



The ERA-CAPS “ABCEED” CONSORTIUM

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Aims

The project integrates work in *Arabidopsis thaliana*, *Capsella* sp. *Camelina sativa* and *Brassica napus*, aiming to identify genetic variation and genes controlling seed number, seed size, and seed quality traits.

Work Packages

1. Control of seed size in *Arabidopsis* and *B. napus*
2. Control of ovule number in *Capsella* and *Arabidopsis*
3. Seed composition and quality in *Arabidopsis*, *Camelina* and *B. napus*

Deliverables

Identification of genetic variation and candidate genes controlling seed number, size and composition in *B. napus*

Integrated multi-scale phenotyping of seed maturation in Brassicaceae

Comparative analyses of gene function in seed formation in Brassicaceae

Work Package 1

Genetic control of seed size in *Arabidopsis* and *B. napus*
Mike Bevan lead, with M. Lenhard and L. Lepiniec

Research here is based on associative transcriptomics analyses of a diverse population of Brassica species to identify genetic variation associated with seed size and number, seed pod length, seeds per pod and pods per plant. The functions of candidate genes will be tested in transgenic *Arabidopsis*. RNAseq of developing *B. napus* seeds will be generated to provide additional sources of genetic variation and data for Gene co-expression analyses in WP 3



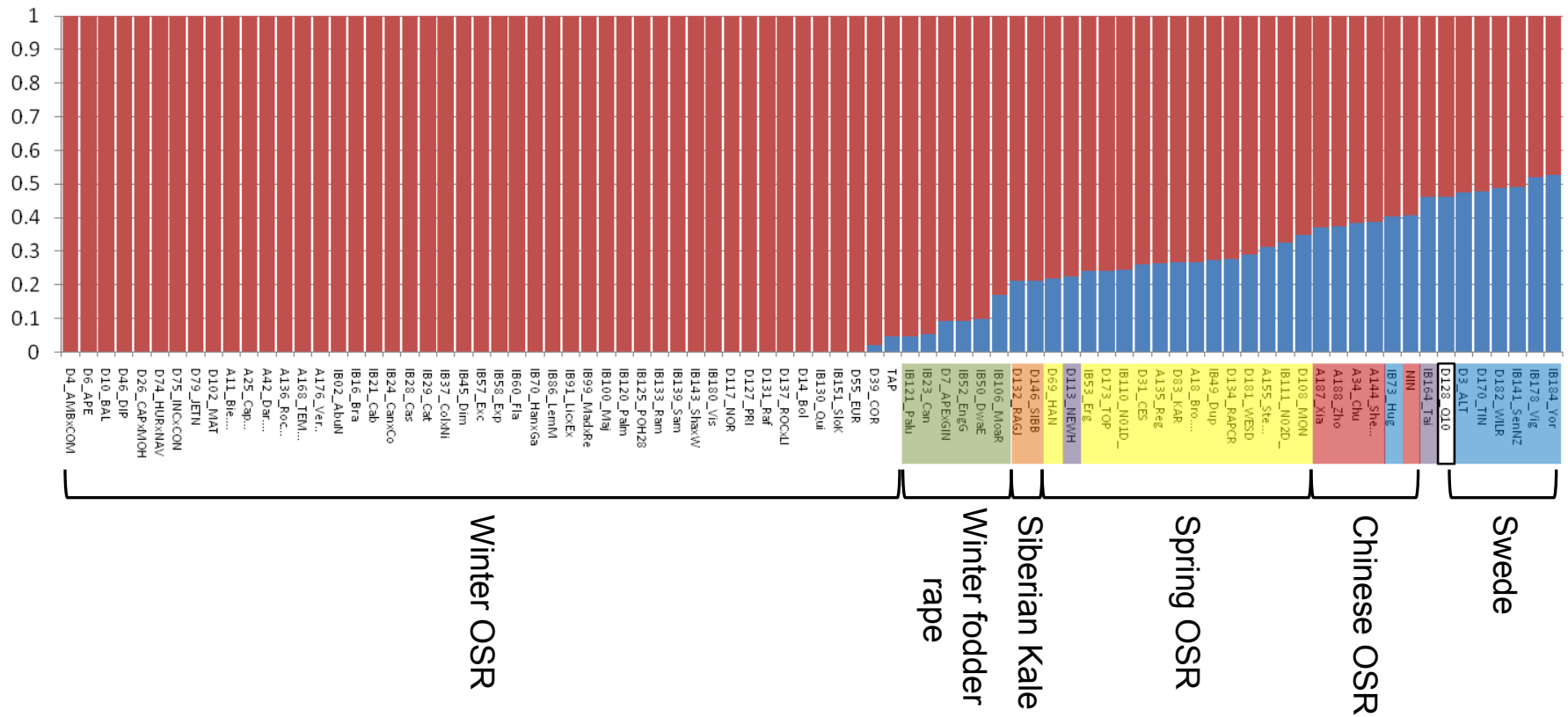
2014 Brassica phenotyping trial of 104 varieties



