

Overview of Brassica Breeding and Genomics Research at AAFC

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National Research Priorities

1. Enhance human health and wellness through food, nutrition and innovative products
2. Enhance the quality of food and the safety of the food system
3. Enhance security and protection of the food supply
4. Enhance economic benefits for all stakeholders
5. Enhance environmental performance of the Canadian agricultural system
6. Enhance understanding of Canadian bioresources and protecting and conserving their genetic diversity
7. Develop new opportunities for agriculture from bioresources

Scientific Programs

- Brassica Breeding:
 - Bifang Cheng – *Brassica juncea* & *Sinapis alba*
 - Kevin Falk – *Brassica rapa*, *B. carinata* & *Camelina sativa*
 - Gerhard Rakow – *Brassica napus*
 - Ginette Séguin-Swartz – *Brassica napus* & *Camelina sativa*
- Brassica Genomics:
 - Hossein Borhan – *Brassica pathogen genomics*
 - Margie Gruber – *Functional genomics of Brassica and Arabidopsis*
 - Ali Hannoufa – *Functional genomics of seed secondary metabolism*
 - Dwayne Hegedus – *Seed protein, insect and disease resistance*
 - Derek Lydiate – *Brassica genetics & genomics*
 - Isobel Parkin – *Brassica genetics & genomics*
 - Kevin Rozwadowski – *Recombination biology*
- Brassica Genetic Resources:
 - Lone Buchwaldt – *Brassica genetics and genomics*
 - Richard Gugel – *Crucifer genetic resources*



Cheng - Condiment Mustard Breeding

Yellow mustard (*Sinapis alba*):

- Development of high-yielding yellow mustard by exploiting heterosis in synthetic varieties
- Improving the uniformity of agronomic characters by developing self-compatible elite inbred lines (OP varieties)
- Mapping and identifying molecular markers linked to self-(in)compatibility, quality (high protein and high mucilage contents) and yield traits for MAS



Cheng - Condiment Mustard Breeding

Brown and oriental mustard (*Brassica juncea*):

- Broadening the genetic base of brown and oriental condiment mustard germplasm and developing elite breeding lines with high yield and improved quality traits (large seed size and low fixed oil content)
- Transferring the Ogura CMS hybrid system into brown and oriental condiment mustard for development of high-yielding hybrid varieties
- Establishing heterotic gene pools
- Disease resistance breeding: white rust resistance



Cheng - Canola *B. juncea* Breeding

- Developing canola *B. juncea* germplasm with broad genetic base, high yield and desirable quality profiles, disease and pod shattering resistance
- Improving the Ogura CMS hybrid system in canola *B. juncea*
- Establishing heterotic gene pools
- Developing canola *B. juncea* germplasm with high stability specialty oil (high oleic and low linolenic acid contents)



Falk: *B. rapa* breeding

- Pollination control system development.
Ogura based un-restored hybrids.
Transferring Rf gene from *B. juncea* using MAS.
- Develop cultivars resistant to brown girdling root rot (BGRR), blackleg and white rust.
Traditional disease nursery selection (Klein-Gebbinck) linked to marker development.
Blackleg resistance. Currently working with two durable sources .
- Development of clubroot resistant germplasms.
Collaborative effort - Peng (AAFC) and Rahman (UofA).



Falk: *B. carinata* breeding

Early maturing strains & industrial platforms.

- High oil/protein content and high glucosinolates - bio-refinery.
- Second generation germplasms have been developed and are being evaluated by industry. Focus on bio-lubricants not fuel.

Novel fat profiles.

- Low erucic acid forms (interspecific transfer) field tested (stable).
- Low C22:1 strains are currently being used by industry partners.

Future direction/work.

- Work continues to identify early maturing/adapted lines and DHs.
- Focus on meal (high protein fish feed) and high/low erucic acid oil.
- Hybridization system - maximize yield and improve uniformity.
- Value added products - using new technologies or processes (Wanasundara).



Falk: Camelina breeding

- Great potential as bio-lubricant or molecular farming platform.
- Develop cultivars adapted to western Canada.
- High oil/protein content.
- Industrial oil platform.
- Large seed size. Increase KWT by 50% (Gugel).
- Disease resistance - downy mildew. Collaboration with Claude Caldwell at NSAC.
- Herbicide tolerance (Hall at U. of A., Johnson at AAFC - Scott).



Rakow - *Brassica napus* (canola) germplasm

Gene pool development for breeding of highly productive hybrid cultivars.

