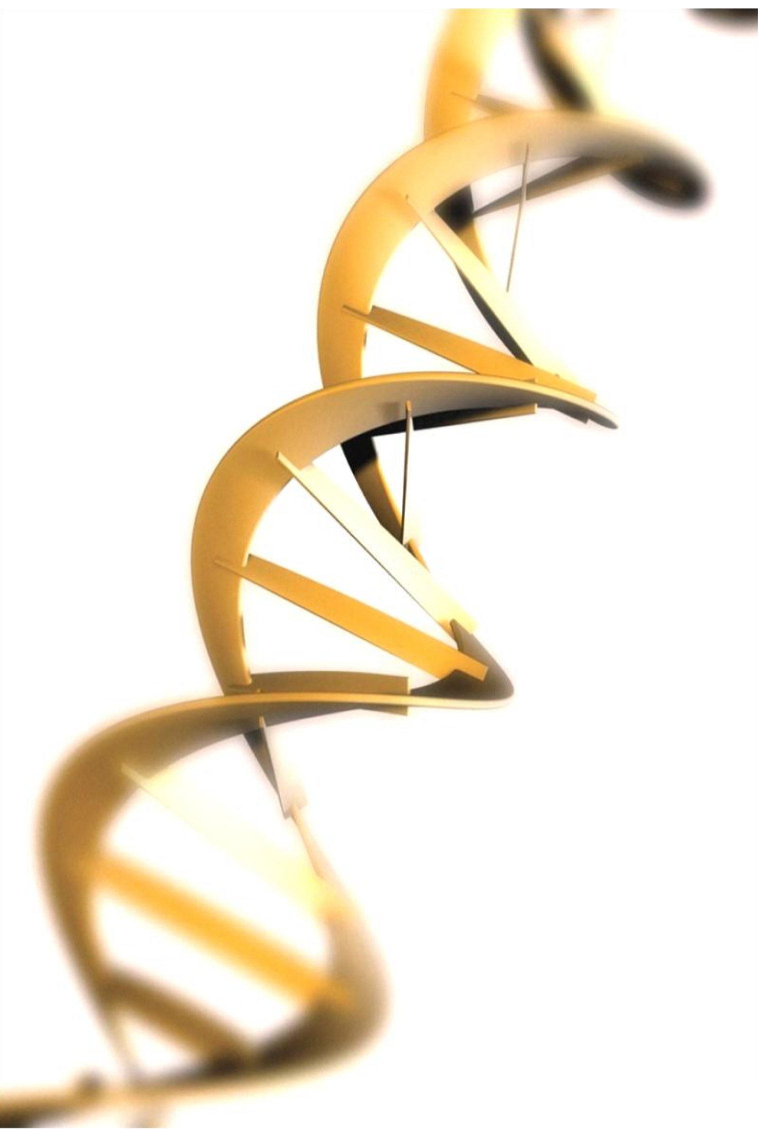

Designing Oilseeds for Tomorrow's Markets

Legacy Report

January 2011

GenomeCanada

GenomeAlberta

An introduction to the DOTM project and this document

The Designing Oilseeds for Tomorrow's Markets (DOTM) project was initiated in June 2006 as a four-year project in collaboration with the German YelLowSin initiative. The Canadian component was funded by Genome Canada (Competition III), Manitoba Energy, Alberta Innovation and Science, Saskatchewan Ministry of Agriculture (Agriculture Development Fund), Agriculture and Agri-Food Canada, National Research Council (Genomics and Health Initiative-3), and Alberta Innovates and Technology Futures (formerly Alberta Research Council).

The objective of the DOTM project was to improve the value of canola meal. Canola is primarily cultivated for its premium oil. However, the seeds are also naturally rich in protein, vitamins, and minerals and yet canola meal is an under-appreciated and poorly utilized commodity. This is evident from the historically consistent discounted price for canola meal at about 40% relative to soybean meal over an almost 20-year period. This is mainly due to the presence of anti-nutritional factors (ANFs) associated with canola meal that detract from realizing the full value of the meal. These ANFs include fibre, phytate, sinapine, and glucosinolates. Fibre increases the feed bulk but reduces the metabolizable energy levels for monogastric animals while phytate binds minerals and proteins, lowering their bioavailability. Sinapine is a bitter substance that is the causal agent of the fishy off-flavour in brown-shelled eggs when the laying hens are fed with canola meal. Glucosinolates inhibit thyroid production and have a variety of negative effects on animals.

Research for the DOTM project was divided into three major themes. Theme One focused on developing a strategy for lowering the fibre content of the seed coat of *Brassica napus*. Theme Two aimed at identifying the genetic mechanisms underlying a yellow seed coat phenotype in *B. napus.* Yellow-seeded canola is an elite line that has good yield, high oil content and less fibre than traditional (black seeded) lines. The goal of Theme Three was to reduce sinapine, phytate and glucosinolates and enhance the carotenoid content, thereby augmenting the nutritional profile of the seed coat. Carotenoids, including carotenes and xanthophylls, are health-promoting substances, and are used as essential supplements in animal and fish feed formulations. A modified canola that accumulates these would be a major advance towards improving canola meal value, especially when the ANF contents of canola seeds are reduced. Underpinning these initiatives was canola meal testing in animals and the GE^{3}LS component as well as services including TILLING, and Bioinformatics.

This document summarizes the work conducted in the project and highlights the resources developed that are available to the scientific community now and after the completion of the project. Inquiries can be made to Dr. Disa Brownfield-Walker, project manager for DOTM (disa@ales.ualberta.ca), Laurie Hayes, project manager (Laurie Hayes (Laurie.Hayes@nrc-cnrc.gc.ca) or to the project leaders, Dr. Randall Weselake (Randall.Weselake@ales.ualberta.ca) and Dr. Gopalan Selvaraj (Gopalan.Selvaraj@nrc-cnrc.gc.ca). A complete list of all investigators (and their contact information) involved in the project can also be found in Appendix 1 of this document.

