Johan Bucher<sup>1</sup>, Richard Visser<sup>1</sup> and Guusje Bonnema<sup>1</sup>

1. Laboratory of Plant Breeding, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands
2. Institute for Genetics, Heinrich-Heine University, University Street 1, D-40225 Düsseldorf, Germany
3. Groningen Bioinformatics Centre, University of Groningen, 9751NN Haren, The Netherlands
4. Plant Research International, Business Unit Bioscience, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

We followed a genetical genomics approach to identify candidate genes for six biosynthetic pathways: carotenoids, tocopherols, folates, glucosinolates, flavonoids and phenylpropanoids, based on the co-localization of metabolic QTLs and expression QTLs. A *B. rapa* doubled haploid population from a cross between a yellow sarson and a pak choi was profiled for metabolite content and variation through targeted and LC-MS untargeted approaches. Additionally, the same population was profiled for transcript variation using a 60-mer oligo community microarray comprising 95.000 probes assembled using EST sequences mainly from three species: *B. napus*, *B. rapa* and *B. oleracea*. Colocalization of mQTLs and eQTLs lead us to successfully identify candidate genes for the carotenoids, tocopherols and glucosinolates. Using the glucosinolates pathway as our model pathway our results revealed the colocalization of eQTLs of a cluster of co-regulated genes and mQTLs for short (3C-5C) chain aliphatic glucosinolates with modified side chains around *AOP* in linkage group A09 and the colocalization of eQTLs for *MAM* genes and mQTL for long chained aliphatic glucosinolates in linkage group A03. Further work is still needed to identify candidate genes for mQTLs found in A07 for flavonoids. The application of this type of studies in *Brassica rapa* and the future validation approaches for the identification of *cis* and *trans* regulation with the soon available *B. rapa* genome sequence are discussed.

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**SESSION 3:**  
**COMPARATIVE &**  
**FUNCTIONAL GENOMICS**  
**ORAL PRESENTATION ABSTRACTS**



**MOLECULAR AND GENETIC ANALYSIS OF BIOMASS- AND METABOLITE-HETEROSIS IN *ARABIDOPSIS THALIANA***

**Rhonda C. Meyer<sup>1</sup>, Berit Ebert<sup>2</sup>, Barbara Kusterer<sup>3</sup>, Jan Lisec<sup>4</sup>, David Riewe<sup>1</sup>, Romy Schmidt<sup>2</sup>, Matthias Steinfath<sup>4</sup>, Kathleen Weigelt<sup>1</sup>, Albrecht E. Melchinger<sup>3</sup>, Joachim Selbig<sup>2,4</sup>, Lothar Willmitzer<sup>4</sup>, and Thomas Altmann<sup>1</sup>**

1. Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany
2. University of Potsdam, Germany
3. University of Hohenheim, Germany
4. Max-Planck-Institute of Molecular Plant Physiology, Potsdam-Golm, Germany

The molecular basis of heterosis is analysed in the C24 x Col-0 cross of *Arabidopsis thaliana* accessions that exhibits strong mid-parent heterosis (MPH) of vegetative growth / biomass accumulation. Using QTL-mapping in combination with metabolite profiling and gene expression profiling, loci responsible for the heterosis have been identified and heterosis-associated gene expression / metabolite composition has been analysed. Genomic regions involved in heterosis were identified by QTL, generation means, and mode-of-inheritance classification analyses using 429 recombinant inbred lines (RILs) and 140 introgression lines (ILs) backcrossed to the two parental accessions. Biomass accumulation and contents of 181 metabolites (by GC-MS) were determined at 15 days after sawing. MPH of shoot dry weight in the RILs ranged from -31% to 99%. Treating MPH and augmented dominance effect as quantitative traits, ten genomic positions involved in biomass-heterosis were detected, that explain between 2.4% and 15.7% of the phenotypic variation. While overdominant gene action was prevalent in heterotic QTL, the results suggest that a combination of dominance, overdominance and epistasis is involved in biomass heterosis in this *Arabidopsis* cross. Similarly, 232 heterotic metabolite QTL were detected. A strong heterotic biomass QTL region of chromosome 4 that co-locates with a cluster of 23 metabolic heterotic QTL was narrowed down to 14 genes by using subILs and segregating IL families. This genomic region is further investigated by comparative sequence analysis (C24 vs. Col-0) and the candidate genes are studied using k.o. mutants subjected to crosses and by transformation of alleles into the opposite genotype.

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# SESSION 3A: COMPARATIVE & FUNCTIONAL GENOMICS

TUESDAY SEPTEMBER 7<sup>TH</sup>

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## **LASER CAPTURE MICRODISSECTION AND RNA PROFILING OF *ARABIDOPSIS* SEED REVEALS DYNAMIC CHANGES IN GENE ACTIVITY WITHIN AND BETWEEN SEED COMPARTMENTS OVER TIME**

**Mark Belmonte<sup>1</sup>, Ryan Kirkbride<sup>2</sup>, Siobhan Brady<sup>2</sup>, John Harada<sup>2</sup>, and Bob Goldberg<sup>3</sup>**

1. Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada, R3T 2N2
2. Department of Plant Biology, University of California – Davis, Davis, CA, USA, 95616
3. Department of Molecular and Developmental Biology, University of California Los Angeles, Los Angeles, CA, USA, 90095

The *Arabidopsis* seed is a complex structure consisting of three major regions; embryo, endosperm and seed coat; and compartments within these regions including the embryo proper, suspensor, micropylar, peripheral and chalazal endosperms and chalazal and distal seed coats. Growth of the seed requires the coordinated development of each of these compartments and regions, but little is known of the gene expression programs that underlie these events. We have identified all of the genes expressed in each compartment of the seed across all stages of seed development using laser capture microdissection and DNA microarray technology. Our work provides the most comprehensive profile of gene activity across seed development to date. Compartments that have traditionally been grouped together based on ontogenetic lineage such as the three endosperm compartments fail to group together based on gene activity early in the seed lifecycle. These surprising results suggest gene activity and not lineage takes precedence in specifying biological function for individual compartments of the seed. Importantly, our data now provides the opportunity to take a more directed approach in manipulating specific compartments through the modification of gene activity in the endosperm, embryo or seed coat for improved seed performance. Finally, we studied how these compartments are transcriptionally regulated using *in silico* algorithms and identified a number of transcriptional networks hypothesized to be responsible for biological processes found within rarely studied seed compartments. Taken together, patterns of gene activity reveal putative functions of understudied seed compartments and identify novel mechanisms for the transcriptional regulation of biological processes.

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**DEVELOPMENT AND ANALYSIS OF COMPARATIVE GENOMICS PLATFORMS FROM CLOSELY RELATED SPECIES: ORYZA AND BRASSICACEAE AS CASE STUDIES****Rod A. Wing**

Arizona Genomics Institute, University of Arizona, Tucson, AZ 85721

Sequencing technology is progressing at such a blazing pace that it's safe to say that "full genome sequences" will be available for all crop plants, and their pathogens and pests within the next five years. Unfortunately, recent trends in the use of these new technologies has emphasized quantity over quality, which have led to the production of a number of poor quality "reference sequences" that in some cases are missing more than half of a genome. What price will researchers have to pay when the genome sequence of their research crop of choice is a mess and claimed to be complete?

Once a community has a high quality and trusted reference genome, the next question is: "Is a single reference genome sequence enough for a given species, genus or family?" For example, given in importance of rice and its role in solving world hunger, is a single RefSeq sufficient to gain a comprehensive understanding of biology and evolution of rice and its closely related wild relatives.

After completion of the rice (*Oryza sativa* ssp. *japonica*) genome in 2004, our consortia has focused on the development and implementation of a comprehensive genomics research platform for the genus *Oryza* to address grand challenge questions in food security, plant biology and evolution, under the rubric – the International *Oryza* Map Alignment Project (I-OMAP). *Oryza* is composed of 2 domesticated and 22 wild species, 10 genome types (6 diploid, 4 polyploid), and a 3.6 fold genome size variation. The wild relatives of rice contain a virtually untapped reservoir of genes that could be used to improve cultivated rice. The I-OMAP resource is composed of a set of 17 BAC libraries, 13 BAC-based physical maps from 13 *Oryza* species aligned to the rice RefSeq, and a set of five Chr3 short arm RefSeqs.

Using these resources we made the following key discoveries: 1) Analysis of structural variation between the rice RefSeq and its AA & BB genome relatives revealed that despite extensive observed colinearity, each genome is in "flux" and can differ by at least 40-80Mb or more (>10% of total genome size)!; 2) Large-scale sequence analyses of four biologically important regions revealed dynamic evolution of the *Oryza* genomes within the last 15 MY fashioned by a variety of lineage-specific structural rearrangements. More surprisingly, we uncovered a striking flux in gene content even among the most closely related species (AA and BB genomes).; 3) Analysis of the transposable element fraction of the *Oryza* enabled the discovery of an ancient retrotransposon family "RWG" which was shown to be responsible for the over two-fold genome size variation found in the diploid species *O. granulata* [GG] and *O. australiensis* [EE].

Given that even the most closely related *Oryza* species contain significant differences in genomic content and organization, we developed a cost-effective method to generate high-quality *de novo* reference sequences of large plant genomes by combining "old school" physical maps with next generation sequencing technology.

Based on these key biological findings and technological advancements, we argue that it is critical to first generate high-quality RefSeqs for all 8 AA, and 1 representative of the 9 other *Oryza* genome types, using a NextGen approach and then utilize re-sequencing methods (i.e. Illumina or SOLiD) to capture species-specific allelic variation.

Data will be presented on recent analysis of the genus *Oryza* and the status of an international effort to sequence the collective *Oryza* genome. I will also introduce a new international initiative (co-organized by J.C. Pires, D. Weigel and R.A. Wing) aimed at development a similar genomics platform for the *Brassicaceae*.







**SESSION 4:**  
**RESPONSE TO THE**  
**ENVIRONMENT**  
**ORAL PRESENTATION ABSTRACTS**





# SESSION 4A: RESPONSE TO THE ENVIRONMENT

TUESDAY SEPTEMBER 7<sup>TH</sup>

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## TREASURE FROM THE DESOLATION ISLANDS AND BEYOND

**Steve Robinson, Rong Xiao, Robert Wood and Isobel Parkin**

Saskatoon Research Centre, Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK. CANADA

*Pringlea antiscorbutica* (Kerguelen cabbage) originates from the Desolation Islands of the Antarctic Ocean. Its value was first reported by Captain Cook’s surgeon William Anderson, who recognised the plant could counteract the effects of scurvy among the crew of HMS Endeavour. This antiscorbutic nature of *Pringlea* was later confirmed almost 200 years later through the isolation of high levels of ascorbic and dehydroascorbic acids (Hatt, H. H. *Nature* 1949, *164*, 1081–1082). *Pringlea* has adapted to a short growing season that is uniformly cold with a mean annual temperature of ~4°C and mean summer and winter temperatures of approximately 10°C and -5°C respectively. Survival among these unfavourable environmental conditions has selected for many metabolic and physiological adaptations. As well as an abundance of proline (Hennion, F.; Bouchereau, A. *Polar Biol.* 1998, *20*, 281–291), the species produces high levels of glucose, glucosinolates and most interestingly forms of polyamines which are largely restricted to animal species (Hennion, F.; Martin-Tanguy, J. *Physiol. Plant.* 2000, *109*, 232–243).

Additional members of the *Brassicaceae* have adapted to unfavourable environmental conditions and accumulate anti-oxidative compounds these include *Lepidium sativum* (Garden cress), *A Armoracia lapathifolia* (Horseradish), *Capsella bursa-pastoris* (Shepherd’s Purse), *Cochlearia officinalis* (Scurvy grass), *Hesperis matronalis* (Dame’s rocket), *Thlaspi arvense* (Pennycress), *Isatis tinctoria* (Woad), *Lepidium meyenii* (Maca), *Stanleya pinnata* (Desert prince's plume) and *Sisymbrium officinale* (Hedge-mustard). We present basic low temperature physiology for these species and report on the development of an EST sequence resources derived using Roche 454 (Titanium) sequencing as these exotic *Brassicaceae* species enter the genomics era.

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**IDENTIFICATION OF *VERTICILLIUM* RESISTANCE LOCI IN *B. ALBOGLABRA*****S. Konietzki <sup>1</sup>, Didier Socquet-Juglard <sup>2</sup> and E. Diederichsen <sup>1</sup>**

1. Freie Universität Berlin, Angewandte Genetik, Albrecht-Thaer-Weg 6, 14195 Berlin, Germany
2. ETH Zürich, Institut f. Integrative Biologie, Universitätstrasse 2, 8092 Zürich, Switzerland

*Verticillium spp.* are soil-borne pathogens infecting many dicotyledonous crop plants. The pathogen penetrates host roots and spreads within the xylem vessels to colonise the whole plant. Infections on *Brassicaceae* are predominantly caused by *V. longisporum*. The disease gained significant relevance in many European oilseed rape growing areas. Our aim is to identify resistance sources and resistance genes to support breeding and general studies of this pathosystem.

Greenhouse resistance tests revealed *Brassica* accessions showing extreme differences for susceptibility. Resistant accessions were identified based on scoring a complex of symptoms to calculate the AUDPC. In addition, the systemic colonisation was determined by recording the fungal outgrowth of apical stem segments, and plant morphology was measured as shoot fresh weight and shoot length. The developmental progress was recorded to study its implications in pathogenesis. Strongly contrasting *B. albobglabra* genotypes were crossed to create a F2/F3-mapping population.

Continuous variation for AUDPC in F3 indicated oligo- or multigenic inheritance. F1 progenies were as resistant as the resistant parent in respect to AUDPC and systemic spread, indicating that both resistance traits are inherited in a dominant manner. Only a few F3 families were strongly colonised, indicating that several dominant genes control this trait. Most resistance parameters were correlated as expected. Slow developing genotypes were often more resistant, although this correlation was not very close.

A genetic map was created and QTL analysis performed. We identified several chromosomal regions that control different *Verticillium* resistance traits. QTL for these traits did show some overlap, whereas QTL for development were genetically independent from resistance QTL.

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**SESSION 5:  
BREEDING  
&  
TRAIT GENETICS  
ORAL PRESENTATION ABSTRACTS**



**EXPLOITING GENETIC AND GENOMIC INFORMATION - NEW CHALLENGES AND NEW OPPORTUNITIES FOR *BRASSICA* BREEDING**

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Whole genome marker-assisted selection has increased the rate of genetic progress in animal breeding, but most plant breeding programs do not incorporate genetic information (such as pedigrees or whole genome markers) in field trial analysis. Plant breeding lines are tested in replicated field trials in many different environments separated in time and space. Efficient analysis of multi-environment trials (MET) will involve estimation of spatial variation within trials, and modeling of patterns of genotype x environment (GxE) across trials. Ideally, this would be done in a single analysis from which the genetic value of lines may be estimated in various target environments. An example will be given where multiplicative mixed models were developed for the analysis of MET data for canola oil and grain yield. Information on pedigrees was included in order to model additive and non-additive genetic effects in different environment groups. Pedigree data improved estimates of genetic effects and improved the modelling of GxE. As a result, better selections were made as parents for crossing and as lines for promotion in target environments. Whole genome marker-assisted selection will further improve estimates of the breeding value of lines, and improve models of GxE. Soon, high density SNP arrays will be available at a reasonable price for several of the world's major crop plants. Their use will accelerate genetic progress, but care must be taken to avoid loss of genetic diversity. Ironically, rapid exploitation of this genetic diversity may quickly isolate small populations in the breeding program, following which genetic progress will be slowed. Methods must be developed to avoid loss of genetic diversity in breeding programs. Whole genome marker-assisted selection will stimulate a new revolution in plant breeding in the 21<sup>st</sup> century.

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## MOLECULAR DISSECTION OF PHENYLPROPANOID-FLAVONOID-PROANTHOCYANIDIN PATHWAY AND ITS ASSOCIATION WITH YELLOW SEED TRAITS IN *BRASSICA*

You-Rong Chai<sup>†</sup>, Jia-Na Li<sup>†</sup>, Kai Zhang, Li-Juan Ma, Yu Feng, Juan Du, Bo Lei, Jun-Lu, Yu Ni, Bo Li, Jia-Ming Yin, An-He Chen, Di Zhang, Shu-Ling Di, Wen-Jun Zhao, Ben-Bo Xu, Rui Yin, Ling-Li Xie, Nan Yan, Yan Chen, Xiao-Chun Lu, Yun-Liang Wei, Hong Leng, Hua-Lei Huang, Bei-Bei Lin, Ying Liang, Kun Lu, Li Chen, Rui Wang, Zhang-Lin Tang, Qing-Yuan Zhou, Lie-Zhao Liu, Xin-Fu Xu, Wei Qian, Na Lin, Yan-Zhen Dong, Rui-Xi Zhang, Ting Cao, Li-Jun Hu, Min Shen, Yu-Ming Wang, Fan-Min Meng, Jie Sun, Wei-Ran Zhong, Yun-Tao Li, Cheng-Jun Yang, and Zi-Chao Zhu

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<sup>†</sup>These authors contributed equally to the study

Phenylpropanoid pathway produces numerous secondary metabolites and influences plant pigmentation, cross pollination, adversity tolerance and quality. 218 genes from 27 functional loci of the phenylpropanoid-flavonoid-proanthocyanidin pathway were isolated from amphidiploid *B. napus* (*Bn*) and its parental species *B. rapa* (*Br*) and *B. oleracea* (*Bo*), and molecularly, functionally and comparatively characterized.

- 1) 2-12 *Bn*, 1-6 *Br* and 1-6 *Bo* orthologs correspond to each *Arabidopsis thaliana* (*At*) gene, supporting the “triplication” assumption of Brassiceae ancestor.
- 2) Some triplicated *Brassica* paralogs experienced “gene death”. Basic *Brassica* genome keeps about 3 paralogs of *PAL1*, *PAL2*, *C4H*, *CCR2*, *CHS/TT4*, *CHI/TT5*, *LDOX/TT18*, *TT16*, *PAP*, *MYB4*, 2 paralogs of *PAL3*, *CCR1*, *F3H/TT6*, *BAN/ANR*, *Lac15/TT10*, *TT19*, *AHA10*, *TTG1*, *TTG2* and *MYBL2*, and just one copy of *TT1*, *TT2*, *TT8*, *PAL4*, *DFR/TT3*, *F3'H/TT7* and *TT12*. Gene loss, partial deletion, insertion, premature stop codon, single-base deletion and exchange are the death mechanisms.
- 3) In most cases, *Bn* contains total sets of genes donated by *Br* and *Bo*.
- 4) Brassicaceae ancestor had two *C4H*, *CHS* and *CHI* genes, both triplicated later in *Brassica*, whereas *At* kept just one.
- 5) Most *Brassica* orthologs encode proteins comparable to *At* genes, but organ-specificity diverges fast.
- 6) Both intra-allelic homologous and inter-allelic homeologous exchanges are very frequent.
- 7) Alternative transcription start sites and alternative poly(A) tailing sites are very common, alternative splicing and 5'UTR regulation also exist, especially in regulatory genes.
- 8) For the tested yellow-seeded stocks, above loci encode normal proteins but the pathway is down-regulated especially at *TT1*, *TT3*, *TT8*, *TT10*, *TT12*, *AHA10*, etc., and different *Brassica* species have different major loci.
- 9) Transgenic suppression of more than 10 of above loci in black-seeded *B. napus* reduced pigment or lignin contents in seed coat, RNAi more than antisense. However, metabolic engineering of multiple loci is necessary to generate an applicable real “yellow seed”!







## SESSION 5B: BREEDING & TRAIT GENETICS

WEDNESDAY SEPTEMBER 8<sup>TH</sup>

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### SEARCHING FOR THE GENETIC BASES OF THE CAULIFLOWER PHENOTYPE

**Sandra Londoño, Camila Avila, and Marilyn Cruz-Alvarez**

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Cauliflower (*Brassica oleracea* var. *botrytis*) and Rbo (Rapid cycling *Brassica oleracea*) exhibit apparent morphological and developmental differences. While Rbo plants show normal flower development, cauliflower is characterized by the formation of a head or curd as a result of apical meristem proliferation and arrested flowering. In order to better understand the phenotypic differences and the underlying genetic differences between cauliflower and Rbo, we have produced F1 and F2 hybrids and analyzed their phenotype and genotype. The hybrids showed an intermediate phenotype between the two varieties. Phenotypic variables such as the length of internodes in the main stem and flowering time were significantly different in the F1 hybrids than in Rbo plants. The F1 hybrids also showed an increased number of aborted flowers, with lack of development of floral organs, with respect to Rbo. The F2 generation showed a wide distribution of phenotypes and a lack of correlation between the different phenotypic variables among plants, indicating the contribution of several segregating genes to the phenotypic differences. We are analyzing expression of floral meristem identity genes as well as of genes that show variety-specific expression in the F2 population to determine their contribution to the observed phenotypic differences.

The floral meristem identity gene *CAULIFLOWER* (*CAL*) has a non-sense mutation in the cauliflower variety suggesting that lack of a functional *CAL* protein is either solely or partially responsible for formation of the curd. However, genotypic analysis of the F2 population has not revealed so far any correlation between expression of the *CAL* gene and any of the observed phenotypic variables.

The *CAULIFLOWER CURD EXPRESSION 1* (*CCE1*) gene shows variety-specific expression in *Brassica oleracea*; it is expressed in cauliflower, but not in Rbo. We have conducted further characterization of the *CCE1* gene aimed at elucidating its function and possible contribution to developmental differences in *Brassica oleracea* varieties. An antibody has been produced against a peptide within the deduced *CCE1* protein sequence. Western blot analysis with this antibody revealed the presence of the predicted protein in the curd of the cauliflower and confirmed variety-specific expression of the gene. Three almost identical copies of *CCE1* have been identified in the cauliflower genome. These three copies lack introns and have poly A tails on their 3' ends, suggesting these sequences may have undergone transposition in the genome. While no ortholog has been detected by Southern blot or sequence data analyses in *Arabidopsis thaliana*, *CCE1* coding sequences seem to be conserved among varieties of *Brassica oleracea* and in other members of the *Cruciferae* family. We are investigating the possibility that transposition of these sequences in the *Brassica oleracea* genome led to production of a novel protein and contributed to the developmental characteristics of cauliflower.





**SESSION 6:**  
**GENETIC DIVERSITY**  
**ORAL PRESENTATION ABSTRACTS**













# SESSION 6B: GENETIC DIVERSITY

WEDNESDAY SEPTEMBER 8<sup>TH</sup>

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## MOLECULAR TOOLS FOR UNDERSTANDING FLOWERING TIME IN CAULIFLOWER HYBRID SEED CROPS

**Stephen Ridge<sup>1</sup>, James L. Weller<sup>2</sup>, Philip H. Brown<sup>1</sup>, Valérie Hecht<sup>2</sup>, Cameron J. Spurr<sup>3</sup>, Ronald G. Driessen<sup>4</sup> and Arie Baelde<sup>5</sup>**

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Synchronous flowering between parent lines is essential for pollination and seed set in commercial hybrid cauliflower seed production. However, weather-related variation in reproductive development and attempts to produce novel hybrids by crossing parents with markedly different flowering phenology make synchronous flowering difficult to achieve. Molecular technology presents an opportunity to understand and manage this variability. In this study, we investigated the contribution of the *FLC* gene family to flowering time variation and examined the vernalisation-regulated expression of key flowering genes.

Fifty-four homozygous cauliflower lines ranging from tropical to winter varieties were screened for polymorphisms in the vernalisation-mediated *FLC* genes. A functional allele of the *FLC2* gene was identified, along with an allele disrupted by a frameshift mutation in exon 4. A strong correlation between flowering time and genotype was observed in field and glasshouse trials. In a segregating F2 population derived from a cross between late-flowering (functional *FLC2*) and early-flowering (mutated *FLC2*) lines, the *FLC2* genotype was found to account for approximately 57% of flowering time variation.

RT-qPCR was used to study the effects of vernalisation on the expression of *FLC* genes and the flowering signal integrator *FT*. Vernalisation downregulated *FLC* and upregulated *FT*, with colder temperatures amplifying this effect. In early-flowering lines with mutated *FLC2*, overall expression of the *FLC2* was lower and *FT* expression significantly higher supporting the idea that *FLC2* plays a key role in maintaining the vegetative state.

