
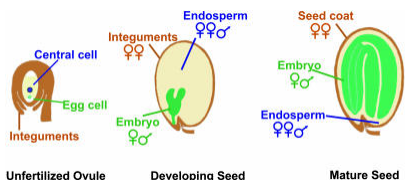


Gene analysis of the Canola seed endosperm

Jitao Zou


National Research Council Canada-Plant Biotechnology Institute



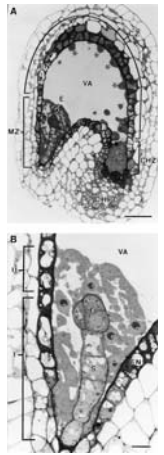


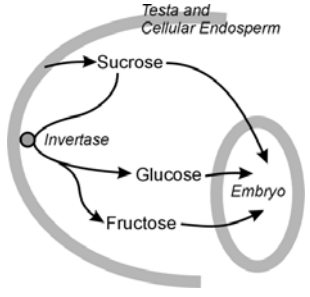
Schematic sketch of seed development in the model plant *Arabidopsis*

Genetically, endosperm is a dead-end


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But functionally essential for embryo development





Schematic view of sugar movements within a developing seed
Hill et al, Plant Physiol 2003 131:228

Otegui, M., et al. *Plant Cell* 2000;12:933-947


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endosperm \longleftrightarrow [?] Integument \longleftrightarrow Seed size

Cell number/size

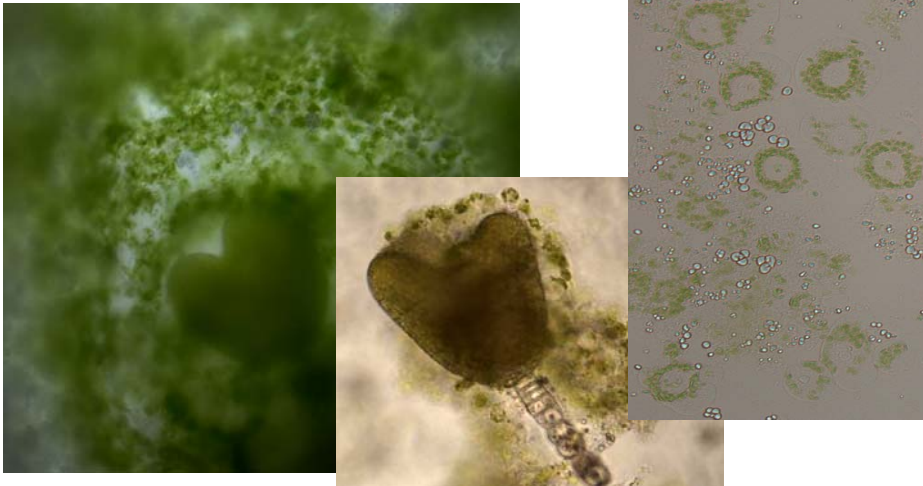
The size of the seed is the result of three different growth programs: those of the diploid embryo, the triploid endosperm, and the diploid maternal ovule.

Proc Natl Acad Sci U S A. (2005) 102: 17887


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- Carbon stored as Oil in the *Arabidopsis* endosperm is used to fuel seedling establishment (Penfield et al., 2004)
- The endosperm has been shown to exert control over germination by secreting cell wall loosening enzymes that weaken the mechanical resistance of the micropylar endosperm cap to radicle protrusion (Bewley, 1997b)

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B. napus endosperm tissue sampling