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Theme 1: Functional Genomics of the Seed Coat

Subproject 1.1 – Investigating the transcriptome of the seed coat

Milestone 1: Preparation of seed coat RNA and completion of 8 microarray hybridizations in *Brassica* and 8 microarray hybridizations in *Arabidopsis*

We conducted global expression profiling of genes of three developmental stages of *Brassica napus* (DH12075) seed coats for comparison with the expression profile of *Arabidopsis thaliana* at comparable stages of seed coat development. Developmental time points of 3, 7 and 11 days post-anthesis (DPA) were selected for *A. thaliana* RNA sampling. A method for extracting RNA from the seed coats of *Arabidopsis* seeds was developed. Using this novel method, expression profiles of two *Arabidopsis* seed coat mutants displaying altered seed coat developmental programs (transparent testa 16 -1 (*tt16-1)* and *apetala2-7 (ap2-7*)) and their respective wild type ecotypes (*Westar-2* and *Columbia-2*) were determined and selected on the basis of seed coat development. Expression profiles of *B. napus* (DH12075) seed coats at the same developmental time periods (3, 7 and 11 DPA) indicate that the largest differences in gene expression are in the biosynthesis of phenylpropanoids and flavonoid metabolism. A platform that contains an updated EST assembly has been created and will be available to the scientific community. This platform also includes all miscellaneous genomic survey sequences that have become available for *Brassica* spp. The *A. thaliana* microarray data is currently accessible by the public from the NRC-PBI website. Work is also progressing to have the information from the microarray displayed on the University of Toronto Bio-Array Resource for Plant Functional Genomics. A publication describing the microarray analysis and methods in the *Arabidopsis* work has been submitted for publication. A publication describing the gene expression dynamics inferred from the microarray analysis of *B. napus* seed coat is in preparation.

Milestone 2: Bioinformatic analysis of seed coat microarray data; selection of 100 interesting target genes

The microarrays completed in Milestone 1 were analyzed using bioinformatics. Target genes were selected based on the differences observed in the expression levels of the *tt16* (transparent testa) and *ap2-7 (apetela 2-7)* mutants (with altered seed coat developmental program) compared to the wild type. A revised microarray platform based on an updated EST assembly has been compiled. Upon completion of the project, the functional characterization of selected *Arabidopsis* genes related to seed coat development will be published and the seed stocks will be sent to the *Arabidopsis* seed stock centre.

Milestone 3: Analysis of 5 selected genes to determine the specificity of seed coat expression

Candidate genes from *Arabidopsis* showing differential expression in the seed coat were identified and analyzed using quantitative real-time PCR (qRT-PCR). Candidate genes included those involved in mucilage synthesis/modification that are differentially expressed during the early stages of seed coat development (3-7 DPA), and those genes involved in secondary cell wall formation/modification which may be upregulated during the later stages of seed coat development (7-11 DPA). Five genes potentially involved in the reduction of seed coat mucilage were discovered: *At5g63180*, *At2g26440*, *At3g14720,*

MAP Kinase 19 (*MPK19*), *At4g19420* and *At4g02050*. *MPK19* was shown to have a role in protein and mucilage development in the seed coat.

Milestone 4: Reverse genetic analysis of 50 selected target genes in *Arabidopsis*

Reverse genetic analysis for 50 genes that were selected based on their expression profile in the microarray analyses was completed. Activities focused on the characterization of the *MPK19* mutations because *MPK19* product is a protein kinase likely involved in signaling and mutations in the *MPK19* gene result in a defective seed coat. Transgenic lines with a variety of constructs are currently being produced. Seed from the transgenic lines will be stored long-term at the Plant Gene Resource Centre, Agriculture and Agri-Food Canada, Saskatoon.

Milestone 5: Transgenic analysis of 10 selected seed coat gene targets in *B. napus*

Transgenic analyses of *TT*16 in yellow-seeded *B. napus* and in DH12075 were completed and showed no evidence of genetic complementation suggesting that this locus does not contribute to the yellow seed coat phenotype. Analysis of other *B. napus* and *Arabidopsis* genes is ongoing. New transgenic lines with selected gene constructs have been developed and this work will be published if there are phenotypic consequences to the perturbation of gene expression. Seed from the transgenic lines will be stored long-term at the Plant Gene Resource Centre, Agriculture and Agri-Food Canada, Saskatoon.

Sub-project 1.2 – Investigating the Transcriptome of the Seed Coat

Milestone 139: Completion of the cell biological characterization of YN01-429

YN01-429 is the most advanced canola-quality, yellow-seeded germplasm in *B. napus*. The low fibre content of the seeds in this line is a desirable feature. Through characterization of YN01-429, the temporal and spatial aspects of seed coat (source of fibre) development have been determined. The developmental window, as well as the specific cell layers, has been identified for the targeted manipulation of fibre content. A publication is in preparation on the molecular aspects of seed coat development in the two contrasting types of *B. napus* and an additional publication comparing the proteomes in the two seed coat types will be published upon completion of the analyses.

Milestone 140: Construct 8 seedcoat cDNA libraries (isolated seed coat where possible)

Fifteen cDNA libraries were constructed and provided a source of physical clones of gene sequences of interest. These libraries are currently held at NRC-PBI, Saskatoon where sequences can be compared and contrasted for the detection of polymorphic markers for marker-assisted breeding.

Milestone 141: Complete a total of 100,000 ESTs

The clones from the DOTM EST collection are available to the public through NRC-PBI, Saskatoon. Interested parties can submit a requisition and a Material Transfer Agreement (MTA) will be required.

Milestone 143: Microarray analysis of black and yellow seed at various stages of development

RNA from black- and yellow-seeded *B. napus* lines were analyzed at four stages of development (12, 15, 18 and 21 DPA). Global gene expression patterns in developing seed coats of black- and yellow-seeded lines provided gene networks that are differentially regulated in these two morphological types. This provided an encyclopedic reference for future manipulation of the fibre-reducing networks. A comprehensive array was designed based on all expressed sequences available. The comprehensive

microarray designed and validated through this work will be available through NRC-PBI, Saskatoon. After publication, the data on microarray hybridization will be available as an open source for further data mining.

Milestone 144: *In situ* analysis of the expression of 10 selected genes

The *in situ* analysis was replaced with qRT-PCR analysis and will use genetic ablation experiments with cytotoxic genes to confirm activity in specific tissues. Fifteen candidate genes were identified through the experiments conducted in milestone 143. Through qRT-PCR, genes were identified that revealed a reduction or near-loss of expression over the course of seed coat development. The selected key genes involved in seed coat metabolism include *Arabidopsis* Response Regulator 22 (*ARR22)*, *cinnamate 4 hydroxylase* (*C4H)*, *chalcone synthase (CHS)*, *flavanone 3B-hydroxylase (F3H)*, *dihydroflavonol 4-reductase (DFR)* and *BANYULS* (*BAN)*. These analyses will be included in the manuscript describing the microarrays.

Milestone 145: Transgenic modification of 10 selected genes

Transgenic lines have been developed using the genes identified in milestones 143 and 144 to evaluate the impact on seed coat phenotype. This transgenic work will be published if insightful perturbations in seed coat thickness are observed. Mutations have been identified in one of the target genes and additional mutations are being screened in this gene (*DFR*) and another gene (*TT16*). A homozygous *DFR* mutant has been obtained. The work on Targeting Induced Local Lesions in Genomes (TILLING)-based mutants will be published should the mutants show a phenotype upon combining mutations in homeologous genes. Seed from the transgenic lines developed will be available; an MTA will be required. The TILLING-based mutations (*DFR* and *TT16*) will be available after the confirmation and characterization is complete; an MTA will be required.