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**POSTER  
PRESENTATIONS  
LIST & ABSTRACTS**



**1 – Seed Quality**

- 
- 1 Kerry Boyle, Wentao Zhang, Erin Higgins, Larissa Ramsay, Christine Sidebottom, Nirmala Sharma, Andrew Sharpe, Isobel Parkin and Pierre Fobert:  
QTL Analysis of Seed Quality Traits in Spring-Type *Brassica napus*
  - 2 Kuljit Cheema, Berisso Kebede and M. Habibur Rahman:  
Genetic Relationship Between Yellow-Seeded *Brassica napus* from Two Different Sources
  - 3 Faouzi Bekkaoui, and Brittany Scarrow:  
The Improvement of Oil Content and Yield of *Brassica napus* using Genomics and Biotechnology Approaches
  - 4 Ram Kumar Basnet, Sokrat Monakhos, Johan Bucher, Steven Groot, Chris Maliepaard and Guusje Bonnema:  
Genetics of Seed and Seedling Quality Traits in *Brassica rapa*
  - 5 Shunsuke Okamoto, Koji Sakamoto, Atsuo Saito, Hiroyasu Kitashiba, and Takeshi Nishio:  
Variety Identification and Seed Purity Analysis of Japanese Radish “Daikon” Using Dot-Blot-SNP Technique
  - 6 Van L. Ripley, Zoe Ehlert, Lasantha Ubayasena, Michelle Beath and Manju Gupta:  
Modified Fatty Acid *Brassica juncea* Development through Introgression of *FAD A* Genome Gene Mutations from *B. napus*
  - 7 Congguang Shi, Yana Zhu, Weijun Zhou, and Lixi Jiang:  
DNA Allelic Variation at the Loci Putatively Implicated in Seed Oil Formation among *Brassica* Oilseed Cultivars
  - 8 Anna Stein, Wolfgang Friedt, and Rod Snowdon:  
Characterization of a Seed Meal Quality Locus on Chromosome A9 of *Brassica napus*
  - 9 Qian Zheng, Kede Liu, and Jitao Zou:  
Isolation and Characterization of *Brassica napus* cDNAs that Encode Lysophosphatidylcholine Acyltransferase by Complementation of Yeast *Lca1Δ* Mutant

# POSTER LIST

---

## 2 – Development

---

- 10 Camila Avila and Marilyn Cruz-Alvarez:  
Transposition of the *CCE1* Gene Sequences in the *Brassica oleracea* Genome May Have Resulted in Expression of a Novel Protein and Contributed to the Emergence of the Cauliflower Variety
- 11 Panjisakti Basunanda, Bertha Salazar, Irina Zaharia, Sue Abrams, and Rod Snowdon:  
Comparative Mapping of QTL for Hormone Metabolites and Developmental Traits in Oilseed Rape Seedlings
- 12 M. Gruber, J. Holowachuk, U. Alahakoon, J. Soroka, and P. Bonham Smith:  
New Hairy Canola and Hairy Mustard Lines and their Potential for Resistance to *Phyllotreta* Flea Beetles
- 13 P. Kamiński, H. Berniak, and M. Kamińska:  
'*Candidatus* Phytoplasma Asteris' Identified in Brussels Sprouts and its Possible Association with Flower Bud Failure in Poland
- 14 Shao-Lun Liu, and Keith L. Adams:  
Dramatic Change in Function and Expression Pattern after Gene Duplication Created a Gene Regulating Zygote Development in the Brassicaceae
- 15 Sandra Londoño and Marilyn Cruz-Alvarez:  
Differences in Expression of the *Cauliflower* (*CAL*) Gene Do not Correlate with Phenotypic Differences in the F2 Progeny of Cauliflower and Rapid Cycling *Brassica oleracea* (Rbo) Hybrids
- 16 N.K. Nayidu, X. Li, and M.Y. Gruber:  
Phytochemical and Transcriptome Analysis of *Brassica villosa* vs *B. napus*
- 17 Xianzhong Wu, Fengling Li, Allan Kolenovsky, Allan Caplan, Yuhai Cui, Adrian Cutler, and Edward W.T. Tsang:  
Characterizations of an *Arabidopsis* Mutant Deficient in S-Adenosylhomocysteine Hydrolase1
- 18 Jianjun Zhao, Dong Xiao, Vani Kulkarni, Dunia Pino Del Carpio, Johan Bucher, and Guusje Bonnema:  
Genetic Dissection of Flowering Time of *Brassica rapa* in Different Environments

**3 – Comparative and Functional Genomics**

- 19 Mei Guan, Xun Li, and Chun-Yun Guan:  
Analysis of Differentially Expressed Genes in Oleic Acid Synthesis of *Brassica napus* by Gene Chip
- 20 Pablo Cardenas, Humberto Gajardo, Hector Urbina, Terry Huebert, Ada Lopez-Emparán, Isobel Parkin, Federico Iniguez-Luy, and Maria Federico:  
Molecular Evolution of the PSY Gene Family in Three Brassica Species
- 21 Julio Cordero, Andrew Sharpe, Christine Sidebottom, Wolfgang Friedt, and Rod Snowdon:  
Ultradeep Genome and Transcriptome Mapping in *Brassica napus* using Next-Generation Sequencing
- 22 Rudolph Fredua-Agyeman and Habibur Rahman:  
Identification and Characterization of NBS-Encoding Resistance Genes in *Brassica napus*
- 23 Daiqing Huang, Kevin Koh, Allan Feurtado, Youlian Pan, Andrew Sharpe, Ed Tsang and Adrian Cutler:  
Genome-Wide Transcript and Small RNA Analyses during Seed Maturation in *Brassica napus* by Microarray and Deep Sequencing
- 24 Musrur R. Jaradat, Max Ruegger, Andrew Bowling, Holly Butler, Yuejin Sun, Tom Skokut, and Adrian J. Cutler:  
A Comprehensive Transcriptome Analysis of Silique Development and Dehiscence in *Arabidopsis* and *Brassica* Integrating Genotypic, Interspecies and Developmental Comparisons
- 25 Johann Joets, Christine Lelandais, Martin Crespi, and Karine Alix:  
Impact of Allopolyploidy on Small RNAs: A Bioinformatic Survey in Resynthesized *Brassica napus* Allotetraploids
- 26 Graham J King, Stephen Amoah, Clare Hopkins, Andrew Stoute, Smita Kurup:  
Exploring and Generating Epigenetic Variation In Brassica
- 27 Natalie Labbé, Anne Johnston, Jas Singh, and Johann Scherthaner:  
The Unfolded Protein Response (UPR) in Microspore-Derived Embryos (MDEs) from *Brassica napus*
- 28 Zahra Navabi, Terry Huebert, Carmel O'Neill, Ian Bancroft, and Isobel Parkin:  
Physical Mapping of Brassica B-Genome of *B. nigra* in Correspondence to the FCA Genomic Region of *Arabidopsis* Chromosome 4
- 29 Zahra K. Navabi, Kiersten E. Stead, J. Chris Pires, Zhiyong Xiong, Andrew Sharpe, Isobel A. P. Parkin and Allen G. Good:  
Development of B-Genome Chromosomes Introgression Lines from Interspecific Hybrids of *Brassica napus* × *B. carinata*
- 30 Inge Roewer, Darrin Klassen, Janet Condie, Christine Sidebottom, Carling Tallon and Andrew Sharpe:  
Next Generation Sequencing Platforms at NRC-PBI: - Recent Up-Grades and Future Improvements
- 31 Bertha Salazar, Aysha Kiran, and Rod Snowdon:  
Digital Gene Expression Analysis of Seedling Development Traits in Winter Oilseed Rape
- 32 Nidhi Sharma, Fred Peng, Saleh Shah, Raju Datla and Randall J. Weselake:  
Transcriptional Profiling of *Diacylglycerol Acyltransferase 1* -Suppression in *Brassica napus*

## POSTER LIST

---

- 33 Rod Snowdon, Andrew Sharpe, Sue Abrams, Irina Zaharia, Pierre Fobert, Isobel Parkin, Benjamin Stich, Janet Higgins, and Ian Bancroft:  
"ERANET ASSYST" – Associative Expression and Systems Analysis of Complex Traits in Oilseed Rape / Canola
- 34 Carling Tallon, Matthew Links, Kevin Koh, Isobel Parkin and Andrew Sharpe:  
Method for Simultaneously Generating 20 and 40 Kb Paired End Libraries for Roche 454 GS FLX Titanium Sequencing
- 35 Shunxue Tang, Michelle Wiggins, Rick Nipper, Jenna Gribbin, Eric Johnson, Nathan Lillegard, Thomas Greene, Steve Thompson, and Siva Kumpatla:  
SNP Discovery using Restriction-Site Associated DNA (RAD) Longread Sequencing in *Brassica napus*, a Polyploid Species
- 36 Martin Truksa, Nidhi Sharma, Michael S. Greer, Wei Deng and Randall J. Weselake:  
Molecular Characterization and Expression Of *DGAT1* Genes in *Brassica napus*
- 37 Jeroen Wilmer, Nicolas Ribière, Jérôme Pauquet, Laurent Hanneton, Jean-Pierre Martinant and Philippe Blanchard:  
High Density SNP Maps – a Tool to Study Homoeologous Exchange?
- 38 Limin Wu, Aliaa El-mezawy, and Saleh Shah:  
Seed Coat Specific-Promoters for Canola

**4A – Response to the Environment**

- 39 Marc Champigny, Robin Cameron and Elizabeth Weretilnyk:  
*Thellungiella salsuginea* – A Robust Physiological and Genetic Model for Environmental Stress Tolerance
- 40 Olesya Kharenko, Jason Boyd, Sue Abrams, and Michele Loewen:  
From Chemical Proteomics to Functional Characterization - Elucidation of Novel Mechanisms of Plant Hormone Action
- 41 X. Li, J. Holowachuk, M.Y. Gruber:  
Tolerance of Ethiopian Mustard to Sodium Sulphate Salinity
- 42 Brett McVittie J. Sergio Moroni, Neil Wratten John Harper and Harsh Raman:  
Characterisation of Canola (*Brassica napus* L.) Germplasm for Manganese Tolerance
- 43 Susanne Schmidt, Michaela Zietz, Monika Schreiner, Sascha Rohn, Rita Zrenner, Lothar W. Kroh, and Angelika Krumbein:  
The Influence of Temperature during Cultivation on the Flavonoid Profile in Kale (*Brassica oleracea* Var. *sabellica*) and the Expression of Key Enzymes in Flavonoid Biosynthesis
- 44 Nirmala Sharma, Yarnel Bender, Kerry Boyle, Kasi Williams, and Pierre R. Fobert:  
*Arabidopsis HSI2* (HIGH-LEVEL EXPRESSION OF SUGAR-INDUCIBLE GENE 2) Affects Drought Tolerance and Response to ABA during Germination
- 45 Melanie Wiesner, Rita Zrenner, Angelika Krumbein, Hansruedi Glatt, and Monika Schreiner:  
Influence of Signaling Molecules on Indole Glucosinolates of *Brassica rapa* var. *chinensis*

**4B – Biotic Stress**

- 46 Antoine Gravot, Louis Grillet, Geoffrey Wagner, Mélanie Jubault, Vincent Bouguenec, Christine Lariagon, Carole Deleu, Régine Delourme, Alain Bouchereau and Maria Manzanares-Dauleux:  
Deciphering the Genetic Link between Tolerance to Trehalose and Partial Resistance to Clubroot in *Arabidopsis thaliana*
- 47 Muhammad Jakir Hasan, Stephen E. Strelkov and M. Habibur Rahman:  
Screening of *Brassica* Germplasm for Resistance to *Plasmodiophora brassicae* Pathotypes Prevalent in Canada
- 48 H.R. Kutcher, D. Cross, B. Marquer, C.L. Kirkham, A.-M. Chèvre, R. Delourme, M.-H. Balesdent, T. Rouxel and H. Brun:  
The Race Structure of *Leptosphaeria maculans* in Western Canada
- 49 Nicholas J. Larkan, Sarah Kuzmicz, Fengqun Yu and Derek J. Lydiate:  
Genetic Evidence for the Recognition of the *Leptosphaeria maculans* Avirulence Gene *AvrLm1* by Two *Brassica napus* Resistance Genes; *Rlm1* and *LepR3*
- 50 Roeland E. Voorrips, Greet Steenhuis-Broers, Marjolein Tiemens-Hulscher, Edith T. Lammerts van Bueren and Ben Vosman:  
Plant Traits Affecting Thrips Damage in Cabbage
- 51 Geoffrey Wagner, Sophie Charton, Nathalie Marnet, Raphaël Lugan, Christine Lariagon, Alain Bouchereau, Régine Delourme, Maria J. Manzanares-Dauleux, and Antoine Gravot:  
The Metabolic Response of Oilseed Rape to *Plasmodiophora brassicae* Infection
- 52 Lipu Wang and Pierre R. Fobert:  
The Role of *Arabidopsis* Clade I TGA Transcription Factors in Modulating Plant Defense Responses

## POSTER LIST

---

### 5 – Breeding and Trait Genetics

---

- 53 Ravneet Behla, Dilantha Fernando, Peter McVetty, and Genyi Li:  
QTL Mapping of Tolerance Against Sclerotinia Stem Rot in *Brassica napus* L.
- 54 Pankaj Bhowmik, Joan Dirpaul, Patricia Polowick, and Alison Ferrie:  
An Efficient High Throughput *Brassica napus* Microspore Culture System: Influence of Percoll Gradient Separation and Precise Bud Selection on Embryogenesis
- 55 Holger Budahn, Herbert Peterka, Evelyn Klocke, Otto Schrader, and Michaela Schlathöfner:  
Radiation Hybrid Mapping of Radish Chromosome D in Rapeseed Background
- 56 Zhengying Cao, Fang Tian, Nian Wang, Congcong Jiang, Bing Lin, Wei Xia, Jiaqin Shi, Yan Long, Chunyu Zhang and Jinling Meng:  
Analysis of QTLs for Erucic Acid and Oil Content in Seeds on A8 Chromosome and the Linkage Drag between the Alleles for the Two Traits in *Brassica napus*
- 57 Bifang Cheng and David Williams:  
Development of Elite Inbred Component Lines for Synthetic Hybrids in Yellow Mustard (*Sinapis alba*)
- 58 Lei Cui, Dengxia Yi, Jie Zhang, Zhihong Lang, Yumei Liu, Mu Zhuang, Yangyong Zhang, Youjun Zhang, Dafang Huang, Zhiyuan Fang, and Limei Yang:  
Transformation and Expression of BT Gene *cry1ia8* in Cabbage (*Brassica oleracea*)
- 59 Elke Diederichsen:  
Race-Specificity of Canadian Clubroot Isolates
- 60 Christina Eynck, Christine Sidebottom, Wayne Clarke, Andrew Sharpe, Ginette Séguin-Swartz, and Isobel Parkin:  
SNP Discovery in *Camelina sativa* – Towards Identifying Molecular Markers for Resistance to *Sclerotinia sclerotiorum*
- 61 Krishna K. Gali, Alison Ferrie, Janice Schmidt, Lasantha Ubayasena, Van Ripley and Andrew Sharpe:  
Introgression of Clubroot Resistance into Elite Canola Germplasm via Re-Synthesis
- 62 Céline Hamon, Stéphane Boury, Manuelle Bodin, Sonia Hallier, and Serge Mabeau:  
Optimizing Brassica Species Breeding Programs
- 63 Yongju Huang, Christophe Jestin, Sue Welham, Graham J. King, Bruce D.L Fitt, and Regine Delourme:  
Effects of Environment on Stability of QTL for Resistance to *Leptosphaeria maculans* in Oilseed Rape (*Brassica napus*)
- 64 P. Kaminski:  
Improvement of Cauliflower Male Sterile Lines with *Brassica nigra* Cytoplasm
- 65 Naoko Kitamoto, Susumu Yui, Yoshihito Takahata, and Shuji Yokoi:  
Expression Analysis of Genes for Flowering in a Late Bolting Breeding Material, “Leafy Green Parental Line No.2 (*Brassica rapa*)” that Requires Long Days Instead of Vernalization for Flowering
- 66 Tom Kubik, Chibwe Chungu, Gerhard Rakow and Steve Thompson:  
Development of Low Fiber, Yellow Seed Coat Omega-9 *Brassica napus*
- 67 Xiao Nan Li, Nirala Ramchiary, Su Ryun Choi, Hyeon Kook Yang, and Yong Pyo Lim:  
Mapping of Quantitative Traits for Morphological Traits in *Brassica rapa*
- 68 Chaozhi Ma, Tingdong Fu, Jinzing Tu, and Jinxiong Shen:  
Development Of PCR-Based Markers for Breeding Self-Incompatible Lines in *B. napus* L.

- 69 Maja Mosch, Frank Marthe, and Hendrik Winter:  
A New Blackleg Resistance Transfer Project for *Brassica napus* Based on the *Brassica* B-Genome
- 70 Annaliese Mason, Guijun Yan, Matthew N. Nelson and Wallace A. Cowling:  
Unreduced Gamete Production in *Brassica*: Genetic and Environmental Effects
- 71 Suhyoung Park, Moo-Kyung Yoon, Haeyoung Na, Min-Young Park and Yong Pyo Lim:  
Finding Effective Conditions of Microspore Culture in Radish (*Raphanus sativus* L.)
- 72 Gary Peng, Kevin C. Falk, Brian James, and Richard K. Gugel:  
Evaluation of Brassica Germplasm for Resistance to Clubroot of Canola
- 73 M.M. Rahman, H.R Kutcher, S. E. Strelkov, and M.H. Rahman:  
Introgression of Blackleg Resistance into *Brassica napus* from *Brassica carinata*
- 74 Rosy Raman, Belinda Taylor, Steve Marcroft, Paul Eckermann, Ata Rehman, Kurt Lindbeck, David Luckett, Neil Wratten, Jiri Stiller, Jacqueline Batley, David Edwards, and Harsh Raman:  
Genetic Map Construction and Localisation of Qualitative and Quantitative Loci for Blackleg Resistance in Canola (*Brassica napus* L.)
- 75 Otto Schrader, Holger Budahn, and Herbert Peterka:  
Detection of Radish Chromosomes in 9 Disomic Rape-Radish Addition Lines via Fluorescence *In Situ* Hybridization
- 76 Motoki Shimizu, Hiroya Tomita, Hidetaka Hori, and Keiichi Okazaki:  
Fine Mapping of Clubroot Resistance Gene, *pb-Bo(Anju)1*, in *Brassica oleracea*
- 77 Yu Si, Guo-ju Chen, Bi-hao Cao, En-you Feng, and Jian-jun Lei:  
Diversity of Glucosinolates and Its Combining Ability and Heredity Parametric in Chinese Kale
- 78 Jun Wang, Derek Lydiate, Isobel Parkin, Cyril Falentin, Regine Delourme, Ambrose Andongabo, Pierre Carion, Chris Love, Graham J King:  
Large Scale Genetic Map Integration in Brassica Via Database Interaction
- 79 Ke-Yun Wei, Wen Zhai, Jian-Ming Ding, Shi-Kai Li, Qian Wang, Xiaowu Wang, and Jian Wu:  
Naturally Occurring Deletion Mutation in the Oil-Type *Brassica rapa* *FLC2* Is Associated with Variation in Flowering Time
- 80 Huixian Zhao, Zhanjie Li, Shengwu Hu, Genlou Sun, Jianjun Chang, and Zehua Zhang:  
Identification of Cytoplasm Types in Rapeseed (*Brassica napus* L.) Accessions by a Multiplex PCR Assay
- 81 withdrawn

## POSTER LIST

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### 6 – Genetic Diversity

---

- 82 Rick Bennett, Rong-Cai Yang, Ginette Séguin-Swartz, and M. Habibur Rahman:  
Broadening Genetic Diversity in Canola: Development of Double-Low Quality Recombinant Inbred Lines from a *Brassica napus* x *B. oleracea* Cross
- 83 Frédérique Eber, Marta Cifuentes, Marie-Odile Lucas, Maryse Lodé, Anne-Marie Chèvre and Eric Jenczewski:  
Repeated Polyploidy Drove Different Levels of Crossover Suppression between Homeologous Chromosomes in *Brassica napus* Allohaploids
- 84 Frédérique Eber, Sophie Paillard, Maryse Lodé, Virginie Huteau, Olivier Coriton, Bathilde Auger, Nathalie Nési, and Anne-Marie Chèvre:  
Extraction of the Diploid A Genome and of C Chromosomes from the Allopolyploid *Brassica napus* (AACC, 2n=38)
- 85 Mahmood Gholami, Daniela Zeltner, Renate Schmidt, Wolfgang Ecke, and Rod Snowdon:  
Multiplexed 454 Amplicon Sequencing For High-Throughput SNP Discovery in *Brassica napus* Candidate Genes for Oil Content
- 86 Yang Jun, Yi Bin, Wen Jing, Tu Jin-xing, Ma Chao-zhi, Fu Ting-dong, Shen Jin-xiong:  
Screening of SSR Core Primers and their Application in Rapeseed (*Brassica napus* L.)
- 87 Patrick Kabouw, Benyamin Houshyani, Harro Bouwmeester, Wim van der Putten, and Arjen Biere:  
Root and Shoot Metabolic Profiles of *Arabidopsis* and White Cabbage: Predictive Value for Intra-Specific Variation?
- 88 B. Kebede, K. Cheema, and M.H. Rahman:  
Construction of a Genetic Linkage Map of *Brassica rapa*
- 89 Adrian E Platts, Stephen I Wright, Mathieu Blanchette, Paul M Harrison, Eef Harmsen, Daniel J Schoen, and Thomas E Bureau:  
Initial Results of Sequencing, Assembly and Annotation of Three Brassicaceae Genomes
- 90 Carol Powers, Fabiano Pita, Shunxue Tang, Michelle Wiggins, Yonghe Bai, Fang Lu, Van Ripley, Greg Gingera, Christof Stoll, Ralf Larsen, Jan Erik Backlund:  
Evaluation of Population Structure in Canola Breeding Germplasm
- 91 R. Raman, B. Taylor, A. Rehman, B. McVittie, C. Beeck, N. Wratten, D. Lockett, W. Cowling, D. Edwards, J. Batley and H. Raman:  
Molecular Diversity and Relationships amongst Germplasm of *Brassica napus* L. Using SSR Analysis
- 92 Christine Sidebottom, Wayne Clarke, Rong Li, Erin Higgins, Isobel Parkin and Andrew Sharpe:  
SNP Discovery In *Brassica napus* using 454 Titanium Sequencing
- 93 Ravinder Singh, Erin Higgins, Richard Gugel, Terry Huebert, and Isobel Parkin:  
Development of Genomic Resources for *Camelina sativa*
- 94 Laiqiang Song, Lunlin Chen, Xiaoyun Zou, Xiaofen Zou, and Shuyu Li:  
Developing a Core Collection (*Brassica napus* L.) for Recurrent Selection Based on SRAP Marker
- 95 Yu Takahashi, Shuji Yokoi, Hiroyuki Iwata, Makoto Kawase and Yoshihito Takahata:  
Genetic Diversity and Population Structure of *Brassica rapa* L. Based on Chloroplast and Nuclear Microsatellites

- 96 Michelle Wiggins, Shunxue Tang, Fang Lu, Yonghe Bai, Carol Powers, Lasantha Ubayasena, Zoe Ehlert, Tom Kubik, Gregory Gingera, Christof Stoll, Van Ripley, Thomas Greene, Steve Thompson, and Siva Kumpatla:  
High-Throughput Single Nucleotide Polymorphism (SNP) Discovery and Marker Validation in *Brassica napus*
- 97 Gabriel Wong, Erin Higgins, Kevin Falk, Colin Morgan, Ian Bancroft, and Isobel Parkin:  
Developing Genetic Resources for *Brassica carinata*

**Additional Abstracts**

---

- 98 Heather Ray, Cheryl Bock and Fawzy Georges:  
“Transgenic Coffee Alpha-Galactosidase in Oilseed *Brassica napus* Reduces Stachyose in Seed”
- 99 Surinder Banga:  
“Alien Introgressions for Germplasm Enhancement in *Brassica juncea*”



**1. QTL ANALYSIS OF SEED QUALITY TRAITS IN SPRING-TYPE *BRASSICA NAPUS***

**Kerry Boyle<sup>1</sup>, Wentao Zhang<sup>2</sup>, Erin Higgins<sup>2</sup>, Larissa Ramsay<sup>1</sup>, Christine Sidebottom<sup>1</sup>, Nirmala Sharma<sup>1</sup>, Andrew Sharpe<sup>1</sup>, Isobel Parkin<sup>2</sup>, and Pierre Fobert<sup>1</sup>**

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2. Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK, S7N 0X2, Canada

The genetic variation underlying many seed composition traits is complex and governed by multiple interacting genetic loci that have quantitative effects. QTL represent a significant proportion of the variation important to understanding agronomic traits, including those controlling seed composition; however, the poor resolution of QTL mapping combined with the complex (polyploid) nature of *Brassica* genomes make the discovery of causative genes a challenge. We are combining traditional QTL mapping with genome-wide transcript profiling of developing seeds to better dissect the genetic architecture of complex loci contributing to seed composition traits in *B. napus*. Here we report on QTL mapping of seed composition traits.

Two large double-haploid populations of spring-type *B. napus* were generated and mapped using molecular markers. The black seeded canola-quality line, DH12075, from which most of NRC-PBI and AAFC's genomic resources are derived, is a parent in both populations: BnaYBDH (generated by Dr. Gerhard Rakow, AAFC-SRC), is derived from a cross with a high-yielding, yellow-seeded canola line that is high in seed oil and low in fibre, while BnaSGDH results from the hybridization of DH12075 with a newly resynthesized *B. napus* line high in glucosinolates and erucic acid. In replicated field trials near Saskatoon during 2008 and 2009, both populations displayed variation for a number of seed composition traits, including oil, protein, fibre, and seed colour. The BnaSGDH population also displayed variation for glucosinolates and fatty acid composition. QTL mapping identified loci contributing to several of the traits measured. While some loci may correspond to those previously reported in the literature, others appear to be novel, indicating that the populations analyzed may contain new genetic variation for seed composition traits in *B. napus*.

**2. GENETIC RELATIONSHIP BETWEEN YELLOW- SEEDED *BRASSICA NAPUS* FROM TWO DIFFERENT SOURCES**

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Increased oil and protein contents are the important objectives of rapeseed breeding. Yellow seeds generally have thinner seed coat than black and brown seeds and associated with reduced fibre content in the seed meal. Two yellow seeded *Brassica napus* lines, YN01-429 and CH5034 were crossed and 122 doubled haploid (DH) lines were produced. The DH lines were evaluated in replicated field trail in 2008, and self pollinated seeds were assessed for seed color and analysed for oil, protein, glucosinolate and fibre content. Seed color of the DH population varied from bright yellow to black, despite both parents were yellow-seeded. About 1.6% of DH lines produced brighter color seed compared to the two parents i.e. were transgressive segregants. The results suggest that genetic control of yellow seed color in the two yellow seeded lines is distinctly different. The fibre content is negatively correlated with seed color ( $r = 0.7$ ,  $P \leq 0.001$ ).