Phenotyping for seed traits will start in July 2014 at JIC

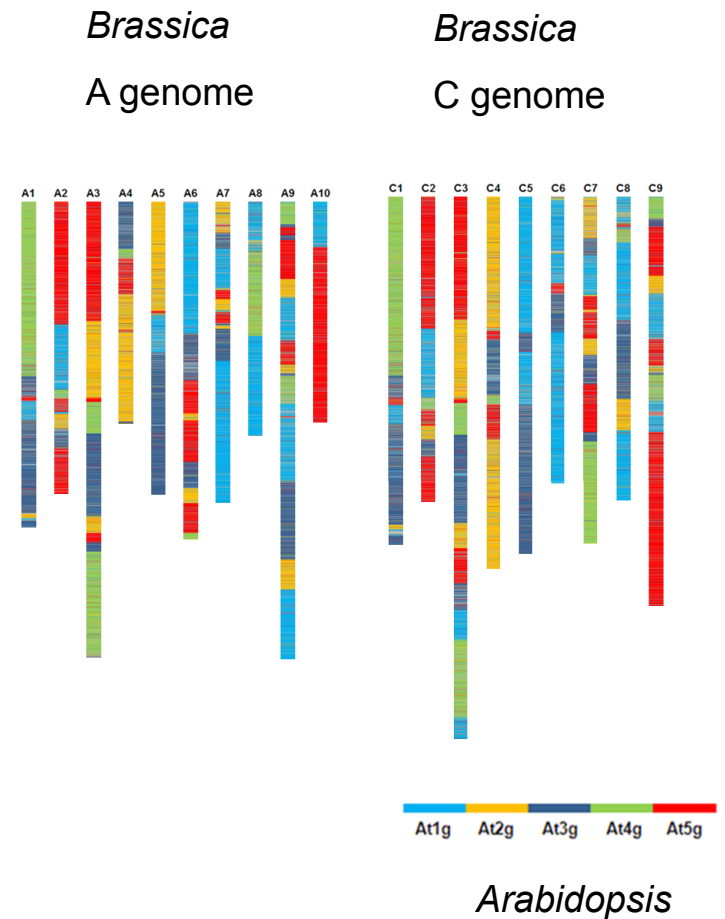
Natural variation in rapeseed – Association transcriptomics

Distribution of SNP alleles to show population structure and relatedness



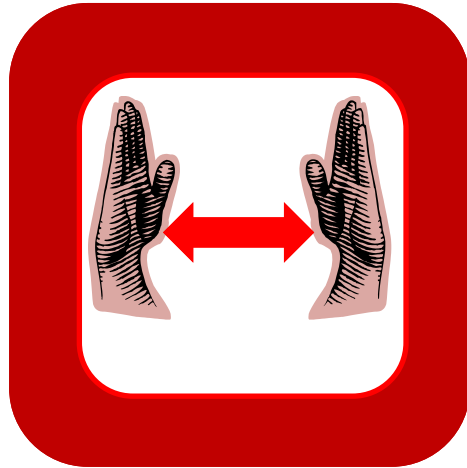
Marker order

- Pseudomolecules
- Developed using *B. rapa* and *B. oleracea* genome sequences
- Genome assembly corrected via:
 - Unigene order in Tapidor x Ningyou7 high density transcriptome SNP map (9,169 unigenes)
 - Synteny with *A. thaliana*



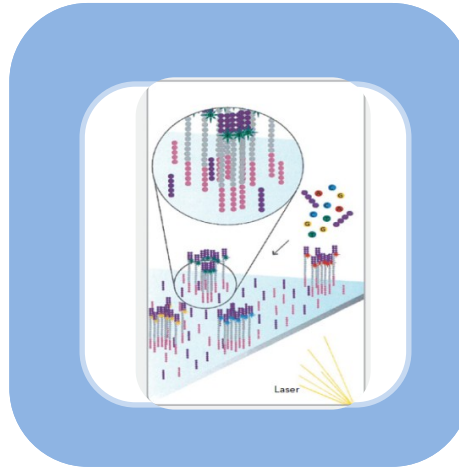
AT workflow

1. Trait data



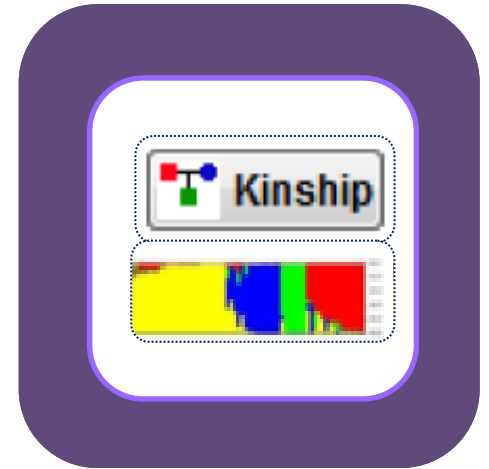
Phenotypic measurements across panel of accessions