Sub-project 3.4 – Constructs with selected seed-coat expressed genes and generate transgenics

Milestone 162: Constructs with selected seed-coat expressed genes and generate transgenics

This Milestone is encompassed in Milestone 5 and Milestone 145 that deal with *Brassica napus* transgenics. The legacy of Milestone 162 is contained in the sections under Milestone 5 and Milestone 145.

Sub-project 3.1.7 – Molecular approach to target seed coat cell wall biosynthesis

Milestone 203: Identification of 200 seed coat cell wall genes of *Brassica napus*

Over 200 genes were identified as being involved in the seed coat cell wall in *B. napus*. The data is currently published in two sources (Jiang 2010 *et al.* 2010, Chen *et al*. 2010). Additional manuscripts reporting on all other activities will be published upon completion and will be available to the public.

Milestone 204: Identification of 5 seed coat-specific promoters in *B. napus*

Seed coat-specific promoters in *B. napus* have been identified. These promoters will be useful to express relevant seed coat genes to modify canola meal. Four promoters have currently been identified as seed coat specific (*AtLAC15 (A. thaliana Laccase 15), A. thaliana BANYULS (AtBAN), A. thaliana*

vacular processing enzyme (AtVPE), and A. thaliana Gamma Interferon-responsive Lysosomal Thiol reductase (AtGILT). Information on the promoters is currently published in two sources (El-Mezawy et al. 2009 and Wu et al. 2010). Two additional manuscripts reporting on all other activities are in preparation. All promoters will be available to researchers; an MTA will be required.

Milestone 205: Construction of 32 RNAi plasmids with selected cell wall gene sequences and genetic transformation of *B. napus*

Eight novel sequences for targeting by RNA interference (RNAi) to reduce seed coat thickness in *B. napus* were selected. A manuscript describing the gene discovery work has been published (Jing and Deyholos 2009). Sixteen RNAi constructs have been developed and transformed into *B. napus* in order to evaluate the effect of RNAi on target gene expression. The data is included in the manuscript described in Milestone 206.

Milestone 206: Evaluation of transgenics for cellular characteristics and overall phenotype

The transgenic plants developed from Milestone 205 were analyzed. Transgenic germplasm with diminished (RNAi) expression of the *Cellulose synthase A (CesA)* family, *TT12 (transparent testa 12), TT16 (transparent testa 16), alpha carbonic anhydrase (ACA), alpha-galactosidase (aGAL), cytochrome P450 (CYP), gibberellin-regulated family protein (GAS), glycosyltransferase 18 (GT18) , (1-4)-beta-mannan endohydrolase (MAN), UDP-glucose 4-epimerase (UG01) and yellow stripe-like (YLS),* as well as phenotypic information (including transcript profiles) were developed for *B. napus* seeds. Phenotypic results will be available to the public through publications upon completion of the project. Commercially significant improvements in seed quality will be patented. Germplasm will be available to researchers through the University of Alberta; an MTA will be required.

Milestone 207: TILLING of 5 key genes for seed coat cell walls and evaluation of cellular characteristics and phenotypes

This Milestone was deleted with the approval of the Scientific Advisory Board (SAB).

Theme Two: Conventional and Molecular Genetic Investigations into the Yellow-Seeded Phenotype in *Brassica*

Sub-Activity 3.1.1 – Genetic analysis of yellow seeded phenotype and breeding population development

Milestone 132: Make crosses and backcrosses between YN01-429 (yellow-seeded) and DH12075 (black seeded), begin DH production from F_1 crosses; grow parents and make backcrosses, produce F_2 seed.

Crosses and backcrosses between varieties resulted in 3,574 double haploid (DH) plants produced from F_1 and BC_1F_1 generations. Field progeny testing was completed on 2,987 of the DH plants. F_2 plants (7,023) were grown and seed collected for analysis. Upon publication, the data will be available to all researchers. The seed is available (with an MTA) from Agriculture and Agri-Food Canada, Saskatoon until the end of the project through Dr. Ginette Séguin-Swartz and through the Plant Genome Resource Centre thereafter.

Milestone 134: Phenotyping for 3 quality traits (seed color, fibre content and an ANF). Similar tests in year 2 for progeny and parents. Genotyping of DH lines with phenotypic characterization

Seed colour and fibre analysis was completed using reflectance method and ANKOM method digestion assay respectively. The methodology for these procedures is in place at Agriculture and Agri-Food Canada, Saskatoon and is available by request. Upon publication, all information will be available to the public.

Milestone135: Establishment of NIRS for fibre and meal substances (year 1: calibration set development, year 2 application of methodology)

Previous research from Agriculture and Agri-Food Canada, Saskatoon was the basis of the current calibration sets for the FOSS NIRS. This includes calibrations for fibre (acid detergent lignin, acid detergent fibre and neutral detergent fibre) and total glucosinolates. The methodology is in place at Agriculture and Agri-Food Canada, Saskatoon and is available by request. Upon publication, all information will be available to the public.

Milestone 136: Grow F_2 and backcross plants, produce DH plant and increase seed; grow F_2, BC and DH populations in field and determine seed colour. Retest interesting selection from Q5-8 in Q9-12

DH plants were produced and grown in the field. Seed will be increased in the greenhouse. Upon publication, all information will be available to the public.

Milestone 137: Field testing of F_2, BC and DH lines (Q9-12) and confirmation of observation (Q13-16) for the various quality traits under study using NIRS

Field evaluations were completed on more than 600 DH lines and upon publication all information will be available to the public.

Sub-Activity 1.2.2 (3.1.2) - Comparative genetic study on yellow-seeded *B. napus* and identification of molecular markers for yellow seed color genes of *Brassica* A-genome

Milestone 198: Crossing of *B. rapa* and *B. napus*, assessment of polymorphism in the parental *B. rapa* by 60-100 microsatellite (SSR) markers, and initiate DH production from F_1

BC4S1 (590) plants were developed from *Brassica rapa* cross Sampad x 3-0026.27 for mapping of the seed colour genes. Near isogenic lines for seed colour (brown/yellow) were generated. Seed colour, seed quality and agronomic data from greenhouse and growth chamber trials were collected. BC4S1 plants and the near isogenic lines are available to DOTM members. Other interested groups may obtain seed subject to negotiation and an agreement with the University of Alberta. The agronomic data as well as other information collected from the trials will be made available through publications upon completion of the project.

Milestone 199: Production of approximately 100 DH lines from *B. rapa* crosses and evaluation for seed color

Ninety-four recombinant inbred lines were developed from a *B. rapa* cross. Seed colour, seed quality and agronomic data from greenhouse and field trials were collected. All data will be made available through publications upon completion of the project.