- genetic diversity and heterosis
- combining ability
- utilization of winter rape in summer rape improvement
- interspecific approaches, resynthesis

Rakow - Brassica napus (canola) germplasm

Reducing production risks

- better adapted earlier maturing inbreds one week earlier than current germplasm
- resistance to blackleg disease
other diseases?
- shatter tolerance, interspecific approach
- standability



Rakow - Brassica napus (canola) germplasm

Oil and meal quality improvement

- increase oil and protein content
- high stability specialty oil
low 18:3, high 18:1
- low total saturated fat (<5%)
- yellow seed (high oil, low fibre meal with increased meal energy content)
- zero glucosinolate

combine all quality traits with yellow seed trait in agronomically superior, early maturity lines, with high yield, resistance to blackleg and superior combining ability.



Rakow - *Brassica napus* (canola) germplasm

Yellow seeded *B. napus* canola as a new standard for Canada.

- adapted early maturing disease resistant, shatter tolerant hybrids for farmers.
- high quality seed (oil and meal)
- identity preserved production platforms to guarantee quality attributes



Séguin-Swartz - Brassica breeding

• Oilseed brassicas

- Identification of new sources of resistance/tolerance to canola and mustard diseases, *e.g.*, blackleg & sclerotinia stem rot, to ensure durable resistance/tolerance.
- Development of germplasm of *B. napus* and *B. rapa* tolerant to sclerotinia stem rot for crop improvement by Drs. G. Rakow and K. Falk.
- Studies of the gene flow potential between genetically modified canola and other *Brassica* species, *e.g.*, *B. carinata* (Ethiopian mustard), in field and controlled environments; determination of the fertility of interspecific hybrids.
- Development of doubled haploid technology and lines of oilseed brassicas (mainly *B. napus* & *B. juncea*) for crop improvement and genetic & genomics studies by researchers at AAFC and other institutions.



Séguin-Swartz - Camelina

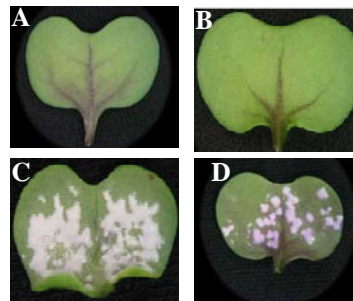
- ***Camelina sativa* (false flax)**

- Development of germplasm tolerant to disease, currently sclerotinia stem rot, for crop improvement by Dr. K. Falk.
- Studies of the mechanism of stem rot tolerance and development of molecular markers associated with the tolerance.
- Assessment of the potential for gene flow between false flax and oilseed brassicas (*B. napus*, *B. rapa*, *B. juncea* & *B. nigra*), weedy *Camelina* relatives (*C. microcarpa*, *C. alyssum* & *C. rumelica*) and common crucifer weeds, e.g., *Capsella Bursa-pastoris* (shepherd's purse).
- Development of doubled haploid technology for *C. sativa*.



Borhan: Genomic study of resistance to Brassica pathogens

- Identifying resistance genes and transferring to Brassica crops to improve disease resistance
Example: White rust resistance using the WRR4 gene from *Arabidopsis thaliana*.