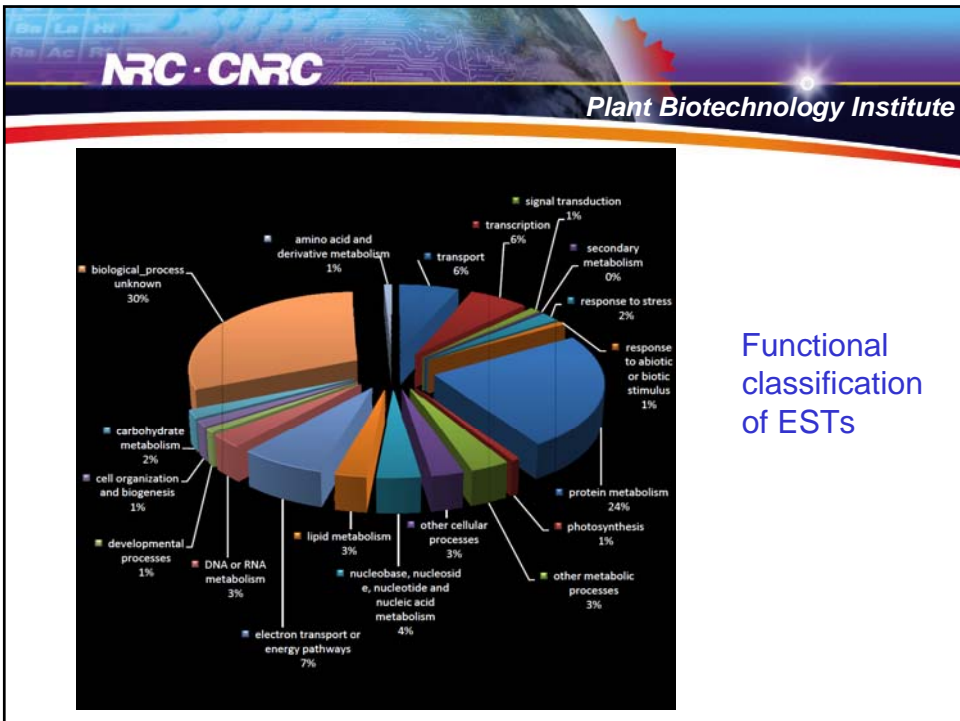
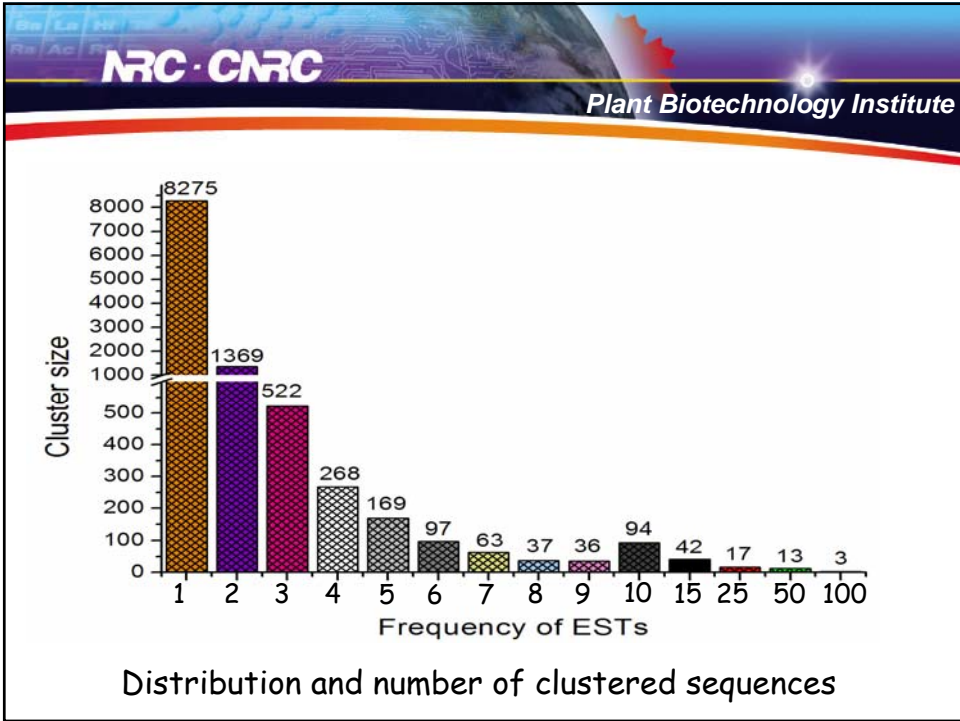
cDNA library construction


- Creator SMART cDNA Library Construction Kit (cat#634903, Clontech).
- Vector: pDNR-LIB
- Sequencing :from 5' end



Endosperm EST summary


Total ESTs	30431
Total high-quality ESTs	23235
Success index (%)	76.4
Average insert size (kp)	1.1
Average sequence size (bp)	467
Number of contigs	2730
Number of singletons	8275
Number of unigenes	11005