2. SNP data

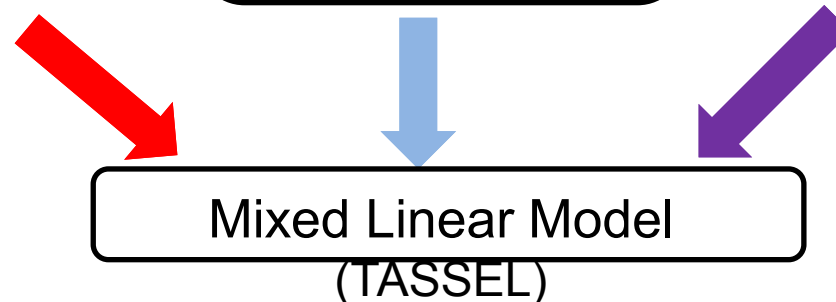


Transcriptome sequencing of accessions, allowing detection of SNPs ordered onto pseudo-molecules

3. Population data



STRUCTURE analysis and kinship analysis carried out to correct for population structure and relatedness



Work Package 2

Control of ovule number in *Capsella* and *Arabidopsis*

Michael Lenhard lead with M. Bevan and L. Lepiniec

Capsella species provide an excellent system for identifying genetic variation controlling ovule number. *C. rubella* has reduced flower size and increased ovule numbers per flower compared to the outcrosser parental species *C. grandiflora*. Genetic mapping populations will be screened to identify QTL and underlying genes identified. Genetic variation in *B. napus* orthologs will be characterised.

Selfing *Capsella* species have reduced flower size,...



