TILLING (Targeting Induced Local Lesions IN Genomes)

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TILLING (Targeting Induced Local Lesions IN Genomes)

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Christine Saffray, Marion Dalmais, Abdelhafid Bendahmane (IPS Paris-Saclay)

TILLING: A method of producing and identifying mutations in any gene of interest

- EMS-generated population of point mutants
- Make DNAs from each plant (~4500), arrange in pools
- Screen for lines of interest by gene-specific detection
- Purify away from other mutations by back-crossing

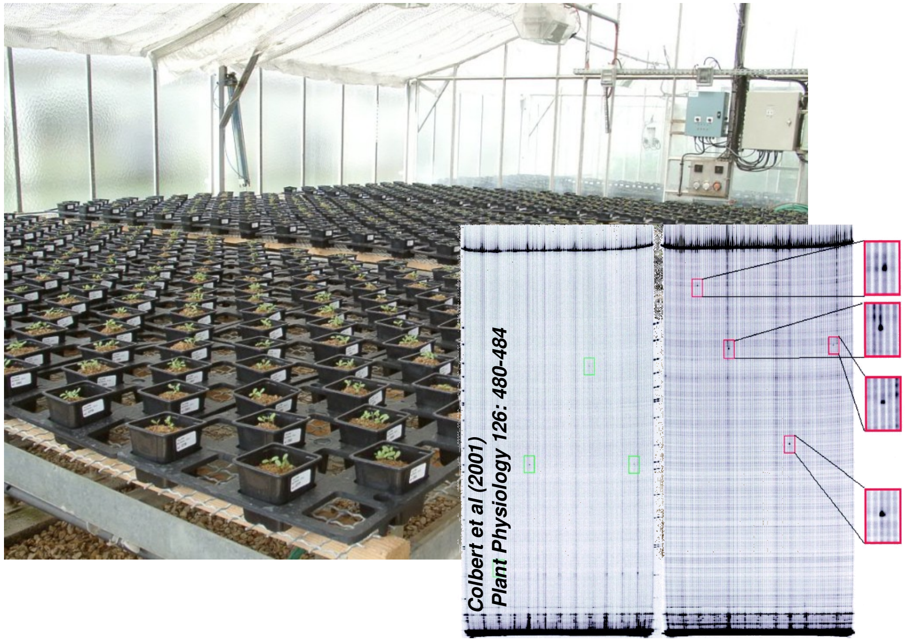


Photo serre URLEG-INRA Dijon C. Le Signor

Colbert et al (2001)
Plant Physiology 126: 480-484

TILLING

Targeting Induced Local Lesions in Genomes

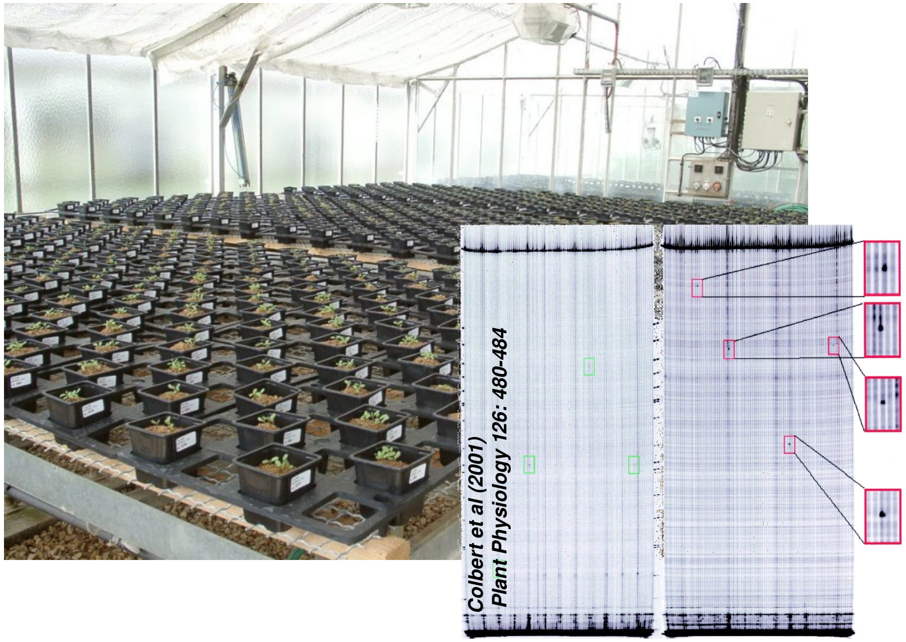


Photo serre URLEG-INRA Dijon C. Le Signor

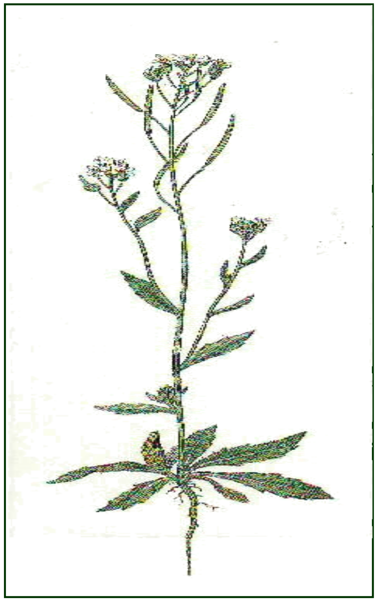
Colbert et al (2001)
Plant Physiology 126: 480-484

Method of producing and identifying mutations in genes of interest

Origin of TILLING

Henikoff's lab

Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, USA.



Arabidopsis
(génome 125 Mb)

Targeted screening for induced mutations.

McCallum et al.

Nat Biotechnol. 2000; 18(4): 455-7

High-throughput TILLING for functional genomics.

Till BJ et al.

Methods Mol Biol. 2003; 236:205-20.

Application of TILLING

Cereals:

Rice

Génome de 500 Mb

- *Crop Pathology and Genetics Research UC- Davis, USA*

Maize

2500 Mb

- *Henikoff's lab Seattle, USA*

Legumes:

Lotus

470 Mb

- *John Innes Centre Norwich, UK*

Medicago

Jemalong A17

520 Mb

- *D. Cook's lab UC Davis, USA*
- *INRA Dijon France*

Pois

4000 Mb

- *INRA Dijon et URGV- INRA Evry France*

Advantages of TILLING:

- Applicable to all organisms (small or large genomes)

fi utilises a chemical mutagen which generates an allelic series of point mutations (mutation frequency 1 mutation/300 Kb).

- Easily set up:

fi Rapid screening of a collection of mutagenized plants for the identification of point mutations in genes of interest

Steps in TILLING procedure

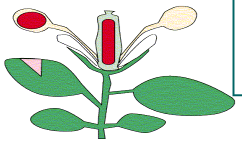
- Mutagenesis of genome using Ethyl Methane Sulfonate (EMS)
- Production of an M2 population
- Screening of the M2 population
- Isolation of lines bearing mutations in gene of interest and back-crossing to eliminate unwanted mutations

Assessment of mutation efficiency

M1

Mutation efficiency:

percentage of embryos aborted in pods of M1 plants ~15 à 20 %

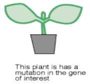


Chimaera

All the mutations are in the heterozygous state

Recessive mutations (99%) - no phenotype associated

M2

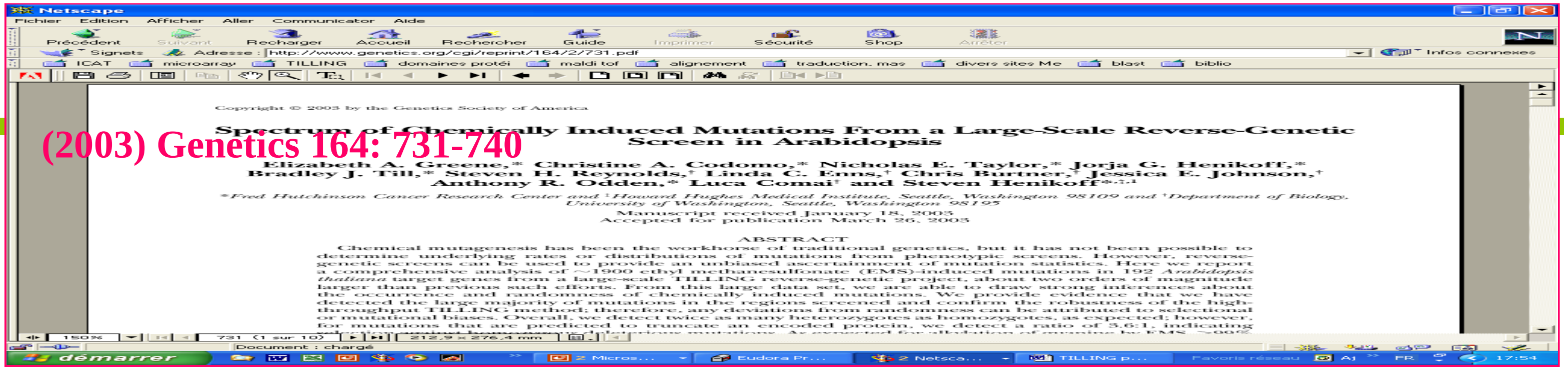


M2 plant carrying one or several mutations in its genome

50% mutations heterozygote
25% mutations homozygote
25% homozygote wild-type

For recessive mutations: 25% of plants show phenotype

For dominant mutations: 75% of plants show phenotype



(2003) Genetics 164: 731-740

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Spectrum of Chemically Induced Mutations From a Large-Scale Reverse-Genetic Screen in Arabidopsis