Most abundant ESTs from 10K

EST #	Anotations
101	copper ion binding / electron transporter; putative nodulin
37	seed specific protein Bn15D80B [Brassica napus]
28	LEC1 (LEAFY COTYLEDON 1); transcription factor
23	seed specific protein Bn15D33A [Brassica napus]
21	At1g10370 glutathione S-transferase
18	myo-inositol 1-phosphate synthase; INO1_BRANA (MI-1-P synthase) (IPS)
18	lipid binding [Arabidopsis thaliana]
17	"glutathione dehydrogenase (ascorbate) Putative GSH-dependent dehydroascorbate reductaseputative
17	unknown protein [Arabidopsis thaliana]
16	unknown protein; emb CAB80455.1; emb CAB37530.1 putative protein [Arabidopsis thaliana]
15	cystatin domain containing protein [Brassica oleracea]
14	lipid transfer-like protein [Brassica napus var. napus]
14	histone H4 [Hyacinthus orientalis]
14	senescence-specific cysteine protease [Brassica napus]
13	pollen coat protein [Brassica oleracea]
12	chloroplast chlorophyll a/b binding protein [Brassica napus]
12	"At4g32110 transferring glycosyl groups [Arabidopsis thaliana]
12	"ribulose-1,5-bisphosphate carboxylase /oxygenase, small subunit;




Endosperm specific genes

Identified through searching the PBI EST data collections

- 133 putative endosperm-specific genes
- A large proportion of these genes were highly expressed in endosperm based on Arabidopsis data


A Resource for endosperm promoter



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Proteomic analysis of endosperm with heart stage embryo


Endosperm in ovules containing heart embryos exhibit all phases of endosperm development (Brown et al., 1999)



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Proteome of the *B. napus* endosperm


• Total proteins identified:	809
• Chloroplast gene products	17
• Mitochondrial gene products	11
• Genes with no ests (matching BNAEN3GH)	149
• Genes with 4 or more EST members	348



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Notable entries in the proteome

- Cell wall invertase
- Invertase/pectinase inhibitor
- cytosolic beta-amylase
- myo-inositol 1-phosphate synthase
- Various components of Golgi vesicle-mediate transport
- Heme transporter
- Lipid metabolism enzymes: fatty acyl desaturase; acyltransferase
- 13 entries correspond to embryo defective genes




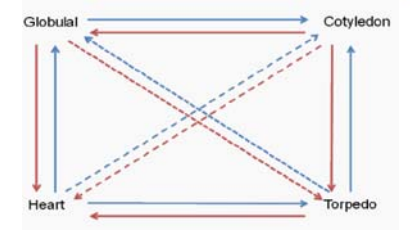
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Global expression profile of endosperm with microarray

The Arabidopsis thaliana Genome Oligo Microarray (Version 3.0 , Arizona) contains:


- a) 29,110 longmer probes and represents 26,173 protein-coding genes,
- b) 28,964 protein-coding gene transcripts, and 87 microRNA genes from A. thaliana.
- c) Pseudo genes are excluded from oligo design.
- d) The design is based on the ATH1 release 5.0 of the TIGR A. thaliana genome annotation database and release 4.0 of the miRNA Registry at the Sanger Institute and allows detection of alternative splicing variants using common, partial common, or individual transcript oligos.
- e) For probe design we use state-of-the-art methodology and proprietary software. An amino linker is attached to the 5' end of each oligo.