Capsella grandiflora

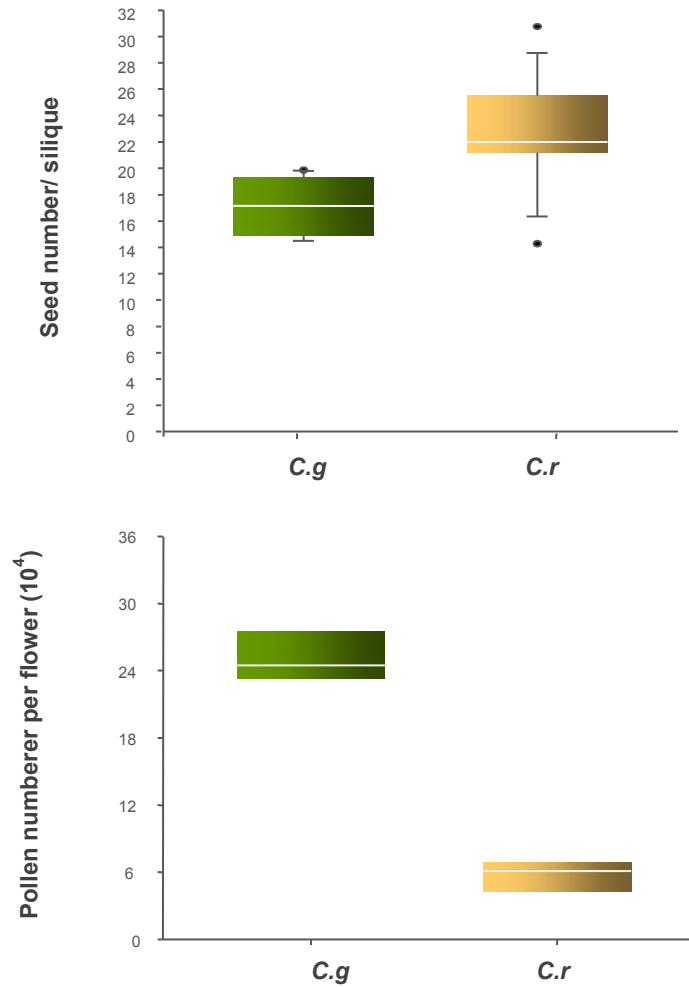


Capsella rubella

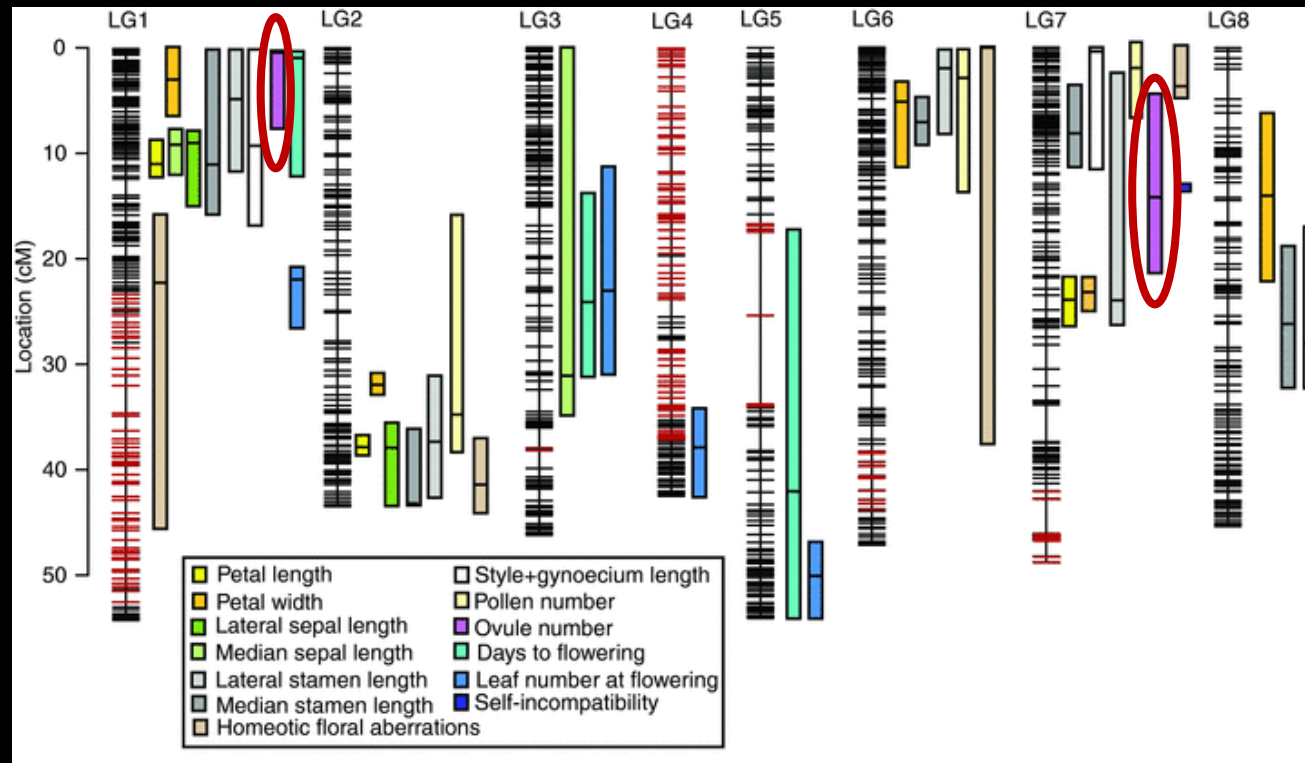


Capsella orientalis

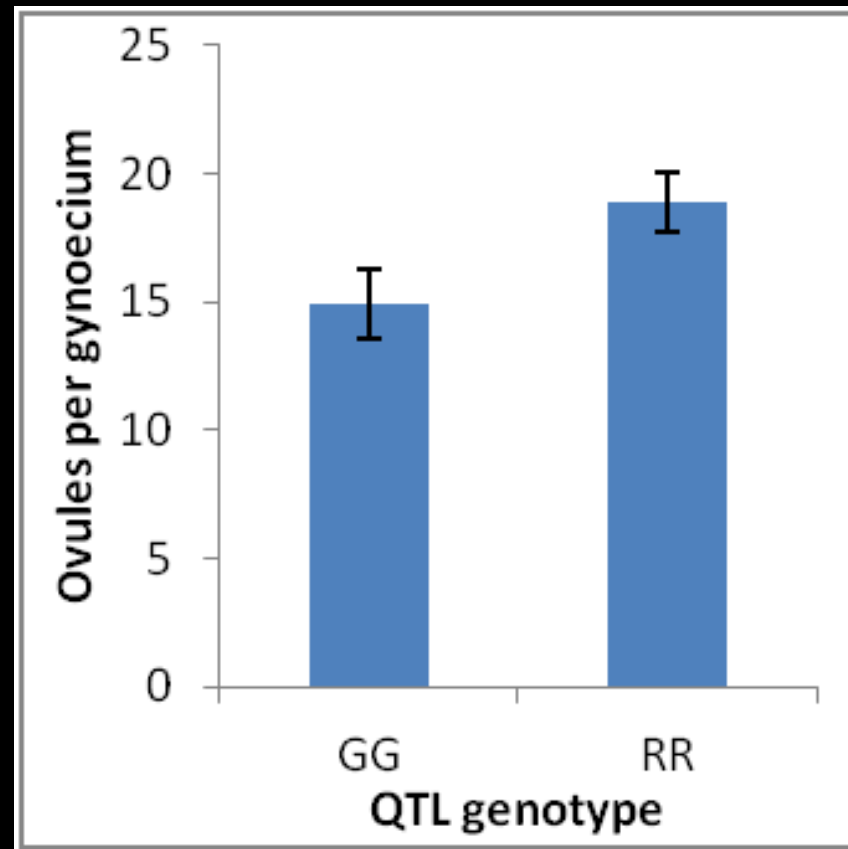
..., more ovules per flower and less pollen.