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Bradley J. Till,* Steven H. Reynolds,† Linda C. Enns,† Chris Burtner,† Jessica E. Johnson,†
Anthony R. Odden,* Luca Comai† and Steven Henikoff*,‡,1

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ABSTRACT

Chemical mutagenesis has been the workhorse of traditional genetics, but it has not been possible to determine underlying rates or distributions of mutations from phenotypic screens. However, reverse-genetic screens can be used to provide an unbiased ascertainment of mutation statistics. Here we report a comprehensive analysis of ~1900 ethyl methanesulfonate (EMS)-induced mutations in 192 *Arabidopsis thaliana* target genes from a large-scale TILLING reverse-genetic project, about two orders of magnitude larger than previous such efforts. From this large data set, we are able to draw strong inferences about the occurrence and randomness of chemically induced mutations. We provide evidence that we have detected the large majority of mutations in the regions screened and confirm the robustness of the high-throughput TILLING method; therefore, any deviations from randomness can be attributed to selectional or mutational biases. Overall, we detect twice as many heterozygotes as homozygotes, as expected; however, for mutations that are predicted to truncate an encoded protein, we detect a ratio of 3.6:1, indicating

Mutation frequency: 1 mutation/300 kb (M2)

For a genome size similar to M. truncatula **(500 Mb), the frequency would be ~1500 mutations/plant M2)**

Therefore backcrosses to WT line needed

Mutation distribution:

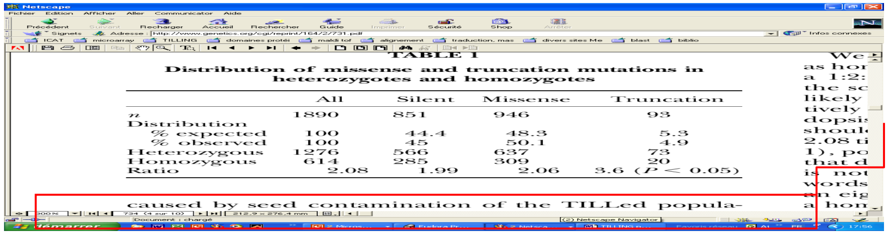


TABLE 1

Distribution of missense and truncation mutations in heterozygotes and homozygotes

	All	Silent	Missense	Truncation
n	1890	851	946	93
Distribution				
% expected	100	44.4	48.3	5.3
% observed	100	45	50.1	4.9
Heterozygous	1276	566	637	73
Homozygous	614	285	309	20
Ratio	2.08	1.99	2.06	3.6 ($P < 0.05$)

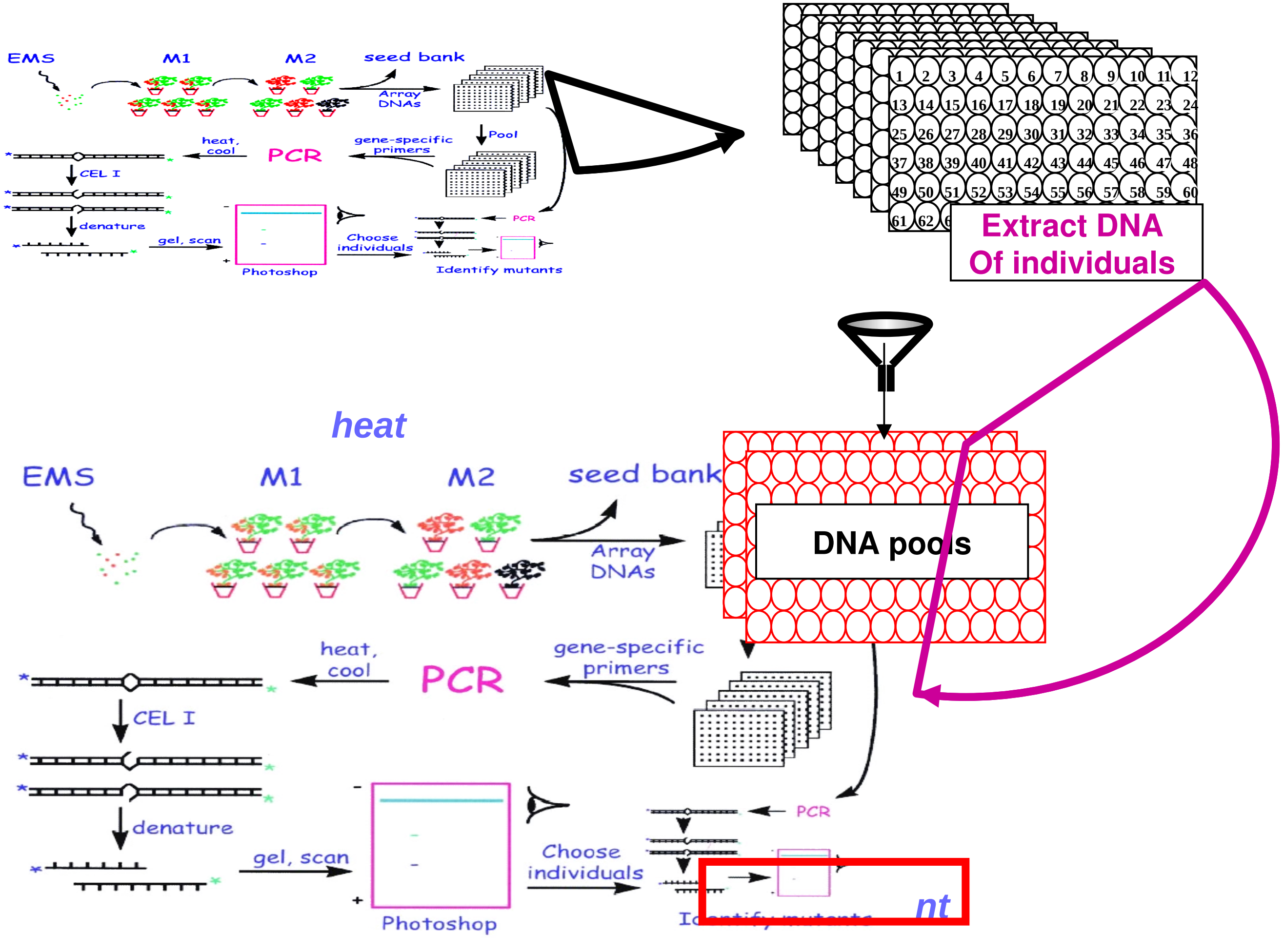
caused by seed contamination of the TILLed popula-

Silent mutations : 45%
Missense mutations : 50%
Stop codon Mutations: 4,9%

Therefore need to isolate several mutants for each gene

Summary of TILLING method

From Colbert et al. (2001). Plant Physiology, 126: 480-484.



Screening carried out on DNA pools from 8-12 individuals on microtitre plates and subsequently on individual DNA samples.

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Dijon
Unité de Recherche

Towards a high throughput screening based on NGS (URGV Paris-Saclay)

2013 : MiSeq detection system – general workflow

2D gDNA pooling

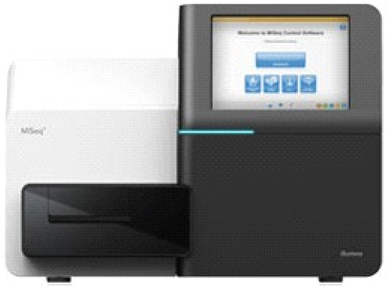
Libraries construction
Indexed PCR
- **384 PCR**
- **Access Array™** Fluidigm ©

Library purification
- **E-Gel ®** **Invitrogen**
- **Nucleobeads** **Agencourt**

Library Quality control
2100 Bioanalyzer™ **Agilent Technologies ©**

Library Quantification
Quant-iT™ PicoGreen ®

Sequencing
Miseq ™ **Illumina ©**



NGS data analysis : Bioinformatic workflow

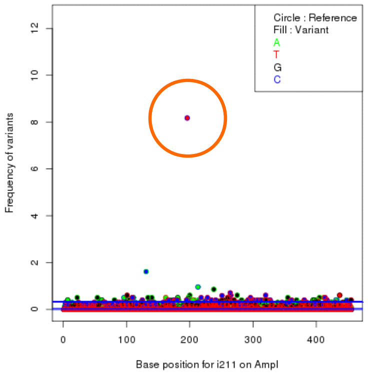
Quality control CLCbio

Alignment, SNP detection
Bowtie II

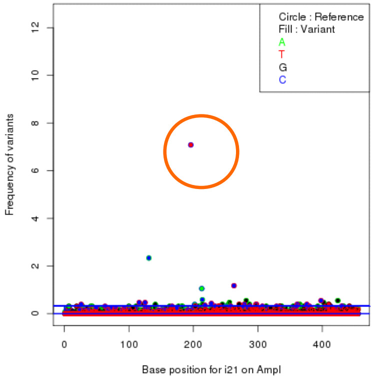
Filter score
Statistical analysis
Python, R, Unix