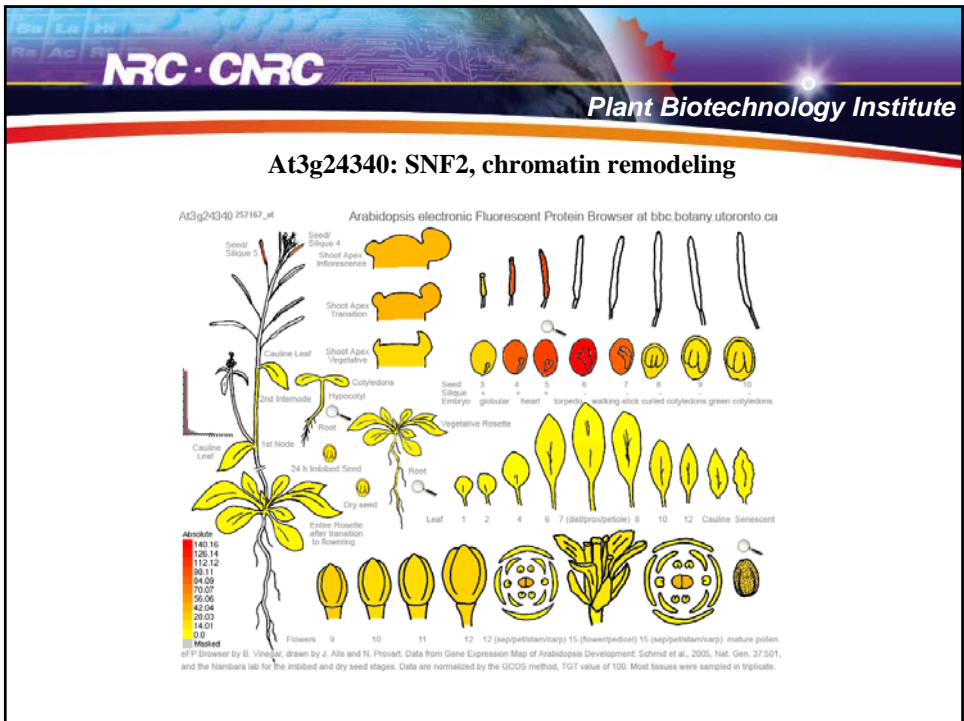
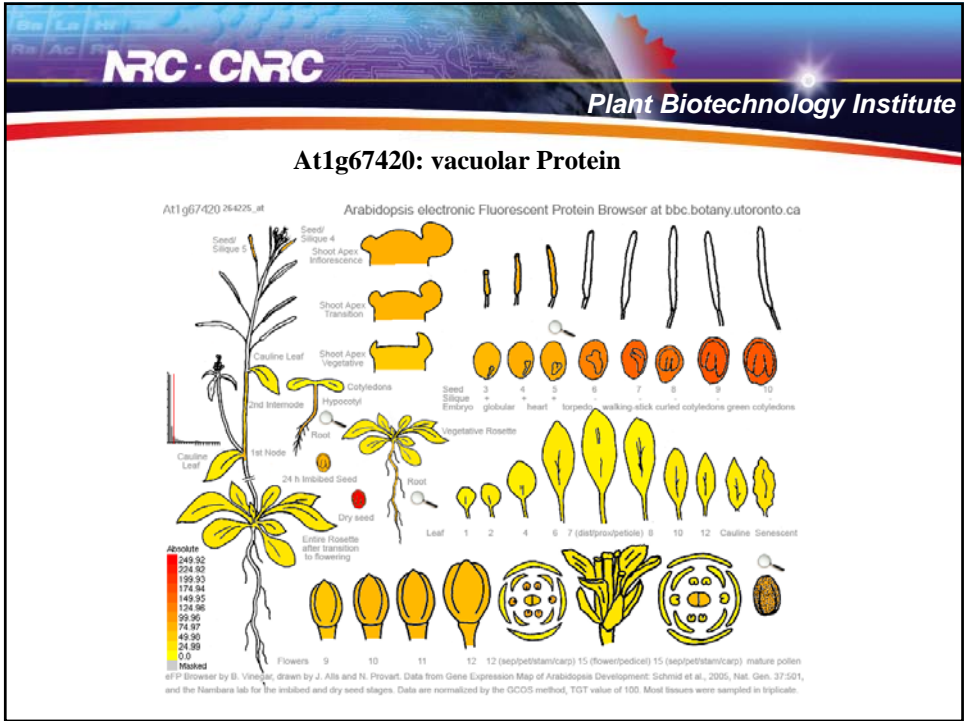
A = heart stage endosperm versus globular stage endosperm
 B = heart stage endosperm versus torpedo stage endosperm
 C = cotyledon stage endosperm versus torpedo stage endosperm
 D =cotyledon stage endosperm versus global stage endosperm



The following four venn diagrams showed numbers of genes significant in each comparison.



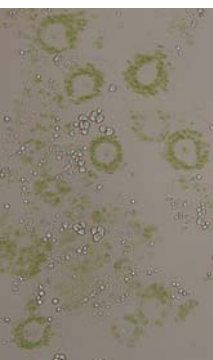
Gene ID	Annotation
At1g04550	auxin-responsive protein
At1g10570	Ulp1 protease family protein
At1g67420	24 kDa vacuolar protein
At2g05380	glycine-rich protein (GRP3S)
At2g17340	pantothenate kinase-related contains Pfam domain
At3g03010	expressed protein
At3g24340	SNF2 domain-containing protein
At3g26090	expressed protein
At3g33494	hypothetical protein
At3g50860	clathrin adaptor complex small chain family protein
At4g04260	bromo-adjacent homology (BAH) domain-containing protein
At5g18500	protein kinase family protein



