POPSEC : molecular bases of acclimation to water deficit in poplar

Marie-Béatrice Bogeat-Triboulot, David Cohen, Didier Le Thiec, Sandrine Balzergue, Marie-Laure Martin-Magniette, Jean-Pierre Renou, Philippe Label, Marie-Claude Lesage-Descauses, Françoise F. Laurans, Isabelle Bourgait, et al.

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POPSEC : molecular bases of acclimation to water deficit in poplar

INRA Nancy - UMR EEF
INRA Bordeaux - UMR Biogeco
INRA Orléans - UGAPF
Université d'Orléans - LBLGC
URGV Evry

Coord. : MB Bogeat-Triboulot

Objectives

- Integrative study of the response of poplar to water deficit

  - combining several approaches : **ecophysiology, transcriptomics and proteomics**

  - studying <u>different tissues in order to focus on different processes</u>
    - mature leaves -> CO_2 assimilation, cell metabolism
    - guard cells -> stomatal conductance regulation
    - wood and growing xylem -> cambial growth
    - root apices -> primary growth

- Identify genes, gene networks and transcription factors involved in drought tolerance

Poplar genomic toolkit

Genomic plateforms
Expertise in transcriptomic
Expertise in proteomic

Diversity in drought tolerance between clones

Expertise in ecophysiology

2 poplar clones: 1 tolerant and 1 sensitive to drought

Controlled water deficit and fine ecophysiology characterisation

Wood formation
Stem mechanics & hydraulics

Root growth

growing root apex

Transcriptome
Proteome

Root hydraulics

mature root

Transcriptome
Proteome

wood

Transcriptome
Proteome

differentiating xylem

Transcriptome

Gas exchange, WUE

leaf

stomata

Leaf T and P
guard cells Transcriptome

Identification of genes involved in drought tolerance

Selection of some candidate genes by:
- Nature of gene
- Regulation in several organs

qPCR on other clones of different drought tolerance

Genetics

Fonctional validation
Transformation?

Experimental design

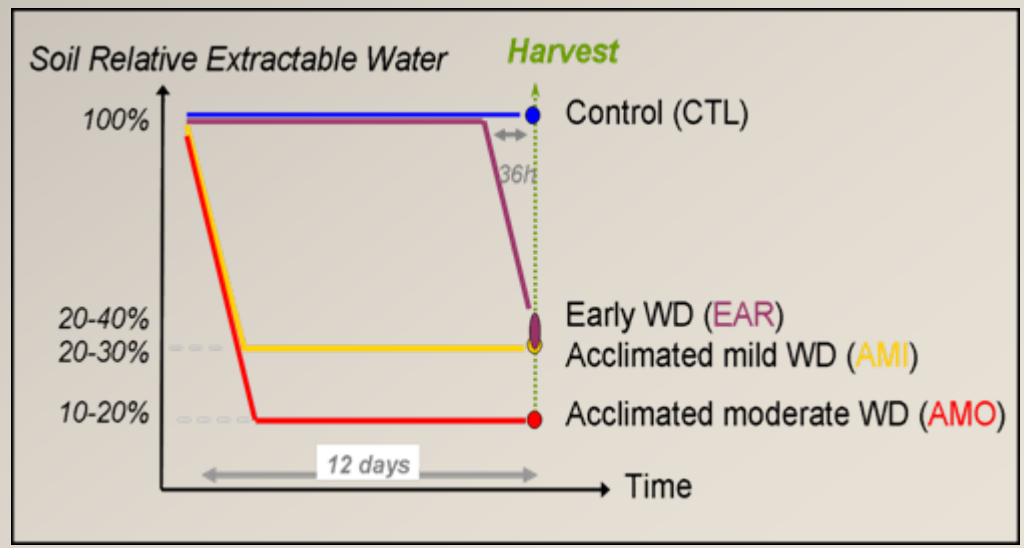
- 2 genotypes *P. deltoides x nigra* : Carpaccio and Soligo
  chosen for
  - similar productivity
  - similar WUE
  - contrasted productivity maintenance under water deficit

- Design
  - 1 batch for repeated ecophysiological measurements
  - 1 batch for molecular analyses
    - 2 biological replicates (pools of 3 plants) per modality