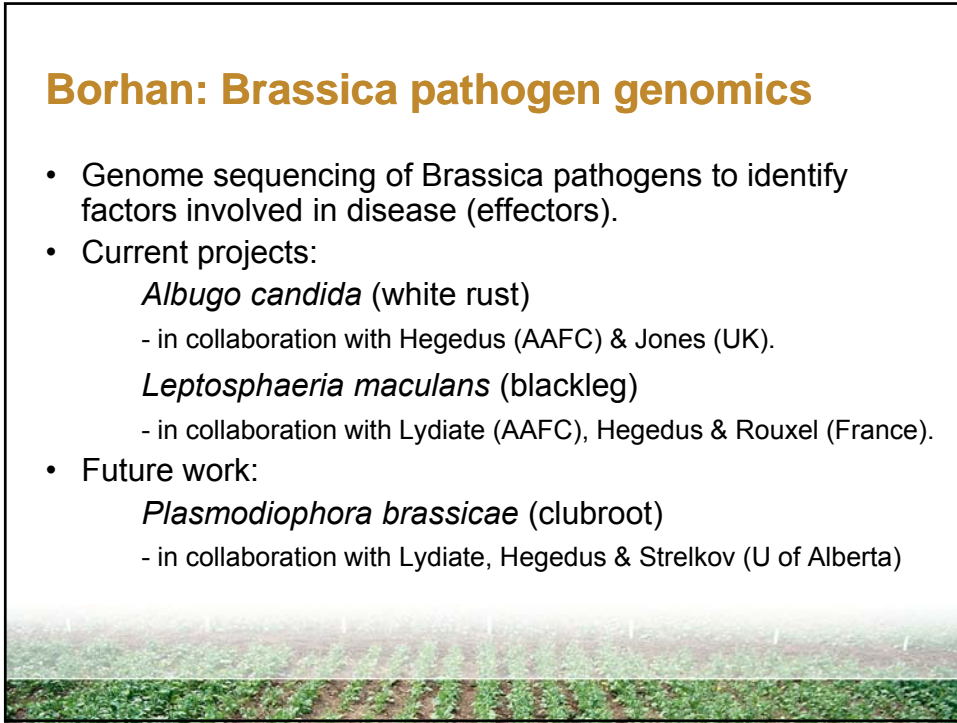


A & B: *B. juncea* and *B. napus*
WRR4 transgenic resistance
C & D: wild type susceptible



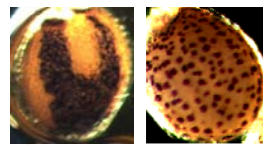
Borhan: Brassica pathogen genomics

- Genome sequencing of Brassica pathogens to identify factors involved in disease (effectors).
- Current projects:
 - Albugo candida* (white rust)
 - in collaboration with Hegedus (AAFC) & Jones (UK).
 - Leptosphaeria maculans* (blackleg)
 - in collaboration with Lydiate (AAFC), Hegedus & Rouxel (France).
- Future work:
 - Plasmodiophora brassicae* (clubroot)
 - in collaboration with Lydiate, Hegedus & Strelkov (U of Alberta)

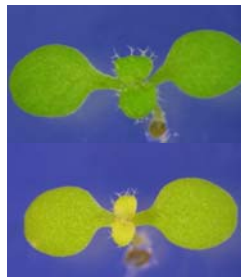


Gruber – Knowledge generation Arabidopsis

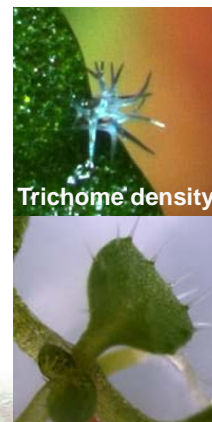
- Functional genomics of trichomes and secondary metabolites (carotenoids and flavonoids)
 - discovery of new Arabidopsis mutants and genes