Milestone 200: Evaluation of seed color in the DH lines from *B. napus* crosses

Many DH lines were created from *B. napus* crosses: 147 from YN01-429 x CH5034 crosses; 108 from resynthesized napus x CH5034 crosses; 32 from CH5034 x Y1; and 81 from CH5034 x Y2. Seed colour, seed quality and agronomic data from greenhouse and field trials were collected. These DH lines can be distributed to DOTM members. Distribution to other interested groups is subject to negotiation with Agriculture and Agri-Food Canada, Lembke, University of Giessen and the University of Alberta. The agronomic data as well as other information collected from the trials will be made available through publications upon completion of the project.

Milestone 201: Bulk segregant analysis of the 100 *B. rapa* DH lines by use of polymorphic SSR primers

A total of 862 SSR markers screened on the parents; 312 polymorphic markers were identified. Polymorphic markers were used for mapping the seed color gene(s). The molecular markers and agronomic data will be available through publications.

Milestone 202: Screening of *B. rapa* DH populations by the unique primers identified form BSA and identification of the markers for yellow seed color. Testing of the markers on the yellow-seeded *B. napus* as well as on its parents

Three SSR markers (351, 591 and 592) on linkage group 9 were identified to be linked with the major seed colour gene (Br_1) in *B. rapa*. Validation of the markers was completed on F_2 populations of Tori-7 (brown) x Sampad (yellow), 3-0026.27 x TR-4-3-3 (yellow) and Anna (brown) x YS49 crosses. One hundred twenty six SSR markers developed from ESTs. The molecular markers and agronomic data will be available through publications.

Sub-Activity 3.1 – Manipulation of seed coat characteristics

Milestone 133: Fine mapping of QTL for seed colour and fibre compounds, including field trials

A new version of the genetic map of the DH population YE2-DH from the cross Express (black seed) x 1012-98 (yellow seed) was generated including SNP, indel and Cleaved Amplified Polymorphic Sequences (CAPS) markers from orthologous *B. napus* loci for *transparent testa* and other relevant candidate genes from *Arabidopsis*. Seed grown in different years and environments were analyzed for phenotypic data for fibre components in the mapping population using the newly-developed NIRS calibrations. This data was used to localise seed fibre quantitative trait loci (QTL) in the new genetic map. *B. napus* ESTs corresponding to each of the candidate genes were used to develop locus-specific primers for qRT-PCR to determine the expression patterns over different seed developmental stages, from seven days after pollination through to seed maturation. All data will be available through publications.

Milestone 138: Cloning and genetic mapping of relevant TRANSPARENT TESTA orthologs in *B. napus*

New NIRS calibrations were used to estimate acid detergent lignin, acid detergent fibre and neutral detergent fibre contents in mature seeds of the 166 DH lines of the YE2-DH mapping population harvested from four different environments. The mean phenotypic data were used to map environmentally stable QTL for seed fibre components in comparison to seed colour QTL.

Theme Three: Elimination of Anti-nutritional Substances and the Enhancement of Value Added Nutritional Substances in Canola Meal

Sub-Activity 3.2 – Reduction of Sinapine content in *Brassica napus*

Milestone 146: Cominatorial transgenes (currently available) and generate transgenics for ANF

Individual constructs were made for RNAi suppression and transgenic lines were developed to curtail the production of sinapine. The following transgene and gene combinations were studied: *Choline Oxidase* (*COX)*, *Sinapoyl choline esterase* (*SCE)*, *Phenylalanine ammonia lyase* (*PAL)*, *Hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase* (*HCT)*, *Sinapoyl glucose transferase* (*SGT)*, *Feruloyl 5 hydroxylase* (*F5H)*, *Reduced epidermal fluorescence 1* (*Ref 1)*, *Cinnamate 4-hydroxylase (C4H), hydroxycinnamoyltransferase (HCT)*, (*COX-SGT)*, (*COX-Ref 1i)*, and (*COX-SCE)*. All data will be available through publications. The seed will be stored at NRC-PBI, Saskatoon and is available after publication upon request; an MTA is required.

Milestone 147: Generate 5 homozygous lines from above; Sinapine analysis

Non-segregating RNAi lines have been developed from *SGT* RNAi lines, *COX* overexpression lines and *COX-SGT* RNAi lines. Lines are showing promising reductions in sinapine content. The seed will be stored at NRC-PBI, Saskatoon and is available after publication upon request; an MTA is required.

Milestone 148: Refinement of gene combinations; sinapine analysis

Many lines have been developed to test the reduction of sinapine levels. Work to choose the best lines that are stable continues. All data will be available through publications. The seed will be stored at NRC-PBI, Saskatoon and is available after publication upon request; an MTA is required.

Milestone 149: Generation of 5 homozygous lines for further stacking

True homozygous lines have been developed for *COX*, *SGT*, *COX-SGT* and *PAL*. The seed will be stored at NRC-PBI, Saskatoon and is available after publication upon request; an MTA is required.

Milestone 150: Development of an improved NIRS calibration for sinapine content

In lieu of an improved NIRS calibration, a sensitive assay such as HPLC was required and has been standardized. With this HPLC method, single seeds can be analyzed. Methodology is available upon request.

Milestone 151: Further development and characterization of the substitution lines

Lines have been analyzed and the data will be available through publications.

Milestone 152: Transformation of *Brassica napus* with suppression constructs based on genes from the sinapine metabolism

Transformation is complete and transgenic lines will be stored at NRC-PBI, Saskatoon and are available upon request; an MTA is required.

Milestone 153: TILLING population development in winter canola

Populations have been developed and are available from NRC-PBI, Saskatoon upon request; an MTA is required.

Milestone 154: Metabolite profiling of 2 currently available low-sinapine lines

Metabolite profiles of three lines (*COX*, *COX-SGT* and *PAL*) have been developed. This information will be available through publications upon completion of the project.

Milestone 155: Genetic crosses for gene combinations and metabolite profiling

Upon completion of the project, data will be available through publications. Seed will be stored at NRC-PBI, Saskatoon and will be available after publication upon request; an MTA is required.

Sub-Activity 3.3 - Reduction of Phytate content in *Brassica napus*

Milestone 156: Cloning of the full length coding gene for *Bn-PLC2* and *PI3-kinase* and production of appropriate transformation vectors

Coding regions for *B. napus phosphoinositide-specific phospholipase C2 (Bn-PLC2)* and *phosphatidylinositol 3-kinase (PI3-kinase)* have been cloned and vectors for plant transformation were developed. Data will be published upon completion of the project.

Milestone 156b: Cloning of the full length coding regions of *Ipk1* and *Ipk2*. Designing of plant transformation vectors for reduction of the level of expression of corresponding genes using antisense or RNAi approaches

In order to complete Milestone 157 which utilized the *inositol pentakisphosphate 2-kinase (Ipk1)* and *inositol polyphosphate 3,6-kinase (Ipk2)* coding regions in the transformation vectors, this milestone was added early in the project. Gene information will be available through publication and clones will be available through NRC-PBI, Saskatoon with an MTA.