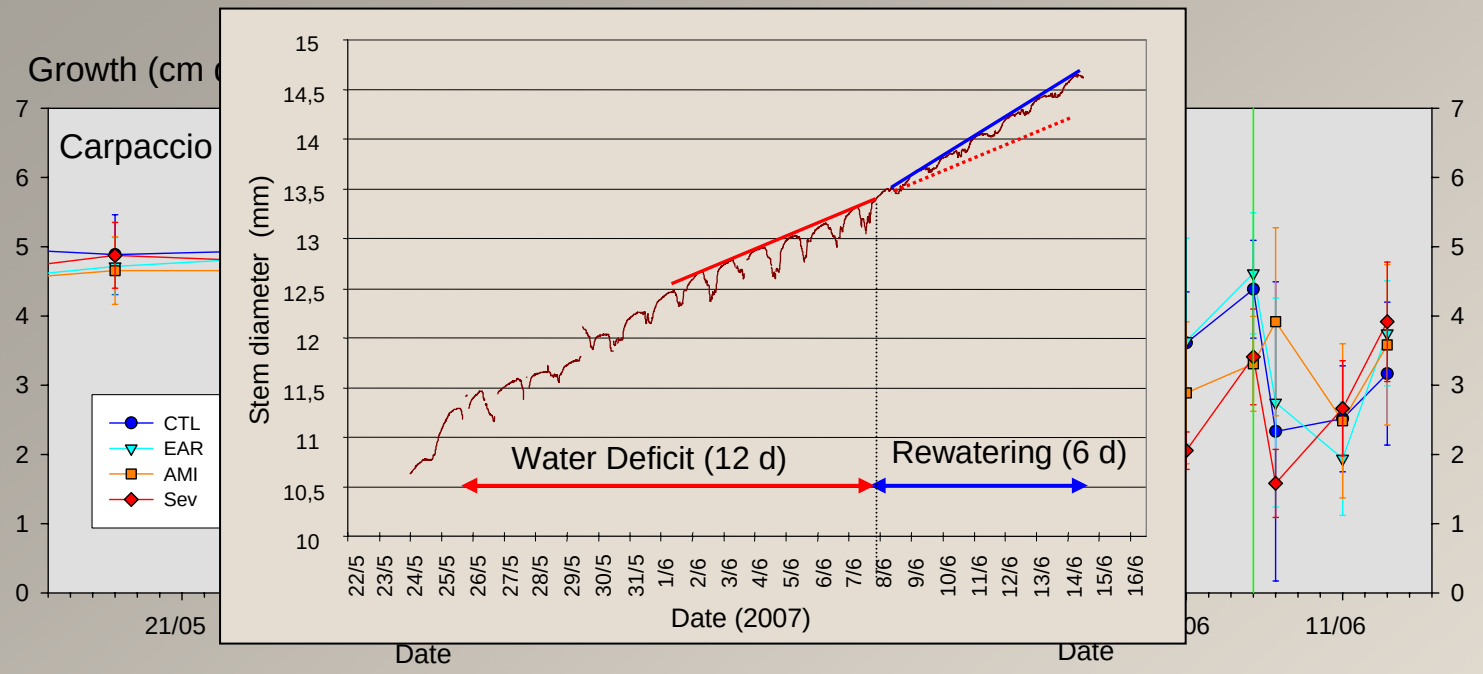
- 3 water deficit treatments
  - Control of soil volumetric water content

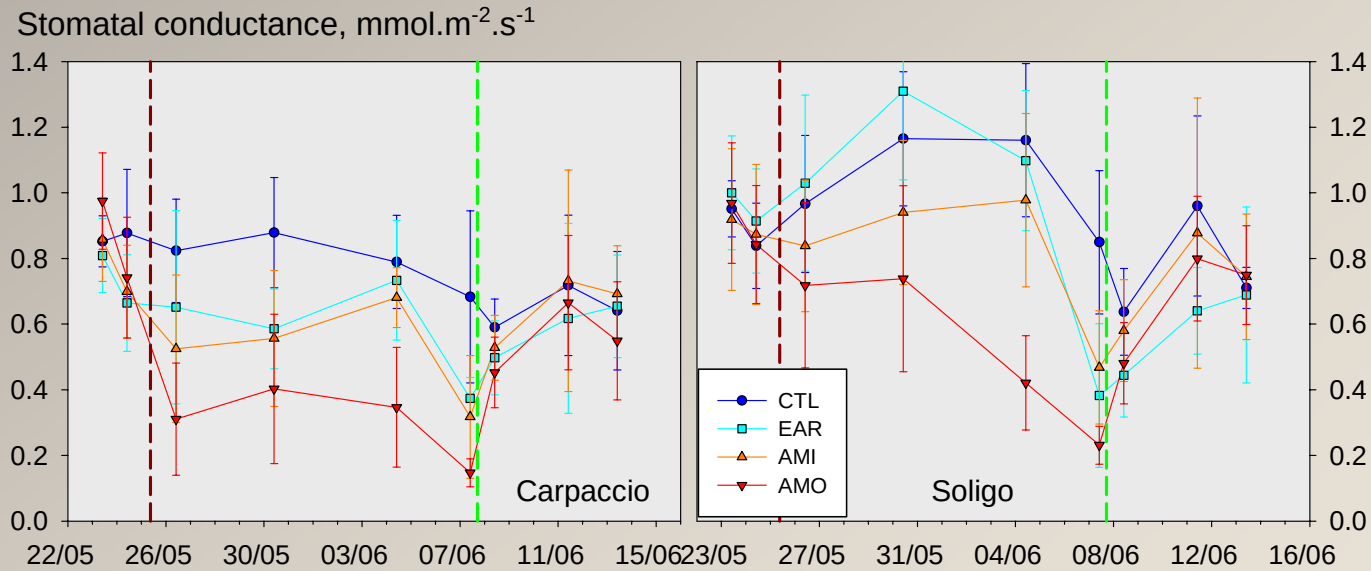
- Characterisation of water status, growth, gas exchange, wood anatomy, δ13C (WUE)