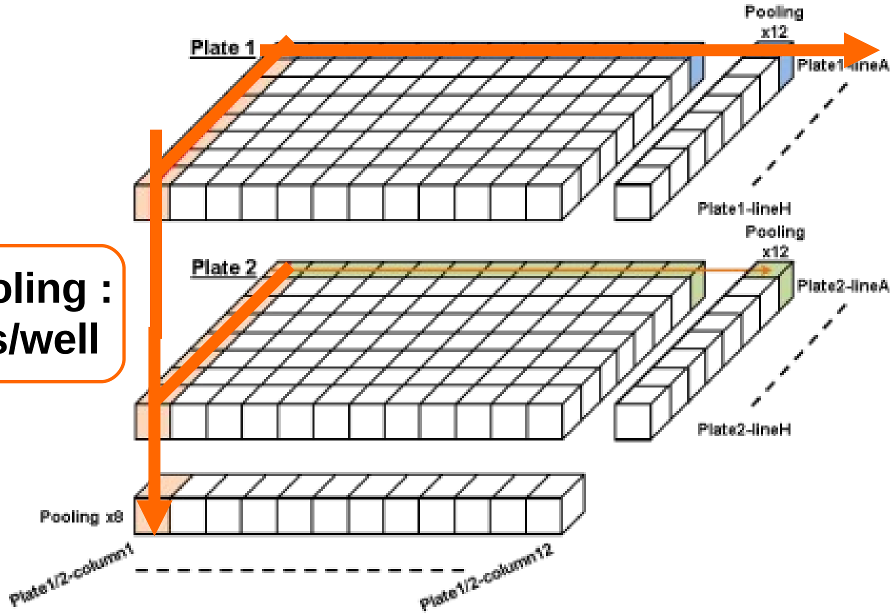
Results

Output in 1st pooling dimension



Output in 2nd pooling dimension





Row pooling : 12 families/well

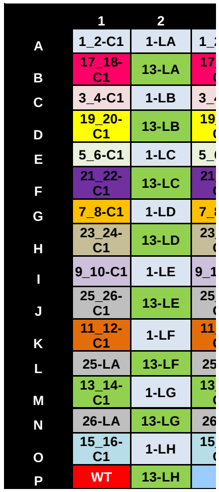
Column pooling : 16 families/well

1 family = 2 coordinates

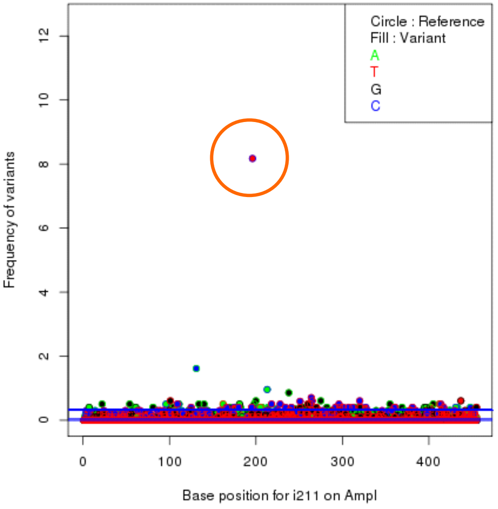
384 well-plate gDNA :

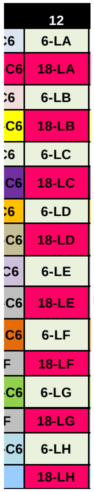
First pooling dimension

Second pooling dimension

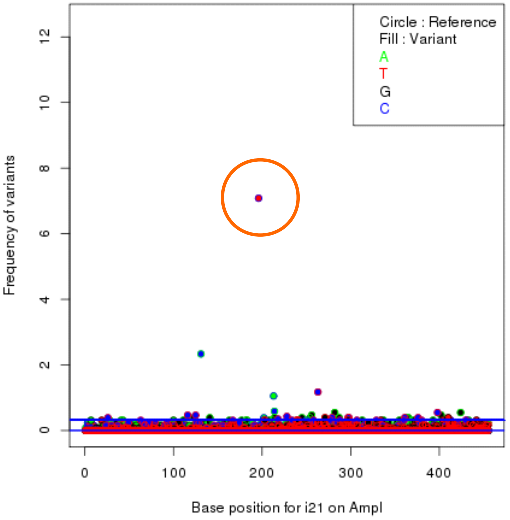


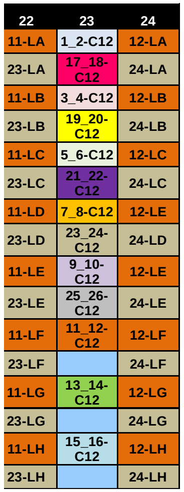
	1	2
A	1_2-C1	1-LA
B	17_18-C1	13-LA
C	3_4-C1	1-LB
D	19_20-C1	13-LB
E	5_6-C1	1-LC
F	21_22-C1	13-LC
G	7_8-C1	1-LD
H	23_24-C1	13-LD
I	9_10-C1	1-LE
J	25_26-C1	13-LE
K	11_12-C1	1-LF
L	25-LA	13-LF
M	13_14-C1	1-LG
N	26-LA	13-LG
O	15_16-C1	1-LH
P	WT	13-LH





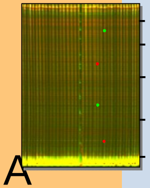
12
6-LA
18-LA
6-LB
18-LB
6-LC
18-LC
6-LD
18-LD
6-LE
18-LE
6-LF
18-LF
6-LG
18-LG
6-LH
18-LH





22	23	24
11-LA	1_2-C12	12-LA
23-LA	17_18-C12	24-LA
11-LB	3_4-C12	12-LB
23-LB	19_20-C12	24-LB
11-LC	5_6-C12	12-LC
23-LC	21_22-C12	24-LC
11-LD	7_8-C12	12-LE
23-LD	23_24-C12	24-LD
11-LE	9_10-C12	12-LE
23-LE	25_26-C12	24-LE
11-LF	11_12-C12	12-LF
23-LF		24-LF
11-LG	13_14-C12	12-LG
23-LG		24-LG
11-LH	15_16-C12	12-LH
23-LH		24-LH

Cel1/Endo1 and NGS TILLING methods compared (URGV Paris-Saclay)

	Cel1/Endo 1	NGS- MiSeq - Illumina	
		PCR 384	Fluidigm
DNA pooling	1D	2D (rows + columns pooling)	
Amplicon	1000 bp	500 bp	500 bp
Detection system	· Endonuclease digestion · Detection of labelled DNA fragments on Li-Cor · Sanger sequençing 	Pair-end sequencing 2 x 250bp □ 2 x 300bp Sequence analysis	
Amplicon/run	1	25	48
Time/ experiment	1 month	3 months	2-3 months
Mutation frequency	1mutant/200 kb	1mutant/130 kb	1mutant/130 kb
Families / screen	5000	384-well plate = 2500 families	384-well plate = 2500 families

Pea TILLING populations

CAMEOR
spring pea collection

4704 M2 families
60 genes screened (80 targets)
1150 mutants
NGS: 1 mutation /130kb

Dalmais *et al*., Genome Biology, 2008
http://urgv.evry.inra.fr/UTILLdb)

Terese *rms4*
Spring pea collection

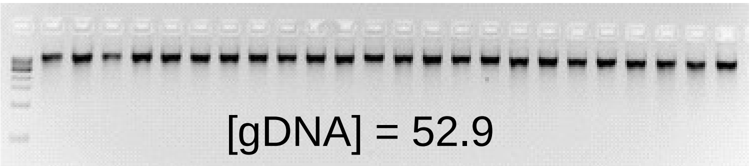
4200 M2 families
10 genes screened
84 mutants
NGS: 1 mutation / 359kb

Hr Ps336/11 genotype
PEAMUST Winter pea collection

4500 M2 families

2014 : DNA samples for 946 low-seeds M2 families

2015 : DNA samples for 960 high-seeds M2 families



[gDNA] = 52.9 ng/µL

2016-2017 : end of population production

Variation in mutation frequency within treated seed lot

→ Comparison of mutation frequency in low- and high-yield families

Hr Ps336/11 genotype

PEAMUST Winter pea collection

2014 : DNA samples for 946 low-seeds M2 families (1-7 seeds/plant) → **Mutation frequency 1/72 kb**

2015 : DNA samples for 960 high-seeds M2 families (20-100 seeds/plant) → **Mutation frequency 1/334 kb**

- Low-yield families had a mutation frequency 4-fold higher, but probability of losing the mutant allele also high → aim for intermediate mutation frequency

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Recovery of mutant line for phenotyping

- TILLING lines contain 100s-1000 or more induced point mutations, so the mutation of interest should be backcrossed to a non-mutagenized parental line (>2 generations)
- Mutant allele is followed using a genotyping method such as dCAPS
- If two different mutant alleles available, an allelism test can help associate mutation and an eventual phenotype

Exploitation of TILLING via new genomics resources in Pea

The Pea Gene Atlas http://bios.dijon.inra.fr/FATAL/cgi/pscam.cgi

jburstin @ Pisum sativum Cameor

PsCam008801_1_AA

Permalink : http://bios.dijon.inra.fr/FATAL/cgi/pscam.cgi?peptideid=PsCam008801_1_AA

ID	PsCam008801_1_AA
AC	PsCam008801_1_AA
IP	Auxin efflux carrier
EC	
GO	Biological Process: transmembrane transport (GO:0055085) Cellular Component: integral to membrane (GO:0016021)
CC	
LN	352 aa

SQ

```
........10........20........30........40........50
         |         |         |         |         |
MISLANVFHVVTKTVPLYVTMFLAYVSIKWFKLFTQEQCSGINKFVAKFS
IPLLSFQIISSNNIYKMSLKLMYADFIQKLLAFLLLTAIIKIRGKGGLKW
IITGLSLSTLPNTLIIGIPVLKAMYNDEAVVLLAQFVFLQSMVWYNLLLF
IYEFDAAKNTLSAPPSETASEIQSKEEEEDEESVGTKRKMKIYPILVTVG
KKLIRNPNTFASLLGIIWSSIHFRWGIHMPEVVNQSIELLSNGGLGMAMF
SIGLFMASQSSIIACGARNTMVAIGLKVLVGPALMALASIVIGLRNTLFK
VAIVQAALPQGIVPFVFAKEYNVHPSILSTAILLGMLIALPVELAFYFLL
AL
```