Milestone 157: Production of transgenic canola lines with *BnPCL2, PI3-K, Ipk1* and *Ipk2* approaches separately and in combination. Transformation and functional analysis

Several transgenic canola lines were produced showing different level of phytate reduction. These include: DH 12075 canola lines with over-expression and RNAi/antisense suppression of *BnPLC2* under a constitutive promoter, and over-expression of *PI3-K,* and with seed-specific antisense suppression of *Ipk1* or *Ipk2* genes. Transgenic seeds where *Ipk1* and *Ipk2* were suppressed simultaneously by seed-specific RNAi-mediated gene silencing showed the highest level of phytate reduction (up to 90%), are also available. Transgenic lines with a combination of RNAi-mediated silencing of 3 genes Ipk1 and Ipk2 and MIPS were developed. TILLING lines of the *Bn-PLC2* and *myo-inositol phosphate synthase (MIPS)* genes have been produced and partially analyzed. Data will be available through publications upon completion of the project and germplasm will be available through NRC-PBI, Saskatoon with an MTA.

Milestone 158: Analysis of three homozygous seed lines for phytate (Q 9-10); metabolite analysis (Q-11-12)

All lines tested for phytate content were also analyzed for inorganic phosphate content. Antisense lines with decreased phytate and increased inorganic phosphate content were propagated toward homozygousity. Data will be available through publication upon completion of the project.

Milestone 159: Field assessment of three selected transgenic lines (A13-14) and biochemical analysis of seeds (Q15-16)

Two years (2009, 2010) of field trial data on *MIPS* lines have been generated. Feeding trials were conducted in 2010. Results from the field assessments, feeding trials and biochemical analysis will be available through publications upon completion of the project.

Sub-activity 3.5 - Gene Manipulation in the Biosynthesis Pathway of Aliphatic Glucosinolates

Milestone 163: 8 Homologous genes of *BoGSL-ALK, Bo-GSL-ELONG* and *BoGSL-PRO* in *B. napus* will be identified, sequenced and put on the high density map through restriction fragment length polymorphism (RFLP) mapping

Homologous genes have been identified and sequenced. Eight loci of *Brassica oleracea glucosinolate-alkenyl* (*BoGSL-ALK), B. oleracea glucosinolate- elongation of aliphatic glucosinolate (Bo-GSL-ELONG)* and *B. oleracea glucosinolate- elongation of 3 carbon side chain aliphatic glucosinolate* (*BoGSL-PRO)* were integrated on two ultra-dense genetic maps. One paper has been published in Theoretical and Applied Genetics (Sun et al. 2007) and one manuscript has been submitted to Nucleic Acids Research.

Milestone 164: 4 required crosses will be made for gene replacement Null alleles of *B0GSL-ALK* and *BoGSL-PRO* from broccoli and null alleles of *BoGSL-ELONG* from cauliflower will be used to replace the functional alleles in *B. Rapa* through additional lines and homoeologous lines

All required crosses for dependent milestones have been completed.

Milestone 165: For gene replacement in glucosinolates, 18 additional lines will be produced

Eighteen additional lines have been developed and used to identify gene replacements in the progeny. Gene introgression or replacement lines for *BoGSL-ALK, Bo-GSL-ELONG* and *BoGSL-PRO* were identified. One *Bo-GSL-ELONG* locus was found to co-segregate with a 5-carbon side chain and total aliphatic glucosinolates, and one locus was identified to control the occurrence of 3-carbon side chain glucosinolates. However, the replacement lines of *BoGSL-ALK* did not showed any change due to two loci in the A genomes that were mapped (see milestone 163). Upon publication, all lines will be available from the University of Manitoba upon request; an MTA is required.

Milestone 166: Transformation will be initiated with 6 constructs for RNAi in glucosinolate biosynthesis

New canola germplasm with very low glucosinolate content (<4 µmol/g seed) was produced with RNAi of the *GSL-ELONG* gene family. All lines are available from the University of Manitoba upon request; an MTA is required.

Milestone 167: Lines with gene replacement will be searched with comparative genetic information and flanking SRAP markers of these 10 homologs of *BoGSL-ALK, BoGSL-ELONG* and *BoGSL-PRO*

Gene replacement lines with mapped gene loci have been identified and glucosinolate profiles were changed in the replacement lines of *Bo-GSL-ELONG* and *BoGSL-PRO*. One manuscript is in preparation. Upon publication, all lines will be available from the University of Manitoba upon request; an MTA is required.

Milestone 168: Transformation will be continued with 6 constructs for RNAi in glucosinolate biosynthesis

Over 200 transgenic lines were produced with RNAi constructs of *BoGSL-ALK, Bo-GSL-ELONG* and *BoGSL-PRO.* These transgenic lines are currently available upon request from the University of Manitoba; an MTA is required.

Milestone 169: 10 *B. rapa* lines with gene replacement will be obtained to check the profile change of glucosinolates

New replacement lines have been found using the molecular markers flanking the mapped gene loci. Upon publication, all lines will be available from the University of Manitoba upon request; an MTA is required.

Milestone 170: Transformants with these 6 RNAi constructs will be produced and analyzed to confirm which genes involved in the pathway of glucosinolates in *B. napus*

One paper has been published in Molecular Breeding (Liu et al., 2010, DOI 10.1007/s11032-010-9444-y) and another manuscript has been submitted to Plant Molecular Biology. All lines are available from the University of Manitoba upon request; an MTA is required.

Milestone 171: Based on the comparative genetic information, all homologs of *BoGSL-ALK, BoGSL-ELONG* and *BoGSL-PRO* will be used to do co-segregation analysis in DH line populations and selfing populations produced from crosses of rapeseed and canola to infer their involvement

Eight genes have been mapped as well as other genes in the biosynthetic pathways of glucosinolates. Data will be published upon completion of the project.

Milestone 172: 3 *B. rapa* lines with changed glucosinolate profiles will be crossed with *B. oleracea* having the corresponding null alleles to produce and artificial *B. napus* with all null alleles of *BoGSL-ALK, BoGSL-ELONG* and *BoGSL-PRO*

Synthetic *B. napus* lines have been developed. Seeds will be crossed with the best RNAi lines to analyze the reduction of glucosinolates. These lines are available from the University of Manitoba upon request; an MTA is required.