- Tissues harvesting for transcriptome and proteome analyses
  - mature leaves
  - wood and growing xylem
  - root apices



Kinetics


Growth (cm
Carpaccio
CTL
EAR
AMI
Sev
21/05
Date
Stem diameter (mm)
Water Deficit (12 d)
Rewatering (6 d)
Date (2007)
11/06
Date


Stomatal conductance, mmol.m-2.s-1
Carpaccio
Soligo
CTL
EAR
AMI
AMO
22/05
26/05
30/05
03/06
07/06
11/06
15/06
23/05
27/05
31/05
04/06
08/06
12/06
16/06

Ecophysiological response (D day)

		Absolute values				p value		
		CTL	EAR	AMI	AMO	Clone	trait	interac.
□pd, MPa	Carpaccio	-0.13	-0.11	-0.11	-0.13	**<0.001**	0.53	0.22
	Soligo	-0.23	-0.26	-0.29	-0.28			
Leaf RWC, %	Carpaccio	92	93	93	93	**<0.001**	0.59	0.19
	Soligo	91	91	91	92			
□FT, mosmol/kg	Carpaccio	627	618	648	667	0.11	**<0.001**	0.27
	Soligo	616	624	676	716			
Primary growth, cm day-1	Carpaccio	4.6	4.8	3.4	3.1	1.0	**<0.001**	0.92
	Soligo	4.5	4.7	3.4	3.5			
Radial growth, mm day-1	Carpaccio	0.168	0.121	0.182	0.146	0.53	**<0.001**	0.24
	Soligo	0.198	0.146	0.182	0.131			
LMA, g m-2	Carpaccio	53.1	54.6	57.9	55	**0.013**	**0.047**	0.59
	Soligo	54.8	57.9	65.6	63.3			
A, µmol m-2 s-1	Carpaccio	12.5	10.9	12.3	9.4	**<0.001**	**0.002**	0.49
	Soligo	19.0	17.4	18.0	12.6			
gs, mol m-2 s-1	Carpaccio	0.68	0.37	0.32	0.15	0.05	**<0.001**	0.7
	Soligo	0.85	0.38	0.47	0.23			
Wi (A/g), %°	Carpaccio	20.7	30.0	43.8	66.7	0.29	**<0.001**	**0.012**
	Soligo	23.2	56.0	41.0	57.0			

Ecophysiological response

- moderate water deficit => small but significant physiological responses
  - primary and secondary growth, gs, CO_2 assimilation rate, $\delta^{13}C$
  - significant effect of EAR on gs
  - differences between AMI and AMO (radial growth, A, gs, WUE, Π_{FT})

- intrinsic differences between clones

- almost no significant interaction genotype x treatment :
  - the difference in WD tolerance not due to large contrast in one or several process(es)
  - tolerance = result of the time integration of weak differences

