

Gene Discovery for Bud Dormancy Induction and Release in Oak by 454-Pyrosequencing and Q-PCR Analysis



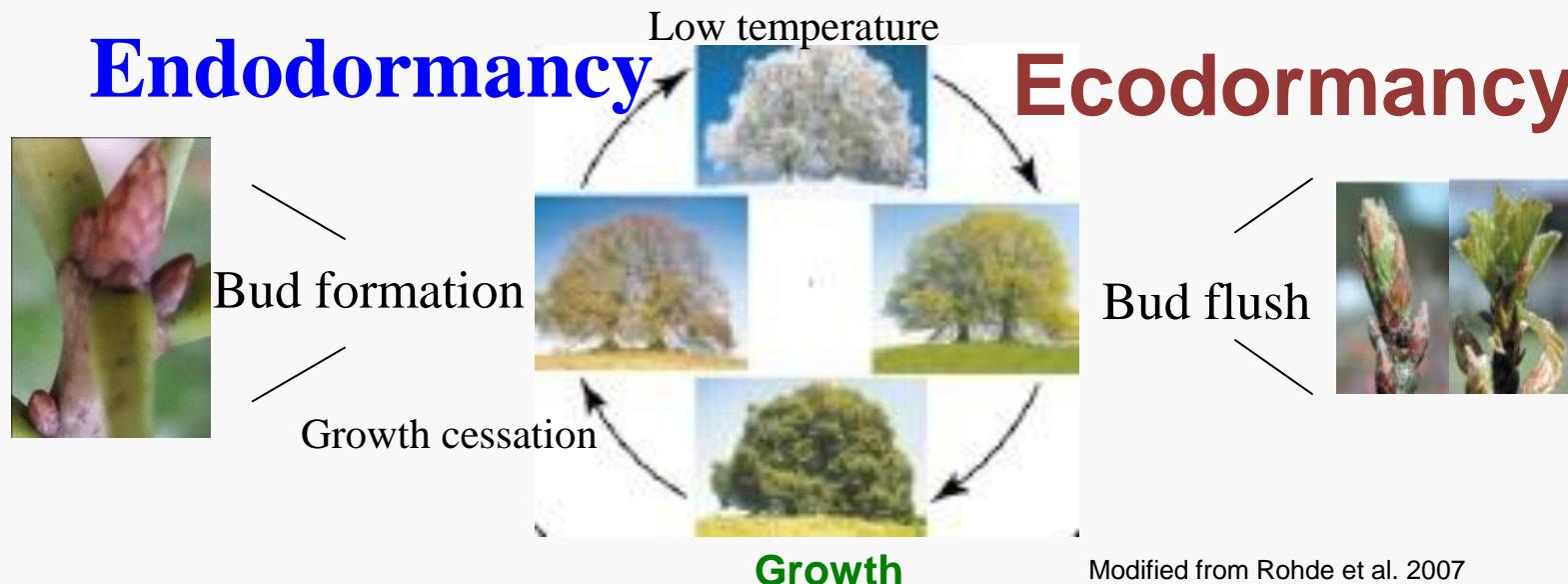
Saneyoshi Ueno



Outline

- Dormancy and bud break phonology in deciduous trees
- *Quercus* (Fagaceae)
- EST catalogue for oaks
- Bud dormancy expression analysis

Growth and dormancy cycle in trees



Endodormancy : is induced by the meristem itself. Growth cessation continues even though environmental condition is favorable

Ecodormancy : is induced by external or environmental factors (eg cold temperatures, nutrient deficiency, water stress ...)

Bud burst timing is critical for deciduous trees

Bud dormancy and climate change

Global warming

- Negative effects on **endodormancy**

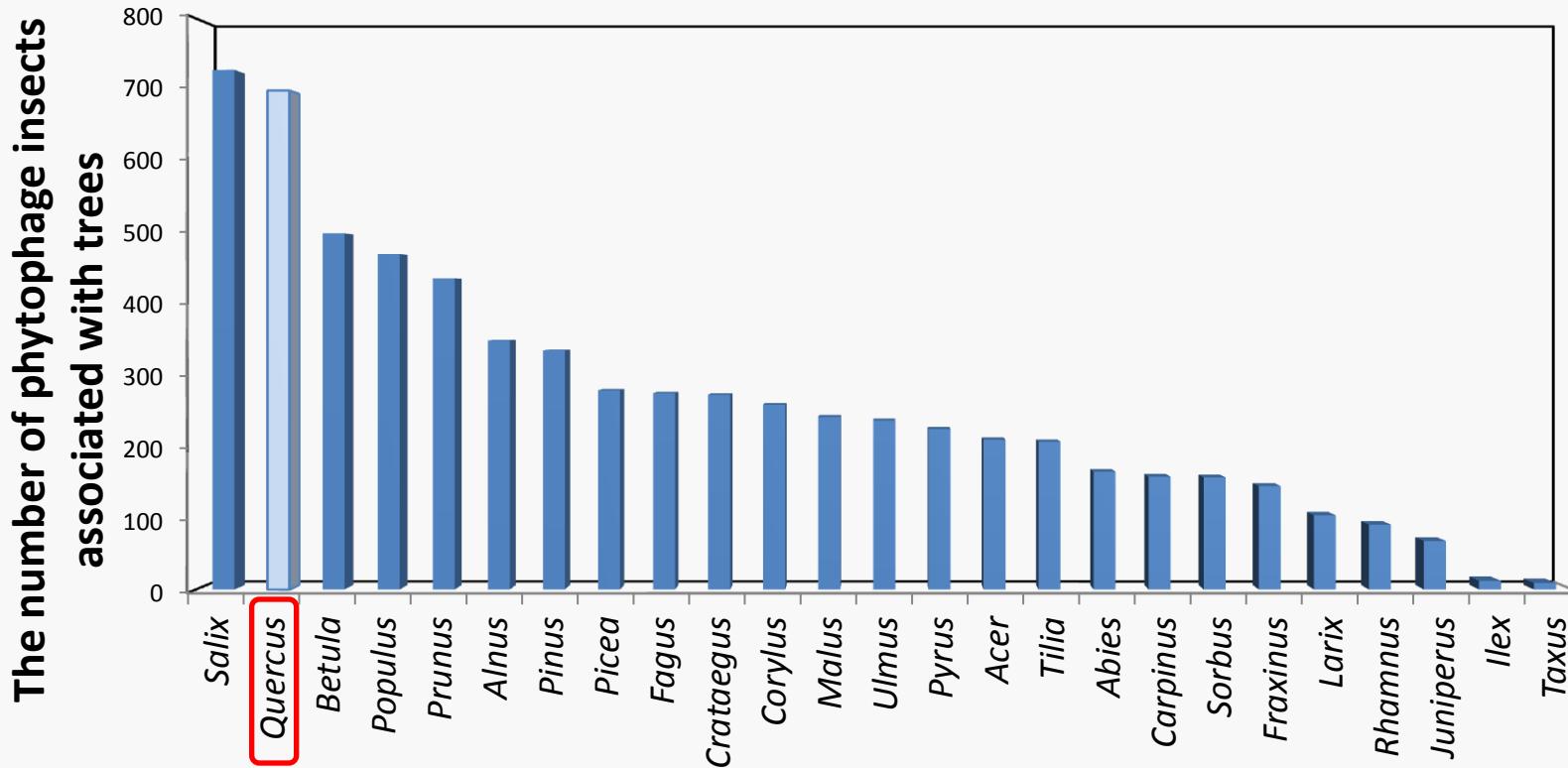
Shortage of low temperature causes delay in dormancy break.