Milestone 173: 30 *B. napus* transformants of these RNAi constructs will be analyzed to find the lines with reduced content of glucosinolates

Over 200 transgenic lines were produced with single RNAi constructs of *BoGSL-ALK, BoGSL-ELONG* and *BoGSL-PRO*. More than 150 crosses between *BoGSL-ELONG* and *BoGSL-PRO* and between two different *BoGSL-ALK* lines were made to analyze double RNAi transgenic lines. Preliminary results from a small scale analysis of double RNAi transgenics, *BoGSL-ELONG* and *BoGSL-PRO,* showed the reduction of total aliphatic glucosinolates in rapeseed with a high content of glucosinolates. Upon completion of the analysis, a manuscript will be prepared and all lines will become available from the University of Manitoba; an MTA is required.

Milestone 174: Co-Segregation analysis will be done and the genes involving the pathway of glucosinolates in canola will be identified

We have found that two loci of *BoGSL-ELONG* and *BoGSL-ALK* co-segregated with two major QTL, which showed that the *BoGSL-ELONG* and *BoGSL-ALK* genes of these loci control glucosinolate content and profile in *B. rapa* and *B. napus*. One manuscript is in preparation for submission to Genome.

Milestone 175: Artificial *B. napus* with null alleles in both A and C genomes will be analyzed to get new germplasm with reduced glucosinolate content

This Milestone was deleted with the approval of the SAB.

Milestone 176: Crosses will be made combine novel lines having these gen replacement and RNAi gene silencing with canola for further reduction of glucosinolate content in canola

This Milestone was deleted with the approval of the SAB.

Milestone 177: New germplasm with reduced glucosinolate content created through a combination of gene replacement and RNAi techniques will be obtained

New rapeseed germplasm with reduced total glucosinolate content (approximately 15 µmol/g seed) was produced through double RNAi of *GSL-PRO* and *GSL-ELONG*. New rapeseed germplasm with enriched anti-carcinogen glucoraphanin (approximately 50 µmol/g) was developed through RNAi of the *GSL-ALK* gene family.

Currently, the new germplasm of canola and rapeseed with reduced glucosinolate content is available to any seed company; an MTA is required. Through publications, double RNAi technology will be offered to the Brassica community for improving different traits in canola and rapeseed.

Milestone 178: 6 constructs for RNAi with homologous genes of *BoGSL-ALK, BoGSL-ELONG* and *BoGSL-Pro* will be prepared for transformation

The constructs were prepared and utilized in milestones 166, 170, 173 and 177.

Sub-Activity 3.6 – Optimizing the levels of carotenoids

Milestone 179: 68,000 *Arabidopsis* mutants with knockouts or activation tags in genes affecting the biosynthesis of carotenoids screened (approx. 17,000/Q)

Two *Arabidopsis* mutant populations were screened. The first was an activation-tagged population (Robinson *et al*. 2009) and the second an APT/ethylene response factor (ERF) over-expression population (Weiste *et al.* 2007).

Milestone 180: 4-8 mutant lines characterized biochemically, and target genes cloned and characterized

A total of eight Norfluazon-tolerant mutants, 14 red seed coat mutants, and 12 other carotenoid-altered mutants were discovered from the activation-tagged population and five carotenoid-altered mutant lines were discovered from the APT/ERF over-expression population. Seven mutants with altered carotenoid profiles were characterized in detail (genetically, chemically and molecularly), including *SK156*, *carotenoid biosynthesis deficient (cbd), KN203, KN3, ABA insensitive 4 (abi4), ethylene response factor1-790 (erf1-790)* and *SW372* (also a trichome mutant). The five novel genes affected in these mutants were cloned and characterized. These are: *microRNA156b, fifty-four chloroplast (FFC), keto acyl coa synthase 19 (KCS 19), RNA binding protein 47C (RBP47C), ABI4, ERF1-790* and *COP9 signalosome* (trichome mutant gene). In addition, to our own screens, 51 additional types of lines (up to 20 lines per type) were generated or obtained from other labs and analyzed to characterize these genes, including complemented lines, over-expression lines, and knock-out mutants (SALK, FLAG, etc.). Data describing these mutants and their characterization will be published upon completion of the project. All

material will be stored at Agriculture and Agri-Food Canada, London, Ontario and will be available with an MTA upon publication of the data.

One of the lines identified in the screen, abi4, was characterized using microarray analysis relative to WT. All microarray data will be submitted to public databases.

In order to identify additional gene targets an EST library from the flower petals of *Adonis aestivalis* was generated (Li *et al*. 2008). *A. aestivalis* accumulates high levels of desirable carotenoids and was therefore a rich source of transcripts (and gene targets) involved in carotenoid biosynthesis. A total of 4,189 ESTs, representing transcripts of most genes involved in carotenoid biosynthesis, were generated and were submitted to GenBank (accession numbers: FL507949-512137). Additional gene targets were also sought by completing microarray analysis on developing *B. napus* seeds to assess changes in gene expression in relation to the accumulation of carotenoids and other seed storage compounds (Yu *et al*. 2010).

Milestone 181: 4-8 *B. napus* lines transformed with genes affecting carotenoid biosynthesis generated and analyzed biochemically and metabolically

Several of the carotenoid mutants identified in milestone 180 were chosen for further investigations in *B. napus*. Seed was developed from *miR156b* over-expression lines in *B. napus* with a constitutive promoter or a seed-specific promoter. Plants over-expressing *miR156* in a constitutive manner showed enhanced branching and elevated seed carotenoid content (Wei *et al.* 2010). Transgenic *Arabidopsis* seed from an *FFC* T-DNA insertion line and transgenic seed of *B. napus* over-expressing the genomic sequence of the *FFC B. napus* homolog have been developed. Over-expression lines exhibit enhanced carotenoid content. US and Canadian patents describing this work will be filed shortly.

The De-Etiolated 1 (*DET1)* regulatory gene has been shown to affect carotenoid accumulation in tomato and was chosen as for further analysis. *B. napus* RNAi seed was developed with the *DET1* suppressed in both a constitutive and a seed-specific manner. Seeds of these transgenic plants had elevated carotenoids and reduced sinapoyl esters (Wei *et al.* 2009).

The lycopene epsilon cyclase gene was also chosen for further analysis based on the literature. Transgenic developing *B. napus* seed with 2 to 42-fold higher carotenoid content was produced (*Yu et al*. 2008). As a result of this work, and subsequent analyses that has not yet been published, a US and Canadian patent has been filed (PCT# CA2008/000344).

Fish and poultry feeding trials using high carotenoid lines are planned post-DOTM in collaboration with Dr. Bogdan Slominski (University of Manitoba) and the data will be published once analysis is completed.

All material will be stored at Agriculture and Agri-Food Canada, London, Ontario and will be available with an MTA upon publication of the data.

Milestone 182: 2-4 *B. napus* lines with altered levels of carotenoids and xanthophylls crossed to lines having reduced levels of antinutritional factors

Seed from the following crosses have been analyzed: high carotenoid pSW05 lines (17, 18, 23) crossed to low sinapine RL lines (RL3 and RL2); high carotenoid pAZ05 lines (-1936, -1854, -1850) crossed to low

sinapine RL lines (RL3 and RL2); and high carotenoid pSW05 lines (-17, -18, -23) crossed to pAZ05 lines (-1963, -1854, -1850).