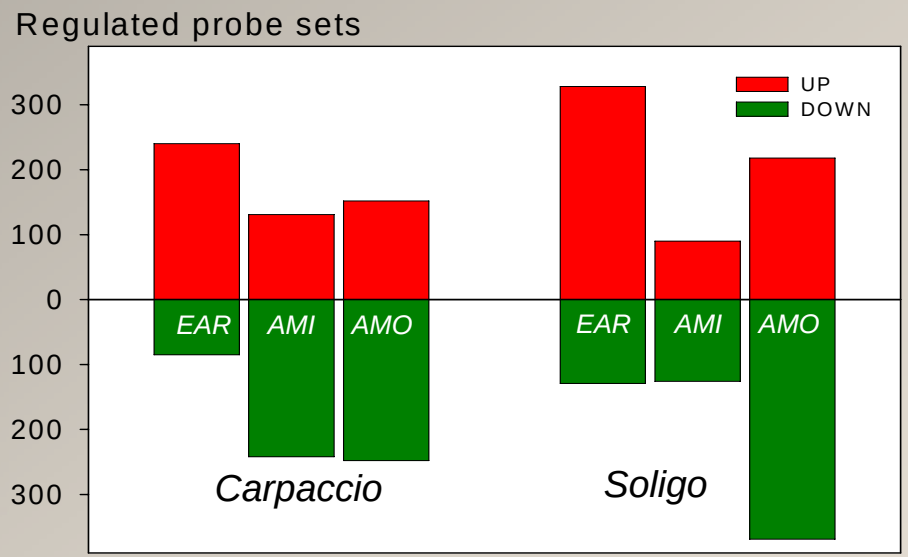
Leaf transcriptome

Affymetrix Poplar GeneChip microarray

partial annotation -> completion of probe set annotation

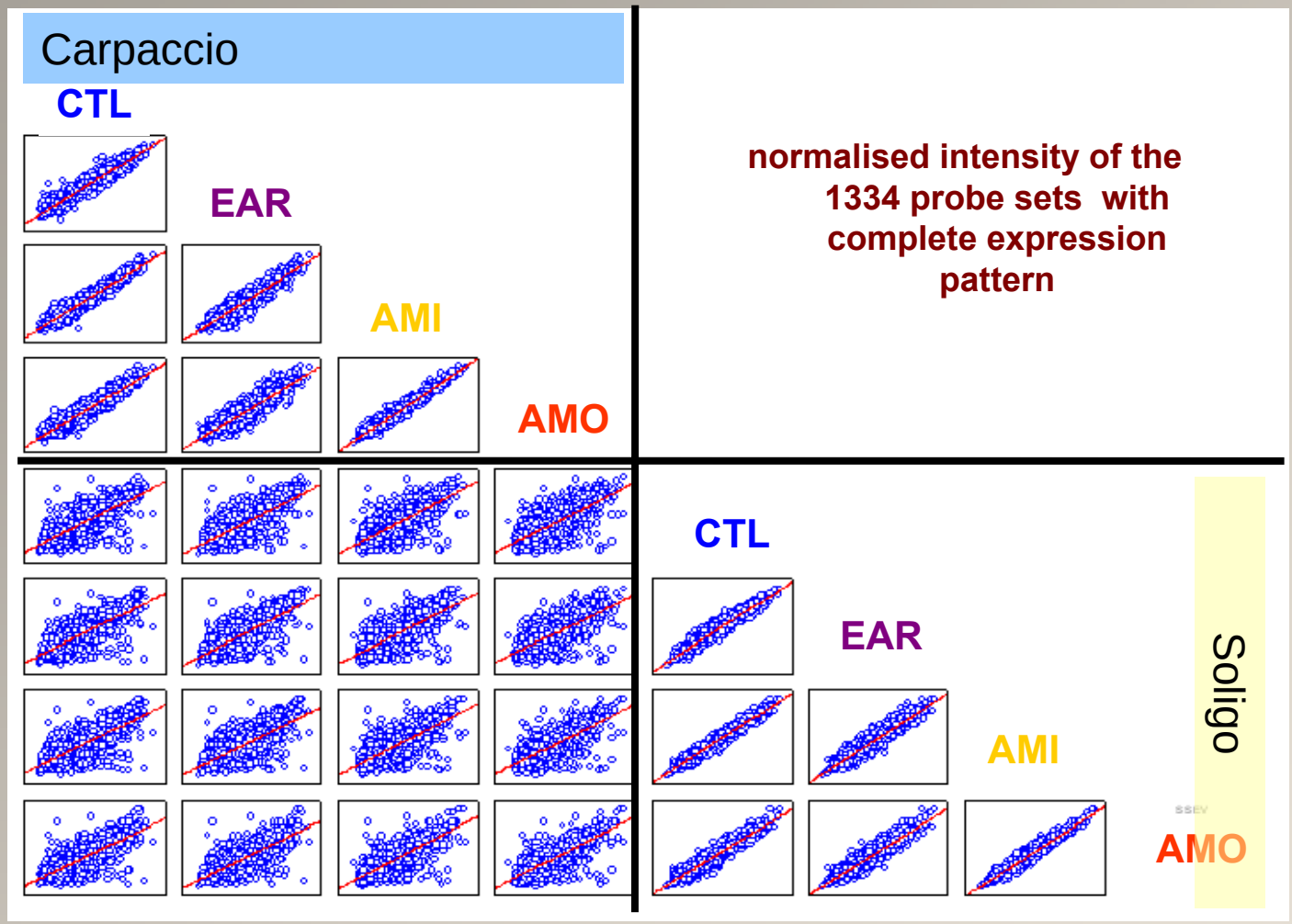
36,687 out of the 61,000 probe sets of the array were validated in our comparative set-up (DNA hybridization, ESTs)

2,195 DW responsive probe sets
(no Log 2 ratio cut-off, p<0.05)



- short term response : mainly up-regulations
- long term response : mainly down-regulations
- genotype specificity : sensitive genotype Soligo showed
  - contrasted response between the two levels of long term WD
  - more numerous gene regulations in comparison with Carpaccio

Leaf transcriptome



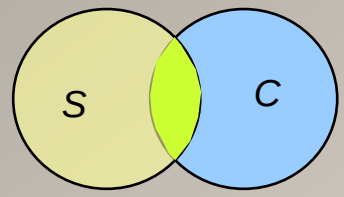
- Expression strongly contrasted between genotypes
- Rank conservation among treatments within genotypes

WD markers and candidates for WD tolerance in leaves

Stress markers

Homeobox-leucine zipper protein*
Galactinol synthase 1
RCI 2A - like
Protein phosphatase 2C (2)*
Xyloglucan endotransglycosylase*

concordant with litterature
(Bray, 2004; Bogeat-Triboulot, 2007)