- Positive effects on **ecodormancy**

Promotion of growth and increase in growing season

- Changes in bud burst phenology may cause **drastic effects** on ecosystems

Oaks as key drivers of terrestrial biodiversity

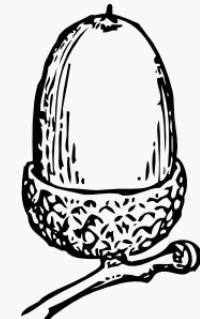


Brändle and Brandl, 2001, *Journal of Animal Ecology* 70:491-504

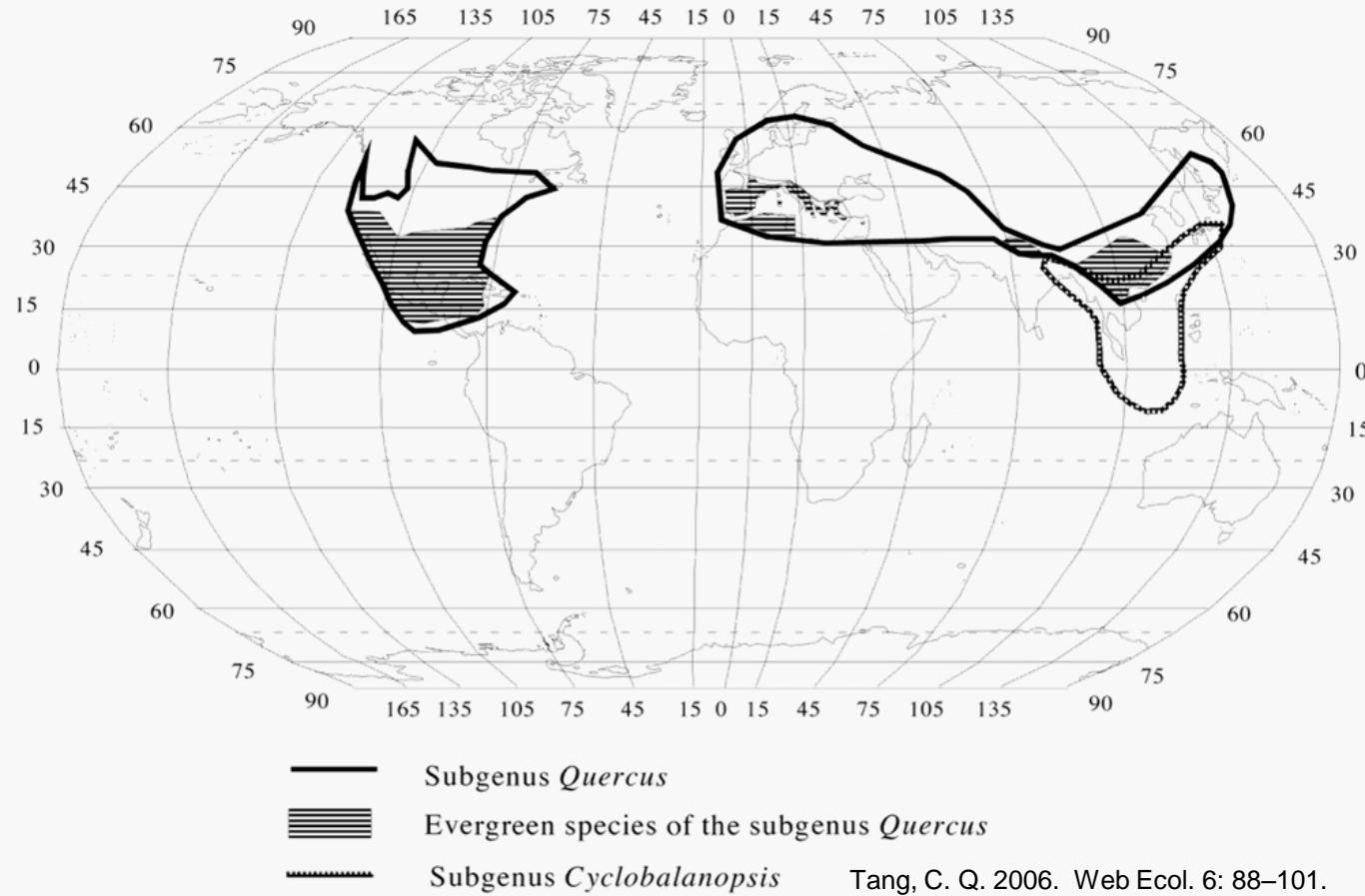
Discovery of genes for adaptive significance in oaks may lead to significant progress in the evolutionary genetics and ecology of whole communities.

Quercus (Fagaceae)

- Bears acorns that provide foods for animals
- Deciduous or evergreen trees
- Pollinated by wind
- Ships, furniture, wine barrel, etc...



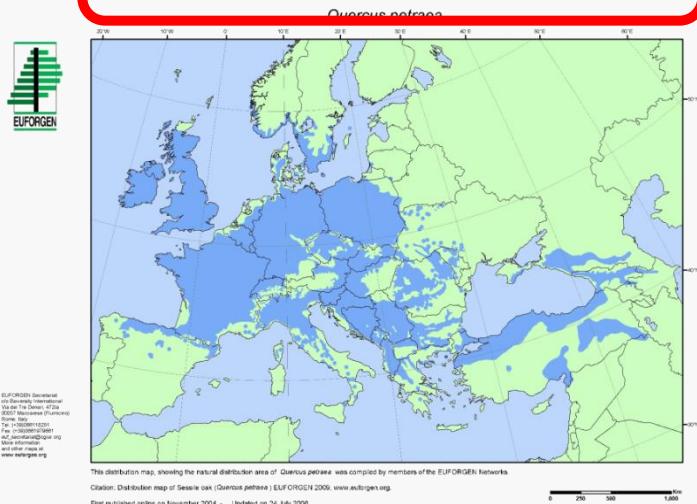
Distribution of oaks



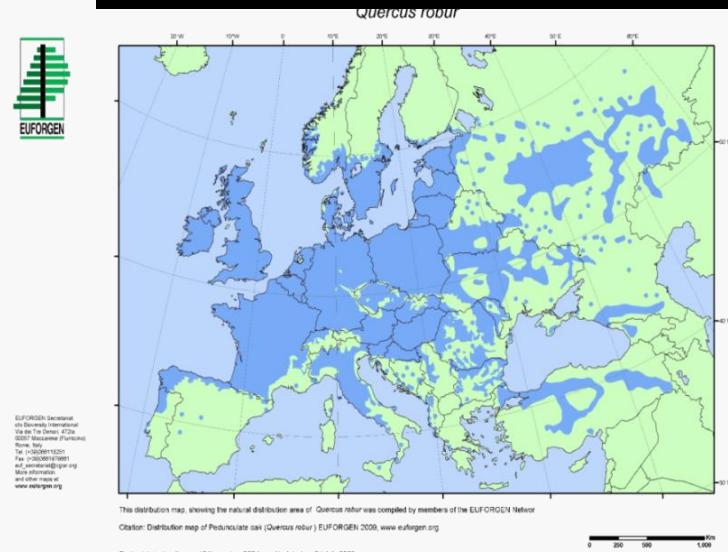
- Several hundreds of species in *Quercus*
- Adapted to various environments

European oaks

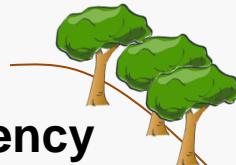
Q. petraea (sessile oak)