### 3. THE IMPROVEMENT OF OIL CONTENT AND YIELD OF BRASSICA NAPUS USING GENOMICS AND BIOTECHNOLOGY APPROACHES

Faouzi Bekkaoui and Brittany Scarrow

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Canola (*Brassica napus*) seed is used for both food and biofuel. It is prized as a healthy oil by consumers because of it is high in monounsaturated fats and low in saturated fats. Canola's unique characteristics make it also an ideal feedstock for biodiesel production, thus contributing a positive environmental impact. In Canada, canola is grown in more than 15 M acres and lead to a production of approximately 12M tonnes generating a \$14 Billion economy. Because of its oil quality and multiple applications, there is a growing demand of canola seeds. The NRC Genomics and Health Initiative program "Biorenewable Oil for Food and Fuel" is using genomic and biotechnology approaches to enhance the productivity of canola by increasing oil content, seed yield and seedling vigour all of which would address the increasing demand for the oil. This three year program involves 17 Principal Investigators from four institutions. The research program is structured as three integrated projects: Gene Discovery, Functional Characterization and Pre-Commercialization. Within the "Gene Discovery" project we are using DNA microarray and next generation sequencing technologies tools to study gene expression (including studies on small, regulatory RNAs) at key stages of seed development and combining gene data with protein and metabolite analyses. Progress was made in these activities which will allow us to integrate and associate genes with the specific traits of interest. In the "Functional Characterization" project, 35 genes that may play an important role in crop improvement are studied in more detail to define their corresponding phenotypes and mode of action more completely using *Arabidopsis thaliana* as a model system. Elite candidate genes are moved into *Brassica napus* and the most successful are incorporated into the pre-commercialization project. This 3rd project involves field evaluation of the most promising candidate genes that have been produced. 12 prototypes were tested in the field with three prototypes showing improvement compared to a control in seed size, oil content and yield.

### 4. GENETICS OF SEED AND SEEDLING QUALITY TRAITS IN *BRASSICA RAPA*

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*Brassica rapa* (2n=20, AA) has been largely used for vegetable and vegetable oil production in the world. *Brassica* species are close relatives of the model plant *Arabidopsis*. Good quality seed plays a crucial role for the crop establishment under different environmental conditions resulting into vigorous plant growth and development. Since *Brassica* seed is also used for oil production purposes, the genetics of seed metabolite content is also equally important to understand the regulation of oil and protein biosynthesis. Diploid *B. rapa* can serve as a model to facilitate the comparative study with the amphidiploid *B. napus*, an economically important species for oil production. In this study, genetics of both seed content and seedling vigor traits will be studied under normal as well as stress environmental conditions, such as drought, salt and high temperature through a genetical genomics approach. A segregating double haploid (DH) population of a cross between Pak Choi (black-seeded) and Yellow Sarson (yellow-seeded) has been used to understand the genomics of seed content and seedling vigor traits by integrating the transcriptional, metabolites profile and seedling vigor traits. We will present the results of QTL analysis on seed metabolites, seed germination and seedling vigor traits.

**5. VARIETY IDENTIFICATION AND SEED PURITY ANALYSIS OF JAPANESE RADISH “DAIKON” USING DOT-BLOT-SNP TECHNIQUE****Shunsuke Okamoto<sup>1</sup>, Koji Sakamoto<sup>1</sup>, Atsuo Saito<sup>1</sup>, Hiroyasu Kitashiba<sup>2</sup>, and Takeshi Nishio<sup>2</sup>**

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Daikon (*Raphanus sativus* L.) is an edible root vegetable of the Brassicaceae family that is grown and consumed throughout East Asia. Most commercial varieties are F<sub>1</sub> hybrids, produced commonly using the self-incompatibility and/or male-sterility mechanisms, as reflected by their uniformity and high yield. These efficiencies are influenced with the environmental conditions during the seed production and thereby increasing the contamination of parental and non-parental seeds. Therefore, it is essential to test each hybrid seed lot for contamination of the inbred and undesired hybrid seeds prior to its release in the market. Grow-out trials, isozyme analyses, and DNA-based molecular markers are used for this purpose, although they are time consuming and labor intensive.

The dot-blot-SNP technique is a labor-saving, cost-effective method for SNP genotyping of a large number of plants. Ninety-four lines originating from F<sub>1</sub> hybrid varieties and landraces were used in this study. Fifty-one SNP markers were designed for detecting allele-specific signals, and all of them were tested; 23 SNP markers were found to be polymorphic. The polymorphism information was used for distinguishing among different lines and evaluating seed purity. Thirteen SNP markers were found to be sufficient for distinguishing all lines from each other, and the result was visualized as a tree-like graph. This was done using the freely available Java software package WEKA (University of Waikato, New Zealand, <http://www.cs.waikato.ac.nz/ml/weka/>).

In this study, we demonstrated that the dot-blot-SNP technique can be used for variety identification and seed purity analysis of commercially available daikon varieties.

**6. MODIFIED FATTY ACID *BRASSICA JUNCEA* DEVELOPMENT THROUGH INTROGRESSION OF *FAD A* GENOME GENE MUTATIONS FROM *B. NAPUS*****Van L. Ripley<sup>1</sup>, Zoe Ehlert<sup>1</sup>, Lasantha Ubayasena<sup>1</sup>, Michelle Beath<sup>1</sup> and Manju Gupta<sup>2</sup>**

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2. Dow AgroSciences LLC, 9330 Zionsville Rd., Indianapolis, IN, USA, 46268

Dow AgroSciences has had great success developing Omega-9 *Brassica napus* (A, C genomes) canola varieties based on our proprietary *fad2* and *fad3* mutations resulting in commercial varieties with high oleic, low linolenic. A project was undertaken to investigate the possibility to develop modified fatty acid profile *B. juncea* (A,B genomes) lines through interspecific hybridization between Omega-9 *B. napus* and *B. juncea* followed by *fad* molecular marker assisted backcrossing to *B. juncea*. Significant improvements to the *fad* markers were developed during the course of the study and ultimately we were successful in development of *B. juncea* germplasm with an increased oleic acid (18:1) and reduced linolenic acid (18:3) profile.

### 7. DNA ALLELIC VARIATION AT THE LOCI PUTATIVELY IMPLICATED IN SEED OIL FORMATION AMONG BRASSICA OILSEED CULTIVARS

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Knowledge about the genes implicated in lipid biosynthesis acquired from the model plant *Arabidopsis* is useful in understanding the formation of seed oil in Brassica oilseeds. In this paper, we report the screening of polymorphic markers at the loci putative for the seed oil formation between two geographically different genotypes: the Chinese cultivar Ningyou-7 and the European cultivar Tapidor. These primer pairs (150) were designed based on 75 Brassica genes that were *Arabidopsis* orthologues implicated in the oil formation. A total of 52 out of the 150 primer pairs associated with 47 of the 75 genes showed polymorphisms between the two genotypes. The type of polymorphisms that could be detected on capillary electrophoresis images and their respective visual futures are described. Further, we selected 34 polymorphic markers to scan allelic variations and found rich DNA polymorphisms among the 54 Brassica oilseed cultivars. On the average, each primer pair resulted in 5.6 alleles at the region that was covered. The correlation between the alleles and seed quality traits revealed that the alleles of *BnFAD7* were related to the variation of linolenic acid ( $C_{18:3}$ ) contents among the cultivars. The allele *FAD7-ics11170 (3/4)-b* that was significantly correlated with high linolenic acid content can be used as an efficient marker for the selection of breeding materials with high linolenic acid content.

### 8. CHARACTERIZATION OF A SEED MEAL QUALITY LOCUS ON CHROMOSOME A9 OF *BRASSICA NAPUS*

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This study describes a forward genetics approach to discover positional candidate genes for antinutritive components in seed meal from oilseed rape (*Brassica napus* L.). Analysis of quantitative trait loci (QTL) in a doubled haploid (DH) population derived from a cross between the black-seeded winter oilseed rape inbred line 'Express 617' and the yellow-seeded line '1012-98' revealed a major QTL on chromosome A9 with strong effects on phenotypic variation for seed colour, neutral detergent fibre (NDF) and acid detergent lignin (ADL), respectively. Exploiting sequence information from *Brassica rapa* led to the development of a number of new, locus-specific molecular markers and helped to saturate the present genetic map surrounding this seed meal quality QTL. Comparative mapping of some of the markers in other rapeseed populations confirmed the importance of the detected QTL. The aim of the current work is to identify potential candidate genes in the QTL region that may be involved in the biosynthesis of fibre components in the seed coat. To enable this a BAC library from 'Express 617' was screened by hybridization to identify clones spanning the QTL region. We are currently doing 454 shotgun sequencing of selected BAC clones to characterize homoelogenous loci corresponding to the meal quality QTL and identify potential candidate genes for seed coat cell wall components. Identification of key genes controlling meal quality will open new possibilities for breeding oilseed rape varieties with a more favourable composition of the meal, enhancing its potential use as an animal feed.

**9 ISOLATION AND CHARACTERIZATION OF *BRASSICA NAPUS* CDNAS THAT ENCODE LYSOPHOSPHATIDYLCHOLINE ACYLTRANSFERASE BY COMPLEMENTATION OF YEAST *LCA1Δ* MUTANT****Qian Zheng<sup>1,2</sup>, Kede Liu<sup>2</sup>, and Jitao Zou<sup>1</sup>**

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Acyl-CoA: lysophosphatidylcholine acyltransferase (LPCAT) is a key enzyme involved in Phosphatidylcholine (PC) turnover. Through modulating fatty acyl incorporation into PC, it not only influences the structure and function of biological membrane, but also affects the fatty acyl pool for triacylglycerol (TAG) biosynthesis. Genetic engineering of LPCAT opens a new way to modify the TAG composition in plant seeds. In this study, we took a biochemical screening approach to identify LPCAT cDNAs from *Brassica napus* by complementing the *Saccharomyces cerevisiae lca1Δ* mutant strain, which is deficient in LPCAT activity and sensitive to lyso-PAF. A cDNA library was constructed with *Brassica napus* seeds from different developmental stages, and then introduced into the *lca1Δ* mutant, followed by selection on lyso-PAF plates. Five *Brassica napus* LPCAT cDNAs were identified. Sequence alignment analysis showed that four of them, named *BnLPCAT1-1*, *1-2*, *1-3*, *1-4*, respectively, are homologous to the Arabidopsis *AtLPCAT1* (At1g12640), and another clone, *BnLPCAT2*, is a homolog of the *AtLPCAT2* (At1g63050). The yeast *lca1Δ* mutant expressing any of the five cDNAs was capable of surviving in the presence of up to 20 μg/ml lyso-PAF. Real-time RT-PCR revealed that both *BnLPCAT1-1* and *BnLPCAT2* were widely expressed in different tissues. We will present enzymatic characterization results on their substrate preference. RNAi transgenic plants and TILLING lines have also been generated to investigate their effects on seed fatty acid composition and oil content.

**10. TRANSPOSITION OF THE *CCE1* GENE SEQUENCES IN THE *BRASSICA OLERACEA* GENOME MAY HAVE RESULTED IN EXPRESSION OF A NOVEL PROTEIN AND CONTRIBUTED TO THE EMERGENCE OF THE CAULIFLOWER VARIETY****Camila Avila and Marilyn Cruz-Alvarez**

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The *Brassica oleracea* *CCE1* (*CAULIFLOWER CURD EXPRESSION 1*) gene was originally identified by its differential expression in the *botrytis* variety. Previous results had shown that the pattern of expression of the *CCE1* at the RNA level is consistent with a role of this gene in the arrest of floral development in cauliflower. We have conducted further characterization of the *CCE1* gene aimed at elucidating its function and possible contribution to developmental differences in *Brassica oleracea* varieties. An antibody has been produced against a peptide within the deduced *CCE1* protein sequence. Western blot analysis with this antibody revealed the presence of the predicted protein in the curd of the cauliflower and confirmed variety-specific expression of the gene. Three almost identical copies of *CCE1* have been identified in the cauliflower genome. These three copies lack introns and have poly A tails on their 3' ends, suggesting these sequences may have undergone transposition in the genome. While no ortholog has been detected by Southern blot or sequence data analyses in *Arabidopsis thaliana*, *CCE1* coding sequences seem to be conserved among varieties of *Brassica oleracea* and in other members of the *Cruciferae* family. We are investigating the possibility that transposition of these sequences in the *Brassica oleracea* genome led to production of a novel protein and contributed to the developmental characteristics of cauliflower.

**11. COMPARATIVE MAPPING OF QTL FOR HORMONE METABOLITES AND DEVELOPMENTAL TRAITS IN OILSEED RAPE SEEDLINGS****Panjisakti Basunanda<sup>1</sup>, Bertha Salazar<sup>1</sup>, Irina Zaharia<sup>2</sup>, Sue Abrams<sup>2</sup>, and Rod Snowdon<sup>1</sup>**

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Seedling vigour is an important trait in winter oilseed rape (*Brassica napus*) due to its influence on seedling establishment before winter and the consequent effects on yield and yield stability. Little is known about the role of hormone metabolism in regulation of seedling developmental traits in *B. napus*. In this study 250 doubled haploid (DH) lines from the cross 'Express 617' x 'V8' were grown in soil in a climate-controlled greenhouse in two randomised replicates of 27 plants per line in 9 pots. The parental lines were included as controls. Measurements of hypocotyl length, shoot fresh weight, dry weight and leaf area were taken during the 28 days after sowing for identification of QTL involved in seedling development and biomass accumulation. Using these data, a reduced set of 96 genotypes with good or poor seedling development, respectively, was selected for UPLC-MS/MS hormone profiling during early seedling development. The method employs isotopically labeled internal standards for accurate quantification of biologically active hormones, their precursors and inactive catabolites. This data was used for correlation analyses of hormone metabolites with seedling traits, to identify QTL for key hormone metabolites, and to compare these loci with positions of QTL involved in seedling development. The results give first insight into the genetic control of seedling vigour in *B. napus* and may help to identify potential selection biomarkers. This work is part of the tri-national ERANET Plant Genomics project "ASSYST: Associative expression and systems analysis of complex traits in oilseed rape/canola."

## 12. NEW HAIRY CANOLA AND HAIRY MUSTARD LINES AND THEIR POTENTIAL FOR RESISTANCE TO *PHYLLOTRETA* FLEA BEETLES

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*Brassica napus* is barren of trichomes and is highly susceptible to feeding damage by *Phyllotreta* flea beetles. *Arabidopsis thaliana glabrous* mutants also are devoid of trichomes and are more susceptible to flea beetle feeding than wild type *Arabidopsis*. In order to induce flea beetle resistance in *B. napus*, we introduced two *Arabidopsis GLABRA* genes *AtGL3* and *AtGL1* into *B. napus* and recovered seedlings with 1000-fold more trichomes on the first three seedling leaves. The trichomes of these plants had uneven length, the cotyledons were cupped, and the plants were dwarfed, but the seedlings showed moderate resistance to flea beetles. A *B. napus TRANSPARENT TESTA GLABRA 1* gene over-expressed in the hairy *AtGL3<sup>+</sup> B. napus* background was lethal and its only two survivors were barren of trichomes and taller than hairy *AtGL3<sup>+</sup> B. napus*. In contrast, two *BnTTG1* RNAi lines in the *AtGL3 B. napus* background showed a new super-hairy leaf phenotype with long trichomes, dense trichome coverage up to the 7<sup>th</sup> true leaf and on the lower stem, only slightly curled leaves, a purple stem, and growth more vigorous than the Westar control plants. The *AtGL3* gene expressed in yellow-seeded Ethiopian mustard, *B. carinata*, also had dense seedling trichome coverage with no penalty on growth or tissue shape, while *AtGL3*, *BnTTG1* or RNAi-*BnTTG1* expressed in the *B. napus* Westar non-hairy background showed no trichome changes or growth differences. Phenotype and molecular analysis of these new plants will be presented.

## 13. *CANDIDATUS PHYTOPLASMA ASTERIS*' IDENTIFIED IN BRUSSELS SPROUTS AND ITS POSSIBLE ASSOCIATION WITH FLOWER BUD FAILURE IN POLAND

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In early spring of 2009 25 Brussels sprout plants of male sterile line CAP with *Ogu-INRA* cytoplasm, growing in the greenhouse, showed previously unknown symptoms. They included stunted growth, leaf chlorosis, severe leaf malformation associated with leaf blade reduction, flower bud failure and root system reduction. Healthy looking plants of the male fertile isogenic lines grown in the same conditions remained symptomless, however some of them produced reduced number of flowers and seeds.

The aim of the work was to describe the disease symptoms and to determine their association with phytoplasma infection and, if so, to identify the pathogen. In attempt to identify the causative factor of the Brussels sprout disease, a standard protocol including extraction of total DNA from symptomatic and asymptomatic tissues, PCR amplification with phytoplasma-specific universal primers and characterization of amplified products by sequence analysis was used. After amplification, the specific bands were obtained for samples of two out of nine tested Brussels sprouts with disease symptoms, two out of three symptomless plants as well as for seedlings of three out of seven tested plants.

Nucleotide sequence analysis of the PCR-amplified 16S rRNA gene fragment of the isolated phytoplasmas revealed that they were closely related to phytoplasma members of 16SrI-B subgroup. Sequences of two phytoplasmas found in *B. oleracea* subsp. *gemmifera* plants (GenBank accession numbers GQ240826 and GQ240827) were identical, and they showed more than 98% identity to the sequences of '*Candidatus Phytoplasma asteris*'. This is the first record on the occurrence of '*Ca. Phytoplasma asteris*' in Brussels sprout.

**14. DRAMATIC CHANGE IN FUNCTION AND EXPRESSION PATTERN AFTER GENE DUPLICATION CREATED A GENE REGULATING ZYGOTE DEVELOPMENT IN THE BRASSICACEAE****Shao-Lun Liu, and Keith L. Adams**

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New gene formation by duplication has been an ongoing process during plant evolution that has contributed greatly to the large number of genes in plant genomes. After duplication, some genes that are retained can acquire new functions or expression patterns. We show that *SHORT SUSPENSOR* (*SSP*) and *Brassinosteroid Kinase 1* (*BSK1*) are paralogs duplicated by a polyploidy event that occurred in the Brassicaceae family about 23 MYA. *SSP* is involved in paternal control of zygote elongation in *Arabidopsis thaliana* by transcription in the sperm cells of pollen and then translation in the zygote, whereas *BSK1* is involved in brassinosteroid signal transduction. Comparative analysis of expression in 63 different organs and developmental stages revealed that *BSK1* and *SSP* have opposite expression patterns in pollen compared with all other parts of the plant. We determined that *BSK1* retains the ancestral expression pattern and function. Thus *SSP* has diverged in function after duplication from a component of the brassinosteroid signaling pathway to a paternal regulator of the timing of zygote elongation. The ancestral function of *SSP* was lost by deletions in the kinase domain. Our sequence rate analysis revealed that *SSP*, but not *BSK1*, has experienced a greatly accelerated rate of sequence evolution and relaxation of purifying selection. This study illustrates a dramatic example of neofunctionalization following gene duplication by complete changes in expression pattern and function, as well as indicating that paternal control of zygote elongation by *SSP* is an evolutionarily recent innovation in the Brassicaceae family.

**15. DIFFERENCES IN EXPRESSION OF THE CAULIFLOWER (*CAL*) GENE DO NOT CORRELATE WITH PHENOTYPIC DIFFERENCES IN THE F2 PROGENY OF CAULIFLOWER AND RAPID CYCLING *BRASSICA OLERACEA* (*RBO*) HYBRIDS****Sandra Londoño and Marilyn Cruz-Alvarez**

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Cauliflower (*Brassica oleracea* var. *botrytis*) and Rbo (Rapid cycling *Brassica oleracea*) exhibit apparent morphological and developmental differences. While Rbo plants show normal flower development, cauliflower is characterized by the formation of a head or curd as a result of apical meristem proliferation and arrested flowering. The discovery of a non-sense mutation in *CAULIFLOWER* (*CAL*), a floral meristem identity gene, in the cauliflower variety suggested that lack of a functional *CAL* protein was either solely or partially responsible for formation of the curd. In order to better understand the phenotypic differences and the underlying genetic differences between cauliflower and Rbo, we have produced F1 and F2 hybrids and analyzed their phenotype and genotype. The hybrids showed an intermediate phenotype between the two varieties. Phenotypic variables such as the length of internodes in the main stem and flowering time were significantly different in the F1 hybrids than in Rbo plants. The F1 hybrids also showed an increased number of aborted flowers, with lack of development of floral organs, with respect to Rbo. The F2 generation showed a wide distribution of phenotypes and a lack of correlation between the different phenotypic variables among plants, indicating the contribution of several segregating genes to the phenotypic differences. Although the genotypic analysis has included a small number of plants so far, it has not revealed any correlation between expression of the *CAL* gene and any of the observed phenotypic variables.

**16. PHYTOCHEMICAL AND TRANSCRIPTOME ANALYSIS OF *BRASSICA VILLOSA* VS *B. NAPUS*****N.K. Nayidu, X. Li, and M.Y. Gruber**

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Trichomes are single cell epidermal structures that frequently function as a first line of defence against insect attack, either by spatial hindrance, or by secreting either toxic or behaviour modifying chemicals. *Brassica napus*, Canada's most important oilseed crop (canola), has very few trichomes and seedlings are highly susceptible to feeding damage by *Phyllotreta* flea beetles. In contrast, the Sicilian mountain species, *Brassica villosa*, is a perennial weed with dense trichome coverage over the leaves, stems, and flowers. Flea beetles are deterred by trichomes in several crucifer species, and *B. villosa* is highly resistant to this pest. To determine whether resistance is due strictly to the mechanical barrier or to additional phytochemical feeding deterrents, we isolated trichomes from the leaves of *B. villosa* grown in a greenhouse. Small leaf pieces were rotated in a 50 mL tube with liquid nitrogen at maximum speed on a Genie 2 vortex mixer for 1 minute. Released trichomes were washed with sterile distilled water and sieved through a 40- $\mu$ m cell strainer. The cell strainer was then inverted into a new 50 mL tube and additional water used to dislodge the trichomes. Trichomes in water were freeze-dried and crushed using liquid nitrogen and then extracted for polar metabolites using 75% methanol. Processed trichome-reduced leaves also were collected and used as a control sample. Filtered methanol extracts were analyzed by HPLC/UV, followed by UPLC/MS and 1D- and 2D-NMR analysis. NMR results indicated several peaks unique to trichomes, one corresponding to inorganic compound(s) and several corresponding to disaccharide derivatives. And the transcriptome analysis from the young true leaves indicate that all the well known trichome related genes were found to be highly expressed in *B. villosa* than *B. napus*.

**17. CHARACTERIZATIONS OF AN ARABIDOPSIS MUTANT DEFICIENT IN S-ADENOSYLHOMOCYSTEINE HYDROLASE1****Xianzhong Wu<sup>2</sup>, Fengling Li<sup>2</sup>, Allan Kolenovsky<sup>1</sup>, Allan Caplan<sup>3</sup>, Yuhai Cui<sup>4</sup>, Adrian Cutler<sup>1</sup> and Edward W.T. Tsang<sup>1</sup>.**

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An *Arabidopsis* mutant, referred to as *sahh1*, was initially recovered from a mutant screen for germination. The mutant shows delayed germination, retarded growth, short root and hairless root phenotypes. Molecular characterization of the *sahh1* mutant revealed that it contained a T-DNA insertion 82 bp 5' to the coding sequence of S-adenosylhomocysteine hydrolase1 (SAHH1). In plants, SAHH1 converts S-adenosylhomocysteine (SAH) to homocysteine (Hcy) which is a precursor for methionine and S-adenosylmethionine (SAM). T-DNA insertion has interfered with transcription resulting in the lower levels of transcripts and corresponding protein. Using the root hairless phenotype of the *sahh1* mutant as a visual assay, the deficiency of SAHH1 was investigated.

**18. GENETIC DISSECTION OF FLOWERING TIME OF *BRASSICA RAPA* IN DIFFERENT ENVIRONMENTS**

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Cultivated varieties of the diploid species *Brassica rapa* can differ based on morphological appearance and consumed organs, like the leafy types Chinese cabbage and pakchoi, turnip types and oil types. Flowering time is a very important developmental trait and wide variation exists among *B. rapa* accessions; it defines the geographical growing area of the different crop types. Flowering time is generally controlled by temperature and/or day length.

Here we describe the effects of vernalization on flowering time in DH populations and on *BrFLC2* expression in selected lines of a DH population in *B. rapa*. The effect of the major flowering time QTL on the top of A02 where *BrFLC2* maps decreases clearly upon vernalization, which points to a role of *BrFLC2* underlying the QTL. In all developmental stages and tissues (seedlings, cotyledons, leaves), *BrFLC2* transcript levels are higher in late flowering pools of DH lines than in pools of early flowering DH lines and *BrFLC2* expression diminishes after different lengths of seedling vernalization in both pools. This reduction of *BrFLC2* expression upon seedling vernalization was strongest at the seedling stage in both early and late flowering DH lines and diminished in subsequent growth stages, which suggests that the commitment to flowering is already set at very early developmental stages. Taken together, these data support the hypothesis that *BrFLC2* is a candidate gene for the flowering time and vernalization response QTL in *B. rapa*.