Data will be published upon completion of the project. This seed will be stored at Agriculture and Agri-Food Canada, London, Ontario and will be available with an MTA.

Reducing anti-nutritional factors in *B. napus* seed

In addition the aforementioned findings, research funded through the matching component has generated *B. napus* seeds with up to 90% reduction in sinapine, 45% reduction in lignin, and 85% reduction in phytate. These findings have been published in peer-reviewed journals (Huang *et al.* 2008, Huang *et al.* 2009, Bhinu *et al.* 2009, Bhinu *et al.* 2009b). Livestock feeding trials using low phytate lines are planned post-DOTM in collaboration with Dr. Bogdan Slominski (University of Manitoba).

Sub-Activity 1.5 – The development of a spring type *Brassica* TILLING population

Milestone 57: Pilot EMS mutagenesis studies in *B. napus* to determine optimal conditions for generating a mutagenized population

Data will be available through publication upon completion of the project.

Milestone 58: Full scale muagenesis and selection of 6000 inbred lines, arraying of DNA for TILLING (1000 every 2 quarters)

The objective of this milestone has been modified to select 4,500 lines. More than 5,100 M_1 plants have been grown, with M_2 seed collected from 5,000 plants. About 4,600 M_2 plants have been grown, with M_3 seed collected from approximately 2,600 plants. Leaf tissue has also been collected from approximately 2,000 M_2 plants. The leaf tissue collected for DNA extraction will be stored at the University of British Columbia.

Milestone 59: TILLING of 10 target genes in *B. napus* to test the arrayed population (2.5 each quarter)

Seed from each of the fulfilled TILLING requests was sent to the requestor and any analysis of the TILLED lines will be available in their publications. Any requests for material should be made to the individual researcher who requested the TILLING lines.

Milestone 60: TILLING of 10 additional target genes in *B. napus* (2.5 each quarter)

Seed from each of the fulfilled TILLING requests was sent to the requestor and any analysis of the TILLED lines will be available in their publications. Any requests for material should be made to the individual researcher who requested the TILLING lines.

Sub-project 3.4 – Gene stacking of selected traits and breeding

Milestone 160: Fine tuning of cumulative gene constructs and transgenics with stacked genes

The concept of a triple-low anti-nutritional factor (sinapine, phytate and glucosinolate) trait was investigated. Transgenic lines low in sinapine and phytate have been developed indicating that these traits can be combined. Low glucosinolate trait is being introduced. Data will be published upon

completion of the project; seed will be stored at NRC-PBI, Saskatoon and available upon request (an MTA is required).

Milestone 161: Metabolite analysis of the above for phenolics

Data describing the phenolic profile of the new lines will be published upon completion of the project.

Sub-Activity 6.1 – Animal testing of new canola varieties with improved meal quality

Milestone 195: Animal testing of new canola varieties with improved meal quality

A comprehensive evaluation of the chemical composition and nutritive value of meals derived from yellow-seeded *Brassica napus* (YN01-429), black-seeded *B. napus* (N89-53), and canola-quality yellow-seeded *Brassica juncea* has been completed. In comparison with its black-seeded counterpart, meal derived from yellow-seeded *B. napus* contained more protein, more sucrose and less dietary fibre. Lower fibre content in yellow-seeded *B. napus* was reflected in lower content of lignin with associated polyphenols. *B. juncea* showed intermediate levels of crude protein, sucrose, and dietary fibre. A seed fractionation study demonstrated that the reduction in fibre content of yellow-seeded *B. napus* was a consequence of a bigger seed size. For yellow- and black-seeded *B. napus* and *B. juncea*, a lower contribution of the hull fraction to the total seed mass, and a lower fibre content of the hull fraction were observed. The nutritive value of canola meal samples was investigated with broiler chickens from 3 to 17 d of age fed corn/soybean meal-based diets containing 30% of canola meals. A significantly higher ($P<0.05$) total ileal digestibility of amino acids was observed in birds fed the yellow-seeded *B. napus* diet when compared with those fed diets containing black-seeded *B. napus* or *B. juncea*. In a second study, the meal apparent metabolizable energy corrected for nitrogen values for yellow- and black-seeded *B. napus*, and *B. juncea* were determined with broiler chickens (from 14 to 19 d of age) and were highest in the yellow-seeded *B. napus* followed by the black-seeded *B. napus* and *B. juncea*. This trend was also observed in a study with turkeys, showing similar energy availability values for yellow- and black-seeded *B. napus* and *B. juncea*.

Several samples developed as part of the DOTM project have been evaluated for their value as low-fibre, high-carotenoid, and low-sinapine feed sources. Neutral detergent fibre (NDF) and fat contents of yellow-seeded lines of canola seed (120 samples) from Dr. Rahman's group have been determined. The analysis of phytate, non-phytate phosphorus and total phosphorus in *MIPS* lines (30 samples) developed by Dr. Georges's group has been completed. The research outcomes related to seed analysis have been forwarded to the respective research groups within the DOTM project. Based on the preliminary evaluation, only the samples with important characteristics and changes made to the content of phytate phosphorus, sinapine or carotenoids will be evaluated in animal trails. As of now, the studies which would qualify for such an exercise would include the following:

1. Growth performance, nutrient digestibility and carotenoid deposition in the egg and meat products using laying hens fed diets containing high carotenoid canola
2. Chemical characterization of low-sinapine lines of canola and their further evaluation with young broiler chickens.
3. Chemical characterization of carbohydrate components (i.e., glucose, sucrose, oligosaccharides, non-starch polysaccharides) of newly developed lines of yellow-seeded canola and their further evaluation with broiler chickens.

Sub-Activity 4.0 - Bioinformatics Services

Milestone 183: Creation of collaborative environment: within the project

The DOTM web portal, along with its links to relevant resources in the project and wiki holding information, have been adapted to a post-project format and will be maintained as the primary access point to project legacy data and information. The portal will be maintained at NRC-PBI after the completion of the project.

Milestone 184: Data acquisition, management and analysis planning starting immediately and ongoing for the entire project

The total number of ESTs processed for the project includes 901k of public sequences (all available *B. napus, B. rapa, B. oleracea, and Brassica carinata* sequences as of April 20, 2010), 296k internal PBI traces, and a 95k array set. The DOTM Fiesta account holds project-generated EST data and has been transferred for permanent storage/use to NRC-PBI. Cumulative and species specific assemblies of these three sets of generated contigs and singletons are available for download through the portal and will continue to be available after the completion of the project. The MAGPIE annotation system provides extensive annotations for the assembled data based on searches against 15 key databases. These MAGPIE projects will provide the interface for searching, analyzing and visualizing the datasets and will continue to be available after the completion of the project.