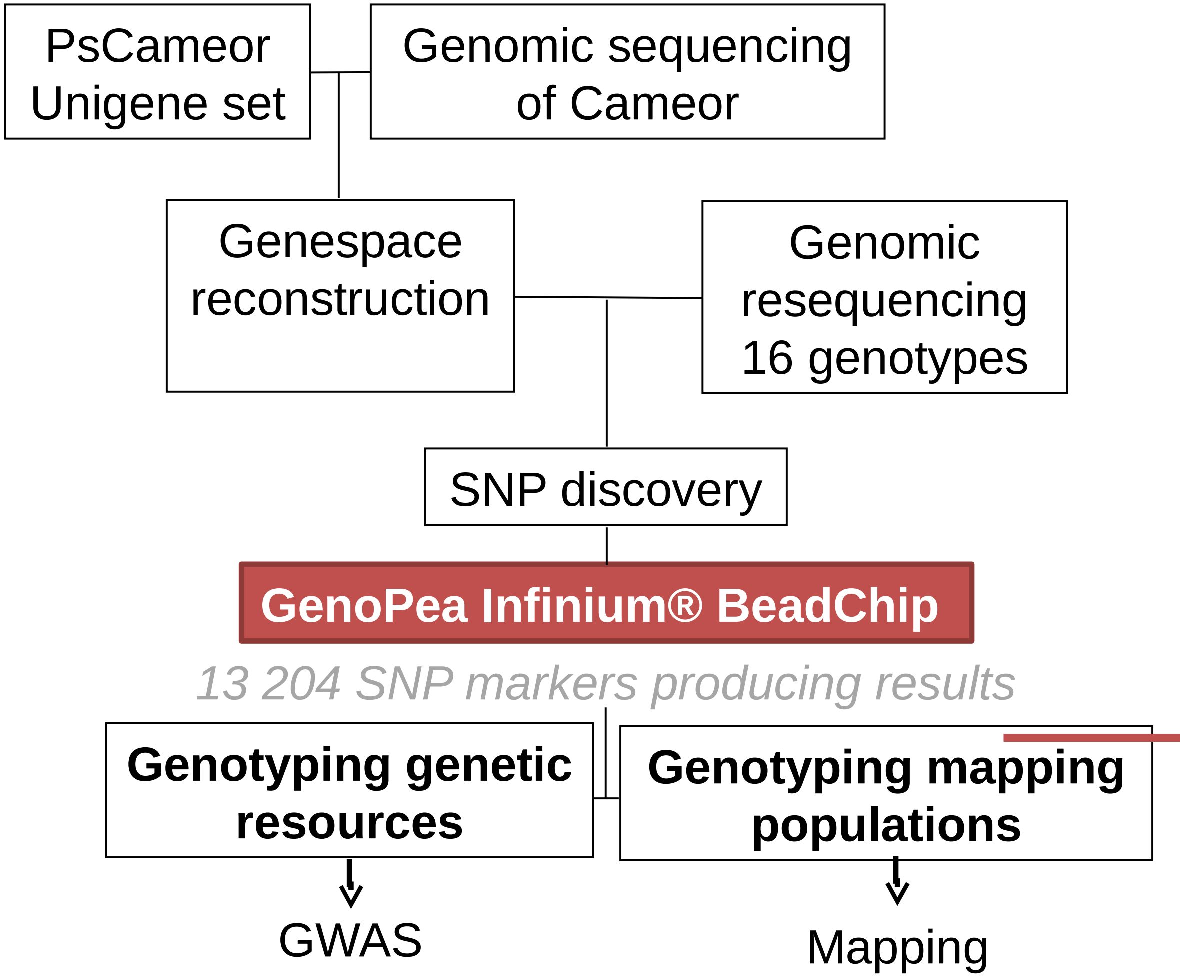
⊞ Domain decomposition

	PsCam016724_1_AA PsCam016724_1_AA	1	352	S=1290 I=70 E=6.52466e-149	IPR004776:Auxin efflux carrier;	pscam
	PsCam007118_1_AA			S=650		

Search any gene of interest

(Judith Burstin et al): Alves-Carvalho Plant

Exploitation of TILLING via new genomics resources in Pea



PsCameor Unigene set

Genomic sequencing of Cameor

Genespace reconstruction

Genomic resequencing 16 genotypes

SNP discovery

GenoPea Infinium® BeadChip

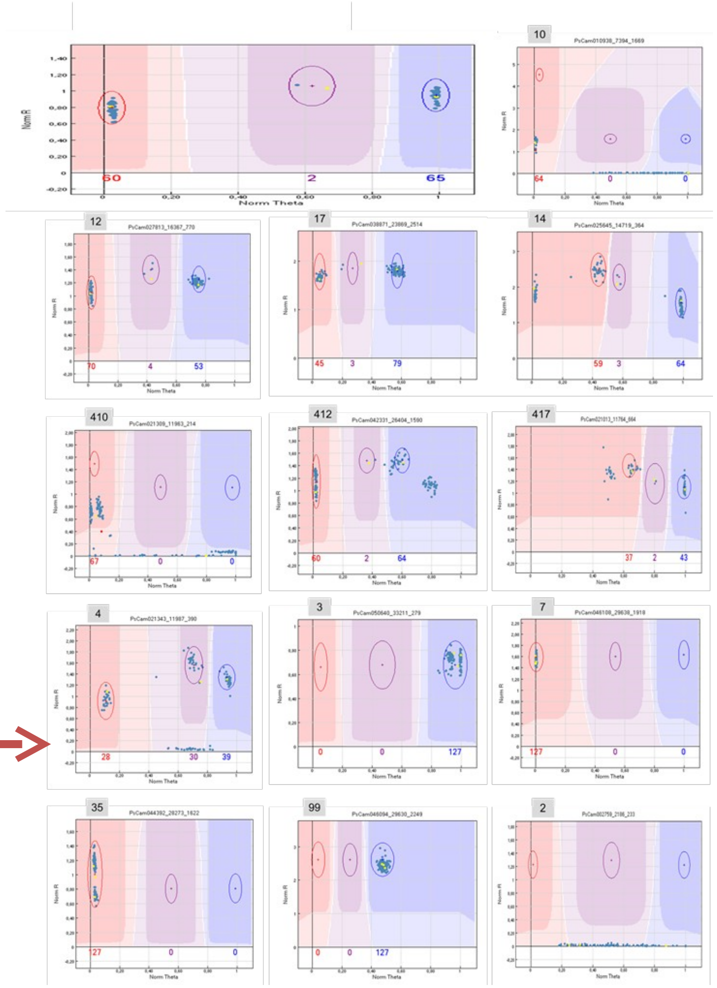
13 204 SNP markers producing results

Genotyping genetic resources

Genotyping mapping populations

GWAS

Mapping



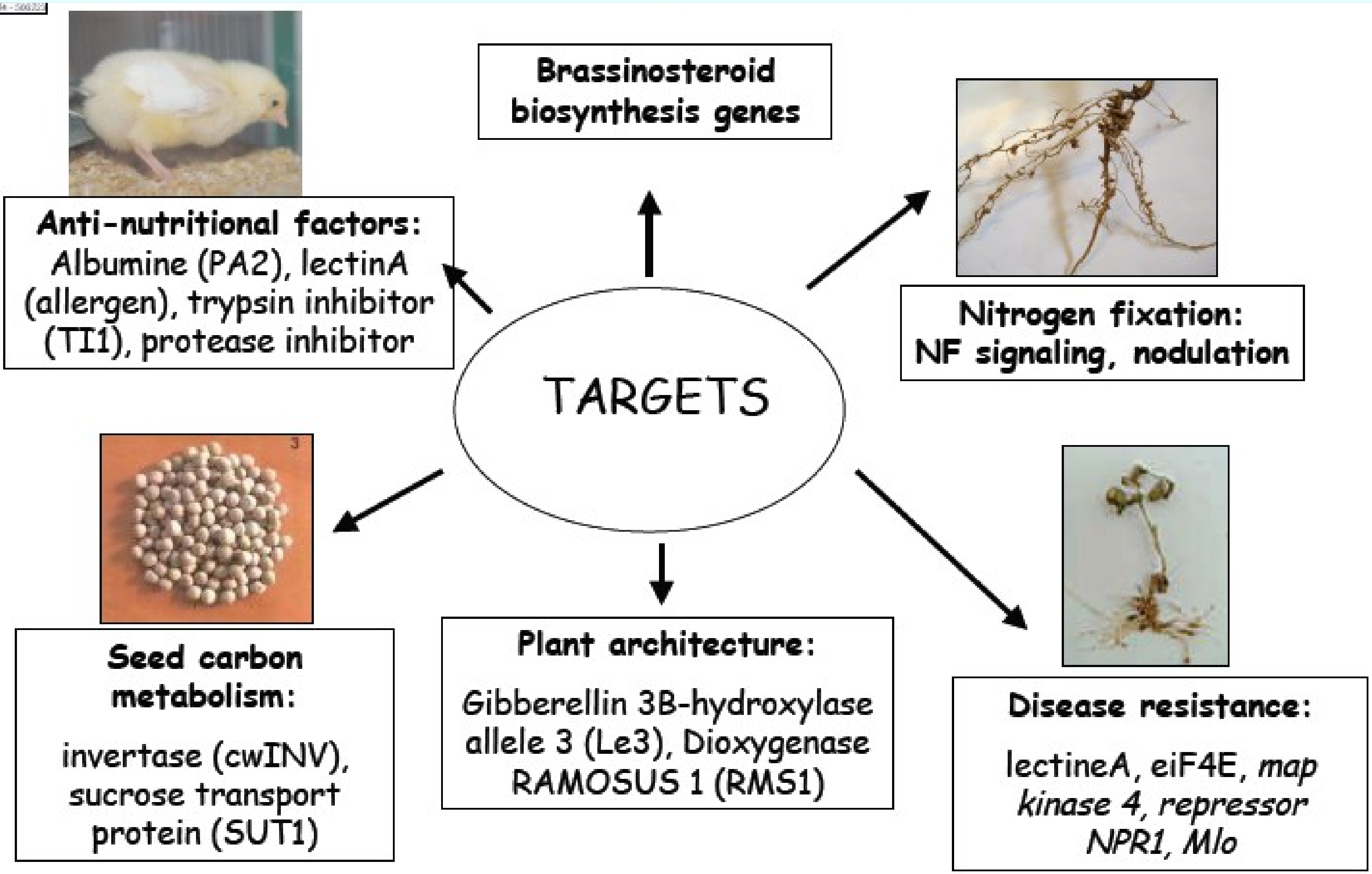
Aluome et al. submitted *Tayeh et al. 2015, The Plant J.*