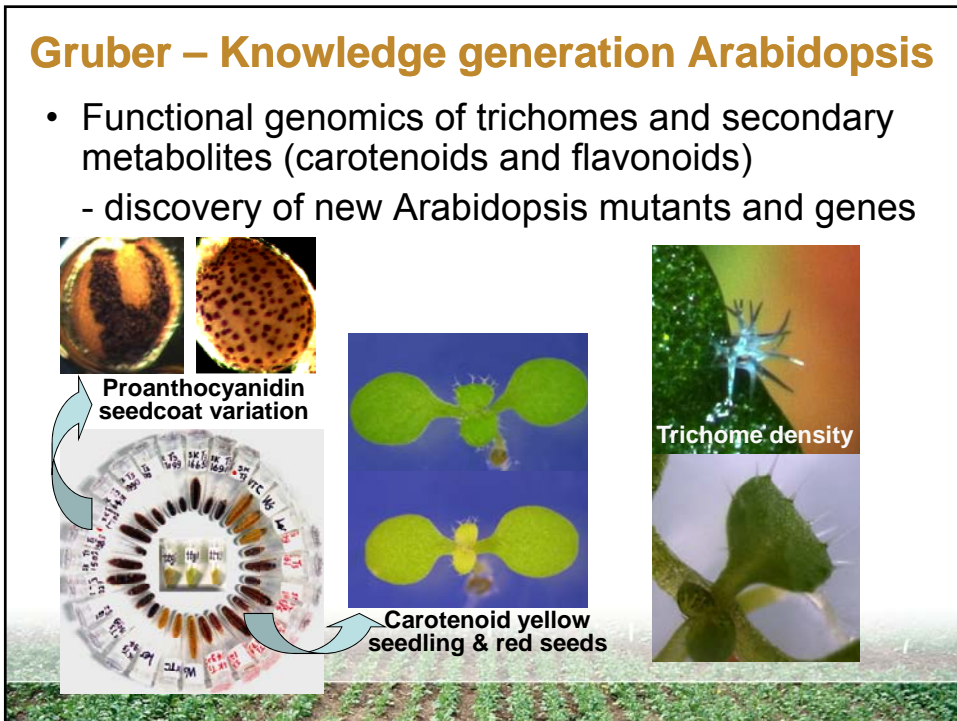
Proanthocyanidin seedcoat variation



Carotenoid yellow seedling & red seeds



Trichome density



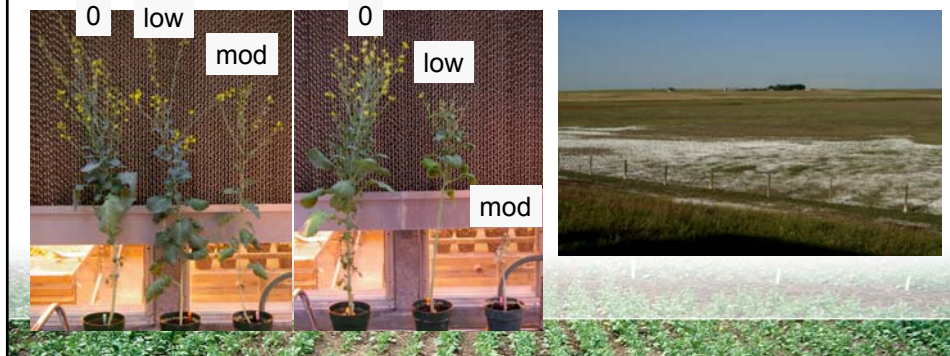
Gruber – Application in *Brassica napus*

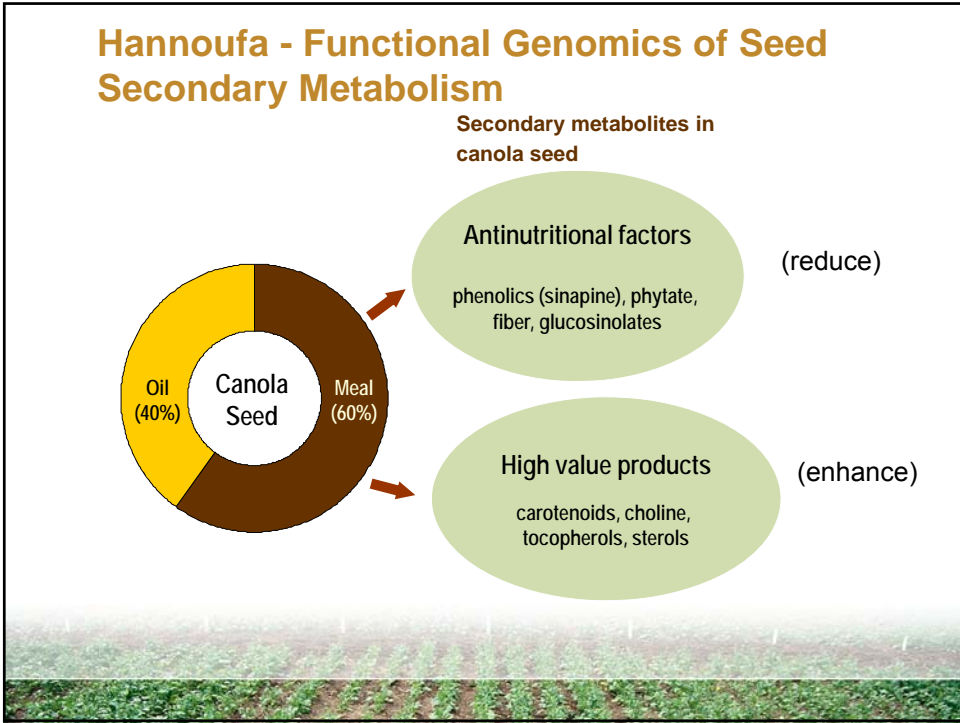
- “Bamboozling the Flea Beetle” with *Hairy Canola* germplasm
- Modifying trichome density and branching using *Arabidopsis* genes
- Discovery and characterization of *B. napus* and *B. villosa* trichome genes
- Enhancing anti-oxidant capacity with flavonoids and carotenoids
- Can canola residue be a bioethanol feedstock? Lignin modification and hydrolytic enzyme expression.