Our phenotypic data also showed that daylength affects flowering time, and this greatly affects the optimal growing area of the different *B. rapa* morphotypes. The flowering time of DH population DH68 (yellow sarson x pakchoi) and several homozygous lines representing different crop types under different daylengths (8 and 16 hrs light) was evaluated in the greenhouse and in climate cells. We will present QTL for flowering time under different daylengths and the expression of the genes CO and FT during short day and long day growth conditions. The effect of daylength on flowering time in *B. rapa* will be discussed.

**19. ANALYSIS OF DIFFERENTIALLY EXPRESSED GENES IN OLEIC ACID SYNTHESIS OF BRASSICA NAPUS BY GENE CHIP**

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The oil from rapeseed with high oleic oil content is insensitive to oxidation and longer shelf life. In daily diet high oleic acid oil can reduce low-density lipoprotein cholesterol levels in blood and prevent arteriosclerosis. In addition, high oleic acid content in edible oil is an important fat indicator. Oleic acid can be effectively converted to methyl ester and is beneficial to produce biodiesel. Oleic acid content in conventional rapeseed is only 17%. However, oleic acid content in low erucic acid rapeseed reaches around 60%. Differential expressed genes of oleic acid have been found in the high oleic acid line (71.71%) and low oleic acid line (55.6%) of *Brassica napus* by gene chip technic. The results showed that there were 562 differential expressed genes relating to high and low oleic acid contents in rapeseed, among them 194 were up-regulated genes and 368 were down-regulated genes. The up-regulated gene (NM\_100489) and the down-regulated gene (NM\_130183) were then taken as the materials. Real time RT-PCR was further used to confirm the results of gene chip, and the results of real time RT-PCR analysis agreed with the results by gene chip. According to the results of gene chip, 562 differentially expressed genes were annotated functionally by Go Annotation System. The molecular function of differential expressed genes mainly included catalysis of enzymes, binding, transcriptional regulation, metabolism and others which were unknown. Some genes relating to sugar metabolism and fatty acid synthesis were identified as differential expressed genes. They are pyruvate kinase, fructose diphosphate, acyl transfer/acyl-ACP S lipase, hydrolase acting on ester bond,  $\Delta 9$  stearoyl acid-N-carrier protein desaturase (ADS1),  $\Delta 9$  acyl-lipid desaturase 2 (ADS2),  $\omega$ -3 fatty acid desaturation enzymes (*fad3*) and so on.

**20. MOLECULAR EVOLUTION OF THE PSY GENE FAMILY IN THREE BRASSICA SPECIES**

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In plants, the first committed step of the carotenoid biosynthetic pathway is catalyzed by the enzyme phytoene synthase (PSY). In *Arabidopsis thaliana*, this enzyme is encoded by a single copy gene (At5G17230), however, the existence of PSY gene families has been documented in horticultural and crop species. In these species, subfunctionalization of the different PSY paralogs provided a mechanism that allowed for the accumulation of high levels of carotenoids in non-photosynthetic tissues and the response to environmental stress. In this study, we describe the existence of a PSY gene family in *Brassica napus* (AACC) and the diploid species carrying both parental genomes, *B. rapa* (AA) and *B. oleracea* (CC). Using an overlapping-PCR strategy, we had identified 5 BnaX.PSY, 2 BraA.PSY and 3 BolC.PSY genes and confirmed these results using DNA-SSCP and Southern blot analyses. Additionally, we had performed rapid amplification of cDNA ends (RACE) and cloned BnaX.PSY cDNAs from a variety of tissues. The gene expression profile of each Brassica PSY was characterized using RT-PCR followed by cDNA-SSCP. Interestingly, one BnaX.PSY is preferentially expressed in petals and another preferentially expressed in green tissues whereas the three remaining BnaX.PSY paralogs are expressed in both photosynthetic and non-photosynthetic tissues. This evidence is suggestive of the existence of a chromoplast-specific carotenoid biosynthetic pathway in Brassicas. Knowledge gathered from this study will aid in the future development of transgenic and conventional *B. napus* cultivars with carotenoid-enriched oil.

**21. ULTRADEEP GENOME AND TRANSCRIPTOME MAPPING IN *BRASSICA NAPUS* USING NEXT-GENERATION SEQUENCING****Julio Cordero<sup>1</sup>, Andrew Sharpe<sup>2</sup>, Christine Sidebottom<sup>2</sup>, Wolfgang Friedt<sup>1</sup>, and Rod Snowdon<sup>1</sup>**

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Next generation sequencing technologies provide powerful new opportunities for mapping-by-sequencing and ultradeep transcriptome analysis. We are performing bulked 454-FLX sequencing in reduced-complexity genomic DNA libraries from 62 doubled haploid lines from our winter oilseed rape mapping population 'Express 617' x 'V8' (ExV8-DH). Segregating SNP haplotypes from the sequence reads will be used to identify and map homoeologous *Brassica napus* loci in a high-density bin map. The ExV8-DH population has previously been used to localize QTL for numerous metabolic, agronomic, developmental and seed traits, and a sequence-annotated SNP map will give us the ability to navigate directly from key QTL to the corresponding genomic sequence region. At the same time we are also using next-generation sequencing for detailed characterization of developing seed transcriptomes in the parental lines 'Express 617' and 'V8'. 454-FLX sequencing of 3'-UTR libraries from different developmental stages will enable sequence polymorphisms among seed-expressed genes to be annotated on the physical genome map. The 3'-UTR sequences will also serve as a reference for annotation of digital gene expression tags isolated from the same libraries and quantified via Illumina sequencing. The aim is to integrate differential gene expression data among the parental lines into the sequence-based genetic map, giving additional information on potential candidate genes localized in QTL for important seed traits.

**22. IDENTIFICATION AND CHARACTERIZATION OF NBS-ENCODING RESISTANCE GENES IN *BRASSICA NAPUS*****Rudolph Fredua-Agyeman and Habibur Rahman**

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The NBS-LRR type *R* proteins have been implicated in the pathogen recognition process leading to disease response in many plants. In this work, we identified 673 candidate NBS-encoding *Brassica napus* disease-resistance genes among 643,947 ESTs downloaded from the NCBI server in June 2010. First, we created a local EST database of the downloaded sequences. Homology searches were then conducted with 202 Arabidopsis NBS-LRR-encoding or related gene sequences downloaded from the NIBLRRS webpage. The query sequences consisted of 93 TIR-NBS-LRR sequences, 54 CC-NBS-LRR sequences, and 55 NBS sequences which lacked the LRR domain. The 202 query sequences were used in BLASTn search of the local *B. napus* EST database. A Perl script was used to retrieve *B. napus* hits for each of the 202 Arabidopsis NBS genes. A sequence was identified to be a hit if the nucleotide identity was greater than 80% at a stringent *E* value cut-off of  $10^{-10}$ . Unique hits from the Perl output were parsed into another file and the number of hits of each unique sequence was obtained. A total of 267 out of the 673 candidate NBS-encoding *B. napus* disease-resistance genes were found to be unique. Phylogenetic analysis of the unique hits shows several conserved motifs of which the NBS-encoding branches are dominant. The existence of such a high degree of diversity among the *R* genes in *B. napus* suggests that many degenerate oligonucleotide primers can be developed to aid in the breeding of resistant lines of this important oil crop.

**23. GENOME-WIDE TRANSCRIPT AND SMALL RNA ANALYSES DURING SEED MATURATION IN *BRASSICA NAPUS* BY MICROARRAY AND DEEP SEQUENCING**

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Seed maturation in canola (*Brassica napus*) involves a complex network of processes that occur from the end of embryo development to the production of mature dry seed. The processes associated with seed maturation affect seed size, oil production, protein content and antinutritional accumulation as well as vigour during seedling growth. In this study, we performed comprehensive transcript and small RNA profiling of whole seeds from the torpedo embryo stage to mature dry seeds (10DAF to 45DAF) using 90K *Brassica* microarrays and next generation sequencing (454, SOLID and ILLUMINA). We also examined tissue specific expression changes through analysis of manually dissected seed parts including: radicle, hypocotyl, cotyledon, embryo, endosperm, and seed coat. Striking changes in gene expression occurred through time that correlated with the physiology and progression of maturation events including chlorophyll loss, oil accumulation, protein deposition, and acquisition of desiccation tolerance. In addition, transcripts that were preferentially expressed in the different tissue components of the seed were identified. Transcription data was supplemented with metabolite analyses obtained from the same time points. Within the small RNA population, more than 200 known microRNAs (miRNAs), as well as potential novel microRNAs, were discovered in canola. Most of the known miRNAs were preferentially expressed in particular tissue types and changed significantly during seed maturation. The spatial and temporal relationships between miRNAs and their putative miRNA target genes were also investigated. This study has established the framework to identify and model the gene regulatory networks that control canola seed maturation.

**24. A COMPREHENSIVE TRANSCRIPTOME ANALYSIS OF SILIQUE DEVELOPMENT AND DEHISCENCE IN *ARABIDOPSIS* AND *BRASSICA* INTEGRATING GENOTYPIC, INTERSPECIES AND DEVELOPMENTAL COMPARISONS**

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Premature silique dehiscence (pod shattering) in *Brassica napus* (canola) can result in significant yield losses. Therefore, a goal for canola crop improvement is to reduce or delay shattering in order to harvest maximum yield potential. We performed a comprehensive transcriptome analysis on *Arabidopsis* and *Brassica* silique tissues that included the dehiscence zone (DZ) and valve of shatter resistant and sensitive genotypes at several developmental stages. Among known *Arabidopsis* dehiscence genes, we confirmed that homologs of *SHP1/2*, *FUL*, *ADPG1*, *NST1/3* and *IND* were associated with shattering in *B. juncea* and *B. napus*. The expression of numerous cell wall-related genes changed during silique development and a correlation between reduced pectin degradation and shatter-resistance was noted. Lignin plays a major role in silique dehiscence and there were differences between genotypes in expression of genes of the monolignol biosynthetic pathway. Light microscopy revealed a clear difference in lignin deposition between siliques of shatter-resistant *B. juncea* and shatter-sensitive *B. napus*. Sustained repression of auxin biosynthesis, transport and signaling in *B. juncea* relative to *B. napus* may play a causative role in producing differences in cell wall constituents. Reduced shattering was generally associated with upregulation of ABA signaling and down regulation of ethylene and jasmonate signaling, corresponding to more pronounced stress responses and reduced senescence and photosynthesis. Expression of seed storage and late embryogenesis associated protein genes occurred in siliques. Overall, we identified 131 cell wall related genes and 112 transcription factors (TFs) that may be involved in silique dehiscence.

**25. IMPACT OF ALLOPOLYPLOIDY ON SMALL RNAS: A BIOINFORMATIC SURVEY IN RESYNTHESED *BRASSICA NAPUS* ALLOTETRAPLOIDS****Johann Joets<sup>1</sup>, Christine Lelandais<sup>2,3</sup>, Martin Crespi<sup>2</sup>, and Karine Alix<sup>4</sup>**

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Polyploidy has played a major role in the evolution of plant genomes; its success can be explained by the fact that a polyploid genome is not the strict addition of the progenitors, but that polyploidisation induces structural and functional modifications which are important sources of novelty. Our plant model is oilseed rape (*Brassica napus*, AACC), an allotetraploid species originating from interspecific hybridization between *B. rapa* (AA) and *B. oleracea* (CC). In a pioneering study, we applied comparative proteomics to early generations of resynthesized *B. napus* allotetraploids, and we showed that numerous proteins exhibited quantitative variations of expression following the polyploidisation event [1]. The transcriptomic analysis of the genes encoding “non-additive” proteins revealed that two thirds of these genes displayed additive transcript levels, indicating that most of the observed differential protein quantity regulation is controlled by post-transcriptional mechanisms [2]. Micro RNAs (miRNAs) have been demonstrated to regulate gene expression through different modes of negative regulation, with post-transcriptional cleavage and translational repression being the predominant mechanisms [3]. Therefore, miRNAs represent a promising pathway to explain the non additive protein quantity patterns observed in the *B. napus* allotetraploids. We constructed and sequenced several small RNA libraries prepared from stems of the diploid progenitors and of two different generations of resynthesized oilseed rapes. Using *in silico* analysis, we compared the distribution of small RNAs in the diploid and allotetraploid genotypes, focusing our study on the 21-nt class which contains most miRNAs. The first trends emerging from this bioinformatic analysis will be presented and discussed.

[1] Albertin et al. 2006. Genetics 173: 1101–1113; [2] Marmagne et al. 2010. New Phytologist 186: 216-227; [3] Brodersen et al. 2008. Science 320: 1185–1190.

**26. EXPLORING AND GENERATING EPIGENETIC VARIATION IN BRASSICA.****Graham J King, Stephen Amoah, Clare Hopkins, Andrew Stoute, Smita Kurup**

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Productivity and quality of crops is determined by cultivar genotype and growing environment. Progress in identifying and characterising genes affecting agronomic traits has been limited by the large size and complexity of many crop genomes. It is now apparent that epigenetic regulation, mediated through marks that affect chromatin structure, may play a major role in the control of development and response of plants to environment. An increasing range of agronomic traits are being shown to be affected to some extent by stably inherited epigenetic modifications. Compared with animal genomes, there are important differences in the prevalence and pattern of DNA methylation marks in plant genomes, where epiallelic variation in methylation is often stably inherited through meiosis. We are generating resources to explore and exploit variation in DNA methylation within *Brassica* genomes, with a focus on the consequences for seed development and composition. Resources developed include lines with knock-outs and an allelic series of DNA methyltransferase 1 mutants, and an ‘epi-TILLING’ population hypomethylated by treatment with 5-azacytidine for forward and reverse genetic analyses. We are adding value to the *Brassica* genome sequence by establishing distribution maps of epigenetic marks at key stages of development, and exploring the effects of stochastic and targeted demethylation of genomic regions on seed traits.

**27. THE UNFOLDED PROTEIN RESPONSE (UPR) IN MICROSPORE-DERIVED EMBRYOS (MDES) FROM *BRASSICA NAPUS*.****Natalie Labbé, Anne Johnston, Jas Singh, and Johann Scherthner**

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Stressed Endoplasmic Reticulum (ER) leads to misfolding of proteins processed in the ER, which in turn induces a response pathway aimed at mitigating the stress. This response is termed UPR. The major characteristics of UPR are increased production of protein folding chaperones and enhanced protein degradation. The use of seed systems as a platform for overexpression of recombinant proteins could potentially result in induction of UPR (Oono et al 2010). UPR can be induced *in vitro* by Tunicamycin, an inhibitor of protein glycosylation. In order to gain insights into the UPR in *Brassica* seeds, we tested the effect of Tunicamycin on *B. napus* (cv Topas) MDEs and analyzed its effect on transcript profiles using the Agilent 105K Brassica microarray. Our analyses indicated that over 600 transcripts were up or down regulated 2-fold or more in the Tunicamycin treated embryos. ER resident chaperones, including BiP, HSP90 (SHEPHERD), calreticulin, calnexin and protein disulfide isomerases were significantly upregulated, reflecting a typical UPR response. However, unlike in vegetative tissues, the UPR in the embryos also down-regulated many ABA dependent stress related genes encoding LEA proteins, genes involved in abiotic and oxidative stresses, genes encoding pathogen related defense proteins and transcription factors and seed storage proteins. These observations suggest that persistent UPR in developing seeds could have implications on seed maturation, stability and seed quality

**28. PHYSICAL MAPPING OF BRASSICA B-GENOME OF *B. NIGRA* IN CORRESPONDENCE TO THE FCA GENOMIC REGION OF ARABIDOPSIS CHROMOSOME 4****Zahra Navabi<sup>1</sup>, Terry Huebert<sup>1</sup>, Carmel O'Neill<sup>2</sup>, Ian Bancroft<sup>2</sup>, and Isobel Parkin<sup>1</sup>**

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The diploid B genome of Brassica species, although known to carry several important traits such as tolerance to various abiotic and biotic stresses, has not been studied as extensively as the related A and C genomes. We have investigated the physical organization of regions of the B genome of *B. nigra* paralogous to a 222 Kb region of *A. thaliana* chromosome 4 that had previously been characterized for both the A and C Brassica genomes. A bacterial artificial chromosome (BAC) library of *B. nigra* (DH No100), with an approximate 10 X genome coverage, was screened with 19 genes from the *A. thaliana* FCA region of chromosome 4 to identify homologous clones. BAC end sequencing was completed for 1,109 positive clones, these data were analysed to identify 500 apparently non-redundant clones. BAC clones were used in Southern blot hybridization to confirm the presence of the *A. thaliana* genes and the identified banding patterns were used to preliminarily assemble contigs. Contig data is being refined and confirmed utilizing the SNaPshot *HICF* fingerprinting method. A comparison between homologous regions in the paleo-polyploid Brassica A, B and C genomes will be presented. In addition, BACs representative of each region are being targeted for sequencing to elucidate the level of micro-scale rearrangements across the Brassica species divide.

**29. DEVELOPMENT OF B-GENOME CHROMOSOMES INTROGRESSION LINES FROM INTERSPECIFIC HYBRIDS OF *BRASSICA NAPUS* × *B. CARINATA***

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*Brassica carinata*, which contains the Brassica B and C genomes, has a number of traits that would be valuable to introgress into *Brassica napus*. Interspecific hybrids were created between *B. carinata* (BBCC) and *B. napus* (AACC) using an advanced backcross approach to identify and introgress traits of agronomic interest from the *B. carinata* genome. We mapped the B and C genomes of *B. carinata* with SSR markers and observed their introgression into *B. napus* through multiple backcross generations, using SSR markers, GISH assays and chromosome counts to study the inheritance of the B-genome chromosome(s). The material developed as part of this backcrossing program includes lines with one or several of the B-genome chromosomes, as addition-deletion and substitution lines. This work provides an analysis of the behavior of chromosomes in a *Brassica* interspecific cross and describes a set of publicly available B genome addition lines which can be further characterized for agronomic traits.

**30. NEXT GENERATION SEQUENCING PLATFORMS AT NRC-PBI: - RECENT UP-GRADES AND FUTURE IMPROVEMENTS**

**Inge Roewer, Darrin Klassen, Janet Condie, Christine Sidebottom, Carling Tallon and Andrew Sharpe**

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The DNA Technologies Laboratory has served researchers working at NRC-PBI, scientists at the University of Saskatoon, many Academic and Governmental Institutes across Canada as well the International Scientific Community since 1990. Staying at the forefront of Genomics Technology, NRC-PBI has acquired several Next Generation Sequencing (NGS) platforms; - a 454 GS FLX from Roche Life Sciences in 2008 and two Genome Analyzers Ix from Illumina in 2009 and 2010.

These NGS platforms have distinct features from which clients can choose to either study whole genomes via *de novo* sequencing or deep re-sequencing, as well as transcriptomic, epigenomic and metagenomic applications. Both platforms are continuously up-graded, increasing throughput and read length therefore lowering the cost of sequencing per base dramatically. The bottlenecks and challenges scientists are now facing is the increasing amount of data to be analyzed.

Technical details of the two platforms will be presented providing a comparison between the two systems and their applications, including contributions to the Canadian Canola Sequencing Initiative (CanSeq).

**31. DIGITAL GENE EXPRESSION ANALYSIS OF SEEDLING DEVELOPMENT TRAITS IN WINTER OILSEED RAPE****Bertha Salazar, Aysha Kiran, and Rod Snowdon**

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Seedling vigour is an important trait in winter oilseed rape (*Brassica napus*) due to its influence on seedling establishment before winter and the consequent effects on yield and yield stability. The aim of this work is to investigate global gene expression during seedling development, with the help of eQTL analysis via digital gene expression. Illumina sequencing of short 3'-EST tag sequences is a powerful alternative to conventional microarray expression analysis, particularly for accurate quantification of low-abundance transcripts and potential identification of unknown genes. We are performing Illumina EST tag sequencing on seedling cDNA libraries from a DH population segregating for seedling developmental traits. Digital expression libraries are being generated from the cross parents 'Express 617' and 'V8', their F1 and 93 ExV8-DH lines that show maximal phenotypic diversity during seedling development. Seeds were sown under controlled conditions in Jacobsen germination vessels before mRNA extraction of whole seedlings at 8 and 12 days after sowing. The use of homogeneous lines enabled pooling of 50 plants per genotype from two biological repetitions. EST tag libraries are generated using a modified LongSAGE protocol involving restriction of immobilised cDNA fragments with *dpnII* and subsequent ligation of Illumina sequencing oligos. Genotype barcoding enables 8-fold multiplexing for the tag sequencing. Quantification of transcript abundance is performed in around 1 million EST tag sequences per sample, while annotation of tags to expressed genes is achieved by using anchored 3'-EST sequences from seedlings of the parental genotypes 'Express 617' and 'V8' as a reference. Besides the eQTL analysis, the results of the digital gene expression study will be combined with detailed hormone profiles and developmental phenotype data in a systems-based approach to identify potential regulatory genes involved in seedling development. This work is being performed within the tri-national ERANET Plant Genomics project "ASSYST: Associative expression and systems analysis of complex traits in oilseed rape/canola."

**32. TRANSCRIPTIONAL PROFILING OF DIACYLGLYCEROL ACYLTRANSFERASE 1 - SUPPRESSION IN BRASSICA NAPUS****Nidhi Sharma<sup>1</sup>, Fred Peng<sup>1</sup>, Saleh Shah<sup>1,2</sup>, Raju Datla<sup>3</sup> and Randall J. Weselake<sup>1</sup>**

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Diacylglycerol acyltransferase (DGAT) catalyzes the acyl-CoA-dependent acylation of diacylglycerol to produce triacylglycerol (TAG). The level of DGAT activity during seed development may have a substantial effect on the flow of carbon into oilseed TAG. Therefore, the enzyme has been extensively studied as a promising target to improve seed oil accumulation. Antisense suppression of *DGAT1* in *Brassica napus* (DH12075) is known to decrease *DGAT1* gene expression, total DGAT activity and seed oil content, which was compensated by an increase in the other seed components including protein. Therefore, a microarray-based transcriptomic analysis of *DGAT1*-suppressed and wild type plants may reveal other molecular events which could be potentially manipulated to enhance oil content. Using the 2-fold up- or down-regulation as a threshold, we found 676 genes to be differentially expressed in the *DGAT1*-suppressed mutant as compared to the wild type. A functional classification using gene ontology (GO) categories of their *Arabidopsis* homologs indicated that these differentially expressed genes are relevant to protein metabolism (12.5%), transport (8.8%), development (5.0%), transcription (3.7%), stress response (3.1%), signal transduction (1.5%), and cell organization/ biogenesis (1.3%). Among the highly induced transcripts in *DGAT1*-suppressed mutants, several genes encoding seed storage proteins, including cruciferin, oleosin and napin exhibited more than 2-fold increase. The down-regulation of genes encoding desaturases in the *DGAT1*-suppressed mutants supports previous observations on the decreased content of polyunsaturated fatty acids in these lines. Other differentially altered genes included those encoding AP2 domain-containing transcription factor, late embryogenesis abundant domain containing protein, auxin responsive protein and several antioxidants. Selected genes are being validated using qRT-PCR. Implications for TAG biosynthesis will be discussed.

### 33. "ERANET ASSYST" – ASSOCIATIVE EXPRESSION AND SYSTEMS ANALYSIS OF COMPLEX TRAITS IN OILSEED RAPE / CANOLA

**Rod Snowdon<sup>1</sup>, Andrew Sharpe<sup>2</sup>, Sue Abrams<sup>2</sup>, Irina Zaharia<sup>2</sup>, Pierre Fobert<sup>2</sup>, Isobel Parkin<sup>3</sup>, Benjamin Stich<sup>4</sup>, Janet Higgins<sup>5</sup>, and Ian Bancroft<sup>5</sup>**

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5. John Innes Centre, Norwich Research Park, Colney Lane, Norwich, NR4 7UH, UK

The tri-national ERANET Plant Genomics consortium ASSYST aims at identifying regulatory genes involved in expression of complex traits in *Brassica napus*. Quantitative gene expression data from well-defined populations of segregating winter and spring genotypes are being integrated with segregating transcriptome data, quantitative metabolite profiles and phenotype data from greenhouse and field trials using a systems genetics approach that combines an analysis of gene co-expression networks with expression QTL approaches. A *B. napus* SNP array is being developed for association analyses in a set of over 500 genetically diverse *B. napus* inbred lines. The work will initially focus on seedling development traits as a case study for a complex interactive system that is genetically very poorly understood, but is agronomically extremely important. The network analysis tools will also be applied for a systems analysis of important seed quality characters to identify key regulatory factors involved in biosynthesis of oil, protein and fibre components. The project incorporates the most recent technological developments in the field of next-generation sequencing for ultra-deep transcription profiling and SNP discovery. The project will attempt to integrate gene co-expression network analysis, classical QTL analysis, genetical genomics and association genetics concepts in a manner that until now has not been used for functional genomics of complex traits in crop plants.

### 34. METHOD FOR SIMULTANEOUSLY GENERATING 20 AND 40 KB PAIRED END LIBRARIES FOR ROCHE 454 GS FLX TITANIUM SEQUENCING

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NRC-PBI is employing a method for simultaneously generating multiple 454 Paired End libraries of two different size spans for *de novo* sequencing of Brassica species for the CanSeq project. We have developed a modified version of the GS FLX 20 Kb Span Paired End Library preparation method using *B. oleracea*, producing four libraries each of 20 and 40 Kb spans. This method allows the user to make two libraries of different size spans by cutting two gel slices at the library span size selection step. With *B. oleracea*, we also took advantage of opportunities to multiply numbers of libraries generated at the DNA circularization and library amplification steps. This development is helping achieve CanSeq's goal to develop a whole genome shot-gun (WGS) sequence of the *B. oleracea* genome using a combination of Illumina and Roche 454 sequencing technologies in collaboration with researchers in the US, UK and France.