Candidates for water deficit tolerance

specific of the short term response (EAR)

(84 + 36)
AREB
ABA-induced-like protein
SNARE-interacting protein
Aquaporin TIP / PIP
NADPH oxidase
Galactinol synthase 2
LEA (4)
ERD protein-related
Rapid alkalinization factor
putative serine protease (2)

common to EAR, AMI, AMO

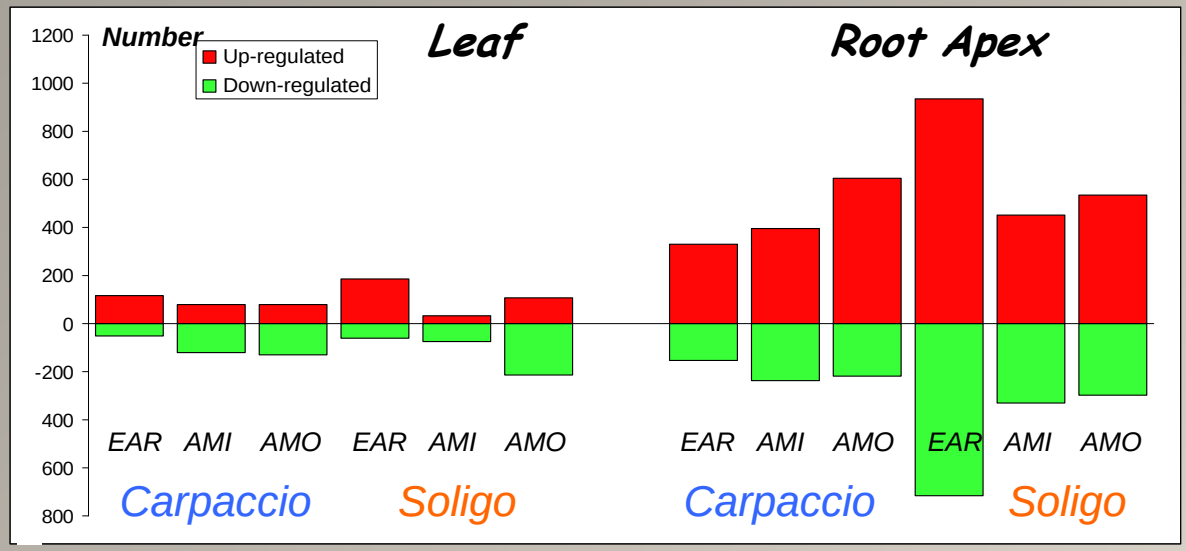
Unknown protein EAR AMI AMO
ABA 8'-hydroxylase (2)
ABC transporter family protein
Receptor-like protein kinase
LRR receptor-like protein kinase
NBS-LRR type

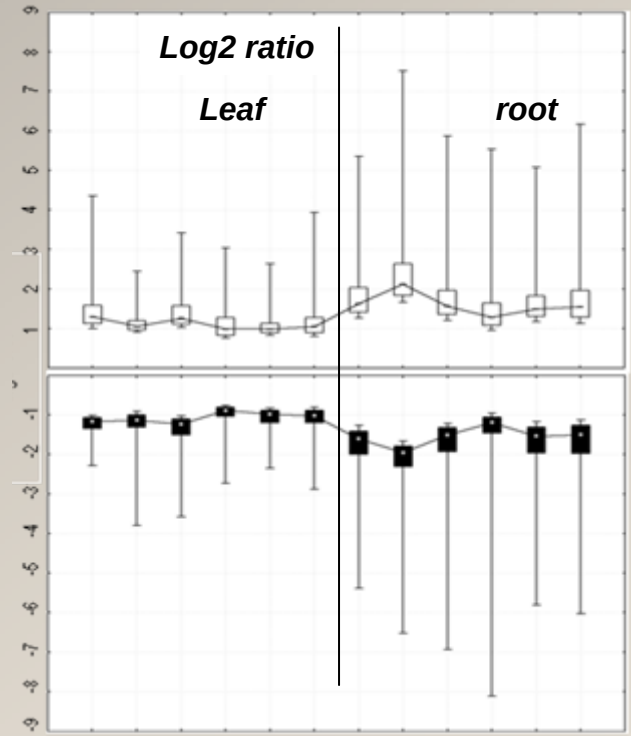
specific of the long term response (AMI et AMO)

(13 + 43)
Polyphenol oxidase
Superoxide dimutase
Alcohol dehydrogenase
WAK-Like (6)
Sucrose synthase (3)
NBS-LRR family protein (6)