Q. robur (pedunculate oak)



**Higher water use efficiency
More drought tolerance**

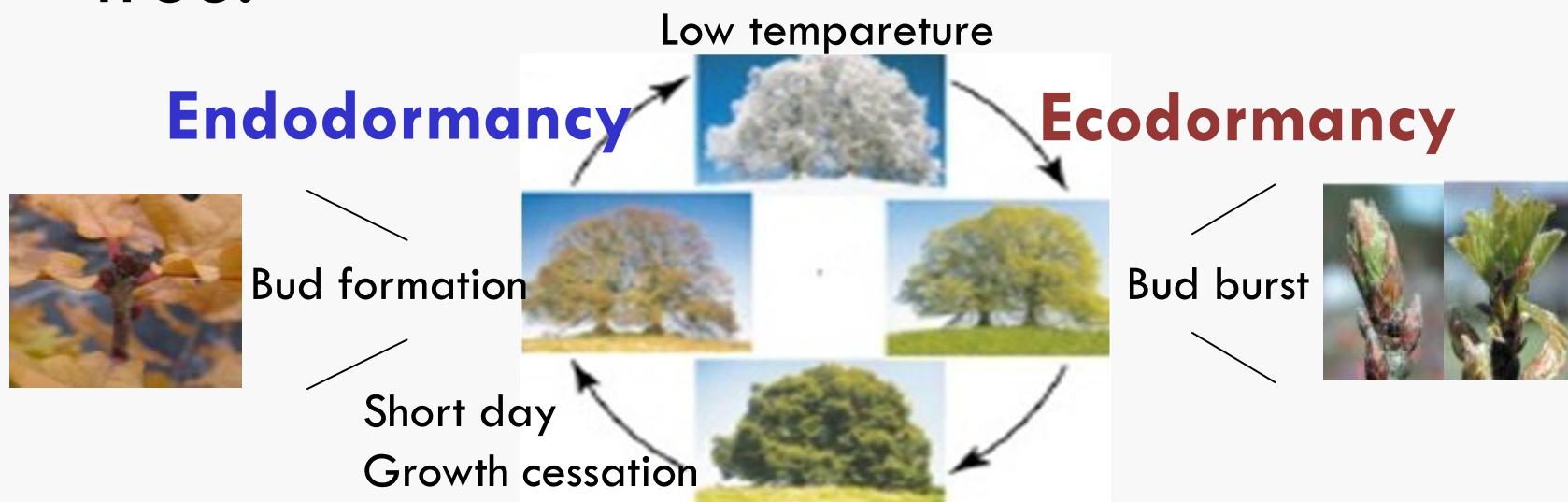


**Lower water use efficiency
More flood tolerance**



Objectives

- Discovering genes that show differential expression between endo- and ecodormancy using *Q. petraea* as a model tree.



Materials



- *Quercus petraea* (sessile oak)
 - Study populations
Saint-Jean (SJ)
Long Champ (LC)
- } Contrasting bud burst phenology

- Seeds were collected and germinated
- Based on forcing tests, RNA was extracted from apical buds for library construction

Forcing tests

➤ Detecting dormancy status for winter buds

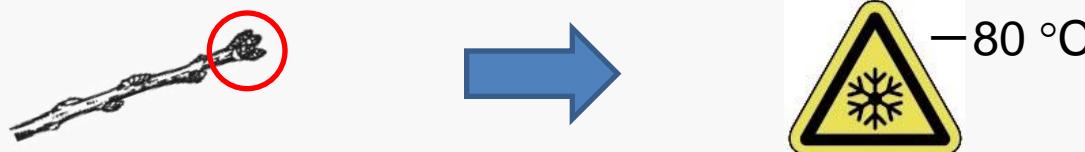
468 and 560 seeds were germinated for LC and SJ population, respectively.

Five seedlings from each population were transferred to a green house.

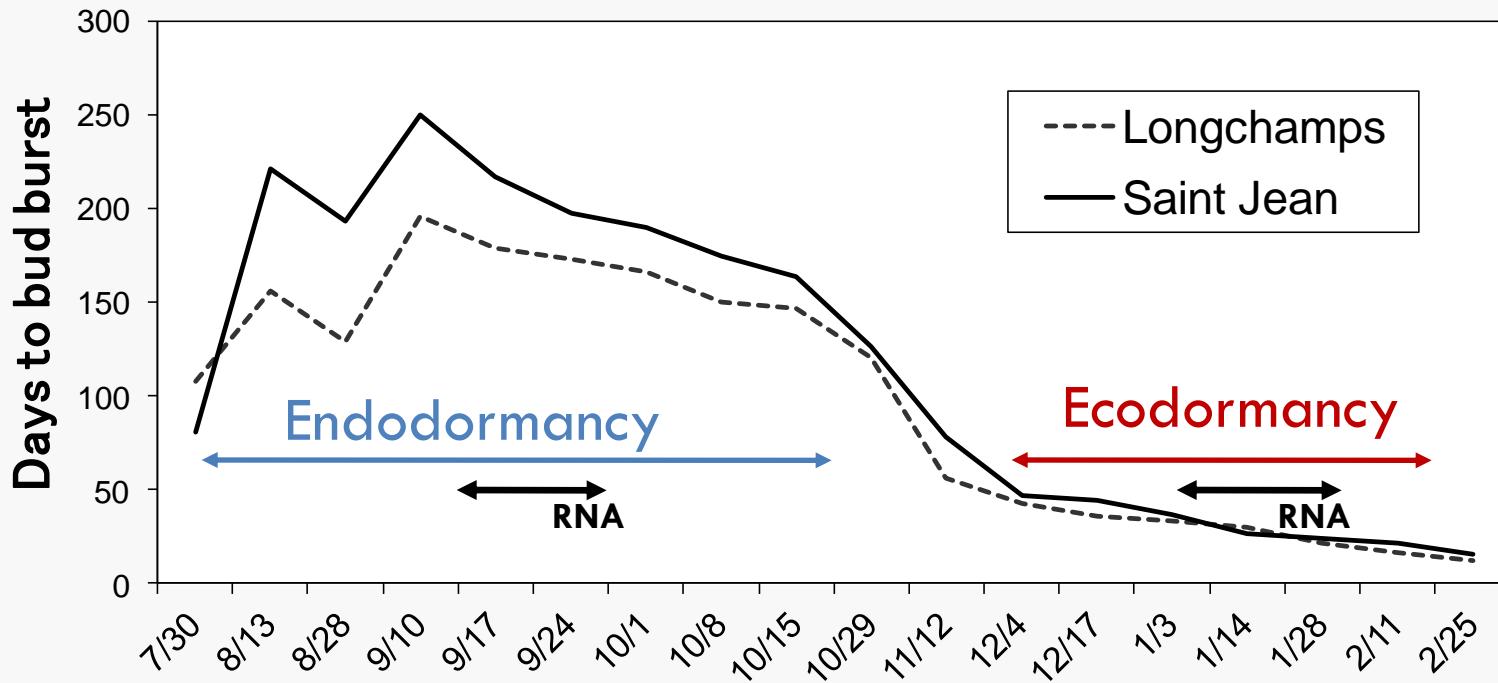


Days to bud flush were recorded.

3 to 5 apical buds were sampled at the same time and stored at -80 °C



Forcing test results



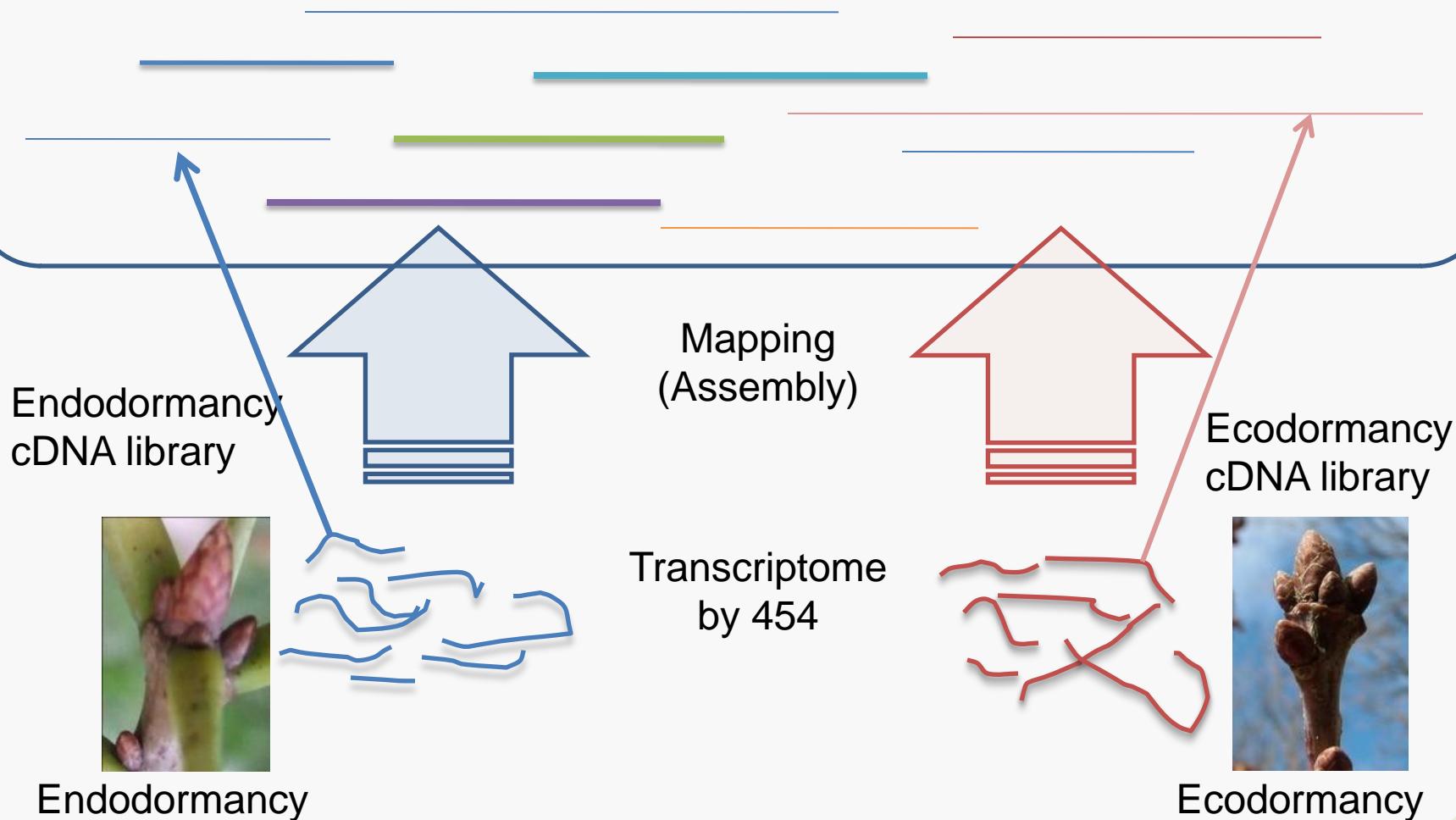
Endodormancy library
(LC1 and SJ1)