Gruber – *Brassica carinata*

- Cellulosic Biofuel Network - supporting development of biorefinery/biofuel crops for marginal land use
- Enhancing stress tolerance for the saline prairies
- Metabolome, cell wall, and transcriptome analysis of Na sulphate tolerant and intolerant lines





Hannoufa - Functional Genomics of Seed Secondary Metabolism

Reducing levels of antinutritional factors

- Comprehensive functional genomics analysis of the sinapine, lignin and phytate biosynthesis pathways in *Brassica napus* and *Arabidopsis*
- Designing and testing of molecular strategies to reduce levels of sinapine, lignin and phytate in *Arabidopsis* and *B. napus*
- Reduction of anti-nutritional factors in *B. napus* seed:

<u>Antinutrient</u>	<u>reduction achieved</u>
Sinapine	90%
Lignin	45%
Phytate	55%

Hannoufa - Functional Genomics of Seed Secondary Metabolism

Enhancing the carotenoid profile of *B. napus* seed

- Isolation and characterization of *Arabidopsis* mutants with altered carotenoid profiles
- Enhancement of the carotenoid content of *B. napus* seed through RNAi silencing of *DET1* and ϵ -*CYC*, and overexpression of miRNA156b.
- Isolation of TILLING mutants of *B. napus* with nonsense and missense mutations of ϵ -*CYC* (collaboration with Dr. G. Haughn, UBC).

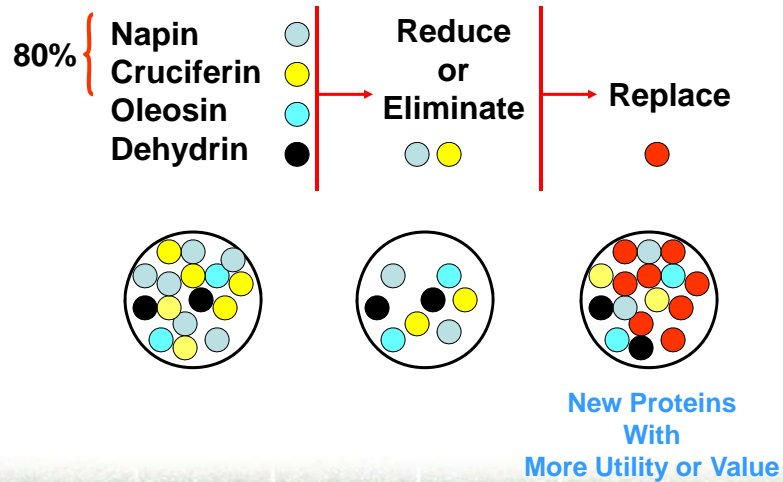


Hegedus – Seed protein production

- Genomic analysis of seed storage protein genes in *B. napus*, *B. carinata* and *Camelina sativa*
- Empty Seed Platform
 - Technology to eliminate and replace endogenous seed storage proteins with those of greater value, with specialized utilities (industrial enzymes) or enhanced nutritional properties (suited to certain livestock or fish)
- Protein targeting
 - Identifying the requirements to target proteins to seed storage compartments to allow accumulation to very high levels
 - In collaboration with Lydiate, Rozwadowski & Wanasundara



Hegedus - The “Empty” Seed Concept



Hegedus - Disease resistance

- Resistance to sclerotinia stem rot in *B. napus*
 - Molecular markers for sclerotinia resistance based on gene expression profiling and association mapping
 - Characterization of *S. sclerotiorum* hydrolytic enzymes and necrosis-inducing proteins and interaction with plant defenses.
 - In collaboration with Buchwaldt, Parkin & Lydiate
- Resistance to other diseases in *B. napus* canola
 - Sequencing of pathogen genomes to identify factors involved in disease and resistance
 - *Plasmodiophora brassicae* (clubroot) genome
 - In collaboration with Borhan, Lydiate and Strelkov (U of Alberta)
 - *Leptosphaeria maculans* (blackleg)
 - In collaboration with Borhan, Lydiate & Rouxel (France)

Hegedus – *Insect Resistance*

- Genomic resources for Canola pests
 - Bertha armyworm bacterial artificial chromosome library
 - Catalogue of genes expressed in gut of bertha armyworm, flea beetle, diamondback moth and root maggot
 - Proteomic analysis of bertha armyworm peritrophic matrix (membrane that regulates nutrient uptake)
 - Developing strategies for insect control by targeting digestive enzymes and proteins involved in nutrient uptake to disrupt digestive processes
 - Improving the efficacy of biological control agents by disrupting barriers to insect pathogen infection in midgut
 - In collaboration with Erlandson, Theilmann (PARC) and Donly (SCPFRC)