Leaf and root transcriptome meta-analysis

Data set: 6725 probe sets regulated at least once among the 12 combinations



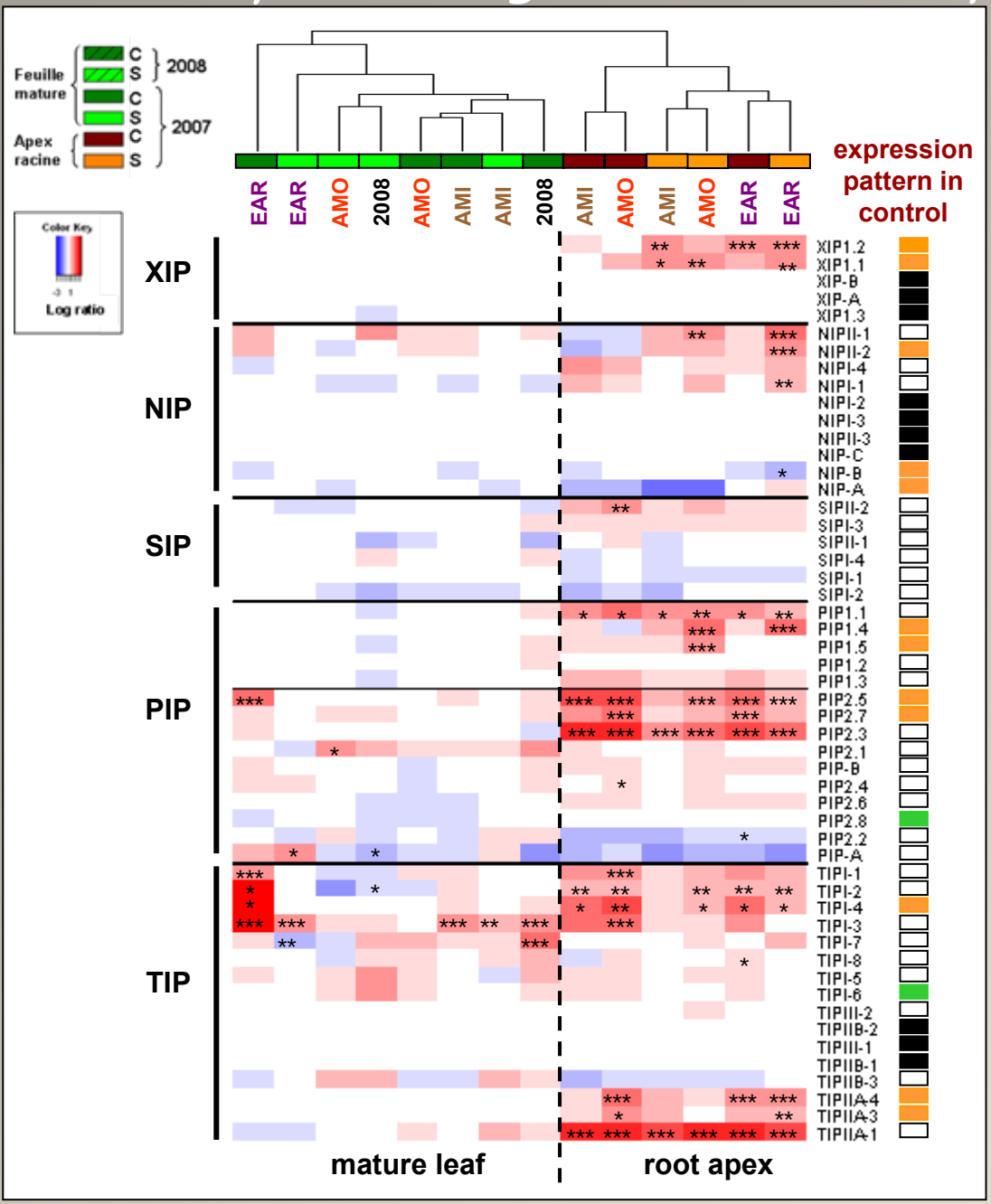


- Root apex transcriptome more responsive than the mature leaves one
  - number of regulations
  - higher fold change

Leaf and root transcriptome meta-analysis

- Leaf and root common regulated genes : common stress marker
  - Homeobox-leucine zipper protein (*atHB12-like*)
  - Protein phosphatase 2C
  - NCED, caroten dioxygenase activity
  - HSP, HSP-binding
  - Bet-v1 allergen (Pyr-like protein)
  - Etc ….
- Leaf specific regulated genes
  - Galactinol synthase-llike
  - B-glucosidase (other photosynthesis-associated gene)
  - Etc ….
- Root specific regulated genes
  - PIP1-4
  - Beta-caroten hydrolase
  - ABC transporter
  - Asparagine synthetase
  - Early responsive to dehydration (AtERD15-like)
  - ACC oxidases
  - Transferases
  - F-Box family
  - Etc…