Agroécologie Dijon Unité de Recherche

AGRO SUP DIJON INRA uB UNIVERSITE BOURGOGNE FRANCHE-COMTE

(Judith Burstin et al)

TILLING: Widely used in Pea since 2008



TARGETS

Brassinosteroid biosynthesis genes

Anti-nutritional factors:
Albumine (PA2), lectinA (allergen), trypsin inhibitor (TI1), protease inhibitor

Nitrogen fixation:
NF signaling, nodulation

Seed carbon metabolism:

invertase (cwINV), sucrose transport protein (SUT1)

Plant architecture:

Gibberellin 3B-hydroxylase allele 3 (Le3), Dioxygenase RAMOSUS 1 (RMS1)

Disease resistance:

lectineA, eiF4E, *map kinase 4, repressor NPR1, Mlo*

(~100 genes screened, supply on average 6 lines per gene screened)

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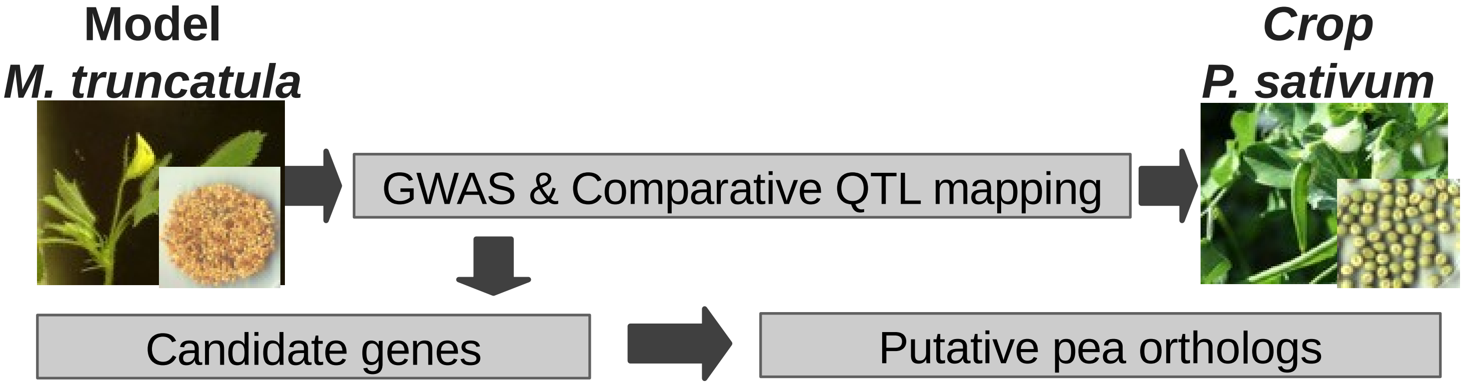
Examples of TILLed genes in our project on seed development and composition



Model
M. truncatula

GWAS & Comparative QTL mapping

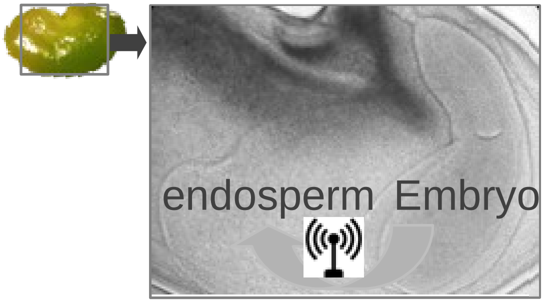
Crop
P. sativum

Candidate genes

Putative pea orthologs

1-seed weight

Seed development



Communication & interaction ?

Two genes with an endosperm-specific expression during embryogenesis determine final seed weight through the regulation of embryo cell division :

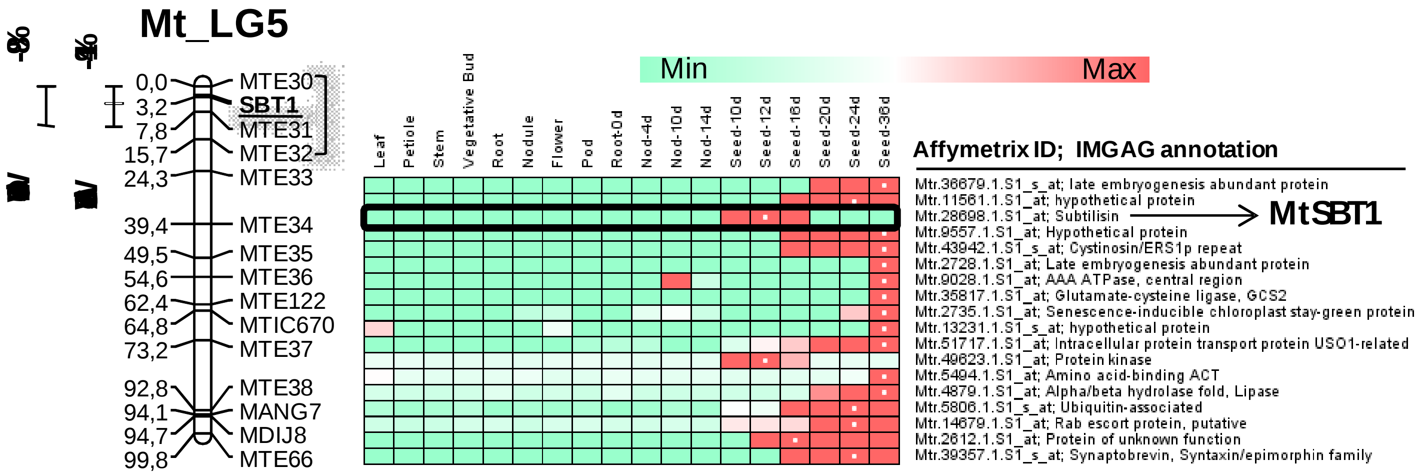
- **a Subtilisin-like serine protease** Phytosulfokines ?

D'Erfurth et al., 2012 New Phytol.

- **a DNA-binding with One zinc-Finger (DOF)-type transcription factor**

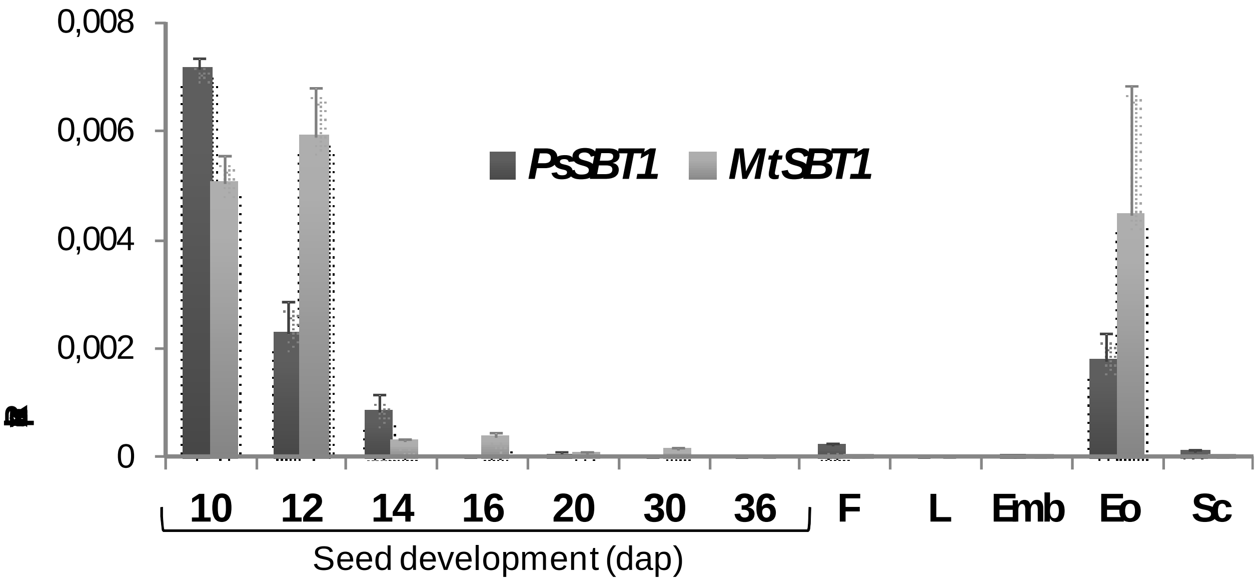
Noguero et al., 2013 Plant Sci.
Noguero et al. 2015 Plant J.