The "Canadian Canola Sequencing Initiative" (CanSeq) is a collaborative project involving the NRC-PBI, AAFC, Genome Alberta and Industrial partners. The goal of the three year project is to develop genomics resources for Brassica crop species (all three genomes: A, B and C).

**35. SNP DISCOVERY USING RESTRICTION-SITE ASSOCIATED DNA (RAD) LONGREAD SEQUENCING IN *BRASSICA NAPUS*, A POLYPLOID SPECIES**

**Shunxue Tang<sup>1</sup>, Michelle Wiggins<sup>1</sup>, Rick Nipper<sup>2</sup>, Jenna Gribbin<sup>2</sup>, Eric Johnson<sup>2</sup>, Nathan Lillegard<sup>2</sup>, Thomas Greene<sup>1</sup>, Steve Thompson<sup>1</sup>, and Siva Kumpatla<sup>1</sup>**

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The combination of next-generation sequencing with genome complexity reduction technologies provides a rapid, efficient and cost-effective approach for SNP discovery in model and non-model organisms, including polyploidy species such as canola (*Brassica napus* L.). Here, we describe SNP discovery in canola through Floragenex RAD LongRead sequencing using the Illumina Genome Analyzer IIx platform. RAD libraries were generated by digesting genomic DNA from two canola lines with *Pst* I. The RAD libraries were sequenced on an Illumina Genome Analyzer IIx using 2 x 54 bp paired-end chemistry. While approximately 20 million raw reads were obtained from both accessions, 10M reads from a single cultivar were used for initial *de novo* genomic assembly. After filtering, a total of 52,048 contigs were obtained, ranging in size from 201 to 631 bp with an average size of 280 bp and with an average contig sequencing depth of 9.0x. All reads were then aligned to assembled contigs and a custom SNP discovery pipeline based on sequencing depth in each canola line at SNP position enabled us to distinguish allelic (intragenomic) variants from non-allelic (intergenomic) variants (nucleotide polymorphisms between paralogs). A total of 1,094 simple (genome-specific) SNPs, 1,634 hemi SNPs, and 6,644 paralog variants with favorable characteristics for Illumina genotyping were identified in 8,389 contigs from a total of 108,551 putative polymorphisms in 27,155 contigs. Accounting for the Illumina reads from both cultivars, the average sequencing depth at the SNP position is 21x. About 95% of the 2,728 simple and hemi SNPs passed the Illumina Assay Design Tool (ADT) and will be used for parental screening and map development efforts. This study demonstrated that RAD LongRead sequencing technology is a cost-effective method for high-throughput SNP discovery in polyploid crop like canola.

**36. MOLECULAR CHARACTERIZATION AND EXPRESSION OF *DGAT1* GENES IN *BRASSICA NAPUS***

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Diacylglycerol acyltransferase (DGAT) catalyzes the final step of triacylglycerol (TAG) synthesis, and is also known to exert substantial control on the overall flux of carbon into seed oil in *Brassica napus*. Despite the biological importance of this enzyme in oil accumulation, relatively little is known about the expression and regulation of genes encoding DGAT1 in *B. napus*. The present study was initiated to identify and characterize the *DGAT1* genes present in *B. napus*. Southern blot hybridization of *B. napus* DH12075 genomic DNA with a 522bp fragment containing a conserved portion of *BnDGAT1* revealed the presence of at least four *DGAT1* genes. Full length cDNAs corresponding to these homologs were cloned using a cDNA library prepared from the developing *B. napus* seeds, and classified into two sub-groups. Our results demonstrated a high degree of polymorphism within the first exon, which encodes the hydrophilic N-terminus. To gain insight about the possible biological relevance of the variable N-terminal regions, we investigated the effect of N-terminal deletions or domain swapping between the N-terminal regions of *BnDGAT1*s from different groups. These recombinant enzymes were expressed in the yeast H1246 strain which is devoid of native TAG synthesis. The recombinant *BnDGAT1* genes have been tested for their protein expression and enzyme activity. This work suggests a potential role of the N-terminal region on post-translational regulation of DGAT1 in *B. napus*.

**37. HIGH DENSITY SNP MAPS – A TOOL TO STUDY HOMOELOGOUS EXCHANGE?**

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In developing our marker base, we analysed SNPs for a large number of amplicons and mapped them on two linked maps. This work has produced a dataset that contains around 400 loci common to the two maps.

We found there are a significant number of amplicons that are common to the two maps that do not simply map back to the homologous location and we will present an analysis of these data as a first example of a global approach to use these completely sequence defined markers as a tool to help understand homoeologous exchange on a local and more global scale.

**38. SEED COAT SPECIFIC-PROMOTERS FOR CANOLA**

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The development of canola (*Brassica napus*) as a nutritionally superior vegetable oil is a widely cited Canadian success story and generates annual economic activity of \$14B. Canada is the world's leading exporter of canola seed, oil and meal. There are excellent opportunities for further improvements of canola, including production of high quality protein meal for feed and reduction of anti-nutrients in meal. One of the problems is the dark polyphenolic pigments that accumulate in canola seed coat layers persisting in meal and interfering with protein utilization. Also, seed coat is the largest source of indigestible fibre in canola meal. Therefore, it is very important to modify the seed coat by regulating seed coat-specific genes for improving canola meal quality.

To regulate the genes involved in seed coat development and metabolism, seed coat-specific promoters are a pre-requisite. To find seed coat-specific promoters for canola, we have tested 21 promoters from various plants. Promoter-GUS constructs were transformed in a double haploid line of canola and analyzed. Four of these promoters have shown seed coat-specific expression in canola and each expressed in specific layer of seed coat. Strength of these promoters, compared to CaMV35S promoter, and their expression pattern at various developmental stages of seed was determined. Our search also resulted in finding several constitutive and other tissue specific promoters for canola.

**39. THELLUNGIELLA SALSUGINEA – A ROBUST PHYSIOLOGICAL AND GENETIC MODEL FOR ENVIRONMENTAL STRESS TOLERANCE****Marc Champigny, Robin Cameron and Elizabeth Weretilnyk**

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Unfavourable weather events and the need to produce more from less land requires the development of new research models to yield gene leads with increased potential to improve stress tolerance in crops. Our research exploits the adaptive traits of a rare native crucifer, *Thellungiella salsuginea*, a plant that thrives in an extreme natural environment featuring highly alkaline and saline soils in the semi-arid, sub-Arctic, Yukon Territory of Canada. Metabolite analyses by mass spectrometry and transcriptome profiling by microarray and next-generation sequencing are approaches being used to identify physiological and genetic mechanisms underlying the tolerance of *Thellungiella* to salinity, drought, and poor soil conditions. Our strategy also exploits the close phylogenetic relationship between *Thellungiella* and *Arabidopsis* as well as the natural variation among *Thellungiella* genotypes for comparative profiling. Stress-responsive qualitative and quantitative changes among metabolites and transcripts are discerned by the use of bioinformatics and this information is associated with differing physiological responses to stress treatments. Among the “tools” available for gene identification are recombinant lines representing crosses between *Thellungiella* genotypes that will enable association-based mapping of loci associated with stress tolerance. Progress to date shows Yukon *Thellungiella* to have the attributes necessary for serving as a genetic model plant with the added advantage of a high innate capacity to withstand a broad array of adverse environments.

**40. FROM CHEMICAL PROTEOMICS TO FUNCTIONAL CHARACTERIZATION - ELUCIDATION OF NOVEL MECHANISMS OF PLANT HORMONE ACTION****Olesya Kharenko, Jason Boyd, Sue Abrams, and Michele Loewen**

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Abscisic acid (ABA) is a plant hormone involved in regulation of growth and development as well as modulation of plant responses to environmental stress. Targeting ABA binding proteins through the use of a biologically active ABA-mimetic photoaffinity probe (Nyangulu, *JACS*, 2005) has now proven successful. Here we describe the identification of two mitochondrial proteins as putative ABA-binding proteins. *In vitro* characterization of a recombinant, purified and reconstituted version of one of these indicates that it translocates ABA across membranes. Further analysis indicated that ABA is taken up into mitochondria at a rate ~ 3 times slower than ATP and that ABA inhibits protein mediated uptake of ATP. Finally, ABA was found to stimulate the mitochondrial ATP synthesizing machinery *in vitro* with a  $K_a$  of 1.7 fM. Phenotypic characterization of *Arabidopsis* T-DNA knockouts of the two proteins showed that both knockouts exhibited hypersensitive growth inhibition in the presence of ABA. Correlation of these effects with ATP pools indicated that application of ABA and osmotic stress induced transient increases in ATP levels in wild type *Arabidopsis*. This effect was delayed in one knockout and abolished in the other. Finally, analysis of ATP content in seeds has shown that ATP production is significantly decreased in seeds in both knockouts compared to wild type. These results suggest a model in which ABA is translocated into the mitochondrial matrix where it modulates ATP synthesis function and thus the availability of cellular ATP.

**41. TOLERANCE OF ETHIOPIAN MUSTARD TO SODIUM SULPHATE SALINITY****X. Li, J. Holowachuk, M.Y. Gruber**

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The Canadian prairies have vast acreages of saline land, which is unsuitable or uneconomical for food crop production. These lands may be suitable for growing industrial crops, if varieties with stress tolerance and low inputs can be selected. Ethiopian mustard (*Brassica carinata*), is a new biorefinery seed crop under development in Saskatoon. If *B. carinata* could be grown successfully on large acreages of marginal land, farmers may have two biofuel opportunities, first to harvest mustard seed for biodiesel and second to contribute mustard residue into stores of biomass feedstock for cellulosic ethanol. To explore the potential of growing *B. carinata* crops on prairie saline land and to determine mechanisms of tolerance to salinity, we conducted a 72 day greenhouse pot study comparing two genetically-related lines (yellow-seeded and brown-seeded) growing in soil amended at day 15 with 50 mM and 100 mM sodium sulphate. By 42 days, growth was compromised more severely in the yellow-seeded line than in the brown-seeded line at 100 mM, even though the yellow-seeded line was smaller than the brown-seeded line without salinity treatment. This salinity-dependent growth suppression was consistent with reduced growth of the yellow-seeded line in lithium chloride (in an earlier study reported by Li et al. 2009. Plant Sci. 177: 68-80). However, the mechanism of tolerance in the brown-seeded line appears to differ between sodium sulphate and lithium chloride. The presentation will include a comparison of growth of the two lines on sodium sulphate lines and will highlight differences between their metabolomes, chlorophyll degradation products, and their transcriptomes as measured by the Agilent *Brassica napus* 105K oligo array.

**42. CHARACTERISATION OF CANOLA (*BRASSICA NAPUS* L.) GERMPLASM FOR MANGANESE TOLERANCE****Brett McVittie<sup>1</sup>, J. Sergio Moroni<sup>2</sup>, Neil Wratten<sup>1</sup>, John Harper<sup>2</sup> and Harsh Raman<sup>1</sup>**

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*Brassica* germplasm tolerant to high levels of manganese (Mn tolerance) is becoming increasingly important in areas of oilseed production with acidic soils. In this study, we evaluated 160 genotypes of *Brassica species* for Mn tolerance using the symptom of chlorosis on the cotyledon as a measure of plant tolerance to high Mn. Evaluation was carried out by nutrient solution culture with a 125µM Mn treatment based on a split plot design. Our findings suggested that genetic diversity exists within canola germplasm. In order to determine the inheritance of Mn tolerance, an F<sub>2</sub> population derived from doubled haploid parental genotypes Mutu (Mn-tolerant) and RSO94-67 (Mn-Sensitive) was evaluated using nutrient solution culture. Genetic variation for Mn tolerance in this intercross population was confirmed to be controlled by a single gene. We are currently constructing a linkage map of the F<sub>2</sub> population from Mutu/RSO94-67 to tag a major locus for Mn tolerance. Genome wide association study is also underway to validate qualitative and quantitative trait loci associated with Mn tolerance in the diverse canola germplasm collected from various parts of the world.

**43. THE INFLUENCE OF TEMPERATURE DURING CULTIVATION ON THE FLAVONOID PROFILE IN KALE (*BRASSICA OLERACEA* VAR. *SABELLICA*) AND THE EXPRESSION OF KEY ENZYMES IN FLAVONOID BIOSYNTHESIS**

**Susanne Schmidt<sup>1</sup>, Michaela Zietz<sup>2</sup>, Monika Schreiner<sup>1</sup>, Sascha Rohn<sup>2</sup>, Rita Zrenner<sup>1</sup>, Lothar W. Kroh<sup>2</sup>, and Angelika Krumbein<sup>1</sup>**

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Kale (*Brassica oleracea* var. *sabellica*) is mainly cultivated in Northern Europe and North America. Compared to other leafy vegetables, like collard greens or rocket salads, kale contains high concentrations of flavonoids. Here we show the influence of low temperature on flavonoid concentrations and the expression of key genes involved. The cultivar 'Winterbor' was grown either for 3 weeks (4-5 leaves, juvenile) or 12 weeks (adult) in greenhouses at ambient conditions. Plants were transferred to growth chambers and treated either for one week (juvenile) or four weeks (adult) with different temperatures. Methanolic extracts were separated using a water/acetonitril gradient in an HPLC-DAD-ESI-MS<sup>n</sup> (Agilent 1100). Real-time qRT-PCR was assayed (Applied Biosystems 7500) with SYBR-Green using *Brassica oleracea* actin (*Act1*) as reference. The main flavonol aglycone in juvenile and adult kale is kaempferol followed by quercetin and isorhamnetin (Schmidt et al. Food Chemistry (2010) 119, 1293-1299). In juvenile plants a decrease in temperature from 15°C to 5°C was associated with an increase of kaempferol whereas quercetin stayed unchanged and isorhamnetin decreased. Interestingly, the total concentration of flavonol aglycones was not changed significantly in young plants. In adult plants decreasing temperature also led to a significant increase of kaempferol. Quercetin and isorhamnetin are increased as well, leading to higher total flavonol aglycones. The molecular investigation was performed after establishing qRT-PCR expression analysis with key genes of flavonol biosynthesis, namely *flavanone 3 $\beta$ -hydroxylase*, *flavonol synthase*, and *flavonol 3'-hydroxylase*. First results with *flavanone 3 $\beta$ -hydroxylase* show that expression is highest in juvenile and adult plants cultivated at 10°C.

**44. ARABIDOPSIS HSI2 (HIGH-LEVEL EXPRESSION OF SUGAR-INDUCIBLE GENE 2) AFFECTS DROUGHT TOLERANCE AND RESPONSE TO ABA DURING GERMINATION**

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*HSI2* (*HIGH-LEVEL EXPRESSION OF SUGAR-INDUCIBLE GENE 2*), also known as *VAL1* (*VIVIPAROUS ABA INSENSITIVE3-LIKE*), is an *Arabidopsis* gene that encodes a putative chromatin remodeling factor and transcriptional repressor. It has been reported that double mutants in *HSI2* and its closest relative leads to the production of embryos on seedling tissues, indicating that *HSI2* represses embryogenic programs in vegetative tissues. Another repressor of embryogenic programs in vegetative tissues, *PICKLE*, arrests germination in the presence of exogenous abscisic acid (ABA). Here we report that loss-of-function mutants in *HSI2*, in contrast to *pickle* mutants, display a decreased sensitivity to ABA during germination as indicated by faster and higher overall germination than in the corresponding wild type in presence of exogenous ABA. We further explored the potential role of *HSI2* in ABA-mediated abiotic stress responses by directly subjecting young juvenile plants to drought stress and, through indirect measurements of the drought response, determined that *hsi2* mutants are more tolerant than their wild type counterparts. Our results implicate *HSI2* as a potential regulator of ABA responses during germination and of drought tolerance in juvenile plants. The mechanisms by which *HSI2* regulates these responses are being investigated using biochemical and molecular approaches such as response to exogenous ABA, hormone profiling and gene expression analyses.

**45. INFLUENCE OF SIGNALING MOLECULES ON INDOLE GLUCOSINOLATES OF *BRASSICA RAPA* VAR. *CHINENSIS*****Melanie Wiesner<sup>1</sup>, Rita Zrenner<sup>1</sup>, Angelika Krumbein<sup>1</sup>, Hansruedi Glatt<sup>2</sup>, and Monika Schreiner<sup>1</sup>**

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*Brassicaceae* are well known for their secondary metabolites the glucosinolates (GS), which are part of the plant defense system. GS are amino acid-derived compounds that are hydrolyzed by the plant enzyme myrosinase upon tissue damage. Hydrolysis products like isothiocyanates, nitriles, and oxazolidinethiones are responsible for the biological activity and toxicity of GS. Plant defense responses including the GS-myrosinase system are modulated by signaling molecules which act synergistically or antagonistically in various signal transduction pathways. It has been shown in *Arabidopsis thaliana* that salicylic acid, jasmonic acid or methyl jasmonate act as such signals in the GS-myrosinase system. The aim of our project is to elucidate the influence of elicitor applications on the GS profiles, especially on the concentration of indole GS in the model plant *Brassica rapa* var. *chinensis* (pak choi).

Sprouts of pak choi were treated with elicitors in different concentrations for 48 h and the GS profile were analyzed by HPLC-DAD using the method of Krumbein (Krumbein et al. 2005, J. Appl. Bot. Food Qual. 79:168-174). GS profiles changed depending on elicitor concentrations and indicate that signaling pathways in the GS-myrosinase system of pak choi may be similar to that in *Arabidopsis thaliana* (Wiesner et al. 2009, 2<sup>nd</sup> Conference on Glucosinolates, Denmark). In order to identify all putative genes involved in the signaling and the biosynthetic pathways of indole GS we compared the *BrassicaDB* sequences with those of *Arabidopsis*. Currently we are establishing qRT-PCR expression analyses for molecular investigations of the indole GS accumulation in pak choi.

**46. DECIPHERING THE GENETIC LINK BETWEEN TOLERANCE TO TREHALOSE AND PARTIAL RESISTANCE TO CLUBROOT IN *ARABIDOPSIS THALIANA***

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Clubroot disease is caused by *Plasmodiophora brassicae* and leads to the development of galls in infected roots of *Brassicaceae*. This physiopathological process relies on the phytohormonal modulation of plant metabolic resource allocation. High accumulation of trehalose has been observed in clubroot infected plants. However, despite the central importance of the trehalose pathway in the regulation of plant primary metabolism, little is known about the implication of trehalose in the mechanisms of resistance / susceptibility to *P. brassicae*. The present work showed that the clubroot partially resistant Arabidopsis accession Bur-0 was tolerant to exogenous trehalose. A QTL analysis on a Bur-0 x Col-0 (susceptible to exogenous trehalose) segregating population led to the identification of one QTL involved in trehalose tolerance that co-localized with a previously identified QTL for quantitative resistance to *P. brassicae*. This result was confirmed by the analysis of near-isogenic lines (NIL). Accumulation of trehalose in tissues of trehalose treated Bur-0 was not drastically lower than in Col-0, suggesting that this tolerance could be related to contrasting downstream response to trehalose rather than to trehalose degradation. Trehalose was accumulated in both parental accessions during clubroot infection, but trehalase enzymatic activity was induced only in the susceptible Col-0 accession. We conclude that tolerance to trehalose in Bur-0 is likely involved in clubroot resistance, and that this tolerance relies on a trehalase-independent mechanism, supporting an original model where a partial resistance would rely on contrasting primary metabolism regulation.

**47. SCREENING OF BRASSICA GERMLASM FOR RESISTANCE TO *PLASMODIOPHORA BRASSICAE* PATHOTYPES PREVALENT IN CANADA**

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Clubroot disease, caused by *Plasmodiophora brassicae*, poses a threat to the Canadian canola industry, and breeding of resistant cultivars is urgent. The objective of this study was to identify *Brassica* germplasm possessing resistance to *P. brassicae* pathotypes prevalent in Alberta, based on greenhouse screening. Germplasm resistant to local pathotype(s) is the prime requirement for breeding clubroot resistant cultivars. Pathotype-specific to broad-spectrum resistance was identified in the diploid species *Brassica rapa* (AA) and *B. oleracea* (CC), and in the amphidiploid *B. napus* (AACC). Among *B. rapa* genotypes, turnip were the most resistant, followed by winter type and spring type oilseed rape. The rutabaga group of *B. napus*, on the other hand, was homogeneous for resistance to Canadian *P. brassicae* pathotypes. The diploid *B. nigra* (BB) also showed pathotype-specific to broad-spectrum resistance. However, the two amphidiploids with *B. nigra* as one of the parental species viz., *B. juncea* (AABB) and *B. carinata* (BBCC), were completely susceptible.

**48. THE RACE STRUCTURE OF *LEPTOSPHAERIA MACULANS* IN WESTERN CANADA**

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Specific resistance genes are an effective means of disease control when the pathogen population is mainly avirulent on the cultivated varieties carrying the corresponding resistance gene. Development of new races of *Leptosphaeria maculans* (Desmaz.) Ces. & de Not., the species responsible for the most damaging symptoms of blackleg disease of canola (*Brassica napus* L.), increases the risk that host genetic resistance may be rapidly overcome. This study aims to elucidate the race structure of *L. maculans* by determining, under controlled conditions, the frequency of avirulence alleles at eleven avirulence loci in pathogen populations collected from nine locations across western Canada. The avirulence allele at *AvrLm6* was present in all isolates tested (63) from three locations. At eight locations >88% of the isolates (423) carried the avirulence allele at *AvrLm2*, but only 37% at another location. For all other avirulence genes (*AvrLm1*, *AvrLm3*, *AvrLm4*, *AvrLm7*, *AvrLm9*, *AvrLmLepR1*, *AvrLmLepR2* and *AvrLmLepR3*), avirulence allele frequency varied from 0 to 99% depending on the loci and the location (300-600 isolates). Knowledge of avirulence allele frequency and the race structure of *L. maculans* in canola producing regions of western Canada will be crucial to develop strategies to maintain the efficacy of resistance genes.

**49. GENETIC EVIDENCE FOR THE RECOGNITION OF THE *LEPTOSPHAERIA MACULANS* AVIRULENCE GENE *AvrLm1* BY TWO *BRASSICA NAPUS* RESISTANCE GENES; *Rlm1* AND *LepR3***

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Advanced backcross populations of *Brassica napus* (canola) segregating for the resistance genes *Rlm1* or *LepR3* were employed to investigate the interaction of these genes with *Leptosphaeria maculans* isolates carrying the avirulence gene *AvrLm1*. Cotyledon infection tests showed the interaction of *AvrLm1* with a single gene in each population. Segregation analysis using SSR markers linked to each R-gene revealed the interaction of *AvrLm1* with two distinct resistance loci on chromosomes A7 and A10, corresponding to *Rlm1* and *LepR3*, respectively. These results suggest that the induction of a resistance response in the canola variety Surpass 400 by *AvrLm1* isolates is due to recognition of the avirulence factor by *LepR3* and not due to the presence of *Rlm1* in the variety as has previously been suggested.

**50. PLANT TRAITS AFFECTING THRIPS DAMAGE IN CABBAGE**

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Cabbage is one of the main organic field crops in the Netherlands. Cabbage grown for storage is often heavily damaged by thrips (*Thrips tabaci*).

Based on field trials with 15 varieties including modern F1 hybrids, classical open-pollinated varieties and two reciprocal experimental crosses over two years and 2 locations, we investigated which plant traits are associated with reduced thrips damage. Observations were made in each year at three or four dates in August-October, of thrips damage and number, maturity, firmness, leaf surface wax, head size, leaf thickness and Brix.

Thrips damage at the final harvest in October was most strongly positively correlated with Brix (i.e. with the amount of sugars) and head development (measured as size, stage of development, or firmness) around end August, and most strongly negatively correlated with the amount of leaf surface wax. In one year also leaf thickness was positively correlated with thrips damage. These traits together explained 75% of the variation for thrips damage between the accessions in both years.

**51. THE METABOLIC RESPONSE OF OILSEED RAPE TO *PLASMODIOPHORA BRASSICAE* INFECTION**

**Geoffrey Wagner<sup>1</sup>, Sophie Charton<sup>1</sup>, Nathalie Marnet<sup>2</sup>, Raphaël Lugan<sup>3</sup>, Christine Lariagon<sup>1</sup>, Alain Bouchereau<sup>1</sup>, Régine Delourme<sup>1</sup>, Maria J. Manzanera-Dauleux<sup>1</sup>, and Antoine Gravot<sup>1</sup>**

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Clubroot, caused by the obligate telluric biotroph *Plasmodiophora brassicae*, is one of the most devastating pathogens in *Brassicaceae*. The disease is characterised by the development of clubs on the root system due to cellular hypertrophy and hyperplasy. Our group focuses on partial quantitative resistance, under polygenic control, described as being more durable than qualitative resistance. Previous work in our team led to the identification of partial quantitative resistance to clubroot in the *Brassica napus* genotype Darmor-*bzh*, which was used as a model to perform a genetic analysis of the architecture of quantitative resistance. Cellular mechanisms underlying this resistance remain however poorly understood, particularly at the metabolic level. In this respect, we have undergone a metabolomic approach i) to characterize the metabolic differences between partial resistant and susceptible genotypes and ii) to identify differentially accumulated metabolites that could be used as reliable metabolic markers of resistance.

A combination of targeted and untargeted metabolite profiles was performed in both root and shoot tissues of Darmor-*bzh* and Yudal (clubroot-susceptible genotype), at several time points after inoculation. We focused our analysis on primary and secondary metabolites: glucosinolates, flavonoids, carbohydrates and amino acids

The results are discussed in the light of current knowledge about source-sink relationships between shoots and roots during clubroot development.