QTL mapping identifies 2 QTL for ovule number per flower.



Mendelization of QTL F



Proposed work on ovule-number control in *Capsella*

- fine-mapping, cloning and functional characterization of ovule-number QTL F
- testing effects of variation in ovule/seed number per flower on overall seed yield and quality
- test the involvement of known factors in ovule-number control in *Capsella*

Work Package 3

Seed composition and quality

Loic Lepiniec with M. Bevan and M. Lenhard

Orthologs of key regulatory genes controlling seed maturation will be identified in *B. napus* and *Camelina sativa*. Their functions will be tested in a high throughput transient expression system for transcription factors, and allelic variation in *B. napus* genes identified and related to seed quality traits

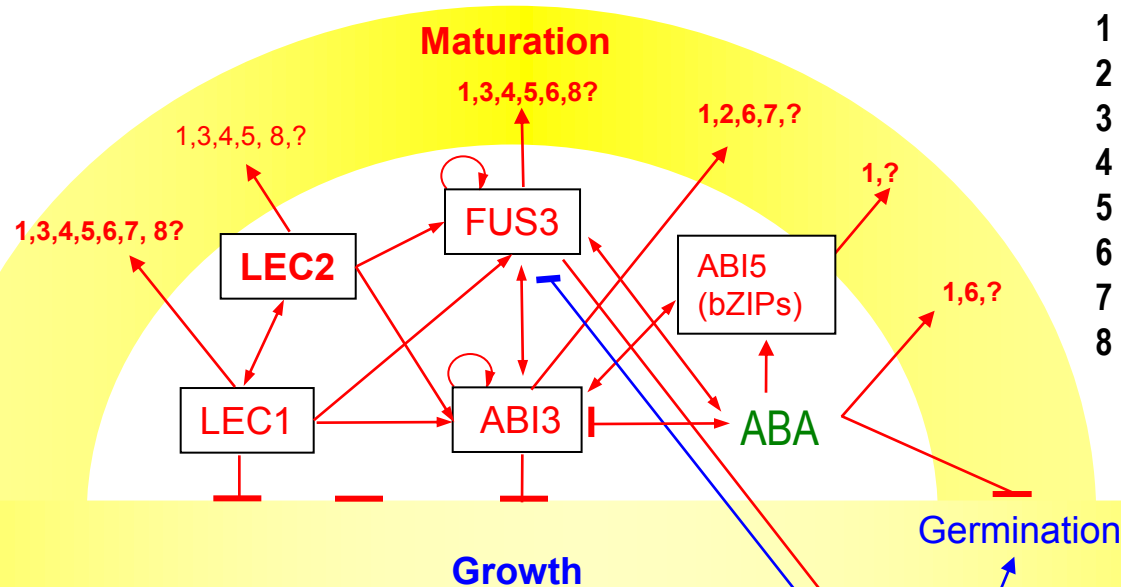
The « AFL » TFs controls seed maturation

- AFL = ABI3-FUSCA3-LEC
- LEAFY COTYLEDON 2 (LEC2) is a seed specific B3-type transcription factor
- FUSCA3 and ABI3 display similar B3 domain
- LEC1 is a seed specific subunit of the CAAT complex (NF-YB)
- mutations have pleiotropic, partially redundant and synergistic effects



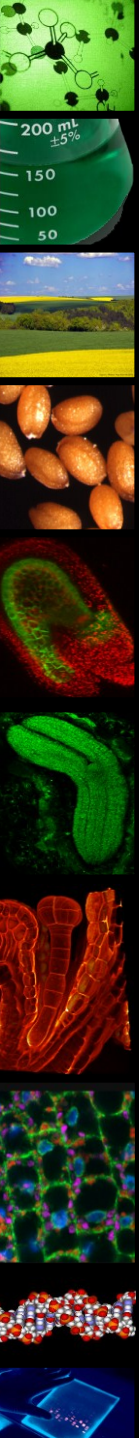
Meinke et al., 1994

- 1 - Seed Storage Proteins
- 2 - Chlorophyll degradation
- 3 - Trichome formation
- 4 - Vascular system repression
- 5 - Anthocyanin repression
- 6 - Dessiccation tolerance
- 7 - ABA sensitivity
- 8 - Oil synthesis



To unravel the network... what are the targets ?