A gene encoding a seed-specific subtilisin colocalizes with a Medicago seed weight QTL and is expressed at the interval embryogenesis-seed filling



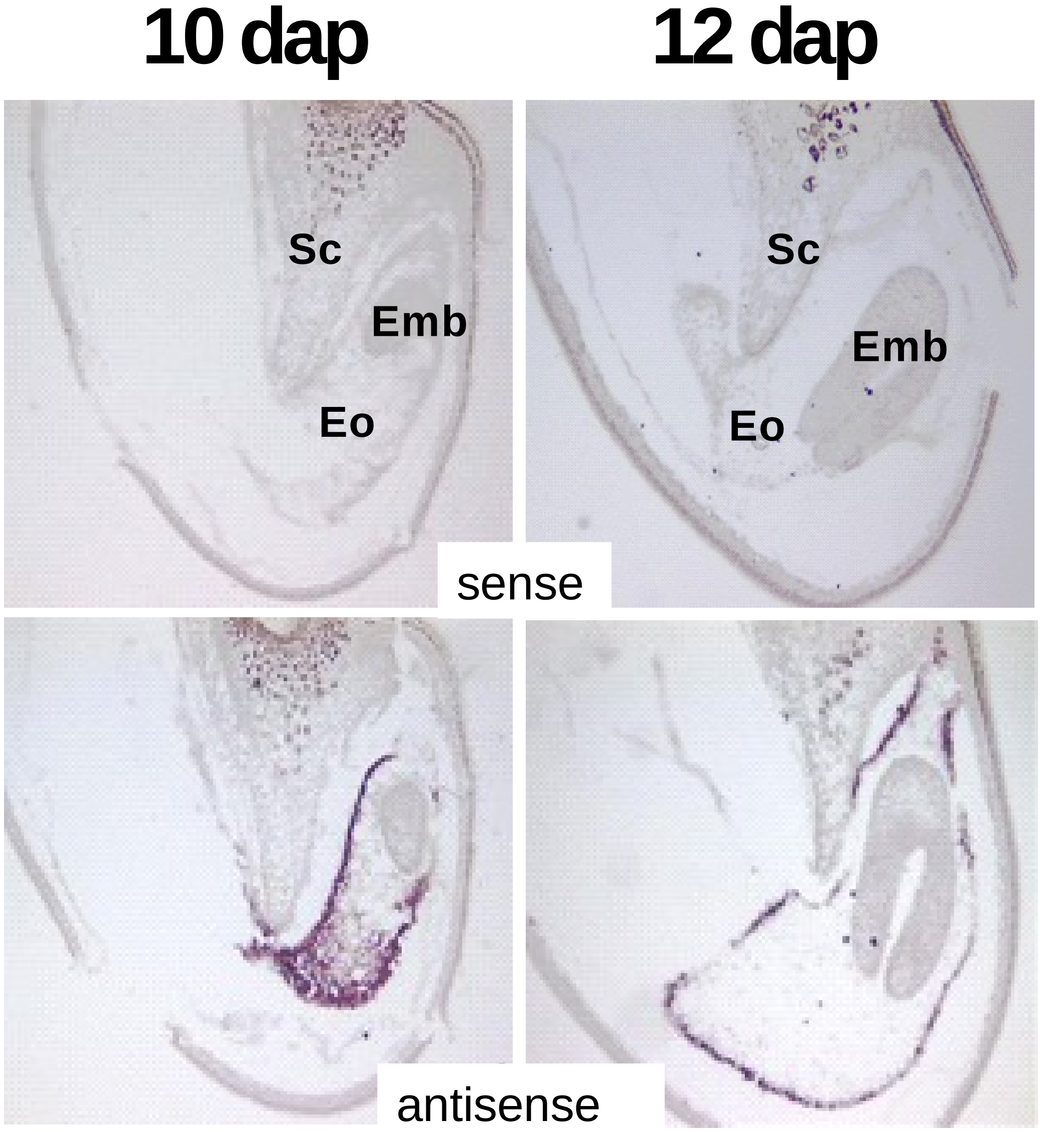
Affymetrix ID; IMGAG annotation

Mtr.36679.1.S1_s_at; late embryogenesis abundant protein
Mtr.11561.1.S1_at; hypothetical protein
Mtr.28698.1.S1_at; Subtilisin
Mtr.9557.1.S1_at; Hypothetical protein
Mtr.43942.1.S1_s_at; Cystinosin/ERS1p repeat
Mtr.2728.1.S1_at; Late embryogenesis abundant protein
Mtr.9028.1.S1_at; AAA ATPase, central region
Mtr.35817.1.S1_at; Glutamate-cysteine ligase, GCS2
Mtr.2735.1.S1_at; Senescence-inducible chloroplast stay-green protein
Mtr.13231.1.S1_s_at; hypothetical protein
Mtr.51717.1.S1_at; Intracellular protein transport protein USO1-related
Mtr.49623.1.S1_at; Protein kinase
Mtr.5494.1.S1_at; Amino acid-binding ACT
Mtr.4879.1.S1_at; Alpha/beta hydrolase fold, Lipase
Mtr.5806.1.S1_s_at; Ubiquitin-associated
Mtr.14679.1.S1_at; Rab escort protein, putative
Mtr.2612.1.S1_at; Protein of unknown function
Mtr.39357.1.S1_s_at; Synaptobrevin, Syntaxin/epimorphin family

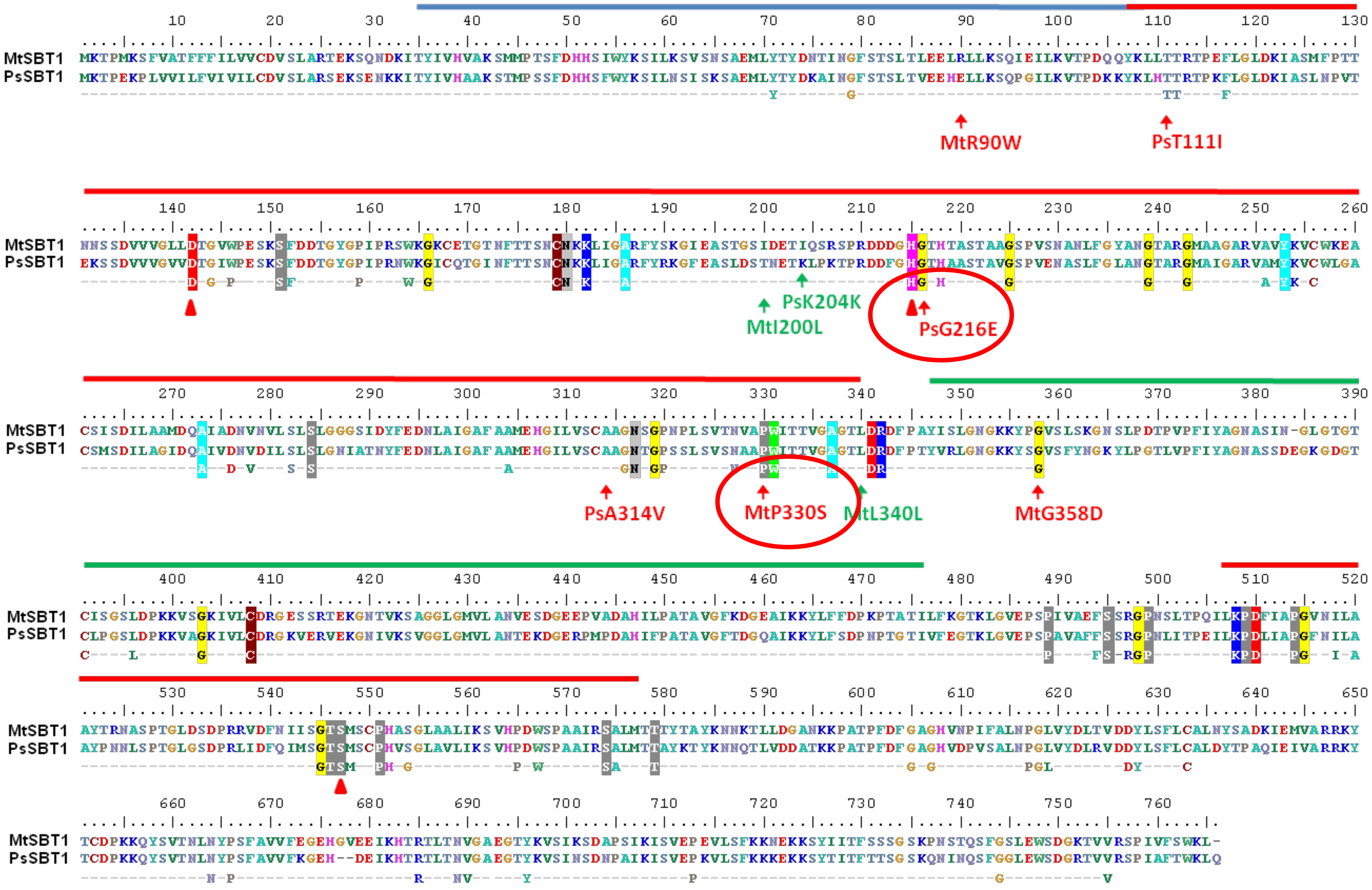


P. sativum	85	82	81	80	72	65	Seed water content (%)
M. truncatula	94	84	82	80	70	59	

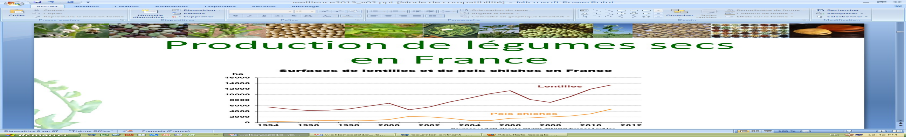
In situ hybridization of MtSBT1.1 to 10 and 12 DAP seeds