Ecodormancy library
(LC2 and SJ2)

- Equimolar RNA mixture was used for sequencing by Roche 454 GS20 in a half plate run.

Analysis scheme

Oak unigene catalogue

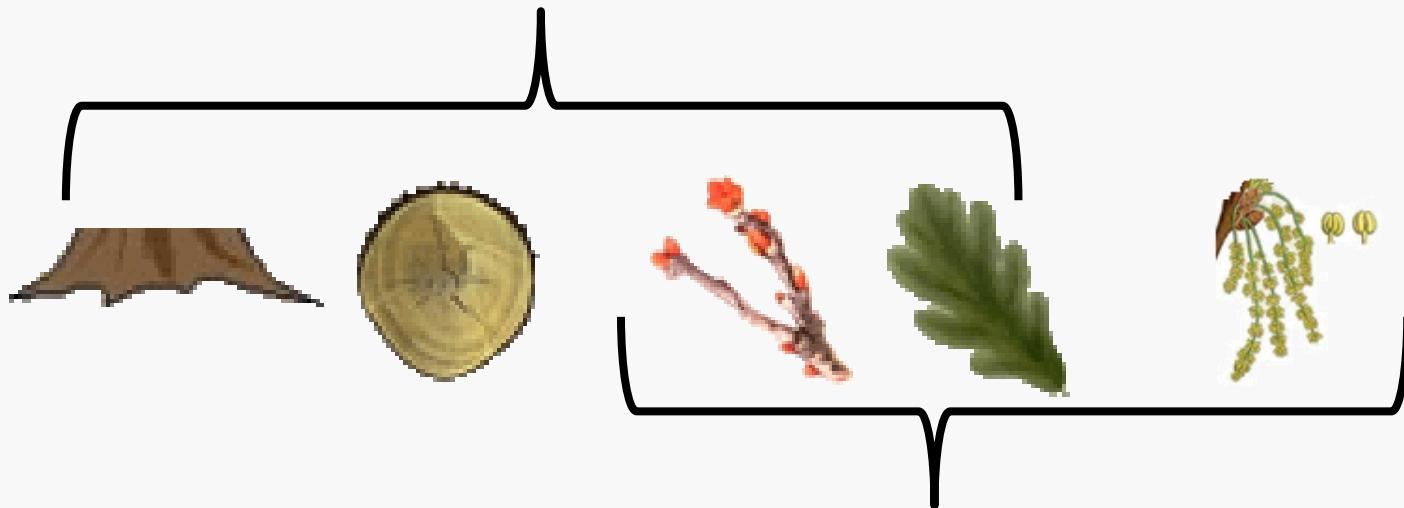


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cDNA libraries for unigene assembly from *Q. petraea* and *Q. robur*

➤ 20 libraries for Sanger sequencing



➤ 14 libraries for pyrosequencing

Hybrid assembly by CAP3

No. Sanger sequences (20 libraries)	125,925
No. 454 sequences (14 libraries)	1,578,192
No. Contigs	69,154
No. Singltons	153,517

Define unigenes/genes = 69,154 contigs.

Oak contig browser

GenoToul Contig Browser Oak ContigView

Search e/Oak Anything ↘
e.g. F0SUT5C01A4ZM.I.qr.1

Based on Ensembl release 40 - Aug 2006

**F0SUT5C01AW4ZM.I.qr.1
1 - 1,979**

- Graphical view
- Features view
- Assembly view
- Blast config
- Blast contig
- Translate - EMBOSS
- Export data about contig
- Export sequence as FASTA

Use Ensembl to...

- Search Contig Browser
- Data mining [BioMart]
- Export data
- Download data
- Blast on your data

Docs and downloads

- About Contig Browser
- Contig Browser data

Other links

- Home
- Plateforme bioinfo

Contig F0SUT5C01AW4ZM.I.qr.1

DNA(contigs) 0 200 400 600 800 1,000 1,200 1,400 1,600 1,800 F0SUT5C01AW4ZM.I.qr.1 >

Detailed view

Features ▾ DAS Sources ▾ Repeats ▾ Decorations ▾ Export ▾ Image size ▾

Jump to region F0SUT5C01AW4ZM.I.qr.1 : 1 | 1979 Refresh

< Window Zoom Window >

Length Forward strand 1,98 Kb

Public ESTs 41 Public ESTs omitted

RefSeq RNA Other contigs

Other TIGR

Pfam RefSeq Protein Swiss-Prot

DNA(contigs)

F0SUT5C01AW4ZM.I.qr.1 >

Grapes:TC74651
TC74651 UniRef100_A/ANLY7 Cluster: Chromosome chr6 scaffold_3, whole genome shotgun sequence; n=1; Vitis vinifera Rep. Chromosome chr6 scaffold_3, whole genome shotgun sequence - Vitis vinifera (Grape), partial (98%)
TIGR cluster TC74651
start | end | score | e-value | %ident
64 | 1397 | 398 | 0 | 82.5

PolymorphismView: FOSUTSC01A0B3Z.lqr.1 - Mozilla Firefox

Fichier Édition Affichage Historique Marque-pages Outils ?

Les plus visités Débuter avec Firefox À la une

Google Rechercher

PolymorphismView: FOSUTSC01A0B3Z... +

Coming from browser data

Other links

- Home
- Plateforme bioinfo

sanger **EBI** **INRA**

Sequence	Position	Consensus	Mutation
FOSUTSC02F8EM4+	61	C...	GTA...
FOSUTSC01B2BDA+	61	.ATCCA...	AAC...
FFYUTAE01CDESG+	61
FOSUTSC02JKS6X+	61	.ATCCA...	ATCCA...
FFYUTAE01B1LQB8+	61
FFYUTAE021MEUL+	61	T...
FFYUTAE02H4H3I+	61	AAC...
d48g0001.m.13_5_1.1-	61	AAC...
FFYUTAE01BIDQX+	61
consensus	61	ACCGGGGA- -TTTATTTCTGCACATAGACTCCAGAGCCAAAC- -TTTTCC	
FOSUTSC01A0B3Z+	121	.	:
FFYUTAE01B9TJL+	121	.	:
FFYUTAE01BHG6-	121	.	:
FFYUTAE01BTWNY+	121	.	:
FOSUTSC01CQ50C	121	.	:
FOSUTSC01EQQ8J+	121	.	:
FFYUTAE02GAZI6+	121	.	:
FFYUTAE02OB3I6+	121	.	:
FOSUTSC01CLH18+	121	.	:
FFYUTAE01ETZFT7+	121	.	:
FFYUTAE02H6LDW-	121	.	:
FFYUTAE02F3EV2+	121	.	:
FFYUTAE01EXDET-	121	.	:
FOSUTSC02F8EM4+	121	.	:
FOSUTSC01B2BDA+	121	.	:
FFYUTAE01CDESG+	121	.	:
FOSUTSC02JKS6X+	121	.	:
FFYUTAE01B1LQB8+	121	.	:
FFYUTAE021MEUL+	121	.	:
FFYUTAE02H4H3I+	121	.	:
d48g0001.m.13_5_1.1-	121	.	:
FFYUTAE01BIDQX+	121	.	:
consensus	121	TTAGGGTTTCCTATTCCCTTCTGTTATTCTCTGCAGGGTAGGCCAACAJ	
FOSUTSC01A0B3Z+		.	-
FFYUTAE01B9TJL+		.	-