GA



Workpackage 3

Objective 1. Identification and characterisation of AFL homologous genes from oilseed rape and *Camelina sativa*.

- Isolation of putative AFL orthologs in *B. napus* and *Camelina*
- Functional analyses by transient expression in moss protoplasts
- Functional analyses by complementation of Arabidopsis mutants

Objective 2. Engineering *AFL* expression for altering seed storage composition.

A self-inducible system containing an *AFL* gene under the control of one of its target promoter will be built and tested in *Arabidopsis* and *Camelina*.

- Complete construction and transformation of Arabidopsis lines
- Complete screening of lines for targeted overexpression of AFL genes and targets

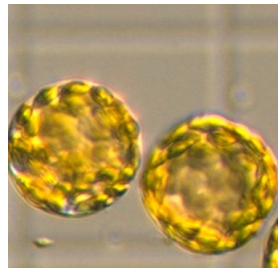
Objective 3. Multi-scale phenotyping of seed maturation

- Phenotyping of *B. napus* and *Camelina* lines
- Complete phenotyping of selected Arabidopsis mutants and transgenic lines

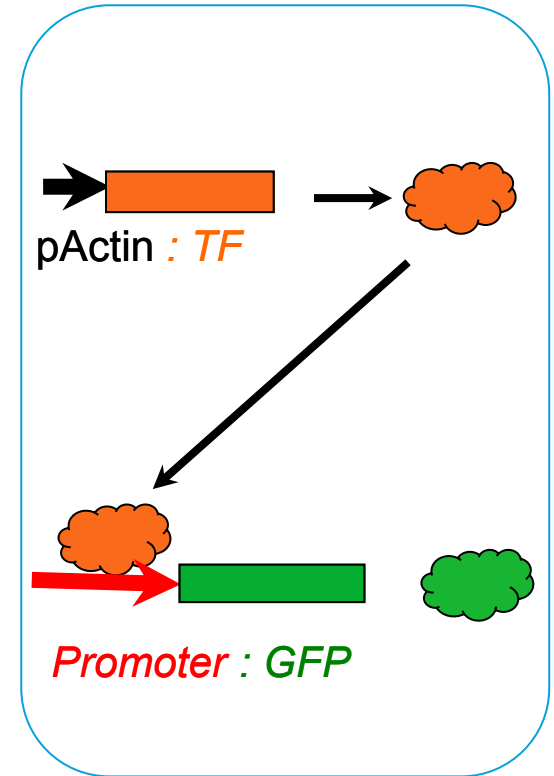
3.1 Developing a transient expression system for testing TF



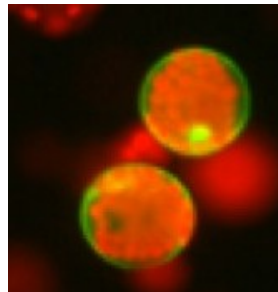
Physcomitrella p.