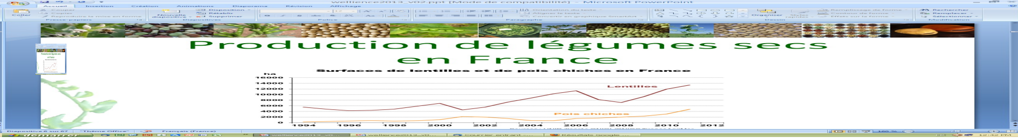


Alignment of MtSBT1.1 AND PsSBT1.1 showing mutants available

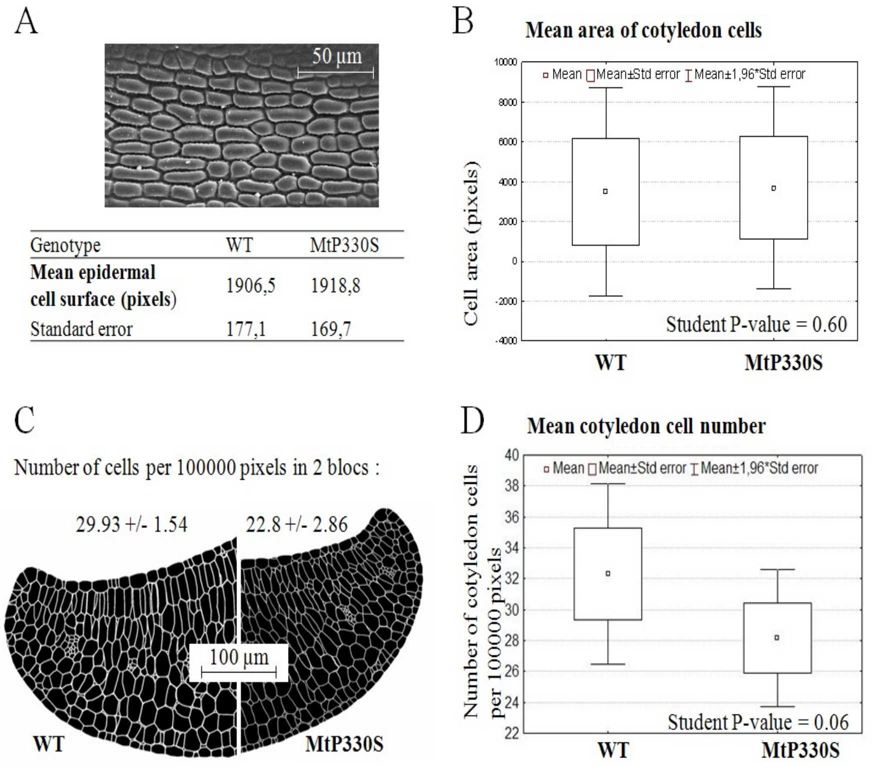


```
               10        20        30        40        50        60        70        80        90       100       110       120       130
       ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
MtSBT1 MKTPMKSFVATFFFILVVCDVSLARTEKSQNDKITYIVHVAKSMMPTSFDHHSIWYKSILKSVSNSAEMLYTYDNTINGFSTSLTLEELRLLKSQIEILKVTPDQQYKLLTTRTPEFLGLDKIASMFPTT
PsSBT1 MKTPEKPLVVILFVIVILCDVSLARSEKSENKKITYIVHAAKSTMPSSFDHHSFWYKSILNSISKSAEMLYTYDKAINGFSTSLTVEEHELLKSQPGILKVTPDKKYKLHTTRTPKFLGLDKIASLNPVT
       ----------------------------------------------------------------------Y-------G-------------------------------TT----F-------------

              140       150       160       170       180       190       200       210       220       230       240       250       260
       ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
MtSBT1 NNSSDVVVGLLDTGVWPESKSFDDTGYGPIPRSWKGKCETGTNFTTSNCNKKLIGARFYSKGIEASTGSIDETIQSRSPRDDDGHGTHTASTAAGSPVSNANLFGYANGTARGMAAGARVAVYKVCWKEA
PsSBT1 EKSSDVVVGVVDTGIWPESKSFDDTGYGPIPRNWKGICQTGINFTTSNCNKKLIGARFYRKGFEASLDSTNETKLPKTPRDDFGHGTHAASTAVGSPVENASLFGLANGTARGMAIGARVAMYKVCWLGA
       -----------D-G--P---SF------P----W-G------------CN-K---A----------------------------HG-H------G-------------G---G-------A-YK-C----

              270       280       290       300       310       320       330       340       350       360       370       380       390
       ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
MtSBT1 CSISDILAAMDQAIADNVNVLSLSLGGGSIDYFEDNLAIGAFAAMEHGILVSCAAGNSGPNPLSVTNVAPWITTVGAGTLDRDFPAYISLGNGKKYPGVSLSKGNSLPDTPVPFIYAGNASIN-GLGTGT
PsSBT1 CSMSDILAGIDQAIVDNVDILSLSLGNIATNYFEDNLAIGAFAAMEHGILVSCAAGNTGPSSLSVSNAAPWITTVGAGTLDRDFPTYVRLGNGKKYSGVSFYNGKYLPGTLVPFIYAGNASSDEGKGDGT
       ------------A--D-V---S-S------------------A------------GN-GP------N--PW-----A---DR---------------G

              400       410       420       430       440       450       460       470       480       490       500       510       520
       ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
MtSBT1 CISGSLDPKKVSGKIVLCDRGESSRTEKGNTVKSAGGLGMVLANVESDGEEPVADAHILPATAVGFKDGEAIKKYLFFDPKPTATILFKGTKLGVEPSPIVAEFSSRGPNSLTPQILKPDFIAPGVNILA
PsSBT1 CLPGSLDPKKVAGKIVLCDRGKVERVEKGNIVKSVGGLGMVLANTEKDGERPMPDAHIFPATAVGFTDGQAIKKYLFSDPNPTGTIVFEGTKLGVEPSPAVAFFSSRGPNLITPEILKPDLIAPGFNILA
       C----L------G----C--------------------------------------------------------------------------------P----FS-RGP--------KPD---PG--I-A

              530       540       550       560       570       580       590       600       610       620       630       640       650
       ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
MtSBT1 AYTRNASPTGLDSDPRRVDFNIISGTSMSCPHASGLAALIKSVHPDWSPAAIRSALMTTTYTAYKNNKTLLDGANKKPATPFDFGAGHVNPIFALNPGLVYDLTVDDYLSFLCALNYSADKIEMVARRKY
PsSBT1 AYPNNLSPTGLGSDPRLIDFQIMSGTSMSCPHVSGLAVLIKSVHPDWSPAAIRSALMTTAYKTYKNNQTLVDDATKKPATPFDFGAGHVDPVSALNPGLVYDLRVDDYLSFLCALDYTPAQIEIVARRKY
       ------------------------GTSM--PH--G---------P-W------SA---T-------------------------G-G---------PGL-------DY----C

              660       670       680       690       700       710       720       730       740       750       760
       ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|.
MtSBT1 TCDPKKQYSVTNLNYPSFAVVFEGEHGVEEIKHTRTLTNVGAEGTYKVSIKSDAPSIKISVEPEVLSFKKNEKKSYIITFSSSGSKPNSTQSFGSLEWSDGKTVVRSPIVFSWKL-
PsSBT1 TCDPKKQYSVTNLNYPSFAVVFKGEH--DEIKHTRTLTNVGAEGTYKVSINSDNPAIKISVEPKVLSFKKKEKKSYTITFTTSGSKQNINQSFGGLEWSDGRTVVRSPIAFTWKLQ
       -------------N-P------------------R---NV-----Y----------------P------------------------------G----------V
```





Surface area and number of cotyledon cells in mature seeds of the homozygous MtP330S mutant versus wild-type.