**52. THE ROLE OF ARABIDOPSIS CLADE I TGA TRANSCRIPTION FACTORS IN MODULATING PLANT DEFENSE RESPONSES****Lipu Wang<sup>1</sup> and Pierre R. Fobert<sup>2</sup>**

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The first layer of plant immune system is pathogen-associated molecular patterns (PAMPs)-triggered immunity (PTI). Recognition of PAMPs, such as bacterial flagellin, activates multiple defense responses including a burst of reactive oxygen species (ROS), callose deposition, and the induction of defense-related genes. Successful pathogens have evolved mechanisms to suppress PTI and promote disease. Specifically, the Gram negative bacterial pathogen *Pseudomonas syringae* uses a type III secretion system (TTSS) to deliver effectors into the plant cytoplasm and the phytotoxin coronatine (COR) to interfere with plant hormone signaling. Although PTI involves massive changes in plant gene expression, relatively little is known about the transcription factors (TFs) involved. Here, we report that TGA1 and TGA4, two members of the TGA family of bZIP TFs, are positive regulators of PTI. The *tga1-tga4-1* double mutant harbors significantly higher titers of various *P. syringae* pv. *tomato* isolates, including strains deficient in TTSS or the production of COR, and *P. syringae* pv. *phaseolicola* that is normally not pathogenic on Arabidopsis. The mutant is also compromised in defense-related callose deposition, and ROS burst triggered by flg22, a derived peptides from flagellin. Analysis of the *non-expressor of pathogenesis-related genes 1 (npr1-1)* mutant and an *npr1-tga1-tga4-1* triple mutant indicates that clade I TGA factors act independently of NPR1 during PTI. Moreover, mutation of clade I TGA factors results in developmental changes, including curly leaves and late flowering. Together, our results demonstrate that TGA1 and TGA4 factors play a unique role in mediating both defense responses and developmental processes.

**53. QTL MAPPING OF TOLERANCE AGAINST SCLEROTINIA STEM ROT IN *BRASSICA NAPUS* L.****Ravneet Behla, Dilantha Fernando, Peter McVetty, and Genyi Li**

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Sclerotinia stem rot (SSR) is caused by a necrotrophic fungus *Sclerotinia sclerotiorum* (Lib.) de Bary. It causes significant yield losses in *Brassica napus* L. Four screening methods; petiole inoculation, cotyledon inoculation, detached leaf and stem inoculation were compared to find the most reliable and convenient method. Petiole inoculation method was identified as most suitable method for screening. This method was used to screen 3 double haploid (DH) populations (H1, H2 and H3). The populations were screened independently three times under the greenhouse conditions. 12 plants per line were screened in each run. Genetic maps were prepared using SRAP (Sequence related amplified polymorphism) markers. 462 SRAP markers were allocated to 19 linkage groups (LG) covering total genetic distance of 1374.2 cM in H1. In H2, 487 SRAP markers covered a total genetic distance of 1333.4 cM in 19 LG. The H3 map with 1055 SRAP markers covered a total genetic distance of 1604.8 cM in 19 LG. We identified QTL against SSR in all 3 DH populations. The phenotypic evaluation of H1 and H2 showed significant differences among the replicates (Rep). QTL analysis for these populations were carried out separately for each replicate. In H1, 5 QTL in Rep1, 4 in Rep 2 and 3 in Rep 3 were identified. In H2 population, 3 QTL in Rep 1, 4 in Rep 2 and 4 in Rep 3 were identified. The phenotypic evaluation of H3 did not show significant differences among the replicates and two QTL were identified on chromosome N12 and N16.

**54. AN EFFICIENT HIGH THROUGHPUT *BRASSICA NAPUS* MICROSPORE CULTURE SYSTEM: INFLUENCE OF PERCOLL GRADIENT SEPARATION AND PRECISE BUD SELECTION ON EMBRYOGENESIS****Pankaj Bhowmik, Joan Dirpaul, Patricia Polowick, and Alison Ferrie**

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Microspore culture for the purpose of developing double haploid plants is routine for numerous plant species; however, the embryo yield is still very low compared with the total microspore population. The ability to select and isolate highly embryogenic microspores would be of great advantage for high embryo yield in microspore culture. To maximize the efficiency of canola microspore culture we followed a combination of precise bud size selection and microspore fractionation using a percoll gradient. This approach has repeatedly given us enhanced embryo yields. Microspores isolated from buds of DH12075 and Topas 4079 in the range of 1.5 to 4.5 mm formed embryos at various frequencies. The 2.0-2.5 mm bud size range of DH12075 and 3.0-3.5 mm bud size of Topas 4079 consistently yielded embryos at very high frequencies. When the microspores from 2.0-2.5 mm buds of DH12075 were carefully layered on top of the percoll gradients (10, 20 and 40%), and subsequently spun through the percoll layers by centrifuging, three discrete bands were formed containing pure populations of staged microspores. The central portion (middle layer) of the gradient contained the late uninucleate and early binucleate microspores that gave us highest embryo yield. The relationship between the bud size, developmental stage of isolated microspores, percoll gradient concentration and the embryogenic frequency of each cultivar will be discussed in detail.

### 55. RADIATION HYBRID MAPPING OF RADISH CHROMOSOME *D* IN RAPESEED BACKGROUND

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Genetic resistance against the beet cyst nematode (*Heterodera schachtii* Schmidt) is present in genus *Raphanus* but absent in rapeseed. An assay for nematode resistance in the complete set of nine disomic rapeseed-radish chromosome additions, *a* to *i*, showed radish chromosome *d* as carrier of resistance gene *Hs1<sup>Rph</sup>*. To induce intergenomic recombination for transfer of *Hs1<sup>Rph</sup>* to rapeseed, a cross resulting in meiotic univalent condition of C and R genomes was carried out. Analysis of chromosome *d*-specific molecular markers in hundreds of progenies has indicated no evidence for the desired recombination. Therefore, hybrids from a rapeseed mother pollinated with X-ray radiated (40 krad) pollen of disomic addition *d* line were produced and screened for chromosomal breakage. From 296 hybrid seedlings tested with six chromosome *d*-specific molecular markers spread over meiotic chromosome *d* map, 61 seedlings (21%) showed partial marker losses. These plants were analyzed for additional 30 chromosome *d*-specific AFLP, RAPD and dpRAPD markers. Retention frequencies for markers/plant varied from 0.09 to 0.88, whereas the frequency of individual markers varied between 0.44 and 0.77. Data of marker presence/absence were loaded into the Carthagene program (INRA). Localization of markers on resulting RH map was compared with that in meiotic map. The presented radish chromosome *d* map is the first example of RH mapping in Brassicaceae. Plants will be tested for nematode resistance and characterized by cytogenetic methods. RH plants with *Hs1<sup>Rph</sup>* and low marker retention will be used to develop beet cyst nematode-resistant rapeseed.

### 56. ANALYSIS OF QTLs FOR ERUCIC ACID AND OIL CONTENT IN SEEDS ON A8 CHROMOSOME AND THE LINKAGE DRAG BETWEEN THE ALLELES FOR THE TWO TRAITS IN *BRASSICA NAPUS*

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Previous research believed that the pleiotropy of *fae1* (*Fatty acid elongase1*) was responsible for the decrease of oil content in the canola rapeseed. However, linkage drag of *fae1* would be another reason for the oil content reduction. TN DH population was developed from a canola cultivar Tapidor and a double high cultivar Ningyou7 to analyze such puzzle. The population had been tested in dozens of environments to map QTLs for erucic acid content (*qEA*) and oil content (*qOC*) in seeds. After *Meta-analysis*, a novel *qEA* on A8 chromosome was revealed at 7 cM away from the known significant *qEA*, *BnA8.FAE1*. Seven independent *qOCs* were detected around the two *qEAs*. Two of the *qOCs* co-localized with the two *qEAs* and the other five frequently detected *qOCs* were independent of *qEAs*. Alleles from Tapidor in this QTL cluster region contributed negative effects to the two traits and showed the same genotypes with that of 'Liho', the origin of low erucic acid content canola rapeseed. It is surprising that Tapidor still held the five inferior alleles for low oil content covering 39 cM after multiple crossing and intensive selection. Ninety cultivars of *B. napus* from 8 countries were used to further analyze such a linkage drag. It showed that 46% of the canola cultivars retained at least one of the haplotypes from 'Liho' for low oil content in seeds. The linked markers developed from this research would help breeders to break such linkage drag in their breeding population, and, therefore, increase oil content of canola rapeseed.

**57. DEVELOPMENT OF ELITE INBRED COMPONENT LINES FOR SYNTHETIC HYBRIDS IN YELLOW MUSTARD (*SINAPIS ALBA*)****Bifang Cheng and David Williams**

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Yellow mustard (*Sinapis alba*) is a cross-pollinated crop due to sporophytic self-incompatibility. Using heterosis in synthetic hybrids is an approach to increasing seed yield. The objective of this research is to develop elite inbred component lines for producing high-yielding synthetic hybrids in condiment yellow mustard.

Open-pollinated populations of yellow mustard comprise extensive heterozygosity and genetic variation. Based on agronomic performance, three yellow mustard accessions (Andante, Ace and YMB05-MY) have been selected as gene sources for developing superior inbred lines via bud-pollination. Open-pollinated plants (S0) of the three accessions showed great variation in growth vigour. The S0 plants with strong growth vigour were bud-pollinated to produce S1 progeny while the weak S0 plants were discarded. The S1 plants exhibited various types and degrees of inbreeding depression as well as great variation in maturity and plant height. They were classified into four groups based on inbreeding depression: 1) Normal type: Plants were comparable to open-pollinated plants in morphology and growth vigour. These plants were further bud-pollinated to produce S2 progeny. 2) Weak type: Plants exhibited reduced leaf size and stunted growth, and were discarded. 3) Abnormal type: Plants had no main inflorescence or had withering leaves, and were discarded. 4) Albino type: Plants were albino and died at seedling stage. The observed inbreeding depression in the S1 progeny is due to the occurrence of homozygosity of the deleterious alleles. Breeding efforts have been directed to develop elite inbred lines tolerable to inbreeding by purging the deleterious alleles. The genetically enhanced elite inbred lines will be used to develop high-yielding synthetic hybrids for yellow mustard.

**58. TRANSFORMATION AND EXPRESSION OF BT GENE *cry1ia8* IN CABBAGE (*BRASSICA OLERACEA*)****Lei Cui<sup>1</sup>, Dengxia Yi<sup>1</sup>, Jie Zhang<sup>2</sup>, Zhihong Lang<sup>3</sup>, Yumei Liu<sup>1</sup>, Mu Zhuang<sup>1</sup>, Yangyong Zhang<sup>1</sup>, Youjun Zhang<sup>1</sup>, Dafang Huang<sup>2,3</sup>, Zhiyuan Fang<sup>1</sup>, and Limei Yang<sup>1\*</sup>**

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As an effective and environment friendly way to produce insect resistant varieties, plant breeding is the best approach for pest control. Unfortunately, the real materials with resistance in cabbage (*Brassica oleracea* var. *capitata* L.) is few. Bt toxin has been used for pest control since 1950s and the diamondback moth (DBM, *Plutella xylostella*) has been reported to have evolved resistance to Bt Cry1Ac in open field conditions. Therefore, the exploitation and utilization of the novel Bt gene (besides *cry1Ac*) are highly important for pest resistant breeding program.

The newly cloned Bt gene *cry1ia8* with its plants expression vector was transferred into cabbage inbred lines CA21-3 and CB24-5 via *Agrobacterium tumefaciens*. In total 125 transformants with kanamycin-resistance were obtained. PCR and Southern blot analysis showed that *cry1ia8* gene was integrated into the genome of cabbage. RT-PCR and Western blot detection demonstrated that *cry1ia8* gene was expressed at RNA and protein levels. The results of the bioassay with the susceptible and resistant diamondback moth displayed that most of the transgenic plants were resistant to the larvae of susceptible DBM and also to the insect strain which is resistant to Cry1Ac toxin. Moreover, T1 seeds and plants of transgenic cabbage with the resistance to DBM were acquired and at least one of the lines showed the homozygous resistance.

**59. RACE-SPECIFICITY OF CANADIAN CLUBROOT ISOLATES****Elke Diederichsen**

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Clubroot has become a serious threat to oilseed rape cultivation not only in Europe, but also for Canadian Canola crops. Control means are poor and mainly restricted to host resistance. *Plasmodiophora brassicae*, the causal agent of clubroot, forms physiologic races that differ for their virulence towards race-specific host resistance, therefore, breeding for clubroot resistant cultivars requires knowledge of the prevailing pathotypes in the cropping area. To determine the race-specificity of Canadian single spore isolates, greenhouse tests were made on a *Brassica napus* differential set that is representing major sources for clubroot resistance in this crop. Opposite to previous studies using the ECD differential set, a high level of virulence towards major clubroot resistance genes was found in Canadian isolates. Possible explanations of this discrepancy will be discussed.

**60. SNP DISCOVERY IN *CAMELINA SATIVA* – TOWARDS IDENTIFYING MOLECULAR MARKERS FOR RESISTANCE TO *SCLEROTINIA SCLEROTIUM*****Christina Eynck<sup>1</sup>, Christine Sidebottom<sup>2</sup>, Wayne Clarke<sup>1</sup>, Andrew Sharpe<sup>2</sup>, Ginette Séguin-Swartz<sup>1</sup>, and Isobel Parkin<sup>1</sup>**

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The crucifer *Camelina sativa* (false flax) has gained renewed interest as an alternative oilseed crop due to its potential value for human and animal nutrition as well as for industrial applications. The species is adapted to canola-growing areas and among other valuable agronomic attributes, it is generally considered to be resistant to many fungal diseases. However, it is susceptible to sclerotinia stem rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary with only a few genotypes showing an enhanced level of resistance. The identification of quantitative trait loci (QTLs) associated with resistance to *Sclerotinia* is yet hampered by limited genetic resources and the lack of molecular markers. In order to discover Single Nucleotide Polymorphisms (SNPs) on a genome-wide scale, the 3' regions of transcripts from two *C. sativa* genotypes with contrasting levels of resistance were captured using targeted restriction digestion and then sequenced using the Roche 454 Genome Sequencer Titanium platform. This approach generates global SNP coverage and allows for the discovery of polymorphisms in genes of interest. SNPs identified between parental alleles of transcripts covering the whole genome as well as putative candidate resistance genes will be assayed within an F2 population. These tools will allow the identification and molecular tagging of QTLs for sclerotinia resistance and the elucidation of whether and to what extent allelic variation in candidate genes contributes to the variation in the level of resistance.

**61. INTROGRESSION OF CLUBROOT RESISTANCE INTO ELITE CANOLA GERMPLASM VIA RE-SYNTHESIS**

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Clubroot caused by *Plasmodiophora brassicae* is one of the emerging threats to canola (*Brassica napus*) production in Canada. Multiple races of the pathogen have been characterized both in Canada and worldwide. Clubroot resistance identified in existing *B. napus* genotypes is more often race-specific. Our objective is to broaden the genetic basis of club root resistance in *B. napus* by using resistant genotypes of progenitor diploid species for re-synthesis and to introgress such disease resistance into elite *B. napus* germplasm. A set of 16 *Brassica* genotypes including *B. rapa* and *B. oleracea* genotypes of European clubroot differentials (ECD) set, and elite lines of *B. napus* were assessed for genetic diversity using a set of 1,000 SSR markers. *B. rapa* var. *rapifera* genotype ECD 04 known to possess complete resistance against *P. brassicae* was used as a major resistance donor. Pair-wise crosses were made between ECD 04 and two moderately resistant *B. oleracea* genotypes ECD 12 (var. *capitata*; cv. Bindsachsener) and ECD 15 (var. *acephala* subvar. *laciniata*; cv. Verheul). Embryo rescue technique was used to obtain interspecific hybrid plantlets. Inheritance of progenitor A and C genomes into the hybrids was confirmed using SSR markers. Colchicine treatment was applied to the hybrids to produce allotetraploids, which are being used to make crosses with elite *B. napus* genotypes to transfer clubroot resistance. Doubled haploid mapping populations will be developed from the crosses between re-synthesized and elite *B. napus* genotypes. Characterization and fine mapping of clubroot resistance in selected DH populations will be carried out by phenotyping with aggressive *P. brassicae* isolates, and SSR and SNP genotyping.

**62. OPTIMIZING BRASSICA SPECIES BREEDING PROGRAMS**

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Cultivated *Brassica* species are important crops worldwide for different purposes. In Vegenov - BBV, our projects aim at developing a range of tools (genetic maps, haplome methods and disease tests) to help breeders in optimizing cauliflower, broccoli (*Brassica oleracea*) and oilseed rape (*Brassica napus*) breeding programs.

For cauliflower, we designed a genetic map from a F2 cauliflower population. The map covers 1843 cM Kosambi and comprises 387 markers, including 71 RAPD, 204 RFLP and 112 SSR markers, distributed over 9 linkage groups. This genetic map is a useful tool to identify genes of interest and molecular markers that can be used in cauliflower marker assisted selection programs.

To shorten the length of breeding process for the different crops, we currently produce dihaploids, mainly through microspore culture. These techniques allow the production of pure lines in approximately one year, in comparison to 6 to 8 for selfing generations.

We also developed disease tests to identify genotype with resistance to diseases: *Leptosphaeria maculans* and *Sclerotinia sclerotiorum* for oilseed rape, *Mycosphaerella brassicicola* and downy mildew for cauliflower, and bacterial diseases on broccoli.

These tools will help cauliflower, broccoli and oilseed rape breeders to (i) identify genes of interest, linked markers and genotypes for new breeding programmes, (ii) improve selection efficiency and (iii) reduce the time and money needed to achieve breeding goals.

**63. EFFECTS OF ENVIRONMENT ON STABILITY OF QTL FOR RESISTANCE TO LEPTOSPHERAERIA MACULANS IN OILSEED RAPE (*BRASSICA NAPUS*)**

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Use of host resistance is the most effective and environmentally friendly way to control plant diseases. To achieve effective control, understanding host resistance is essential. Phoma stem canker, caused by *Leptosphaeria maculans*, is a disease of world-wide importance on oilseed rape (*Brassica napus*), causing serious losses in Europe, Australia and North America. Two types of resistance to *L. maculans* have been identified; major resistance (*R*) gene mediated qualitative resistance and quantitative resistance. *R* gene mediated resistance to *L. maculans* is race-specific and usually loses its effectiveness within three seasons of widespread use in commercial cultivars because of changes in *L. maculans* populations. Quantitative trait locus (QTL) mediated quantitative resistance to *L. maculans* is considered to be race non-specific and durable. Therefore, identification of QTLs for resistance to *L. maculans* is important for breeding resistant cultivars with durable resistance. The work reported uses an oilseed rape mapping population BnaDYDH (Darmor-*bzh* × Yudal) to investigate stability of QTLs for resistance to *L. maculans* under different environments. A total of 11 QTLs involved in quantitative resistance to *L. maculans* were detected over five years at two sites. Genotype × environment interaction analysis suggested that two QTLs (on A2 and C1) were not affected by environmental factors whereas seven QTL that were detected in two to four environments were affected by environmental factors. Further definition of the mode of action of these QTLs will provide a basis for prioritisation of QTL pyramiding in breeding programmes, which will contribute to sustainable management of the disease.

**64. IMPROVEMENT OF CAULIFLOWER MALE STERILE LINES WITH *BRASSICA NIGRA* CYTOPLASM**

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In the Research Institute of Vegetable Crops, Skierniewice, Poland, cytoplasmic male sterile lines of broccoli and broccoli-cauliflower have been maintained, selected and investigated since 1976. In these *cms* plants stamens are transformed into petals.

The aim of the work was the improvement of the seed effectiveness and curd quality of cauliflower genotypes with *B. nigra* cytoplasm. In 1999 four selected cauliflower genotypes with *B. nigra* cytoplasm as well as their maintainers were crossed with three good quality fertile cauliflower lines. In the consecutive generations back-crosses and restoration of sterility in *cms* lines and identification of homozygous, recessive *ms,ms* genes in the maintainer lines followed by the selection of the good quality traits. Simultaneously improvement of *B. nigra* cauliflower lines for flower structure, larger nectarines and screening for effective seed formation through selection process was performed.

In 2009 most of *cms* lines and their maintainers had good morphological and commercial characters in comparison to fertile genotypes. Cauliflower F<sub>1</sub> hybrids obtained by the use of *cms* components with *B. nigra* cytoplasm were vigorous and they had good commercial value.

After twelve years of selection four cauliflower *cms* genotypes with *B. nigra* cytoplasm with reasonably high seed set in comparison to fertile components (40 g./plant) and normal flower morphology was derived.

According to obtained results, *cms* system with *B. nigra* cytoplasm is reliable and feasible to maintain, however, *cms* lines with higher ability for generative propagation should be checked for their stability in consecutive generations.

**65. EXPRESSION ANALYSIS OF GENES FOR FLOWERING IN A LATE BOLTING BREEDING MATERIAL, “LEAFY GREEN PARENTAL LINE NO.2 (*BRASSICA RAPA*)” THAT REQUIRES LONG DAYS INSTEAD OF VERNALIZATION FOR FLOWERING****Naoko Kitamoto<sup>1</sup>, Susumu Yui<sup>2</sup>, Yoshihito Takahata<sup>1</sup>, and Shuji Yokoi<sup>1</sup>**

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Breeding of cultivars with late bolting is important because premature bolting lead to a loss of yield in *B. rapa* crops. The aim of this study is to explore the genes that control flowering time in a late bolting breeding material, “Leafy Green Parental Line No.2 (LG2)” that requires long days instead of vernalization for flowering.

We investigated the bolting days of 7 Chinese cabbage cultivars and 13 selected lines which were made by back crossing the LG2 with Chinese cabbage (*B. rapa* L. ssp. *pekinensis*) several times. Seeds were sown in pots on 10 September 2008 and the plants were transplanted to the open field in Morioka, Northern Japan (39.70N, 141.18E) in October. All Chinese cabbage cultivars had bolted until mid April whereas most of selected lines had bolted around mid May.

Southern blot analysis with *FLC* of *Arabidopsis thaliana* as the probe indicated that there was polymorphism between LG2 and four Chinese cabbage cultivars. Because three plants from selected lines shared same bands with LG2, involvement of *BrFLCs* in the flowering time variation was suggested.

RT-PCR analysis of four *BrFLCs* transcripts showed that the *BrFLC* transcript levels in a mid-flowering cultivar of Chinese cabbage “Musō” were very low after cold treatment for 60 days, whereas the transcript of LG2 still accumulates high, albeit slightly reduced, *BrFLC* expression. Therefore it was suggested that the late bolting of LG2 might result from high transcript levels of *BrFLCs* even after long cold treatment periods.

**66. DEVELOPMENT OF LOW FIBER, YELLOW SEED COAT OMEGA-9 *BRASSICA NAPUS*****Tom Kubik<sup>1</sup>, Chibwe Chungu<sup>2</sup>, Gerhard Rakow<sup>3</sup> and Steve Thompson<sup>4</sup>**

1. Dow AgroSciences Canada Inc., 101-421 Downey Rd., Saskatoon, SK, CANADA, S7N 4L8
2. Dow AgroSciences LLC, 2220 West Lincoln Ave., Olivia, MN, USA, 56277
3. Dow AgroSciences LLC, 9330 Zionsville Rd., Indianapolis, IN, USA, 46268
4. AAFC, Saskatoon Research Station, 107 Science Place, Saskatoon, SK, CANADA, S7N 0X2 (special acknowledgement of our colleague in this work John Phillip Raney, deceased)

While canola oil has generally been recognized as a healthy oil, the meal component of the seed remaining after the oil is extracted is inferior to soybean meal due to its high fiber content. Dow AgroSciences has had a successful history of development of further improvements to canola oil through development of our high stability Omega-9 oil. Dow AgroSciences, in collaboration with AAFC, undertook to develop low fiber, yellow seed coat canola lines in combination with the Omega-9 profile to further improve the overall quality of canola. We report on our efforts to successfully develop low fiber, yellow seed coat lines with Omega-9 oil.

**67. MAPPING OF QUANTITATIVE TRAITS FOR MORPHOLOGICAL TRAITS IN *BRASSICA RAPA*****Xiao Nan Li, Nirala Ramchiary, Su Ryun Choi, Hyeon Kook Yang, and Yong Pyo Lim**

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Rapid cycling *Brassica rapa* is one of the European type that showed totally different phenotype from Chinese cabbage. In this study, we developed a F<sub>2</sub> population by crossing Rapid cycling *Brassica rapa* and Chiifu for constructing linkage map and morphological trait analysis. Two kinds of molecular markers, BAC-end sequence based SSR and IBP (Intron Based Polymorphism) were used to construct linkage map. Until recently, 68 IBP markers and 250 BAC derived SSR markers were located in linkage map covering 1105cM genomic length. A total of 19 QTLs affecting 10 morphological traits were detected on this CRF<sub>2</sub> map, including 2 for pod related traits, 5 for petiol traits, 3 for seed number, 3 for leaf traits, 4 for flowering time and 2 for bolting time. This map will continue to integrate with our *Brassica* CKDH reference linkage map and it is possible to compare the corresponding QTL region.