Hierarchal Sprockets clusters were generated from *Brassica* transcript data and incorporating full length and annotated coding sequence data sets from *A. thaliana* and *Orzya sativa*. They represent models of cross species protein families enabling higher level analysis. These Sprockets clusters are available for browsing through the SprocketsView interface via the web portal which will continue to be available after the completion of the project. However, no new assemblies will be possible after December 31, 2010.

Specific data have been generated from individual requests during the course of the project. This includes the SNP discovery pipeline and online interface for searching SNP sites based on databases of project generated transcript datasets. A SNP collection compendium will also be available for download through the DOTM website and will be maintained after the completion of the project. Data of narrower usage will be archived and available to the community through request via NRC-PBI.

Milestone 185: Development of phenotype and morphology modules for MAGPIE/Bluejay/FIESTA. Bioinformatics tools for mining and management of B napus TILLING poplulation from Q5 to Q16

TILLING datasets from this project have been received and will be accessible through this system via the web portal. TILLING information was provided to the investigator who requested the target. Those interested in a specific gene should contact the investigator.

The GMOD-based PHENOME databases were designed to be the primary legacy databases for this project, incorporating complete genotype-phenotype records. The scope of this database will reflect the total phenotypic information generated by the project. It will be made available through the web portal which will be maintained after the completion of the project at NRC-PBI.

Milestone 186: Development of new module for proteomics analysis

SBEAM has been provided as the proteomic data management system for the project. Any proteomic data submitted by project groups will be available through this system via the web portal and will be maintained after the completion of the project.

Milestone 188: Development of data mining approaches for analysis and integration of ESTs, proteomics, microarrays and metabolomics datasets

Microarray data submitted by project groups will be available within the existing management system BASE (web-based microarray LIMS system). This system will be maintained after the completion of the project but will be available to DOTM internal users only. All data will be available through the publications and all investigators will be depositing microarray datasets into public databases.

Annotated unigenes from EST assemblies and Sprockets clusters generated during the course of the project have been mapped against the *Arabidopsis* genome. A visualization of this through Gbrowse and Bluejay will be maintained online through the website to assist future analysis. The software Bluejay (a Java-based software which can be launched remotely) can integrate expression data with gene function. Bluejay will continue to be available after the completion of the project through the University of Calgary (http://bluejay.ucalgary.ca/install/).

Milestone 189: Data submission to public databanks

100,000 EST sequences have been submitted to GenBank. The information is in the public domain.

Sub-Activity 5.0 – GE3LS

Milestone 190: Recruitment of two graduate students and PDF one each for the analysis of the scientific process and one for the IP program. Ideally we will engage researchers who have completed their course work for their Masters or Doctoral degrees.

One Professional Research Associate (PRA) was hired to undertake and complete the research within this activity (Smyth); we tried to find qualified and interested Masters and Doctoral candidates but we were unsuccessful.

Milestone 191: Analysis of the scientific process and one for the IP program.

A manuscript describing this work has been submitted and is currently under review (Smyth S. J. and R. Gray. Intellectual Property Sharing Agreements in Gene Technology: Implications for Research and Commercialization).

Milestone 192: Hold a workshop on IP

A session entitled 'Round Table on IPRs'(Intellectual Property Rights) was held at the 13th International Consortium on Agricultural Biotechnology Research in Ravello, Italy. Smyth chaired the Round Table Session and participants included: P. Phillips (U of S); R. Gray (U of S); and D. Eaton (LEI, The Netherlands). This Round Table was attended by 35-40 people. The discussion following the presentations resulted in the identification of three substantial challenges facing IP (the impacts of market power, the lack of research funding and the role that the anti-commons might play). Future international collaborations are planned from this Round Table.

Milestone 193: Hold a workshop on regulation

In preparation for this meeting two articles were published: Smyth and McHughen 2008; McHughen and Smyth 2008. These articles were used to help frame the meeting agenda.

This workshop was held on December 6th and 7th, 2009 in Banff, AB. This workshop was attended by approximately 30 people from across Canada. The discussion on regulation and innovation resulted in several areas of importance being identified. As a result of these discussions, a model of regulatory choices and economic impacts was developed and a manuscript is in preparation (Phillips, P., S. Smyth and S. Zhang. Profitable genomic innovations: Strategic regulatory choices).

Milestone 194: Complete primary research in three subject areas and integrate material in unified report

A book describing this work was published (Smyth, Endres, Redick and Kershen 2010). *Innovation and Liability in Biotechnology* introduces and articulates an innovative framework, the Liability Analysis Framework (LAF), which offers a new perspective from which stakeholders and society can assess, manage and communicate about liability in relation to innovation. This book provides a detailed description of the relationship between risk and liability. Risk and liability are not synonymous and the fact that, at times, the terms have been used in very close proximity has resulted in confusion and misunderstandings.

The book begins with an overview of risk and the development of the Risk Analysis Framework, describing how it has evolved from initial concepts to present day form. The book highlights the need for a LAF and provides a diverse examination of the LAF as a means to stimulate further debate. The authors conclude that risk is concerned with hypothetical probabilities whereas liability relates to actual marketplace externalities.

Offering a new conceptualization for the analysis of liability, this book will appeal to academics involved in the fields of law, innovation and business, as well as federal regulators and industry representatives. Agricultural organizations and their lawyers who are attempting to understand the legal liability issues involved in growing and marking transgenic crops and their products, will also find this book of great interest.

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Appendix 1: The DOTM Investigators

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Dr. Fawzy Georges	National Research Council- Plant Biotechnology Institute	Fawzy.Georges@nrc-cnrc.gc.ca	Phytate
Dr. Abdelali Hannoufa	Agriculture and Agri-Food Canada	Abdelali.Hannoufa@AGR.GC.CA	Carotenoids
Dr. George Haughn	University of British Columbia	haughn@interchange.ubc.ca	Seed coat/fibre; TILLING
Dr. Genyi Li	University of Manitoba	G_Li@UManitoba.CA	Glucosinolates
Dr. Peter Phillips	University of Saskatchewan	peter.phillips@usask.ca	GE^3LS
Dr. Habibur Rahman	University of Alberta	Habibur.Rahman@afhe.ualberta.ca	Yellow seed coat
Dr. Gerhard Rakow	Agriculture and Agri-Food Canada	rakowg@agr.gc.ca	Yellow seed coat
Dr. Christoph Sensen	University of Calgary	csensen@ucalgary.ca	Bioinformatics
Dr. Saleh Shah	Alberta Innovates and Technology Futures	shah@albertainnovates.ca	Seed coat/fibre
Dr. Bogdan Slominski	University of Manitoba	slominsk@cc.umanitoba.ca	Feeding trials
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