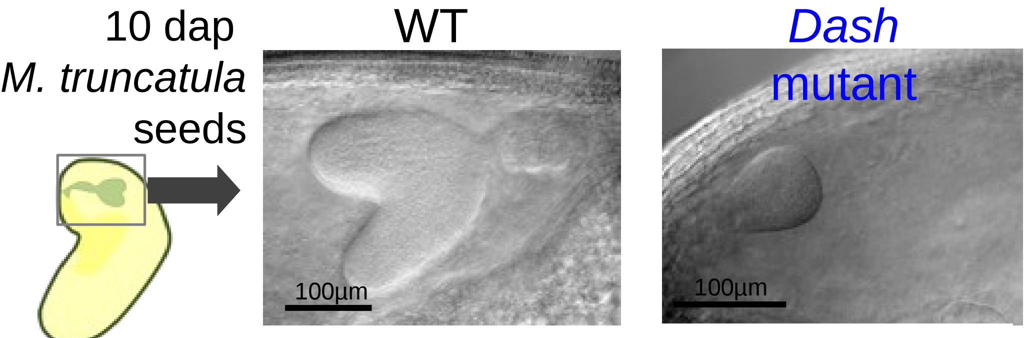


Genotype	WT	MtP330S
Mean epidermal cell surface (pixels)	1906,5	1918,8
Standard error	177,1	169,7

Number of cells per 100000 pixels in 2 blocs :

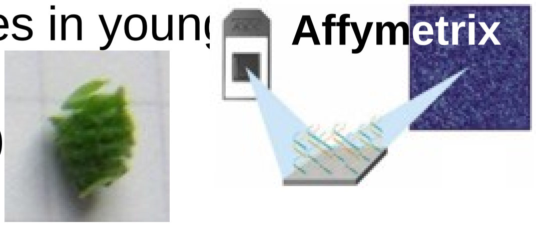
Endosperm-specific genes controlling seed quality

DASH, a DOF TF Acting in Seed embryogenesis and Hormone regulation



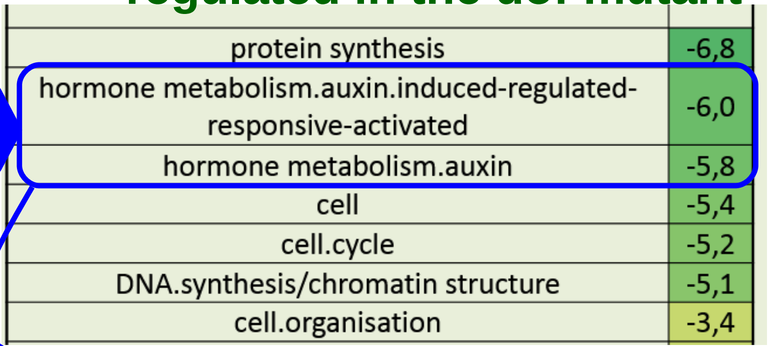
Embryo growth arrest (globular stage)

Transcriptome changes in young pods (8 dap)



defect in transport or distribution of

PageMan display of the functional classes down-regulated in the dof mutant



protein synthesis	-6,8
hormone metabolism.auxin.induced-regulated-responsive-activated	-6,0
hormone metabolism.auxin	-5,8
cell	-5,4
cell.cycle	-5,2
DNA.synthesis/chromatin structure	-5,1
cell.organisation	-3,4

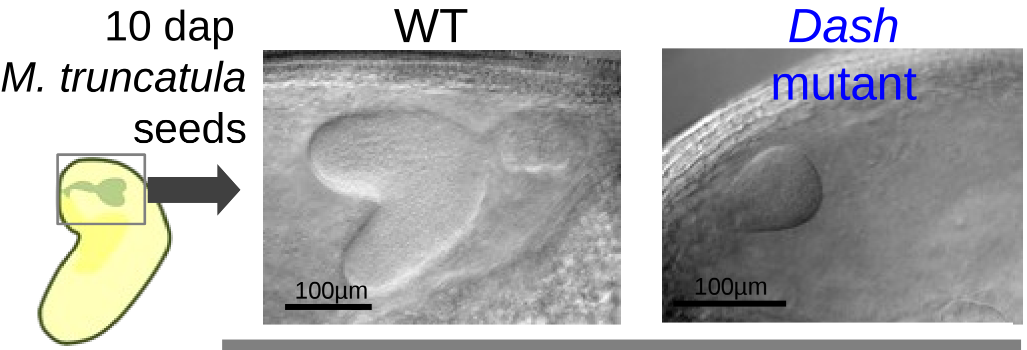
Auxin-transport genes
Auxin-responsive genes

the plant journal

Noguero & Le Signor et al. 2015

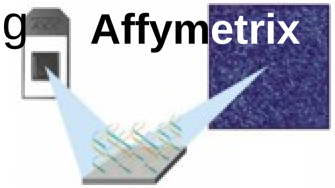
Endosperm-specific genes controlling seed quality

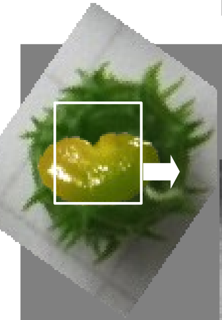
10 dap
M. truncatula
seeds

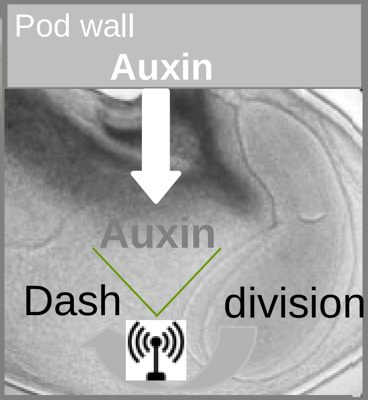


Embryo growth arrest (globular stage)

Transcriptome changes in young pods
(8 dap)







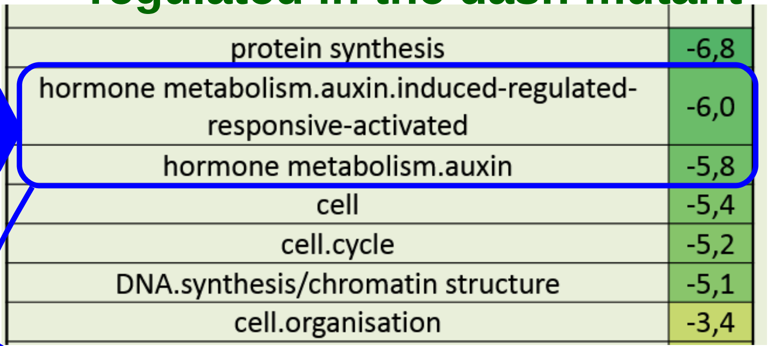
In vitro auxin treatment promotes cell division in young *M. truncatula seeds*

Atif et al. 2013

Physiologia Plantarum
An International Journal for Plant Biology

Compartmentation studies in progress using pea mutants

PageMan display of the functional classes down-regulated in the dash mutant



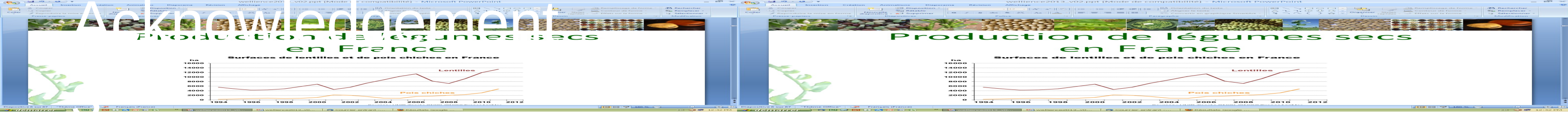
protein synthesis	-6,8
hormone metabolism.auxin.induced-regulated-responsive-activated	-6,0
hormone metabolism.auxin	-5,8
cell	-5,4
cell.cycle	-5,2
DNA.synthesis/chromatin structure	-5,1
cell.organisation	-3,4

Auxin-transport genes
Auxin-responsive genes

the plant journal

Noguero & Le Signor et al. 2015

Acknowledgement


Production de légumes secs en France

Surfaces de lentilles et de pois chiches en France

Melanie Noguero, Christine Lesignor, Brigitte Darchy, Greg Aubert,
Myriam Sanchez, Judith Burstin, Karine Gallardo, (AE, Dijon)
Jérôme Verdier (Shanghai Center for Plant Stress Biology)

C. Saffray, M. Dalmais,

A. Bendahmane (IPS Saclay)

Agroécologie Dijon Unité de Recherche

Agence Nationale de la Recherche ANR

LEGATO LEGumes for the Agriculture of TOmorrow

INRA SCIENCE & IMPACT

Pea/Mtr TILLING: Publications arising

Resource development

- Triques, K., et al. (2008). "Mutation detection using ENDO1: Application to disease diagnostics in humans and TILLING and Eco-TILLING in plants." BMC Molecular Biology 9.
- LeSignor C., (2009) Optimizing TILLING populations for reverse genetics in Medicago truncatula. Plant Biotechnology J. 7 : 430-441
- Dalmais, M., UTILLdb, a Pisum sativum in silico forward and reverse genetics tool. Genome Biol. (2008) 9(2): R43.

Examples of Applications

- Hofer, J., L. Turner, et al. (2009). "Tendril-less Regulates Tendril Formation in Pea Leaves." Plant Cell 21(2): 420-428.
- Laura de Lorenzo, (2009) A Novel Plant Leucine-Rich Repeat Receptor Kinase Regulates the Response of Medicago truncatula Roots to Salt Stress The Plant Cell 21:668-680
- Plet et al, (2011) MtCRE1-dependent cytokinin signaling integrates bacterial and plant cues to coordinate symbiotic nodule organogenesis in Medicago truncatula. Plant J. 65: 622-633.
- Tivendale, N. D., S. E. Davidson, et al. (2012). "Biosynthesis of the Halogenated Auxin, 4-Chloroindole-3-Acetic Acid." Plant Physiology 159(3): 1055-1063.
- D'Erfurth, I., C. Le Signor, et al. (2012). "A role for an endosperm-localized subtilase in the control of seed size in legumes." New Phytologist 196(3): 738-751.
- Noguero, M., C. Le Signor, et al. (2015). "DASH transcription factor impacts Medicago truncatula seed size by its action on embryo morphogenesis and auxin homeostasis." Plant Journal 81(3): 453-466.
- Vernié, T, Camut S, et al (2016) PUB1 interacts with the receptor kinase DMI2 and negatively regulates rhizobial and arbuscular mycorrhizal symbioses through its ubiquitination activity in ago truncatula. Pl. Physiol. In press.

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