Common differentially expressed genes

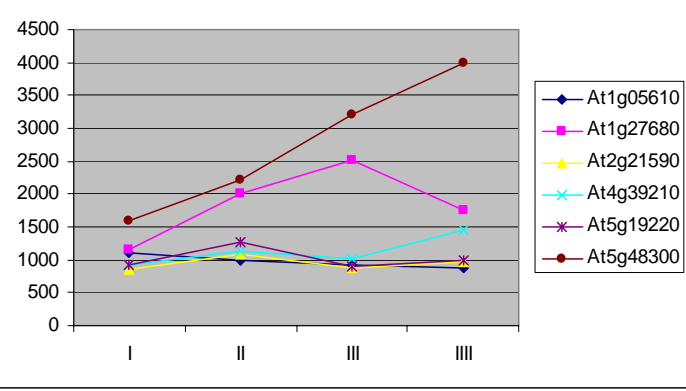


Understanding the role of starch metabolism in endosperm



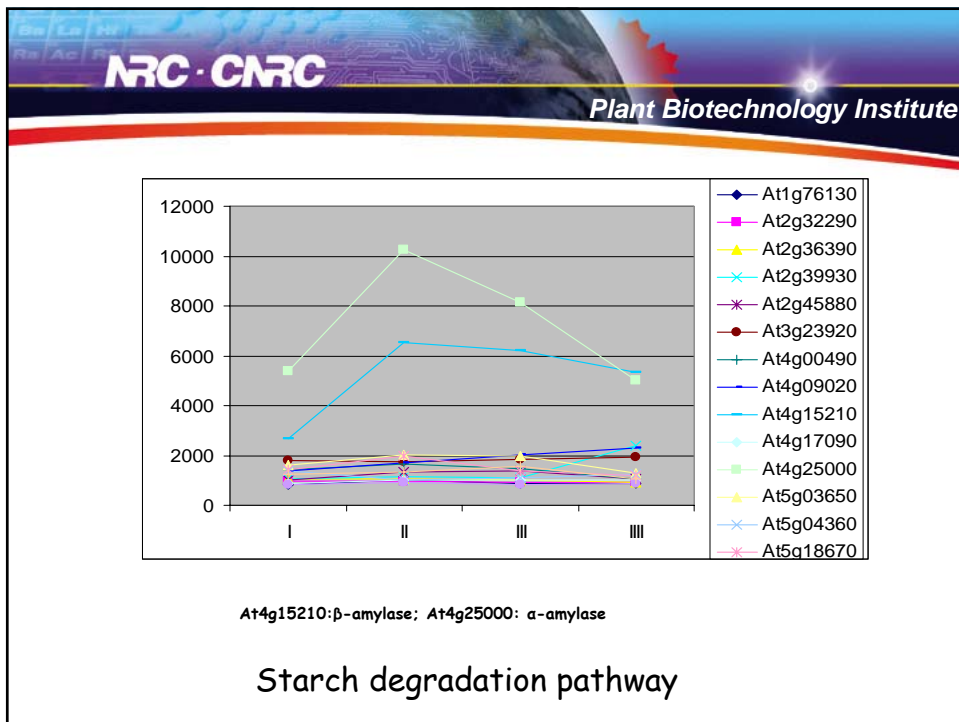






Gene	I	II	III	IIII
At1g05610	~1000	~1000	~1000	~1000
At1g27680	~1200	~2000	~2500	~1800
At2g21590	~1000	~1000	~1000	~1000
At4g39210	~1000	~1000	~1000	~1500
At5g19220	~1000	~1300	~1000	~1000
At5g48300	~1600	~2200	~3200	~4000

At5g48300: ADP-glucose pyrophosphorylase small subunit 1; **At1g27680:** ADP-glucose pyrophosphorylase large subunit 2