Data structure

➤ MySQL table

```
mysql> select * from qrobur_sigenaecontig_est_mrna_dm order by rand() limit 0,9;
```

contig_id_key	est_id	est_name	genbank_accession	type	available	library_name
190568	NULL	F2D1W4Z01CC3VN	F2D1W4Z01CC3VN	454t	GENOTOUL	QS28 Qpe
202041	NULL	F0SUT5C01D6EB0	F0SUT5C01D6EB0	454s	GENOTOUL	FP Qro
182999	NULL	FFYUTAE01AWPJJ	FFYUTAE01AWPJJ	454s	GENOTOUL	LC1-EcoEndoDorm Qpe
107679	NULL	FFYUTAE01E095P	FFYUTAE01E095P	454s	GENOTOUL	LC1-EcoEndoDorm Qpe
25517	NULL	F2H13RQ01AH98Y	F2H13RQ01AH98Y	454t	GENOTOUL	QS21 Qpe
163988	NULL	F0SUT5C02GL00H	F0SUT5C02GL00H	454s	GENOTOUL	10QP-Intersp Qro
184877	NULL	F0SUT5C01E10NK	F0SUT5C01E10NK	454s	GENOTOUL	FP Qro
190077	NULL	Contig3709	Contig3709	mRNA	GENOTOUL	Computed
200080	NULL	F2H13RQ01E3AB0	F2H13RQ01E3AB0	454t	GENOTOUL	A4 Qro

➤ All data can be downloaded from

http://genotoul-contigbrowser.toulouse.inra.fr:9092/Quercus_robur/index.html

(user name: oak , password: querucs33) Ueno et al. (2010) BMC Genomics 650

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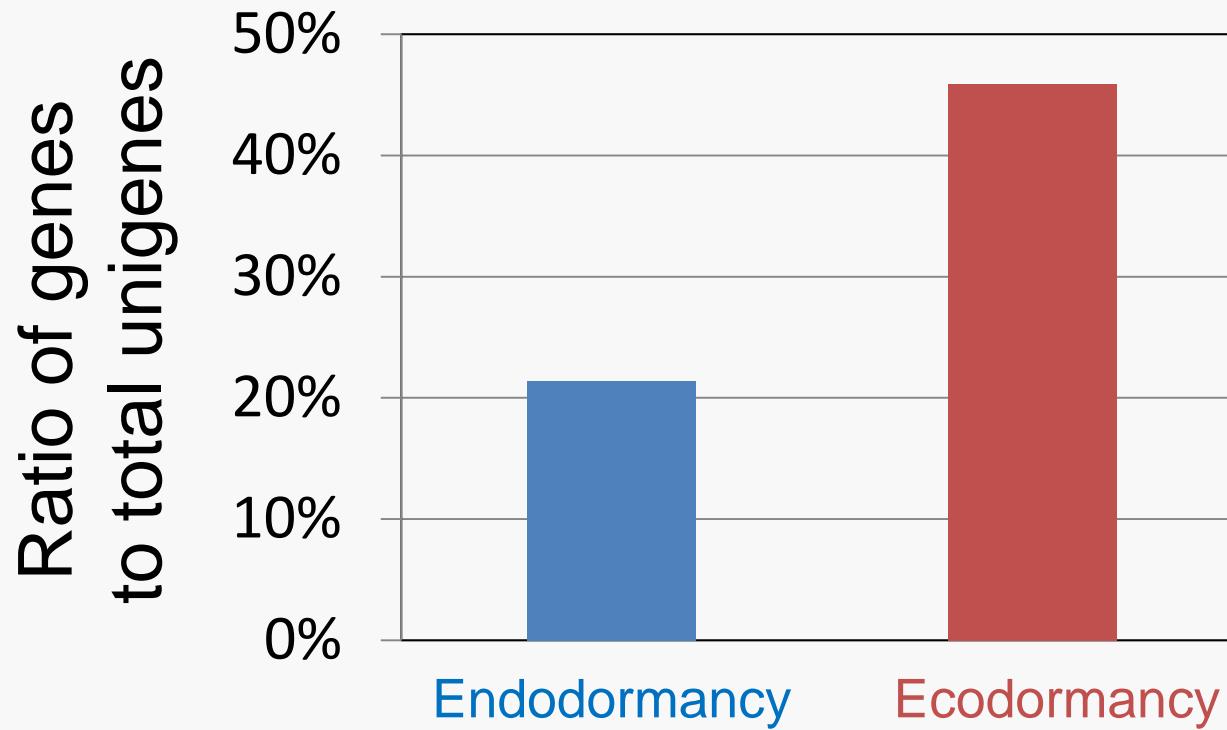
454 pyrosequencing results



Population	Endodormancy	Ecodormancy
Longchamp	LC1 (70,019)	LC2 (98,725)
Saint Jean	SJ1 (44,732)	SJ2 (138,921)

Total: 352,397 reads (half plate)