**68. DEVELOPMENT OF PCR-BASED MARKERS FOR BREEDING SELF-INCOMPATIBLE LINES IN *B. NAPUS* L.****Chaozhi Ma, Tingdong Fu, Jinzing Tu, and Jinxiong Shen**

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Self-incompatibility (SI) is an important pollination system for hybrid seed production in *B. napus*. We developed an SI line '271' by introgressing an S haplotype of *B. rapa* (cv 'Xishuibai') into *B. napus* through interspecific hybridization. Line '271' was improved to become double-low (low erucic acid, low glucosinolates) SI line 'S-1300'. This SI line can be used to produce three-way hybrids (SI line/maintainer/restorer). There are currently no efficient means to increase SI lines on a large scale, and the difficulty of identifying the SI phenotype have limited its utilization in *B. napus* hybrid breeding. We have been developing markers linked to the SI of 'S-1300' since 2003 to use in marker assistant selection for SI. Based on homology-based candidate gene method, five PCR primer pairs were derived from sequence differences of S-locus glycoprotein gene (*SLG*), S-locus receptor kinase gene (*SRK*) and S-locus protein 11 (*SP11*) /S-locus cysteine-rich protein (*SCR*) gene between 'S-1300' and restorers/maintainers, resulting in five SCAR markers that can select plants with the 'S-1300' S genotype. Five multiplex PCR markers were also developed to distinguish the homozygous S genotype of 'S-1300' from heterozygous S genotype and homozygous S genotype of a restorer/maintainer. These markers were validated in F<sub>2</sub> and BC<sub>3</sub>F<sub>2</sub> lines from several 'S-1300'/restorer and 'S-1300'/maintainer combinations. Plants selected using multiple PCR markers indicate that our system is effective in identifying SI lines and could be very helpful for marker-assisted selection of SI in *B. napus* hybrid breeding.

### 69. A NEW BLACKLEG RESISTANCE TRANSFER PROJECT FOR *BRASSICA NAPUS* BASED ON THE *BRASSICA* B-GENOME

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Blackleg caused by *Leptosphaeria maculans* (*Phoma lingam*) is the most significant disease affecting oilseed rape (*Brassica napus*) worldwide. Considering climate change it is expected to become even more relevant in future.

To widen the narrow base of oilseed rape resistance, offspring derived from somatic hybrids *B. oleracea* (+) *B. carinata* and *B. oleracea* (+) *B. nigra*, respectively, are currently characterised and developed towards the *B. napus* karyotype (genome AACC, 2n = 38).

Genomic *in situ* hybridisation (GISH) is a powerful tool for the detection of alien chromatin in interspecific hybrids, although possible limitations in *Brassica* and related genera have to be considered.

The main focus of this study is on blackleg resistance behaviour of selected selfing and backcross offspring produced using embryo rescue techniques. Adult plant resistant individuals of different generations, e. g. F<sub>6</sub>, F<sub>4</sub>BC<sub>1</sub> and F<sub>6</sub>BC<sub>3</sub>, along with susceptible genotypes, were examined cytologically by GISH. These early generations have been developed as a part of a cabbage pre-breeding program using *B. oleracea* as the backcross parent. Furthermore, several of these promising genotypes were selfed again and backcrossed with *B. napus* and *B. rapa*, respectively, to obtain an AACC background. The so developed advanced generations also revealed to comprise several adult plant resistant individuals.

GISH results are compared with those obtained earlier from blackleg resistant addition (2n = 39, 2n = 40) and putative recombination (2n=38, no GISH signals) lines originally derived from interspecific, sexual hybrids between *B. napus* and *Sinapis arvensis*, *Coincya monensis* and *B. juncea*, respectively.

### 70. UNREDUCED GAMETE PRODUCTION IN *BRASSICA*: GENETIC AND ENVIRONMENTAL EFFECTS

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Unreduced (2n) gametes may represent the evolutionary mechanism by which the amphidiploid species *B. napus* (2n=AACC), *B. juncea* (2n=AABB) and *B. carinata* (2n=BBCC) were formed from diploid species *B. rapa* (2n=AA), *B. oleracea* (2n=CC) and *B. nigra* (2n=BB). However, little is known about the genetic and environmental effects on unreduced gamete production in this genus. We assessed male unreduced gamete production in several genotypes of *B. napus*, *B. juncea*, *B. carinata* and in the interspecific hybrids between them. A subset of genotypes was also assessed across four different ambient temperature treatments. Giant pollen frequency and observations at the sporad stage of pollen development were used to estimate unreduced gamete production. Unreduced gametes were produced at higher frequencies in most interspecific hybrid types (0.06 to 3.3% at the sporad stage, and 0.2 to 34% in viable mature pollen) compared to the parental amphidiploid species (0.02% on average). Temperature did not affect unreduced gamete production in any parental genotype, or in 3/5 hybrid genotypes. However, two *B. napus* × *B. carinata* genotypes produced higher frequencies of male unreduced gametes (26% and 9%) in the cold temperature treatment (10°C day / 5°C night) compared to other temperature treatments (0 to 4% at 18°C / 13°C, 25°C / 15°C and 30°C / 20°C). These findings indicate that unreduced gamete production is stimulated in *Brassica* interspecific hybrids, but is influenced by genotype and temperature conditions. A high level of unreduced gamete production in interspecific hybrids may have contributed to allopolyploid formation in this genus.

**71. FINDING EFFECTIVE CONDITIONS OF MICROSPORE CULTURE IN RADISH (*RAPHANUS SATIVUS* L.)****Suhyoung Park<sup>1</sup>, Moo-Kyung Yoon<sup>1</sup>, Haeyoung Na<sup>1</sup>, Min-Young Park<sup>2</sup> and Yong Pyo Lim<sup>2</sup>**

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To make genetically identical inbred in short time, microspore culture is one of popular method used for *Brassica* vegetables breeding. However, in case of radish, one of major *Brassica* vegetable in Korea, only one case of success has been reported. Thus we tried to find effective conditions of radish microspore culture by adjusting culture conditions of Chinese cabbage (*Brassica rapa* L.).

As the optimum condition of microspore embryos induction in Chinese cabbage (*B. rapa*) is addition of small amount of active charcoal, 0.5 mg·L<sup>-1</sup> AgNO<sub>3</sub> in X0.5 NLN medium containing 10% sucrose, we tested active charcoal, AgNO<sub>3</sub>, NLN medium and sucrose concentration in radish (*R. sativus*). While adjusting culture conditions, thirty-two varieties and germplasms collected from Korea, China and Japan were tested and only two varieties produced embryos. About 200 embryos were regenerated from those two varieties and embryos were transplanted to the MS medium for rooting. We found addition of 0.4mg·mL<sup>-1</sup> active charcoal, 0.1 mg·L<sup>-1</sup> of NAA, 0.05 mg·L<sup>-1</sup> of BA, and 0.1 mg·L<sup>-1</sup> AgNO<sub>3</sub> in X1 NLN medium containing 13% sucrose is the most efficient condition of microspore culture of radish (*R. sativus*).

**72. EVALUATION OF BRASSICA GERMLASM FOR RESISTANCE TO CLUBROOT OF CANOLA****Gary Peng, Kevin C. Falk, Brian James, and Richard K. Gugel**

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Clubroot, caused by the protist pathogen *Plasmodiophora brassicae* Woronin, is a serious disease of Brassica crops worldwide and is becoming a serious threat to canola production on the Canadian prairies. The disease was first observed on canola in the Edmonton area in 2003, but has since been found in more than 450 fields in Alberta. Contaminated fields have also been reported in Saskatchewan and Manitoba. Since 2009, clubroot resistant (CR) canola cultivars have been available to producers, but the durability of these cultivars is unknown. CR genes are generally race specific and therefore it is important to establish a broad base of genetic resistance for the development of new cultivars over the long term. In the current study, over 900 accessions of *Brassica* spp. have been evaluated against the predominant *P. brassicae* race (pathotype 3) using a new bioassay system. Ten accessions, mostly from several sub-species of *B. rapa* and *B. juncea*, showed good resistance, reducing clubroot severity by 70–100% relative to susceptible controls. These CR materials are being further characterized.

**73. INTROGRESSION OF BLACKLEG RESISTANCE INTO *BRASSICA NAPUS* FROM *BRASSICA CARINATA*****M.M. Rahman<sup>1</sup>, H.R. Kutcher<sup>2</sup>, S. E. Strelkov<sup>1</sup>, and M.H. Rahman<sup>1</sup>**

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Blackleg, caused by the fungus *Leptosphaeria maculans*, is one of the most damaging diseases of oilseed rape worldwide. Development of resistant cultivars is the most effective way of controlling this disease. The species *B. carinata* is believed to carry resistance to multiple blackleg pathotypes. To transfer blackleg resistance from *B. carinata* into *B. napus*, interspecific hybridization between these two species was done and interspecific hybrids were produced from *in vivo* hybrid seeds as well as through application of *in vitro* ovule culture technique. The F<sub>1</sub> hybrids were recurrently backcrossed to the susceptible *B. napus* with selection for resistance in each generation. Five blackleg isolates PG4#166, PG4#290CDN, BL#03-02RK, BL#05-08RK, and PGT#165 were tested for their virulence on five *Brassica* genotypes: *B. napus* cvs. Westar, Polo, Glacier and Quinta, and *B. carinata*. Amongst the five isolates, 290CDN was found to be most aggressive and hence was used for screening the F<sub>1</sub> and backcross generation populations. All the interspecific F<sub>1</sub> hybrids were 100% resistant. A number of backcross generation plants showed resistance at both cotyledon and adult plant stage, and thus indicate the possibility of introgression of blackleg resistance from *B. carinata* into *B. napus*.

**74. GENETIC MAP CONSTRUCTION AND LOCALISATION OF QUALITATIVE AND QUANTITATIVE LOCI FOR BLACKLEG RESISTANCE IN CANOLA (*BRASSICA NAPUS* L.)****Rosy Raman<sup>1</sup>, Belinda Taylor<sup>1</sup>, Steve Marcroft<sup>2</sup>, Paul Eckermann<sup>4</sup>, Ata Rehman<sup>1</sup>, Kurt Lindbeck<sup>1</sup>, David Luckett<sup>1</sup>, Neil Wratten<sup>1</sup>, Jiri Stiller<sup>3</sup>, Jacqueline Batley<sup>3</sup>, David Edwards<sup>3</sup>, and Harsh Raman<sup>1</sup>**

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A linkage map comprising 255 markers, based upon simple sequence repeats (SSR), sequence-related amplified polymorphisms, sequence tagged sites and EST-SSRs was constructed utilising a doubled haploid population from the Australian canola (*Brassica napus*) cultivars Skıpton/Ag-Spectrum. Markers were well distributed throughout the A and C genomes covering a total distance of 2,672 cM. We also validated intra- and inter-chromosomal duplicated regions on the A and C genome. The linkage map was subsequently utilised to identify loci controlling both seedling and adult-plant blackleg (*Leptosphaeria maculans*) resistance using the whole genome average interval mapping approach. Marker regression analyses revealed that at least 12 genomic regions control resistance to blackleg. Molecular markers explained 8% to 70% of genotypic variation for blackleg resistance at the adult plant stage. A major race specific gene *RlmSkıpton* was tagged on chromosome A7 within 1 cM of an SSR locus. This linkage was further validated in an independent F<sub>2</sub> population comprising 102 lines between Skıpton and Ag-Spectrum. Our results have suggested that SSR markers tracking consistent genomic regions for blackleg resistance both at the seedling and adult plant stages are suitable for routine marker-assisted selection in canola breeding programs.

**75. DETECTION OF RADISH CHROMOSOMES IN 9 DISOMIC RAPE-RADISH ADDITION LINES VIA FLUORESCENCE *IN SITU* HYBRIDIZATION****Otto Schrader, Holger Budahn, and Herbert Peterka**

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Chromosome complements of the complete set of nine disomic rape-radish chromosome additions (*a* to *i*,  $2n = 4x = 38 + 2$ ) were analyzed via fluorescence *in situ* hybridization (FISH). The tandemly repeated sequence pURsN (Hirai *et al.* 1995), 123 bp long, specific for all nine *Raphanus* chromosomes and localized in regions near the centromeres, was used for cytogenetic identification (Budahn *et al.* 2008). The meiotic stability was analyzed especially in line *d*, the carrier of resistance against the beet cyst nematode (*Heterodera schachtii* Schmidt). Analysis in 5 disomic *d* plants showed minimal deviation of the observed pollen mother cells to the expected values in stages of: 20 pachytene, 60 diakinesis, 35 metaphase I as well as 80 Anaphase I and 120 telophase II with one deviation in each.

In two-color FISH experiments with probes of digoxigenin-labelled pURsN (detected with FITC-coupled antibodies in yellow fluorescence) and biotin-labelled 5S or 18/25S rDNA (detected with Cy3-coupled antibody in red fluorescence) the cytological localization of the ribosomal RNA genes in all nine addition lines was tested. It was shown that the 5S rDNA was localized in lines *c* and *h*, as well as the 18/25S rDNA in lines *d* and *h*. The three *Raphanus*-specific chromosomal localizations of rDNA in the rape-radish chromosome additions (*c*, *d* and *h*), with one double-localization in line *h*, were in accordance with presented results in corresponding two-color FISH experiments in *Raphanus sativus*. Possibilities of transfer the resistance against cyst nematode into the rape genome were discussed.

**76. FINE MAPPING OF CLUBROOT RESISTANCE GENE, *PB-BO(ANJU)1*, IN *BRASSICA OLERACEA*****Motoki Shimizu, Hiroya Tomita, Hidetaka Hori, and Keiichi Okazaki**

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A fine linkage map of *Brassica oleracea* was constructed to identify the clubroot resistance (CR) gene. In the previous study, we identified CR-QTLs in  $F_2$  progenies from the cross of a resistant double-haploid (DH) line (Anju) with a susceptible DH line (GC). In the 9 linkage groups obtained (O1-O9), the major QTL, *pb-Bo(Anju)1*, derived from Anju, was identified in O2. Since this region has high genome synteny with the middle arm of Arabidopsis chromosome 5 and BAC clones of *B. rapa*, the new markers were designed based on the genome synteny. As a result, the four markers were mapped to the region harboring *pb-Bo(Anju)1*. Among a population of 1,467  $F_2$  plants, 46 plants showing recombination around *pb-Bo(Anju)1* locus were selected and used for the fine mapping. In the fine map, the marker interval between the closest markers, BRMS-228 and KBrH059L13R, is 1.74 cM, which may be served as useful markers for the marker assisted selection of *pb-Bo(Anju)1*. In order to identify the precise position in the fine map, CR test using recombinant plants is currently underway.

**77. DIVERSITY OF GLUCOSINOLATES AND ITS COMBINING ABILITY AND HEREDITY PARAMETRIC IN CHINESE KALE****Yu Si, Guo-ju Chen, Bi-hao Cao, En-you Feng, and Jian-jun Lei**

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Glucosinolates(GSs) can be degraded by catalysis of myrosinase or bacterial enzyme in gastrointestinal tract, and these degradation products perform active biological and biochemical characteristics, which make the product have a special flavor. It is also a chemical protector that can prevent the occurrence of many types of cancer. The GSs content in 43 varieties were assayed by HPLC in chinese kale. Complete diallel crossing was designed with 6×6 to calculate the combining ability and the main genetic parameters. The content of GSs varied greatly, including anti-cancer glucoraphanin (RAA) and progoitrin (PRO) with inducing goiter. The GCA effect of P<sub>1</sub>, P<sub>2</sub> and P<sub>5</sub> was excellent. They were used as parents to get hybrids, the heterosis of anti-cancer glucosinolates of their hybrid was very high. Among these 15 crosses obtained in this study, 1 × 2 was a perfect hybrid. The RAA content of the their parents was highest and with the lowest content of PRO (result in goiter). The RAA content of the hybrid was the highest, and their content were 3.18μmol / g FW, which were much higher than the anti-cancer vegetables broccoli variety (0.29 ~ 0.88μmol /g) and purple cabbage variety(0.47 ~ 0.67μmol /g) that was popular internationally. At the same time, the SCA and GCA effect of the combinations and their parents in RAA were the highest. So this combination was a desirable combination.

**78. LARGE SCALE GENETIC MAP INTEGRATION IN BRASSICA VIA DATABASE INTERACTION****Jun Wang<sup>1</sup>, Derek Lydiate<sup>2</sup>, Isobel Parkin<sup>2</sup>, Cyril Falentin<sup>3</sup>, Regine Delourme<sup>3</sup>, Ambrose Andongabo<sup>1</sup>, Pierre Carion<sup>1</sup>, Chris Love<sup>1</sup>, Graham J King<sup>1</sup>**

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The value of genetic linkage maps for resolution of loci associated with crop traits is often dependent upon the availability of sufficient polymorphic marker loci that segregate in the relevant reference populations. Integrating different genetic maps helps to increase marker density and create more reliable marker orders. This allows intensive study of the orthologous relationships between *Brassica* and reference species such as *Arabidopsis*, as well as re-calculation of QTL positions associated with valuable crop traits. We describe the generation of a consensus genetic linkage map for the 19 chromosomes of *Brassica napus*, which brings together various genetic maps collated in our genetic source database, CropStoreDB ([www.cropstoredb.org](http://www.cropstoredb.org)). For sequence-tagged marker loci, we also describe the process to extract sequence information from our DNA sequence database, SeqStoreDB, and feed this into an in-house sequence alignment database AlignStoreDB. The genetic (population-level) information from CropStoreDB, along with the sequence-level information from SeqStoreDB and AlignStoreDB is used to explore orthologous/homologous relationships between a query species such as *Brassica* and a reference species and/or any target sequence collection. The integration of data between these three databases also allows the provenance of additional features to be presented within the Ensembl genome browser, BrassEnsembl ([www.brassica.info/BrassEnsembl/index.html](http://www.brassica.info/BrassEnsembl/index.html)).

**79. NATURALLY OCCURRING DELETION MUTATION IN THE OIL-TYPE *BRASSICA RAPA* *FLC2* IS ASSOCIATED WITH VARIATION IN FLOWERING TIME**

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Flowering time is an important agronomic trait, and a wide variation exists among *Brassica rapa*. *FLOWERING LOCUS C (FLC)*, a repressor of flowering, encodes a MADS-domain transcription factor in *Arabidopsis*. There are four *FLC* homologs in *Brassica* species, which are involved in the vernalization pathway. We analyzed sequence variation of *BrFLC2* in nine *B. rapa* accessions with a wide range of flowering time variation.

The analysis revealed a variation of 13 bp inconsistent deletions within a 18 bp region at the end of fourth exon and 44 bp deletion at the start of fourth intron. An In-Del marker designated as *FLC2-Del57* was developed for this locus to distinguish In-Del genotypes. In total, 148 accessions from 10 cultivar groups were screened for the genotype of *FLC2*. The sequence variation of 57 bp deletion was only observed for oil-type *B. rapa*, including *ssp. tricoloris* and *ssp. oleifera*, but not in any other cultivar groups. Open field experiment revealed that the deletion is associated with early flowering in oil-type *B. rapa*.

**80. IDENTIFICATION OF CYTOPLASM TYPES IN RAPESEED (*BRASSICA NAPUS* L.) ACCESSIONS BY A MULTIPLEX PCR ASSAY**

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Cytoplasmic male sterility (CMS) has widely been used as an efficient pollination control system in rapeseed hybrid production. Identification of cytoplasm type of rapeseed accessions is becoming the most important basic work for hybrid-rapeseed breeding. In this study, we report a simple multiplex PCR method to distinguish the existing common cytoplasm resources, *Pol*, *Nap*, *Cam*, *Ogu* and *Ogu-NWSUAF* cytoplasm, in rapeseed. Cytoplasm type of 35 F<sub>1</sub>-hybrids and 140 rapeseed open pollinated varieties or breeding lines in our rapeseed breeding programme were tested by this method. The results indicated that 10 of 35 F<sub>1</sub>-hybrids are the *Nap*, and 25 the *Pol* cytoplasm type which is consistent with the information provided by the breeders. Out of 140 accessions tested, 100 (71.4%), 21 (15%) and 19 (13.6%) accessions possess *Nap*, *Cam* and *Pol* cytoplasm, respectively. All 19 accessions with *Pol* cytoplasm are from China. Pedigree analysis indicated that these accessions with *Pol* cytoplasm were either restorers for *Pol* CMS, including Shaan 2C, Huiyehui, 220, etc. or derived from hybrids with *Pol* CMS as female parent. Our molecular results are consistent with those of the classical testcross, suggesting the reliability of this method. The multiplex PCR assay method can be applied to CMS "three-line" breeding, selection and validation of hybrid rapeseed.

**82. BROADENING GENETIC DIVERSITY IN CANOLA: DEVELOPMENT OF DOUBLE-LOW QUALITY RECOMBINANT INBRED LINES FROM A *BRASSICA NAPUS* x *B. OLERACEA* CROSS**  
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The narrow genetic base of *Brassica napus* (2n=38, AACC genome) is of concern to many researchers, as diversity is critical for continued improvement of the crop. The objective of this research was to broaden genetic diversity in *B. napus* through exploitation of the C-genome of *B. oleracea* (2n=18, CC genome). Sixty-five F<sub>8</sub> recombinant inbred lines (RILs) were developed from a wide cross between *B. napus* 'Hi-Q' (zero-erucic, 10 to 15 µmol g<sup>-1</sup> glucosinolate) and *B. oleracea* var. *alboglabra* (40% erucic acid, >80 µmol g<sup>-1</sup> glucosinolate). The pattern of inheritance of erucic acid (C22:1) observed in F<sub>2</sub> seeds suggests that segregation distortion occurred in favour of the high erucic acid allele originating from *B. oleracea*. Low glucosinolate (<30 µmol g<sup>-1</sup>) genotypes were obtained in the F<sub>4</sub> generation from a relatively small segregating population, and seeds from 68% of the F<sub>6</sub> families evaluated in field trials had low glucosinolate content. Flow cytometry analysis of F<sub>8</sub> plants (Partec value 196.3 ± 4.9) showed no significant difference from the *B. napus* 'Hi-Q' parent (197.4 ± 2.5) (t=0.63, P>0.50). The RIL population was genotyped with 29 polymorphic microsatellite (SSR) markers from the Brassica C-genome. F<sub>8</sub> lines with 0% to 55.2% alleles (avg. 17.9%) originating from the *B. oleracea* parent were identified. These genetically diverse lines will be used in the breeding program for enhancement of specific traits and may have great potential as a heterotic pool in hybrid canola breeding.

**83. REPEATED POLYPLOIDY DROVE DIFFERENT LEVELS OF CROSSOVER SUPPRESSION BETWEEN HOMEOLOGOUS CHROMOSOMES IN *BRASSICA NAPUS* ALLOHAPLOIDS**

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Allopolyploid species contain more than two sets of related chromosomes (homeologues) that must be sorted during meiosis to ensure fertility. As polyploid species usually have multiple origins, one intriguing, yet largely under-explored question is whether different mechanisms suppressing crossovers between homeologues may coexist within the same polyphyletic species. We addressed this question using *Brassica napus*, a young polyphyletic allopolyploid species. We first analyzed the meiotic behavior of 363 allohaploids produced from 29 accessions, which represent a large part of *B. napus* genetic diversity. Two main clear-cut meiotic phenotypes were observed, encompassing a two-fold difference in the number of univalents at metaphase I. We then sequenced two chloroplast intergenic regions to gain insight into the maternal origins of the same 29 accessions; only two plastid haplotypes were found and correlated with the dichotomy of meiotic phenotypes. Finally, we analyzed genetic diversity at the *PrBn* locus, which was shown to determine meiotic behavior in a segregating population of *B. napus* allohaploids. We observed that segregation of two alleles at *PrBn* could adequately explain a large part of the variation in meiotic behavior found among *B. napus* allohaploids. Overall, our results suggest that repeated polyploidy resulted in different levels of crossover suppression between homeologues in *B. napus* allohaploids.

**84. EXTRACTION OF THE DIPLOID A GENOME AND OF C CHROMOSOMES FROM THE ALLOPOLYPLOID *BRASSICA NAPUS* (AACC, 2n=38)**

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There are two strategies available for understanding structural and/or functional modifications which took place during the stabilization of polyploid species. The first involves production of synthetic forms in order to mimic the events occurring during genome stabilization. The second involves the extraction of the polyploid's diploid component for comparison with the present natural diploid species. The oilseed rape model (*Brassica napus*, AACC, 2n=38) is a natural hybrid between *B. rapa* (AA, 2n=20) and *B. oleracea* (CC, 2n=18). We applied two methods to extract the diploid AA genome from *B. napus*. First, AAC F1 interspecific hybrids (produced by crosses between *B. napus* and *B. rapa*) were backcrossed three times to *B. napus*. AAC plants were selected at each generation but the resulting AAC hybrids occurred to be male sterile. It was thus impossible to eliminate the C chromosomes by selfing. Second the initial AAC F1 hybrids were crossed to *B. rapa*. Progenies with only AA genomes (n=20) were selected for, selfed and backcrossed to *B. napus*. The next AAC hybrids were crossed to the selfing progeny of AA plants. After two cycles of crossing, an AA plant carrying expected 56% of *B. napus* A genome was generated. Additionally at each cycle, monosomic addition lines (2n=21) carrying C1, C2, C3, C5, C7, C8 or C9 *B. napus* chromosomes were characterized. This material will allow us to determine the comparative evolution of the progenitor genomes in a polyploid genetic background.