Starch biosynthesis pathway

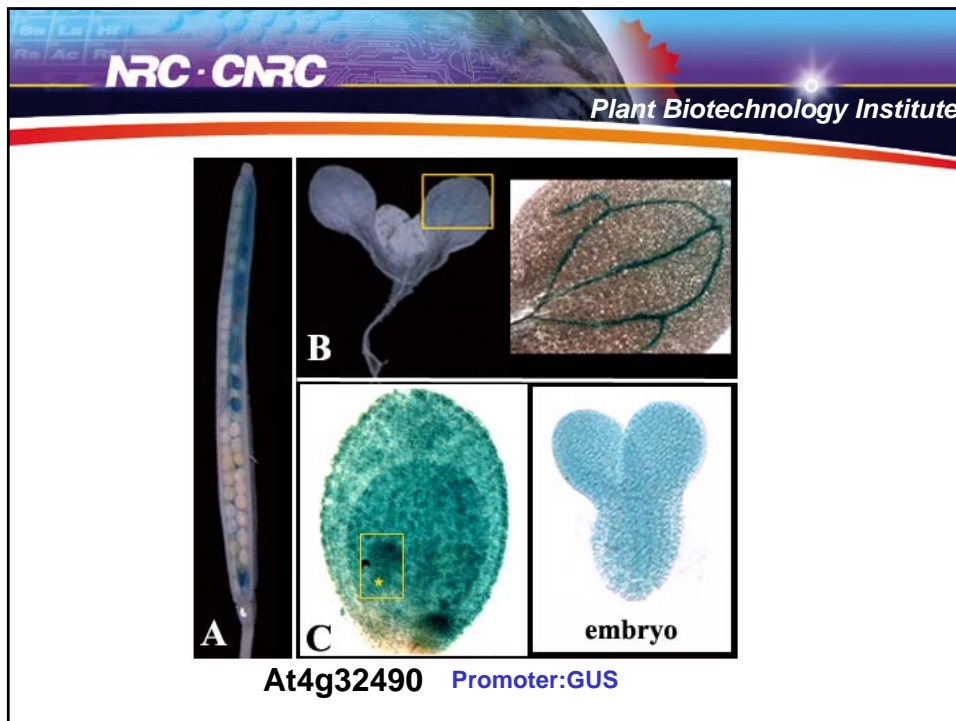
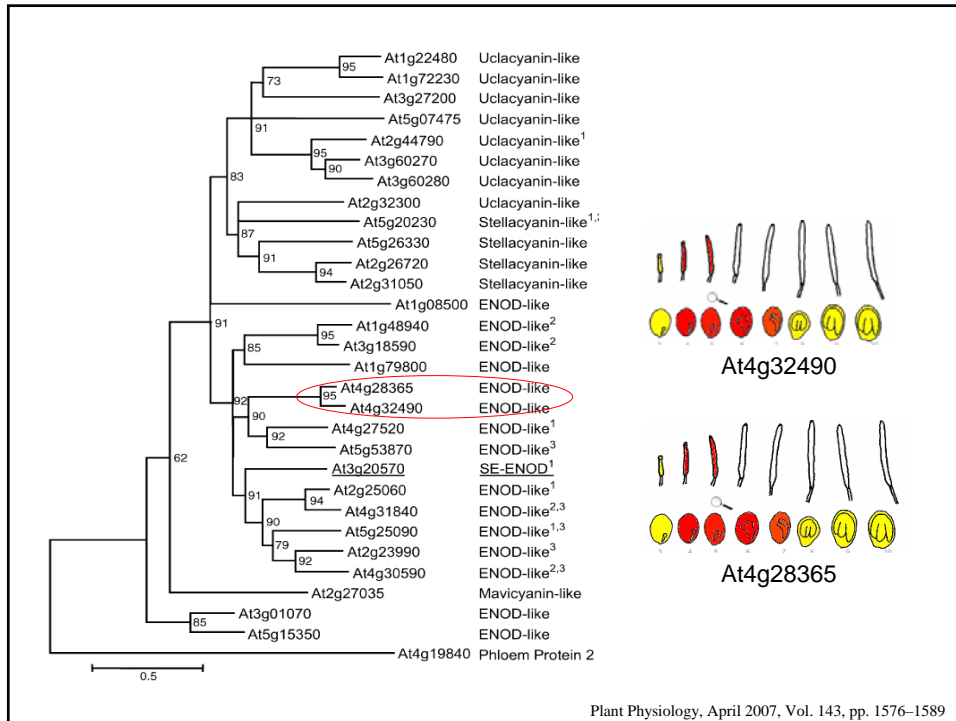