Unigenes in dormancy libraries



More genes were detected
in ecodormancy libraries

41,731 unigenes in dormancy libraries

Endodormancy

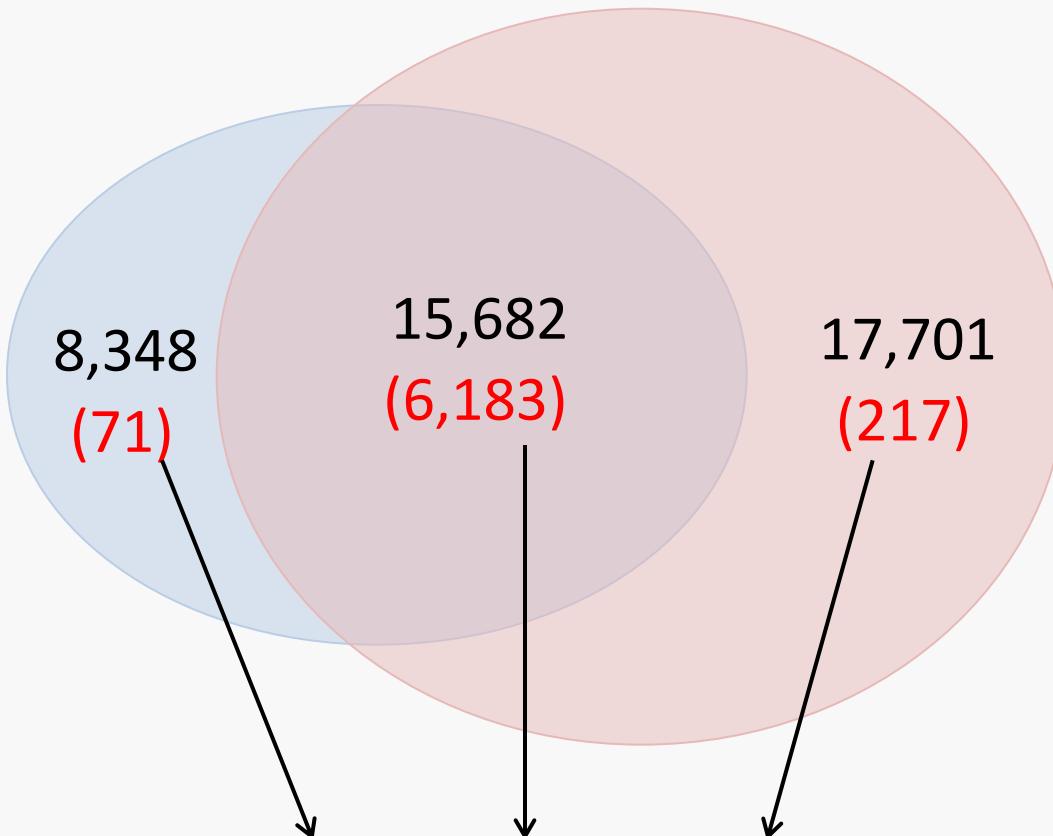


24,030
genes

Ecodormancy



33,383
genes



6,471 genes with reads ≥ 5 for differential expression analysis

Programs used



➤ edgeR

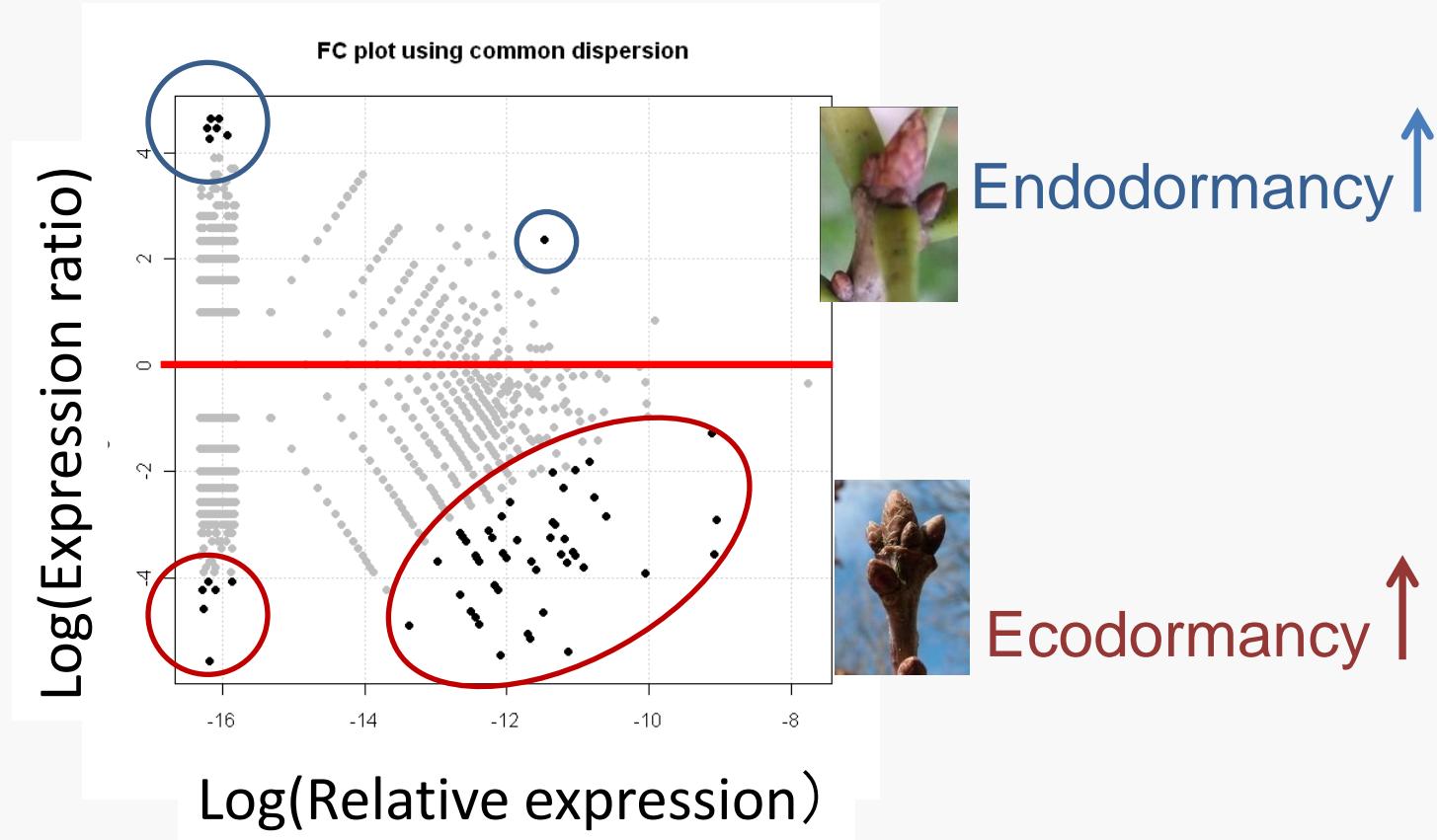
Robinson et al. *Bioinformatics* 2010, **26**(1):139-140.

➤ DEGseq

Wang et al. *Bioinformatics* 2010, **26**(1):136-138

The two populations (LC and SJ) used as a biological replication

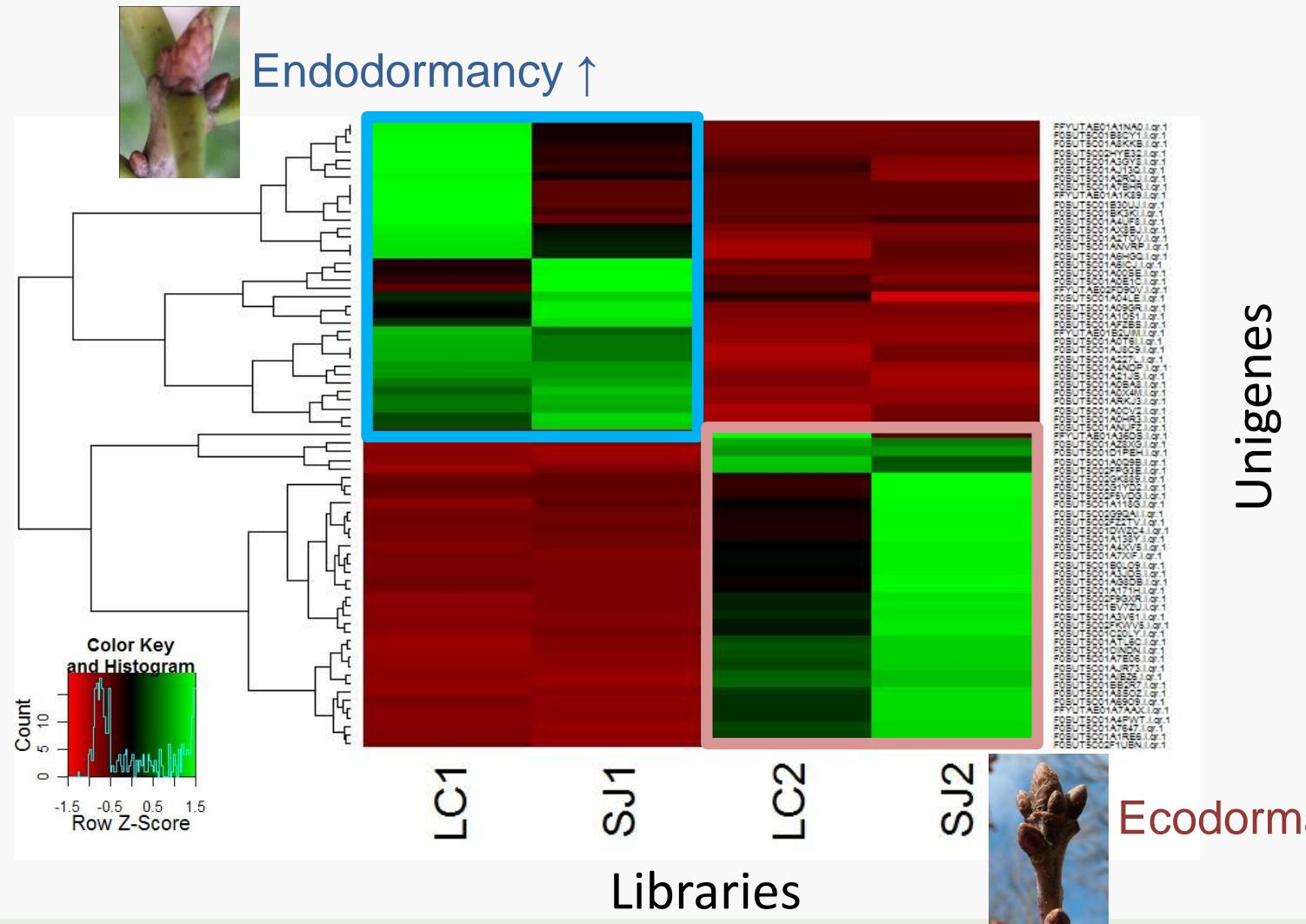
Expression variation MA-plot (edgeR)