**85. MULTIPLEXED 454 AMPLICON SEQUENCING FOR HIGH-THROUGHPUT SNP DISCOVERY IN *BRASSICA NAPUS* CANDIDATE GENES FOR OIL CONTENT**

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Discovery of phenotypically relevant sequence variation in candidate genes for important traits is difficult in *Brassica napus* because locus-specific assays are normally required for conventional DNA sequencing of homoeologous loci. On the other hand, high-throughput next-generation sequencing provides a powerful alternative to simultaneously screen for sequence variants in all homoeologous copies of multiple *B. napus* candidate genes. We have established a method for simple amplification and tagging of PCR amplicons with genotype-specific multiplex identifier (MID) tags for sequencing on the Roche 454-FLX platform. A two-step PCR procedure is used: First, gene-specific amplicons are amplified with target-specific primers that have an M13 overhang at the 5' end of the forward primer and a T7 overhang at the end of the reverse primer. These products are then re-amplified with 454-FLX fusion primers complementary to the M13 and T7 overhang sequences. In addition, the fusion primers have 5' overhangs containing primer sequences for 454 emulsion PCR and sequencing, along with 10 bp MID barcodes that enable each genotype to be specifically tagged. We tested this system for SNP discovery in 20 exotic *B. napus* genotypes with large variation in oil content. 10 MID fusion primer sets were used to amplify and pool a total of 30 amplicons representing around 60 loci from 9 genes with a putative role in seed oil metabolism. Initial results will be presented to demonstrate the power of the method for high-throughput SNP discovery in *B. napus*.

**86. SCREENING OF SSR CORE PRIMERS AND THEIR APPLICATION IN RAPESEED (*BRASSICA NAPUS* L.)****Yang Jun, Yi Bin, Wen Jing, Tu Jin-xing, Ma Chao-zhi, Fu Ting-dong, and Shen Jin-xiong**

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There are a large number of SSR primer pairs in rapeseed (*Brassica napus*). However, not all primer pairs have been demonstrated to be equally informative. Therefore, we should screen and establish a small set of SSR primer pairs that could high effectively determinate the  $F_1$  hybrid seed purity and detect the genetic diversity among germplasms in this crop. Based on this conception, firstly we collected 100 rapeseed genotypes, of which some were landraces from different regions of China and some were elite cultivars (including hybrid cultivars) bred and widely planted in China and the others were introduced from foreign countries. Then SSR markers were employed to detect the diversity of these genotypes. Out of 746 primer pairs used in this study, 40 ones were selected according to their polymorphisms and reproducibility as the preferred primers. These 40 primer pairs nearly distributed across all of the *Brassica napus* linkage groups. Among the 40 primer pairs, 20 ones were selected as the core primers, and the others were as the alternative ones. Each core primer pairs could detect 3 to 9 alleles with the average of 5.05. Totally, 101 alleles were detected. The average of Shannon-Weaver Index was 1.311 and the average of Simpson Diversity Index was 0.637. The PIC of core primer ranged from 0.413 to 0.780 with an average of 0.633. 91 accessions accounted for 91 percent of total varieties could be identified by three pairs of primers in the core primers. At last, the core SSR primer pairs were used to determinate the hybridities of two polima CMS three-line hybrid cultivars randomly selected. The results of the molecular marker identifications were almost equivalent to those of field determinations. This indicated that the core primer pairs were considerably practical and highly efficient both in  $F_1$  hybrid seed purity identification and in varietal genetic diversity detection.

**87. ROOT AND SHOOT METABOLIC PROFILES OF *ARABIDOPSIS* AND WHITE CABBAGE: PREDICTIVE VALUE FOR INTRA-SPECIFIC VARIATION?****Patrick Kabouw<sup>1</sup>, Benyamin Houshyani<sup>2</sup>, Harro Bouwmeester<sup>2</sup>, Wim van der Putten<sup>1</sup>, and Arjen Biere<sup>1</sup>**

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In Brassicaceae metabolic profiles in aerial parts are known to vary intraspecifically, due to genotypic differences and environmental variation. However, it is unknown whether there is similar intraspecific variation in metabolic profiles of roots. We examined if metabolic profiles of roots of *Arabidopsis* and white cabbage showed similar intraspecific and environmental variation patterns as aerial parts. Second, we evaluate if genetic diversity becomes expressed in both shoots and roots.

To address these questions we analyzed glucosinolate profiles from four white cabbage cultivars grown in three environments, as well as untargeted metabolomics profiles measured by LC-MS and GC-MS of nine *Arabidopsis* accessions grown in four environments. To assess genetic diversity we used an existing dataset with 149 single nucleotide polymorphism markers of the nine *Arabidopsis* accessions. Root glucosinolate profiles in white cabbage showed significant intraspecific variation; however, this variation was unrelated to that recorded in shoots. Root profiles were generally comparable between environments, whereas shoot profiles were highly plastic and responsive to changes in environmental conditions. Also for *Arabidopsis*, metabolic profiles of roots showed significant intraspecific variation that was unrelated to the variation in shoots. Shoot profiles in *Arabidopsis* also responded to changes in environment, although variation between accessions was larger than between environments. Shoot profiles among accessions have a small but significant correlation with genetic variation. Root profiles however were uncorrelated with genetic variation.

We conclude that among Brassicaceae variation in shoot metabolic profiles is not indicative of metabolic variation in roots. Cabbage shoots and roots also differed in their responsiveness to environmental conditions. Metabolic differences cannot be used consistently as a reliable predictor for genetic variation between accessions.

**88. CONSTRUCTION OF A GENETIC LINKAGE MAP OF *BRASSICA RAPA*****B. Kebede, K. Cheema, and M.H. Rahman**

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We constructed a genetic linkage map of *Brassica rapa* ( $2n = 20$ ) using 94 recombinant inbred lines (RILs) derived from a cross between 'yellow sarson' cv. Sampad and yellowish-brown seeded line 3-0026.027. Replicated greenhouse and field trials were conducted to generate agronomic, morphological and quality traits using these lines. A total of 750 Simple Sequence Repeat (SSR) markers derived from different public domain were used to detect polymorphisms between the two parents. Two hundred thirty seven SSR markers were polymorphic between the parental lines, out of which 160 markers were used to genotype the lines. A total of 109 markers produced genotypic data. About 16% of the markers showed segregation distortion towards the parent Sampad while 15% showed segregation distortion towards 3-0026.27. The remaining 69% markers showed expected segregation pattern of 1:1. The frame work map was developed using SSR markers, and additional AFLP markers will be used to develop a dense genetic map. Based on the SSR markers, 10 linkage groups were identified and designated as A1 -A10 ( $\approx N1 - N10$ ) through aligning with the published *B. rapa* reference linkage maps. This linkage map can be used for identification and mapping of genes and quantitative trait loci (QTLs) of different morphological, physiological and biochemical traits, and thereby would assist plant breeding and genomic research.

**89. INITIAL RESULTS OF SEQUENCING, ASSEMBLY AND ANNOTATION OF THREE BRASSICACEAE GENOMES****Adrian E Platts<sup>1,2</sup>, Stephen I Wright<sup>3</sup>, Mathieu Blanchette<sup>2</sup>, Paul M Harrison<sup>1</sup>, Eef Harmsen<sup>1</sup>, Daniel J Schoen<sup>1</sup>, and Thomas E Bureau<sup>1</sup>**

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As part of the Value-directed Evolutionary Genomics Initiative (VEGI) we present early de novo sequencing and assembly results for three Brassicaceae (*Leavenworthia alabamica* (LA), *Sisymbrium irio* (SI) and *Aethionema arabicum* (AA)). These Crucifers were initially sequenced with up to 4 lanes of short insert Illumina GAIIx data (2x108, 64nt spacer) per species to a depth of approximately x60 (~18GBase per species). An assembly strategy was optimized a-priori through a series of assembly simulations. Two closely related Brassica genomes (*Arabidopsis thaliana* and *Arabidopsis lyrata*) were fragmented in-silico and jointly reassembled to assess a range of quality parameters including contig length distributions, total assembly characteristics and the probability of assembling chimeric sequences from paralogous sub-regions. Parameters investigated included read filtering, sequencing depths and Kmer values in three popular assemblers: Velvet (EBI), SOAPdenovo (BGI) and Abyss (MSGSC). The optimized contig assembly strategy utilized stringent read trimming (Q<31 -5bp), read depth limits (x5-x180) and a long-Kmer assembly strategy (K:51-61) to assemble contigs approximately three times longer than were generated with an un-optimized strategy. Read trimming and depth limits allowed the assemblies to complete in a reasonable time without generating unduly large or complex de Bruijn graphs. Long Kmers ( $k > 49$ ) were found to be useful in resolving ambiguity in many repetitive and paralogous domains. The contig-sets generated for the three plants had N50s of 13.4 Kbase (AA), 18.8Kbase (SI) and 19.0Kbase (LA) and a consistent maximum contig size of ~260Kbase. The total assembled libraries ranged in size from 149MBase (AA) to 202MBase (SI). Contig quality was assessed post-hoc relative to a set of 458 genes that are deeply conserved across Eukaryota. These were detected through BLAST and then assessed for protein length using FGenesH+ (*thaliana* models). Validation for chimerism and fragmentation suggested that all the highly conserved genes were present in the contigs with only ~1% (4) of the CDS models less than 80% of their length in *A. thaliana*. Assembly size was related to repeat content using a novel repetitive element (RE) assay that generated consensus RE models from the raw read data. This allowed assembly levels into repetitive structures to be tracked and early estimates of RE distributions between the plants to be described. The contig-sets were used to generate initial conservation and synteny results using the UCSC Blast-Chain-Net approach relative to two reference genomes (the derived *A. thaliana* genome and the larger interim reference *A. lyrata* genome). Conservation around several loci are described. Our results to date indicate a promising pipeline for the rapid assembly of high quality contig libraries. Further sequencing to scaffold the contigs using Illumina mate-pair sequencing with a 2-5kb insert size is currently underway. This research is supported by Genome Canada/Quebec

**90. EVALUATION OF POPULATION STRUCTURE IN CANOLA BREEDING GERmplasm**

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As canola breeding programs shift from developing open pollinated cultivars to hybrids, understanding population structure within a breeding program can inform the optimization of hybrid breeding for canola. Here we present an analysis of 222 spring canola and winter oilseed rape lines/cultivars. Each line was genotyped at 1,536 SNPs using GoldenGate SNP assay on the Illumina BeadStation. After quality control of the data, 625 SNPs were utilized for population structure analysis. First, a Dice similarity matrix and corresponding dendrogram were produced. Within the spring germplasm six clades were observed, while five were observed in the winter germplasm. Second, a model-based clustering approach was used to explore possible subpopulations within the germplasm. An admixture model was used to explore two to 13 subpopulations (k). When all lines were analyzed together there was strong separation of the spring and winter germplasm pools, thus further analysis were conducted independently for each subset. Based upon the likelihood values and knowledge of the germplasm we consider five subpopulations in the spring pool and four in the winter pool to be useful definitions of subpopulations moving forward. Understanding subpopulation structure within a breeding program that is in transition from developing open pollinated cultivars to hybrids may influence the creation of test crosses and breeding crosses.

**91. MOLECULAR DIVERSITY AND RELATIONSHIPS AMONGST GERmplasm OF *BRASSICA NAPUS* L. USING SSR ANALYSIS**

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Canola (*Brassica napus* L,  $2n=4x=38$ ) is widely grown oilseed crop in various continents of the world. Determining the level of genetic diversity and genetic relationships is crucial to make continued genetic improvement in canola. Simple sequence repeat (SSR) markers were used to determine genetic divergence in canola germplasm. The genotypes represented 181 accessions of *B. napus*, 1 of *B. rapa*, 3 of *B. juncea* and 2 of *B. carinata* collected from different parts of the world. Initially, two hundred SSR markers that have been previously mapped on to the canola linkage map of the Australian doubled haploid population derived from Skipton/Ag-Spectrum were analysed for polymorphism on twelve elite genotypes of canola. Subsequently, we selected 58 markers on the basis of their genotyping qualities etc and screened for polymorphism among 187 genotypes to estimate allele lengths, allele frequencies. Forward primer of each SSR marker was fluorescently labelled with either HEX, TET or 6-FAM dye phosphoramidites. PCR amplicons were analysed on the automated DNA sequencer (Applied system). Fragments that were separated by less than 0.4bp were binned together. Because some of the SSR primer-pairs amplified both dominant (+/-) and codominant alleles (multialleles), we converted allele sizes into binary scores. Dissimilarity (genetic distances) was calculated as 1-similarity. Both inter- and intra-SSR allelic polymorphism was observed within genotypes. The data from 58 primers was used for analysis. In total 475 loci were amplified with the number of alleles ranging from 2 to 21. A dendrogram based on the dissimilarity matrix (1 and 0) was constructed using unweighted pair group method with arithmetic means using DARWin version 5. The hierarchy cluster analysis and principal co-ordinate analysis based on genetic distance matrices showed that cultivars tended to group according to their origin/pedigrees. Molecular analyses revealed that some genotypes have unique alleles that could be exploited for genetic improvement. Results on relationships between genetic distances based upon SSR marker data and coefficient of parentage will be presented.

**92. SNP DISCOVERY IN *BRASSICA NAPUS* USING 454 TITANIUM SEQUENCING**

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NRC-PBI is employing the Roche 454 GS FLX platform for Single Nucleotide Polymorphism (SNP) discovery in canola (*Brassica napus*) using two approaches. The first approach targets selected gene families of interest and the second approach generates global 3' anchored transcript profiles. The natural genetic information identified by these methodologies will be utilized to develop SNP marker platforms for improving crops via marker-assisted selection (MAS). The two approaches utilize 454 Titanium sequencing technology which is capable of generating over one million 400bp reads or >400Mbp of sequence data per 9hr run.

The employed strategy of the first approach uses PCR primer pairs that amplify multiple members of each target gene family in a range of germplasm. Multiple primer pairs for each target will be generated to ensure maximum gene space is assayed. The strategy for the second approach involves isolating 3' gene transcripts from pooled total RNA of different tissues in a range of germplasm. The transcripts are digested with *AcI* to produce fragments of optimal size for Titanium chemistry. The longer read lengths and throughput of the 454 GS FLX platform provides sufficient coverage and depth to thoroughly characterize each gene family and each isolated 3' transcript. The extended long read chemistry set to be released summer 2010 will further increase gene coverage with read lengths approaching 1Kb. The germplasm panel for both approaches includes the parents of the well characterized and highly polymorphic AAFC DH SG genetic mapping population (DH12075 x PSA12 cross). A streamlined automated analysis pipeline has been developed to carry out SNP discovery and mapping in 3' transcript profiling data and will also be described.

**93. DEVELOPMENT OF GENOMIC RESOURCES FOR *CAMELINA SATIVA***

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Camelina is a member of the Brassicaceae family that has recently attracted attention mainly due to its unique fatty acid profile with potential applications for human nutrition in the food sector and use as industrial oil and bio-fuel in non-food sectors. In an effort to develop genomic resources for Camelina, 22,506 Expressed Sequence Tags (ESTs) were sequenced from two cDNA libraries and assembled into 4,459 non-redundant contigs and 6,097 singletons. To facilitate sequence annotation, the sequence data was integrated into a web-based database hosted by Agriculture and Agri-Food Canada (AAFC). In order to develop molecular markers for Camelina, these contigs and singletons were also searched for the presence of different SSR repeat motifs leading to the discovery of 3,197 SSRs. A total of 250 SSRs were selected for primer design and synthesis, of which 41 were found to be polymorphic between two parental lines of an established mapping population, allowing 46 loci to be linked to the genetic map. A subset of 35 SSRs was also used on a collection of 132 Camelina genotypes to study diversity patterns among Camelina germplasm. This will lead to identification of diverse Camelina genotypes that could be used as potential parents in future breeding programs and gene expression analysis studies. Apart from the genomics resources developed above, efforts are underway to employ next-generation sequencing (NGS) technology and Reduced Representation Library (RRL) methods for partial sequencing and assembly of two Camelina genotypes, 'Lindo' and 'Licalla', parents of the mapping population. The two assemblies will provide a framework for the development of genome-wide SNP markers for linkage mapping and association analysis, and for further investigations into Camelina genome content and comparisons with plants of the Brassicaceae family, the model Arabidopsis and the Brassica crops.

**94. DEVELOPING A CORE COLLECTION (*BRASSICA NAPUS L.*) FOR RECURRENT SELECTION BASED ON SRAP MARKER****Laiqiang Song, Lunlin Chen, Xiaoyun Zou, Xiaofen Zou, and Shuyu Li**

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Recurrent selection is an effective pathway for genetic enhancement of many important traits, especially of the complex quantitative traits. Sampling of the donor parents is a critical step to start recurrent selection. A core collection in a small size but preserving most of the genetic diversity of the original collection increases work efficiency. Evaluation of genetic diversity based on molecular marker has proved to be a practical approach to construction of such a core collection. In our research, 126 accessions of rapeseed breeding lines or parents were employed for SRAP marker analysis, and evaluation of genetic diversity was made with the markers obtained. A stepwise reduce method was used to screen for a core collection. The analysis resulted that a sample with 28 lines, only 21.88% of the entire collection, was chosen as the core collection. The reserved rate of polymorphic loci was 95.3%, and the reserved rate of alleles observed, effective number of alleles, Nei's gene diversity and Shannon's information index were 98.28%, 100.98%, 100.55%, 99.52% respectively. These parameters demonstrated that this collection could excellently represent its initial one. The 28 lines were crossed to a dominant genic male sterility line, and the resulted hybrids were equally mixed to generate a base population which will be applied to recurrent selection for higher oil content and improvement of other important traits.

**95. GENETIC DIVERSITY AND POPULATION STRUCTURE OF *BRASSICA RAPA L.* BASED ON CHLOROPLAST AND NUCLEAR MICROSATELLITES****Yu Takahashi<sup>1</sup>, Shuji Yokoi<sup>1</sup>, Hiroyuki Iwata<sup>2</sup>, Makoto Kawase<sup>3</sup> and Yoshihito Takahata<sup>1</sup>**

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In order to estimate processes of the domestication and divergence to subspecies in *Brassica rapa*, 6 chloroplast microsatellites for 374 accessions and 21 nuclear microsatellites for 189 accessions were analyzed. Accessions were composed of cultivars and landraces from different geographical regions like Europe, Africa, Russia, West Asia, Central Asia, South Asia and East Asia.

Analysis using 6 chloroplast microsatellites revealed that 25 haplotypes were detected in these accessions. The median-joining network analysis of these haplotypes showed that haplotypes could be roughly categorized into four haplogroups. European and South Asian accessions exhibited high value in gene diversity  $H$  for chloroplast haplotypes and Russian and East Asian ones were found low value. The genetic variances among geographic regions were 4 times higher than those among subspecies in the analysis of molecular variance. STRUCTURE analysis and principal coordinate analysis using the nuclear microsatellites showed that 189 accessions could be divided into two major differentiated classes, Europe and Asia.

The region with high-level genetic diversity is considered to be geographical origin of the cultivated crops. Several researches indicated that *B. rapa* species originated from the highland near the Mediterranean Sea and the primitive cultivated type of *B. rapa* originated in Europe or Central Asia based on the morphology and molecular marker. Our results are agreement with previous reports.

#### 96. HIGH-THROUGHPUT SINGLE NUCLEOTIDE POLYMORPHISM (SNP) DISCOVERY AND MARKER VALIDATION IN *BRASSICA NAPUS*

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Canola (*Brassica napus* L., AACCC,  $2n = 4x = 38$ ), an allotetraploid formed from the diploid *B. rapa* (AA,  $2n = 2x = 20$ ) and *B. oleracea* (CC,  $2n = 2x = 18$ ), is the most important vegetable oilseed crop in the world. The development of genomics tools and resources for canola molecular breeding is very challenging because of the highly duplicated nature of the genome. In this poster, we describe high-throughput SNP discovery and marker validation in canola. More than 21,000 *in silico* SNPs were identified from nearly 500,000 canola expressed sequence tags (ESTs). A total of 3,000 putative SNPs with high confidence were selected to design a 1,536-plex Illumina OPA. The 1,536 SNPs were validated with 235 canola lines using the GoldenGate SNP assay on the Illumina BeadStation. More than 90% of the SNP markers produced good SNP clustering patterns, and 42% of the SNP markers were polymorphic among the canola lines. The polymorphic information content (PIC) scores of the informative SNPs ranged from 0.02 to 0.50 with an average of 0.36. Based on the 569 polymorphic SNPs, a Dice similarity matrix and corresponding dendrogram were produced; seven clades and five clades were observed in the spring and winter germplasm, respectively. This study demonstrated the utility of Illumina SNP genotyping platform for high throughput SNP validation in a polyploid crop like canola and also provided useful genotypic information for canola breeding.

#### 97. DEVELOPING GENETIC RESOURCES FOR *BRASSICA CARINATA*

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*Brassica carinata* ( $2n = 34$ , BBCC) (Ethiopian Mustard, Abyssinian mustard) has desirable qualities, including disease resistance (black leg, alternaria leaf spot), insect resistance (flea beetles, aphids) and drought tolerance, suitable for a new Prairie crop platform. The crop could provide new sources of sustainable non-food raw material either biomass to be processed into energy or oil which could be transformed into liquid biofuels or erucic acid derivatives for the plastic industry. Traditional breeding efforts are underway to improve such traits as oil content, yield, crop architecture and introduce early flowering alleles. Since there are currently limited genetic resources available for molecular breeding in *B. carinata*, we are developing a robust set of genome-wide markers for rapid trait introgression. Thirty-nine *B. carinata* lines selected from collections around the world were screened using a set of A/C (*B. napus*) and B (*B. juncea* and *B. nigra*) genome Brassica simple sequence repeat (SSR) markers to determine the genetic diversity represented among the lines. Six of the most genetically distinct lines are being used for deep 3' biased transcriptome sequencing (Roche 454) to identify single nucleotide polymorphisms. (SNPs). Initial sequencing efforts have focused on the parental lines from a segregating doubled haploid population to allow the SNP loci to be placed on a genetic linkage map. The developed SNP markers and associated genetic map will be a powerful tool for marker-assisted breeding in *B. carinata*.

**98. ALIEN INTROGRESSIONS FOR GERMPLASM ENHANCEMENT IN *BRASSICA JUNCEA*****Surinder S. Banga**

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Productivity in *Brassica juncea* is limited by susceptibility to an array of biotic and abiotic stresses. These include alternaria blight, sclerotinia rot, mustard aphid, drought and low/high temperatures. Despite massive germplasm evaluation; it has so far not been possible to identify any transferable source of resistance to these stresses.

Phenotyping a fairly large collection of wild crucifers allowed us to recognize some very important sources of resistance. Four sets of introgression lines have been developed in *B. juncea* for resistance to sclerotinia rot, seedling stage low temperature stress and mustard aphid. These carry introgressions from *Erucastrum cardaminoides* ( $2n = 18$ ), *Diplotaxis tenuisiliqua* ( $2n = 18$ ), *E. abyssinicum* ( $2n = 32$ ) and *Brassica fruticulosa* ( $2n = 16$ ). The general breeding scheme to introgress genomic segments from wild crucifers involved synthesis of intergeneric hybrids, *E. cardaminoides*/*B. rapa*, *E. cardaminoides*/*B. nigra*, *B. juncea*/*D. tenuisiliqua*, *B. juncea*/*E. abyssinicum* and *B. fruticulosa* /*B. rapa*. Chromosome doubling was achieved using colchicine in cross combinations involving monogenomic *Brassica* species to recover fertility. In hybrids of wild crucifers with digenomics, chromosome doubling was not required as these were partially fertile. The synthetic amphiploids or the trigonomic hybrids were subsequently used as pollen/seed parents to hybridize with *B. juncea* ( $2n=36$ ). This was followed by three to four generations of selfing using single pod descent method. Phenotyping of these introgression lines have shown them to be valuable genetic resource base for resistance to the target defensive traits. Introgression lines of *B. napus*, carrying genomic segments from *E. cardaminoides* and showing sclerotinia resistance are also available.

**99. TRANSGENIC COFFEE ALPHA-GALACTOSIDASE IN OILSEED *BRASSICA NAPUS* REDUCES STACHYOSE IN SEED****Heather Ray, Cheryl Bock and Fawzy Georges**

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The utility of defatted seed meal from many crops such as canola (*Brassica napus*) is limited by such antinutritional factors as the raffinose series oligosaccharides. Anaerobic breakdown of these sugars in the digestive tract of monogastric livestock is a major source of farm gases. We used an alpha-galactosidase gene isolated from *Coffea arabica* to transform *Brassica napus* under a 35S promoter, to reduce the accumulation of these oligosaccharides. We found that this approach significantly reduced stachyose accumulation, by more than 80% in some lines. Stachyose reduction was usually accompanied by a proportionate increase in sucrose and effects on myo-inositol and galactinol, but raffinose levels were only slightly changed. In leaf, expression of genes associated with myo-inositol and phytate metabolism was little affected, except that in a few lines the expression of phosphatidylinositol 4-phosphate 5-kinase was increased up to 100-fold. Seed meal from canola lines with reduced sucrose galactosides would be expected to have improved value for livestock feed.



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*\*Registrants as of August 22, 2010*

## Upcoming Meetings:

### ***13<sup>th</sup> International GCIRC Rapeseed Congress***

June 5-11, 2011

Prague Congress Centre

Prague, Czech Republic

<http://www.irc2011.org/information.html>

(Revised) Abstract Deadline: Sept 30 2010

### ***6<sup>th</sup> ISHS International Symposium on Brassicas & 18<sup>th</sup> Crucifer Genetics Workshop***

November 12-16, 2012

Catania, Sicily, Italy

### ***2010 Canola Industry Meeting & 7<sup>th</sup> Applying Genomics to Canola Improvement Workshop***

December 8-9, 2010

Hilton Garden Inn

Saskatoon, SK

<http://brassicagenomics.ca>

17<sup>th</sup> Crucifer Genetics Workshop

# BRASSICA 2+10

September 5th – 9th

Saskatoon, SK