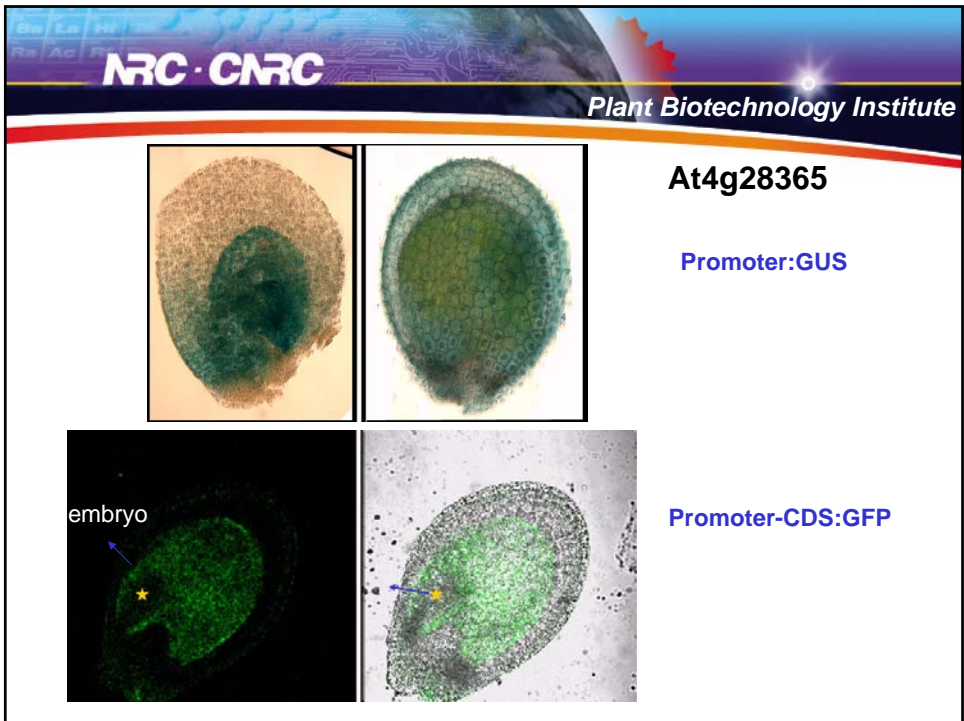
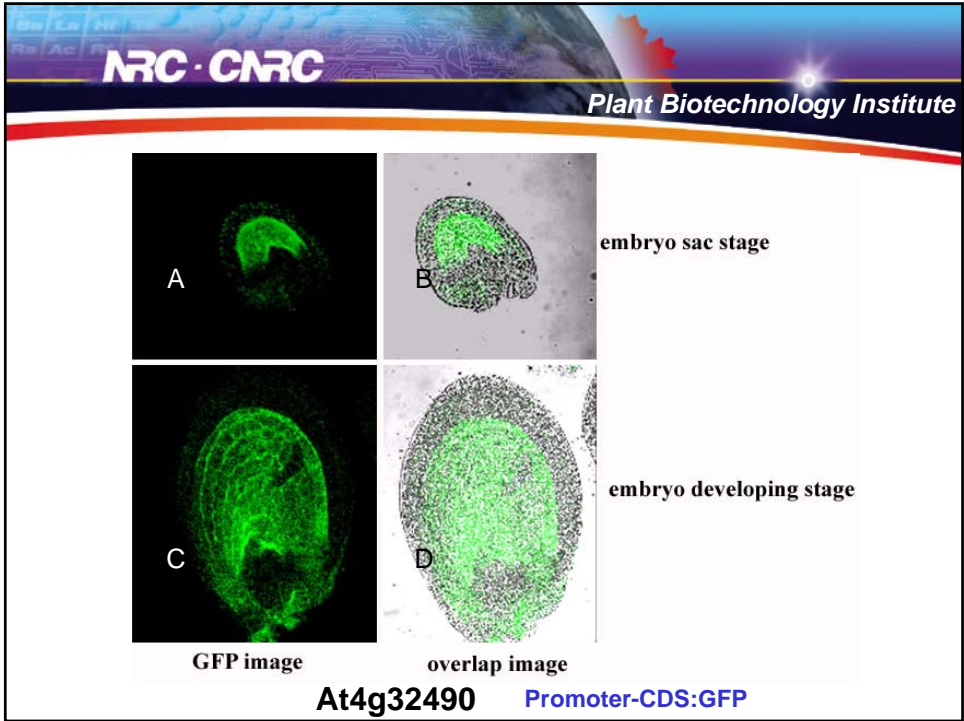


Functional Study of endosperm specific genes

At4g28365
AT4g32490

Highly abundant in EST
 Differentially expressed based on Microarray
 Putative copper-binding protein
 GP_anchored protein (Cell wall protein??)






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Transgenic down-regulation in *B. napus*
An RNAi approach

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RNAi transgenic *Brassica napus*



negative control transgenic line

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Transgenic over-expression of the endosperm gene in Arabidopsis