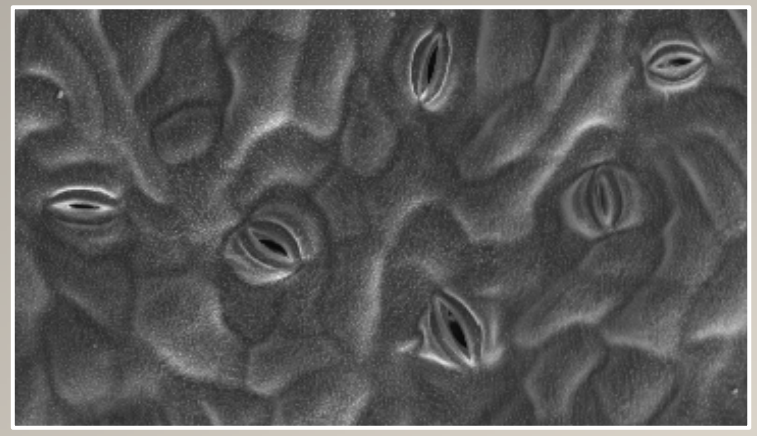
Transcriptional regulation of the aquaporins family

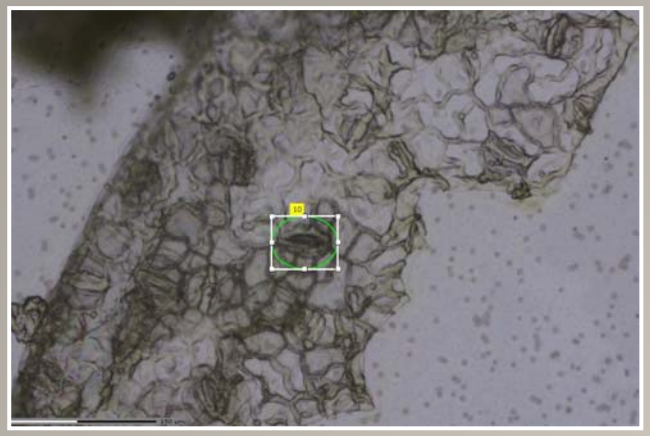


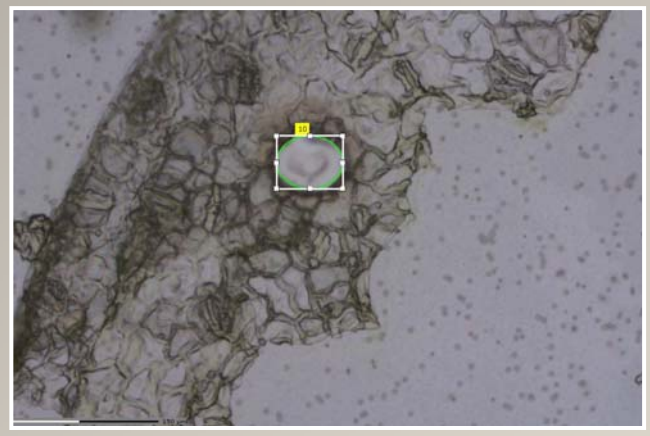
- Expression in controls
  - 10 genes not expressed
  - 12 genes root-specific
  - 2 genes leaf-specific
- More numerous and stronger regulation in root apices as compared to leaves (similar to the whole genome response)
- Co-regulations detection
- Induction detection
  - TIP1.4, PIP2.5 in leaves
- Identification of AQPs potentially involved in drought tolerance

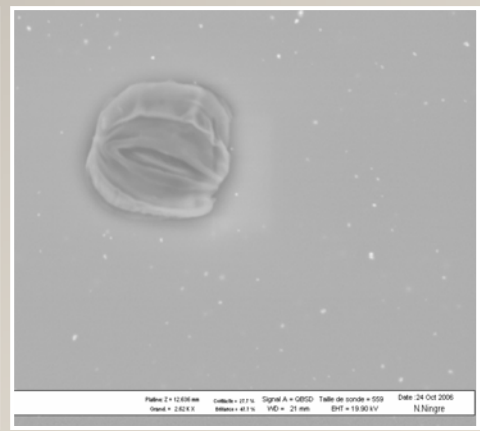
Guard cell transcriptomics

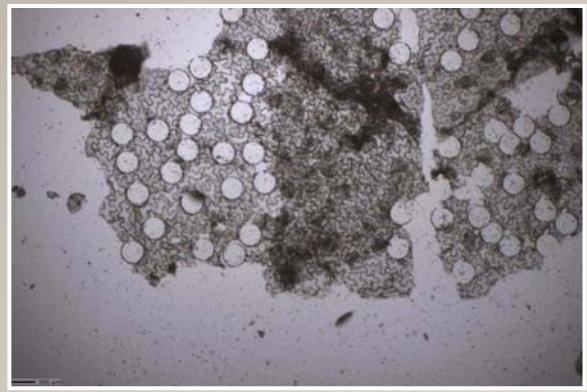
Laser microdissection (1500 guard cell complexes)