Lydiate – *Brassica genetics and genomics*

- Resistance to blackleg in *Brassica napus* canola – collaboration with industry breeders
 - mapping and characterisation of major genes for marker assisted breeding
 - single gene substitutions lines for more predictable disease trials
 - cloning of resistance genes and avirulence genes to enable the future design of new resistance
 - mapping genes for quantitative resistance in Australian varieties
 - genetic characterisation of LepR4 a new recessive resistance gene



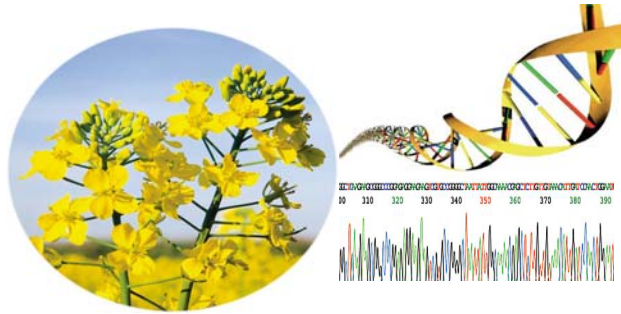
Lydiate – *Brassica* genetics and genomics

- Resistance to other diseases in *B. napus* canola
 - mapping genes for resistance to sclerotinia for marker assisted breeding in collaboration with Dr. Lone Buchwaldt
 - mapping genes for resistance to clubroot in collaboration with Dr. Steve Strelkov (U of A)
- Improving the efficiency of marker-assisted crop breeding
 - developing improved genetic markers for *Brassica* crops
 - SNP markers and genotyping chips in collaboration with Drs. Isobel Parkin and Andrew Sharpe (PBI)
 - producing diagnostic and predictive computer programs to improve the selection strategies in marker-assisted breeding in collaboration with Dr. John Nixon

Lydiate – *Brassica* genetics and genomics

- Improving the quality of canola meal
 - genetic characterisation of a new *B. napus* source of yellow seed
 - modifying the protein profiles of *Brassica* seeds in collaboration with Drs. Dwayne Hegedus and Kevin Rozwadowski
- Enhancing the genetic recombination behavior of *Brassica napus* in collaboration with Dr. Kevin Rozwadowski
 - Stabilizing the amphidiploid genome to improve the uniformity of canola varieties
 - Stimulating homologous recombination to allow the introduction of beneficial genes through interspecific hybridization

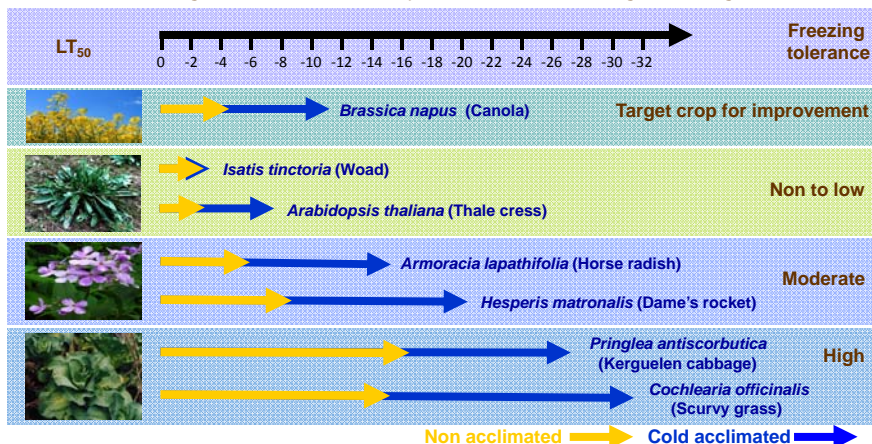
Parkin-Mapping the Genomes of Brassica Species



- **Canadian Canola Sequencing Project (CanSeq)**
 - Collaboration between AAFC and NRC-Plant Biotechnology Institute
- Genome sequences of *Brassica rapa*, *B. oleracea*, *B. nigra* and *B. napus* will be generated
 - Identify the Brassica gene complement
 - Map the genome structure
 - Generate comprehensive genome-wide marker sets