Pp protoplasts



Quantification by Flow cytometry



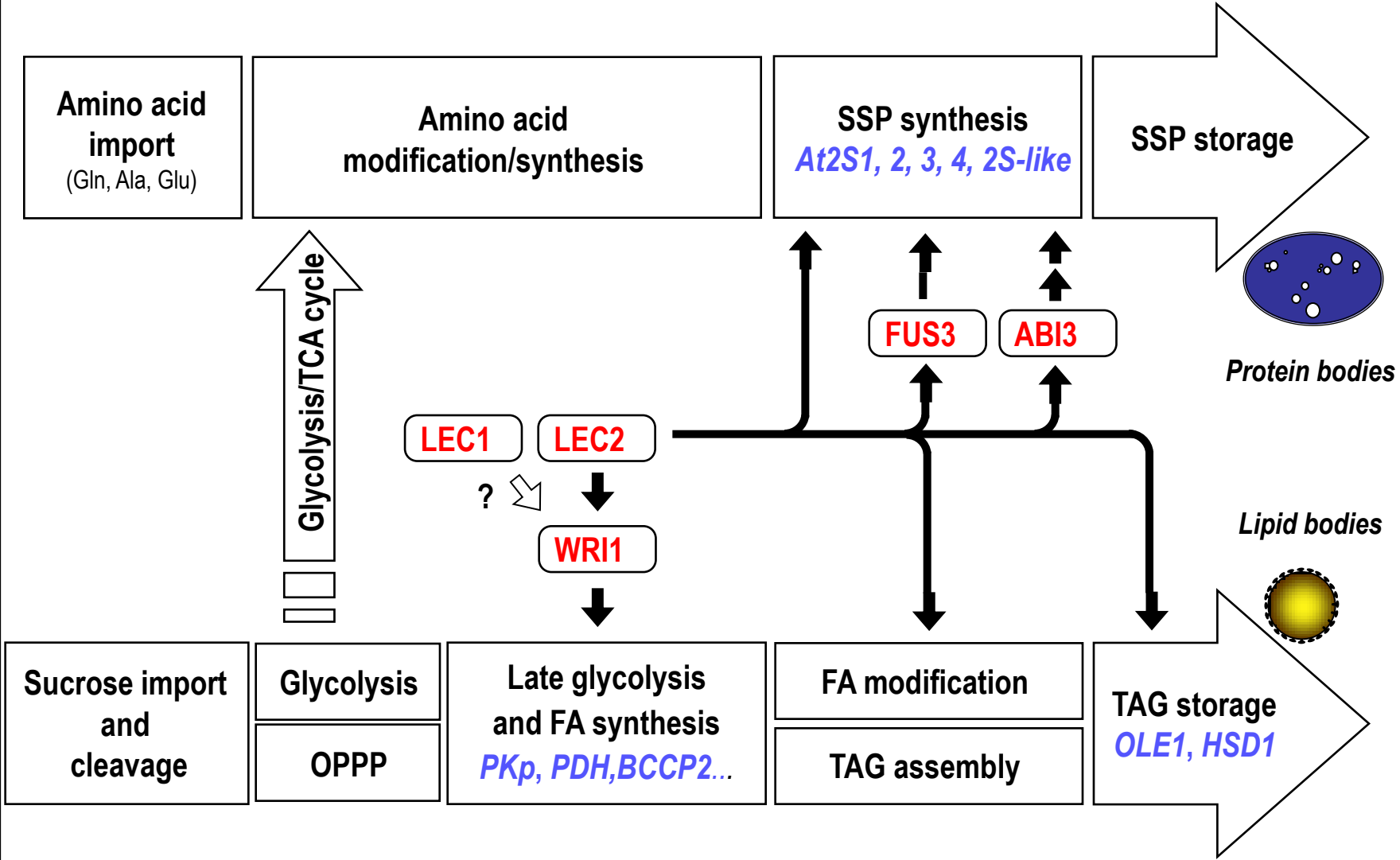
GFP activity



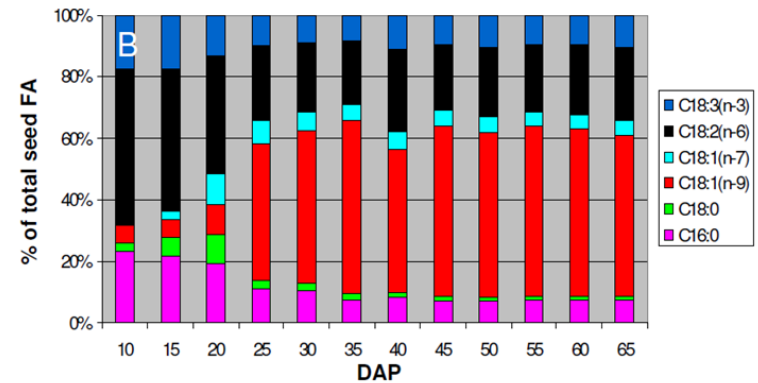
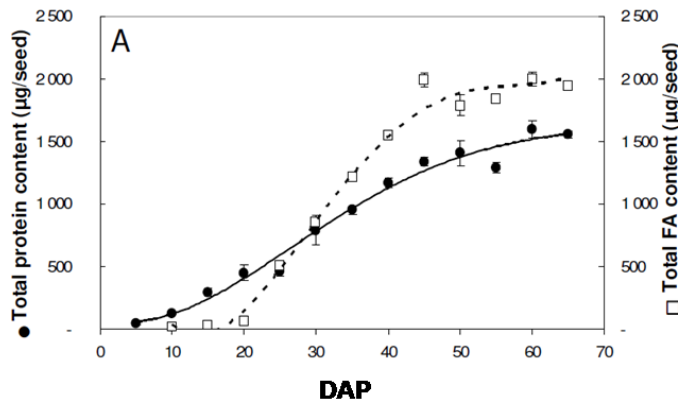
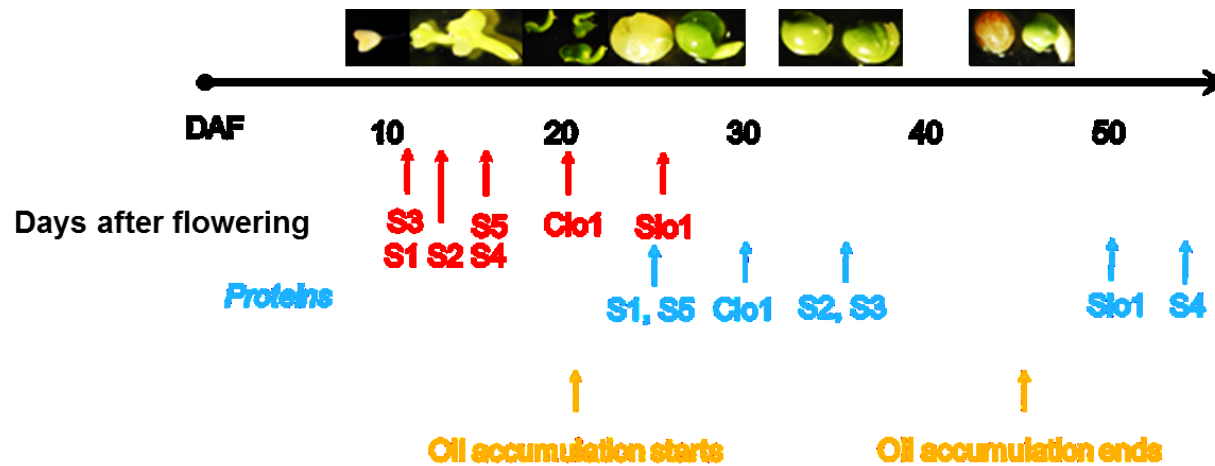
Efficient, fast, and quantitative analyses for simultaneous expression of up to 4 TFs