- 73 genes (●) showed significant variation (FDR5%).
- More expression in ecodormancy

Expression variation

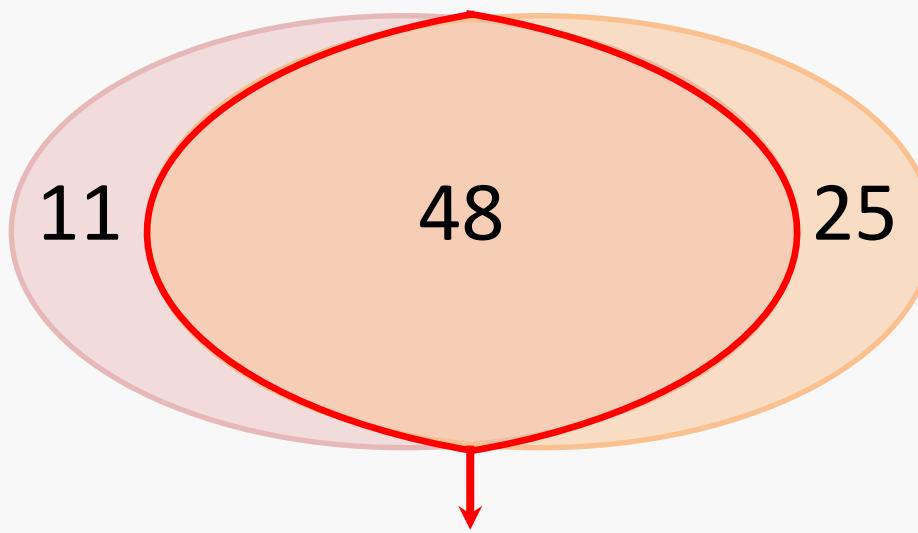
Heatmap for 73 genes by edgeR



Differentially expressed genes

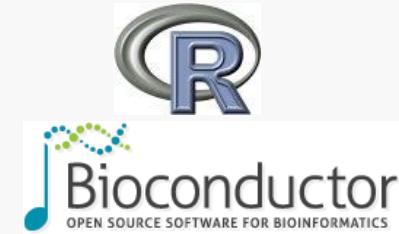
DEGseq (59 genes)

edgeR (73 genes)



- 21 in endodormancy and 27 in ecodormancy up-regulation
- 18 (37.5 %) had hits with proteins in SWISSPROT database
->GO
- 34 (70.8 %) had hits with *Arabidopsis* (TAIR9) sequences
->MapMan

GO for Differentially Expressed 48 Genes (DEG)



➤ Gene ontology for DEG (goseq)
Three GOs statistically overrepresented

- Response to stress
- Response to endogenous stimulus
- Response to abiotic stimulus

Dehydrin, Heat shock protein, Gibberellin receptor

...

16 DEGs on MapMan ontology

Biotic stress

Peroxidase



Glutation S-transferase



Signaling



Hormon signaling

Auxins



Cell wall



Proteolysis



Abiotic stress

Heat



Drought



Misc



Endodormancy ↑



RNA/Protein synthesis

Plastidic



Development



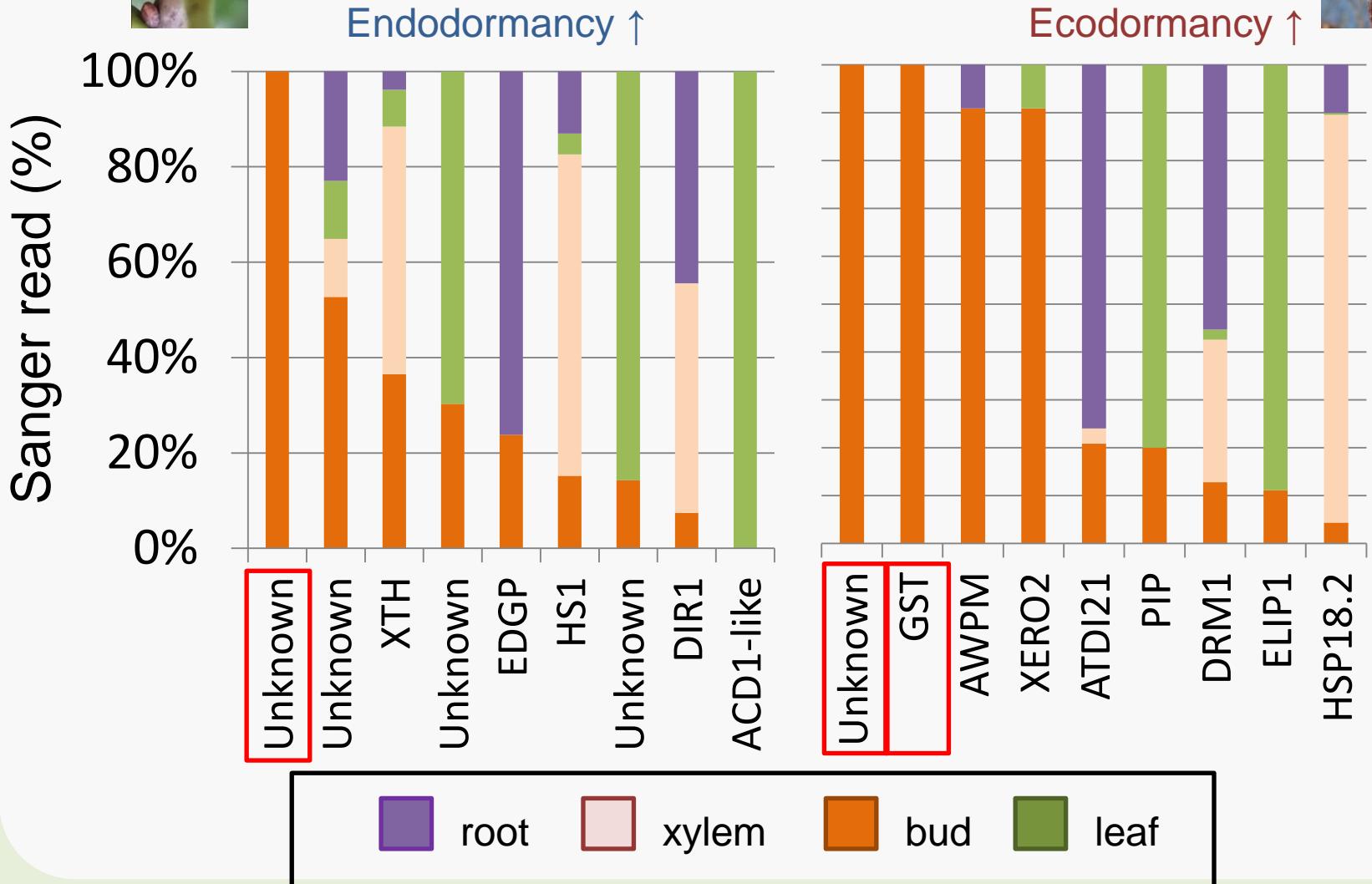
Ecodormancy ↑



Tissue specificity for 48 DEGs

- Sanger cDNA libraries from leaf, bud, xylem and root as tissue references
- *R* statistics (Stekel et al. 2000) was used for tissue specific expression.