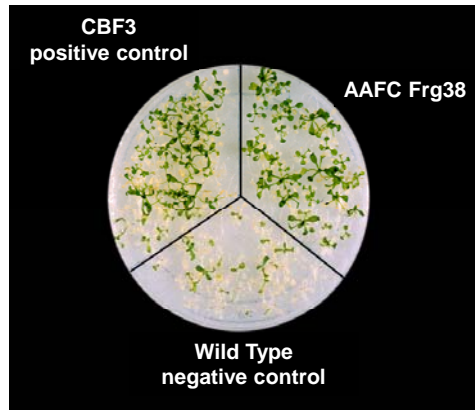
Parkin - Exploiting Genetic Diversity within the Brassicaceae

- comparative genomics to identify factors controlling freezing tolerance



- Combine transcriptome and metabolome data to uncover pathways controlling freezing tolerance
- Discover genes/pathways from related freezing tolerant species to exploit in canola

Parkin - Functional Genomics - exploiting the Arabidopsis model



Plants frozen to -5°C for 12 hours

- Population of 50,000 Arabidopsis mutagenised lines
- Population screened to identify genes controlling:

Freezing tolerance
Trichome development
Accumulation of anti-nutritional compounds

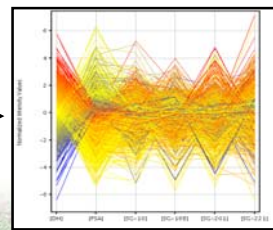
Parkin - Genetical Genomics of Seed Composition

- ERA-PG – collaboration between Canada (AAFC, NRC-PBI), UK and Germany

- Uncover the genetic mechanisms governing variation in complex phenotypic traits (seed composition)
 - combine population structure, marker information, phenotypic data and gene expression data
- Mapping expression QTL (eQTL) – associating the expression of individual genes with key seed quality traits



Assessing phenotype



Monitoring gene expression

Parkin - Developing genetic resources for industrial crop platforms

- *Camelina sativa* and *Brassica carinata*

- Assessing genetic diversity in available germplasm
- Generating deep transcriptome data using next-generation sequencing technologies
 - Identify genes
 - Discover SNP polymorphisms
- Developing robust gene related markers for crop improvement through marker assisted breeding and genomic selection
- Associate markers with traits of interest



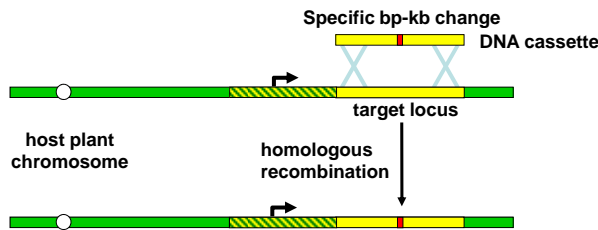
Rozwadowski - Recombination Biology

- Investigation of DNA recombination and repair processes in plants
- Engineering of plants with altered homologous recombination activities in
 - vegetative cells to facilitate gene targeting
 - in meiotic cells to alter genetic linkage



Rozwadowski - Applications in gene targeting: altered recombination and DNA delivery

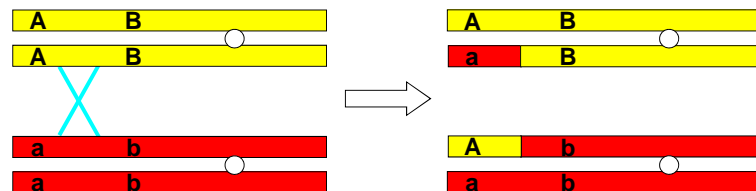
- Gene targeting enables prescribed, specific and predictable alterations of plant genomes



- precisely modified target locus with no second site mutations
- native chromosomal context for predictable expression
- genetically stable for heritable phenotype and commercialisation
- no selectable marker retained to promote regulatory & consumer acceptance
- achieve:
 - *in vivo* protein engineering
 - absolute gene inactivation with FTO
 - gene replacement
 - promoter replacement