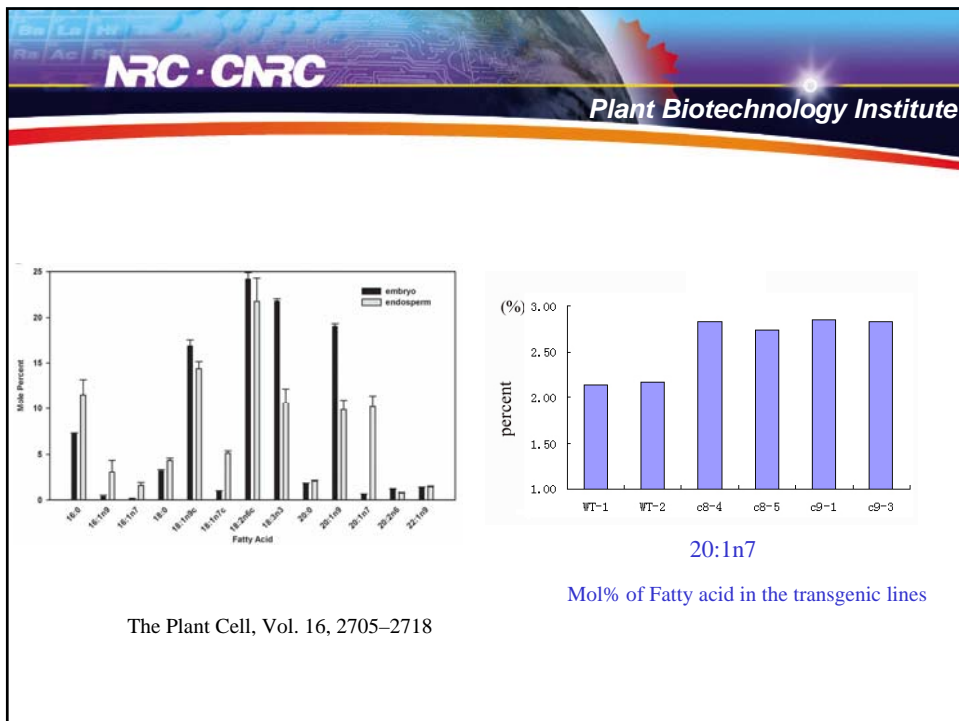
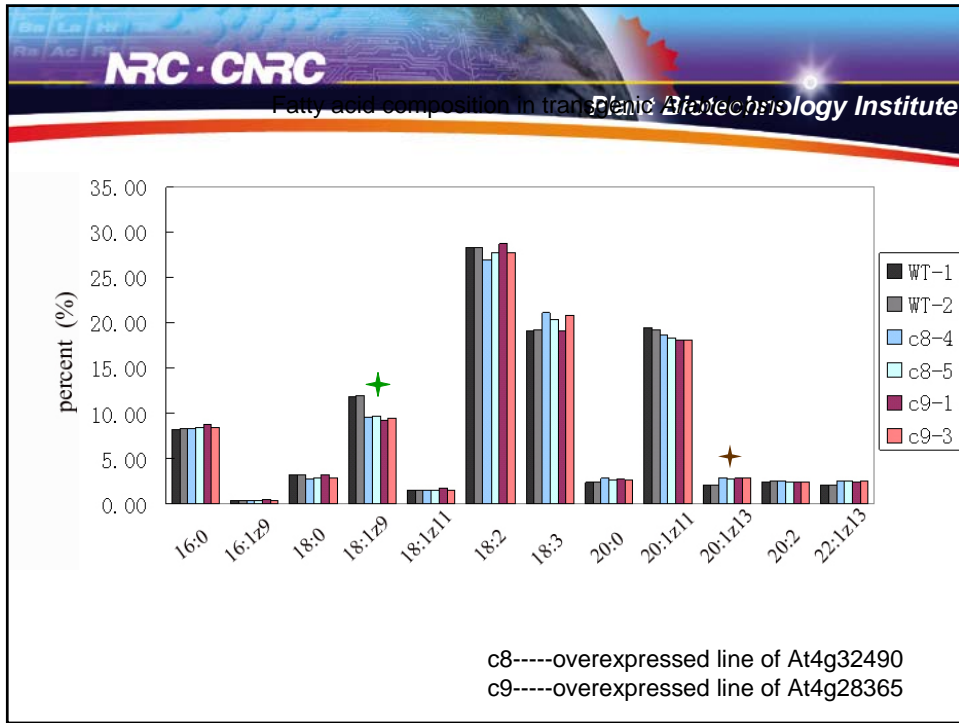
C8 lines: 35S-A+4g32490
C9 lines: 35S-A+4g28365


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Oil content in transgenic *Arabidopsis*

Line	Oil content (%)
WT-1	33.5
WT-2	31.5
c8-4	23.0
c8-5	26.5
c9-1	24.5
c9-3	23.0


c8-----overexpressed line of At4g32490
c9-----overexpressed line of At4g28365





Future Work

- Survey of the genetic network
- Identify key endosperm development genes
- Uncover signature metabolic pathways
- Select gene targets for genetic manipulation
- Assess genetic association of candidate genes with major seed traits in natural (breeding) population



Acknowledgement

EST analysis	Microarray analysis	Functional study
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to **Innovation...**

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