Thévenin et al. (New Phytol. 2012), Xu et al. (New Phytol. 2013), Denay et al. (Development, 2014)

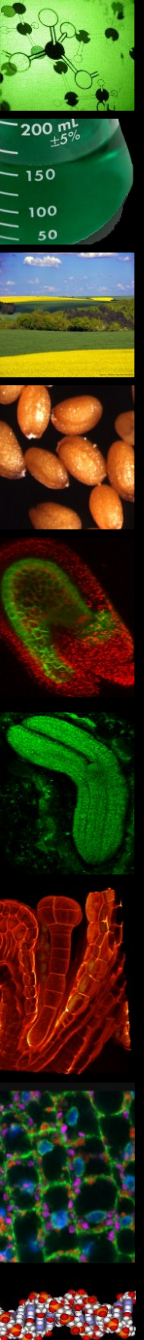
Biological model: Control of storage compound synthesis and accumulation



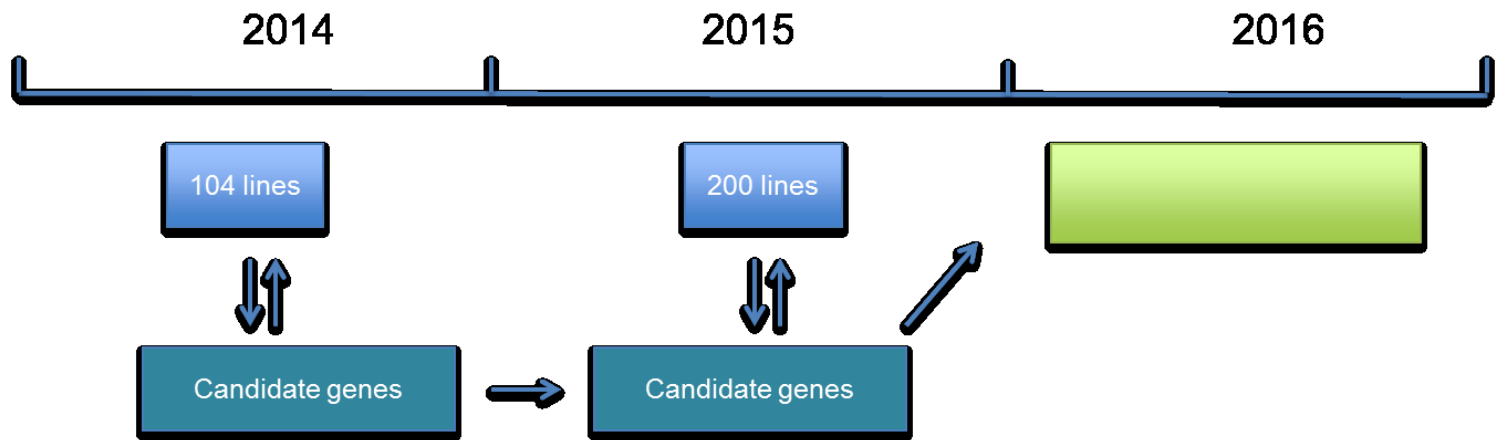
Reserve synthesis and accumulation



Jolivet et al 2011
FTIR (Coll N Nesi INRA Rennes)



Timeline



Associative transcriptomics
phenotyping



Candidate gene identification
and analyses



Seed multiscale
phenotyping





Thanks for your attention

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