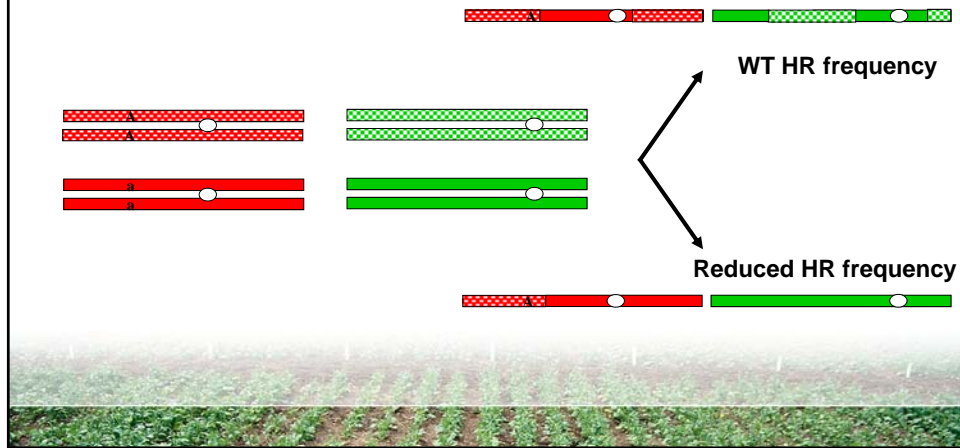
Rozwadowski - Applications in Plant Breeding: increased recombination frequency

- Increasing meiotic recombination frequency breaks linkage between close genes.



Rozwadowski - Applications in Plant Breeding: decreased recombination frequency

- Decreased meiotic recombination frequency suppresses blending of genomes and accelerates recovery of the recurrent genotype.



Buchwaldt – *Brassica* genetics and genomics

- New sources of resistance to sclerotinia stem rot identified in *Brassica napus* from Kashmir, Pakistan, China, South Korea and Japan.
- Molecular markers associated with QTLs for sclerotinia resistance identified in Zhongyou 821 (China); identification of molecular markers linked to resistance in new spring type lines is in progress – in collaboration with Derek Lydiate

Buchwaldt – *Brassica* genetics and genomics

- Candidate defense genes identified in Zhongyou 821 using a 15,000 oligonucleotide microarray platform – verification of phenotypic effect by transformation of *B. napus* and *Arabidopsis* is underway
- Candidate defense genes also pursued in new *B. napus* material by association mapping technologies (90,000 oligonucleotide microarray platform) - in collaboration with Dwayne Hegedus and Isobel Parkin



Gugel – Crucifer genetic resources

- Crucifer genetic resources are conserved by Plant Gene Resources of Canada (PGRC)
 - PGRC mandate: preserve the genetic diversity of crop plants, their wild relatives and native species important to Canadian agriculture and biodiversity
 - Acquire genetic resources and maintain them in a national collection
 - Characterize genetic resources and conduct research on topics related to genetic resource conservation
 - Distribute genetic resources to clients
 - Rejuvenate / increase genetic resources as required
 - Manage and distribute genetic resource bio-information through the GRIN-CA database (<http://pgrc3.agr.ca>)
 - Focus of conservation efforts is currently on adapted germplasm
 - Current crucifer holdings include *Brassica carinata*, *B. juncea*, *B. napus*, *B. nigra*, *B. oleracea*, *B. rapa*, *B. tournefortii*, *Camelina sativa*, *Crambe abyssinica*, *C. hispanica*, *C. glabrata*, *C. filiformis*, *Eruca sativa*, *Raphanus sativus*, *Sinapis alba*, *S. arvensis*, and several other (often weedy) species



Gugel – Crucifer genetic resources

- Characterization of crucifer accessions is a major activity; focus is on traits of interest to plant breeders
 - **Agronomic traits:** maturity, height, lodging, seed size
 - **Seed quality traits:** oil and protein content; fatty acid and glucosinolate profiles; mucilage
- Genetic diversity studies published (collaborators)
 - *Brassica carinata* (Warwick, Falk)
 - *Crambe abyssinica* – *C. hispanica* – *C. glabrata* complex (Warwick)
 - *Eruca sativa* (Warwick, Gomez-Campo)
 - *Sinapis alba* (Katepa-Mupondwa, Fu, Raney)
 - *Brassica rapa* (Fu)
 - *Brassica napus* (Fu)
- Agronomic studies published (collaborators)
 - *Camelina sativa* (Falk)
- On-line resources (collaborators)
 - Guide to wild germplasm of *Brassica* and allied crops (Warwick, Francis)
<http://www.brassica.info/info/publications/guide-wild-germplasm.php>

Gugel – Crucifer genetic resources

- Other activities/collaborations
 - *Camelina sativa*
 - Breeding and agronomy (Falk)
 - Molecular markers (Parkin)
 - Disease resistance (Séguin-Swartz et al.)
 - Aster yellows (Olivier)
 - *Sinapis alba*
 - Proteins (Wanasundara)
 - *Sinapis arvensis*
 - Diversity, fatty acid profiles (Barthet-Canadian Grain Commission)
 - *Brassica rapa*
 - Clubroot resistance (Peng et al.)