Tissue specificity for DEG

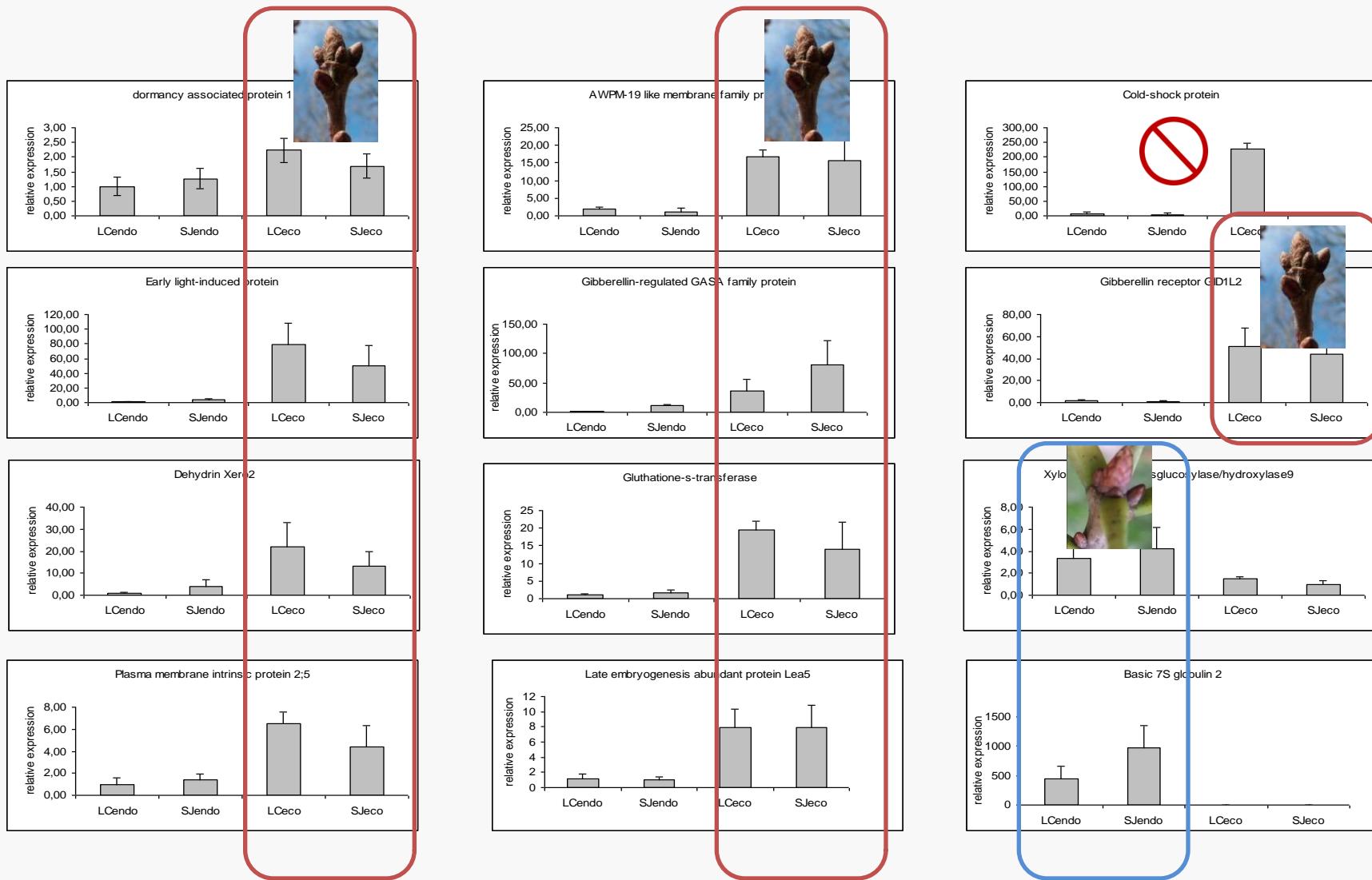


Validation by Q-PCR for DEG candidates

- 13 genes were selected
 - Cold-shock protein (**CS120**)
 - Glutathione-S-transferase tau 19 (**GST**)
 - Dormancy-associated protein (**DRM1**)
 - Gibberellin-regulated GASA/GAST/Snakin family protein (**GASA**)
 - Dehydrin Xero 2 (**XERO2**)
 - Plasma Membrane intrinsic protein (**PIP**)
 - 18.1 kDa class I heat shock protein (**HSP**) → multi-banding pattern
 - AWPM-19-like membrane family protein (**AWPM**)
 - Probable gibberellin receptor GID1L2 (**GID**)
 - Late embryogenesis abundant protein Lea5 (**LEA**)
 - Early light-induced protein (**ELP**)
 - Basic 7S globulin 2 (**Globulin**)
 - Xyloglucan endotransglucosylase/hydrolase9 (**XET**)

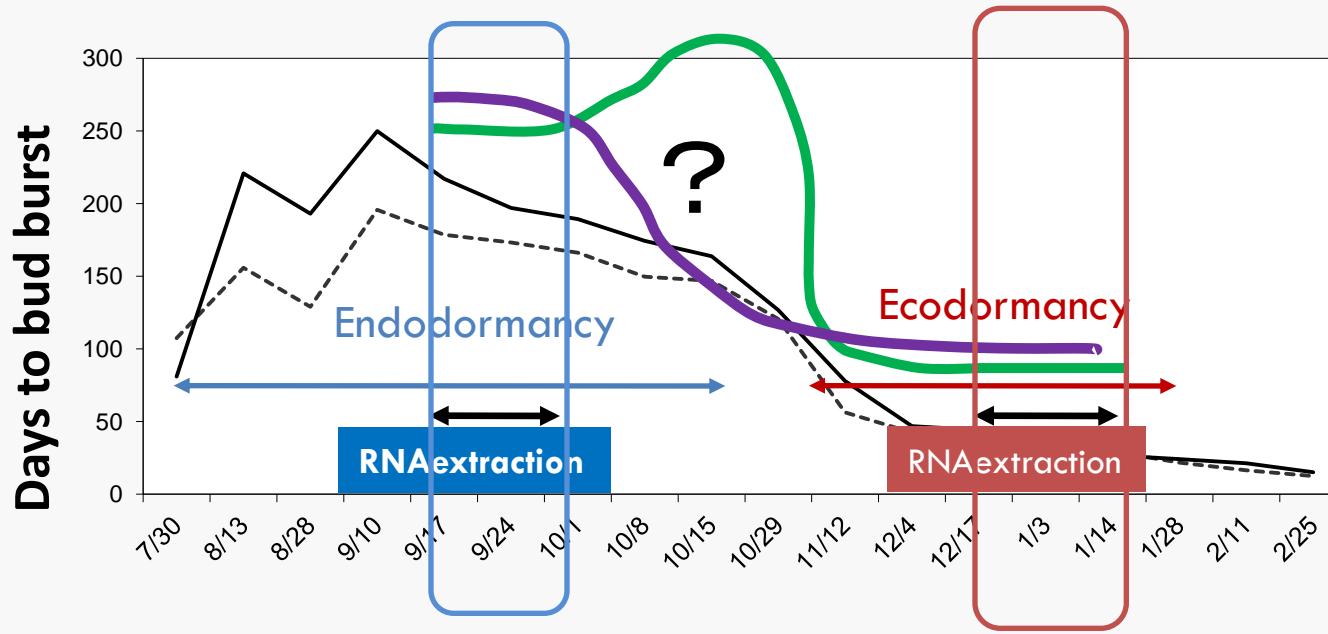


Q-PCR results



Future directions

- Time course analysis of candidate genes



- Metabolic pathway/network analysis
(needs further genomic resources)

Summary

Gene discovery relating to bud dormancy for sessile oak in France

- Maximum endodormancy in September
Ecodormancy after December
- Differentially expressed genes had similarities with
Dehydrin, Heat shock protein, Gibellerin receptor, etc.
 - Response to stress
 - Response to endogenous stimulus
 - Response to abiotic stimulus
- 454-pyrosequencing was effective to discover bud dormancy-related genes which were validated by Q-PCR.

Acknowledgements



INRA Bordeaux

Valérie Léger
Elodie Prince
Antoine Kremer
Christophe Plomion
Jérémy Derory
Grégoire Le Provost

INRA Toulouse

Christophe Klopp
Céline Noirot

Summary

Gene discovery relating to bud dormancy

- Maximum endodormancy in September and ecodormancy after December in sessile oak in France.
- Differentially expressed genes had GOs
 - Response to stress
 - Response to endogenous stimulus
 - Response to abiotic stimulus
(Dehydrin, Heat shock protein, Gibellerin receptor, etc.)
- 454-pyrosequencing was effective to discover bud dormancy-related genes which were validated by Q-PCR

Thank you for your attention