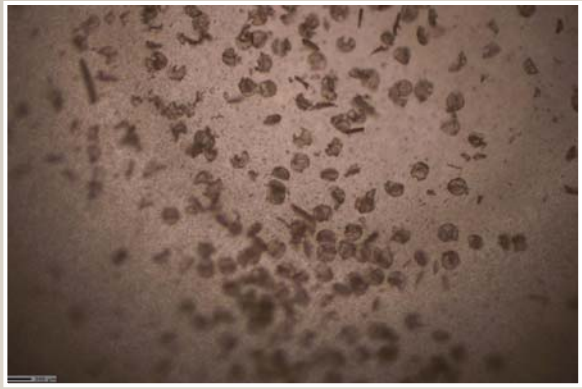










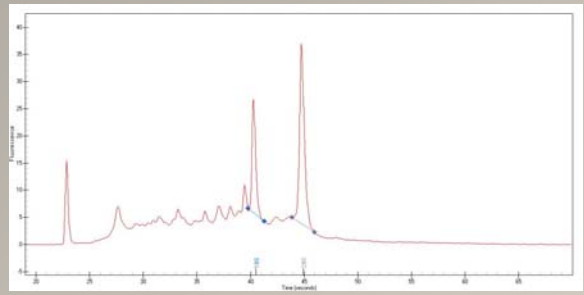


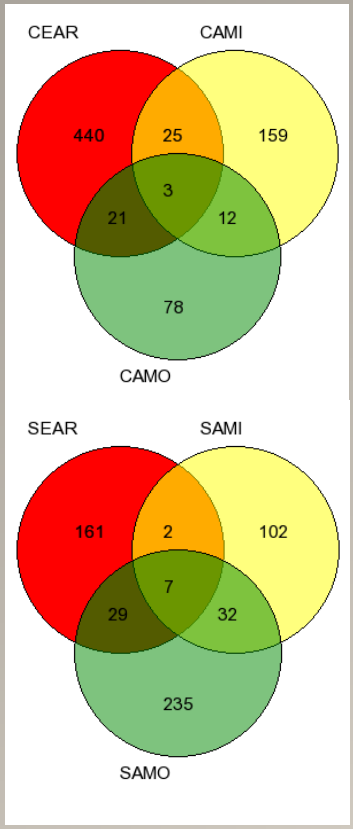
Guard cell transcriptomics

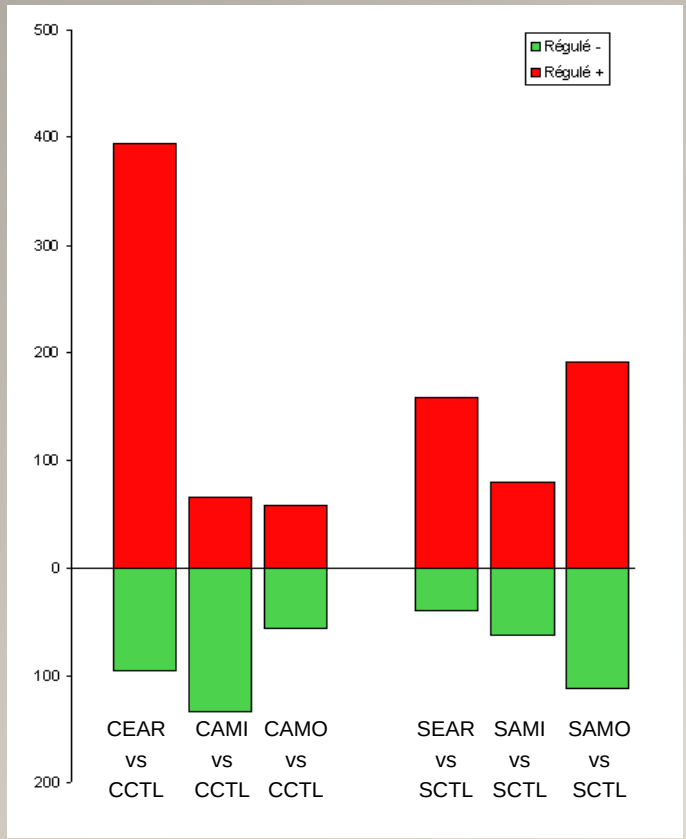
RNA extraction

Amplification

Hybridization on microarrays





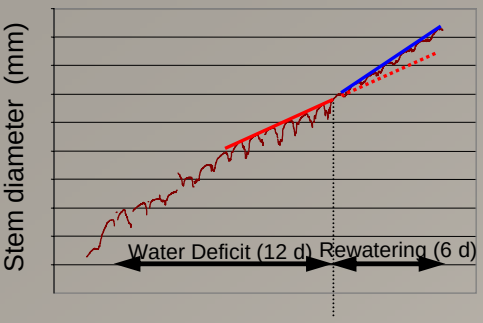


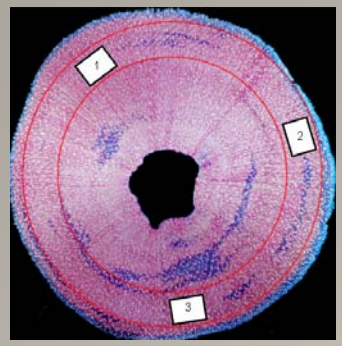
1,196 DW responsive probe sets
(no Log 2 ratio cut-off, p<0.05)

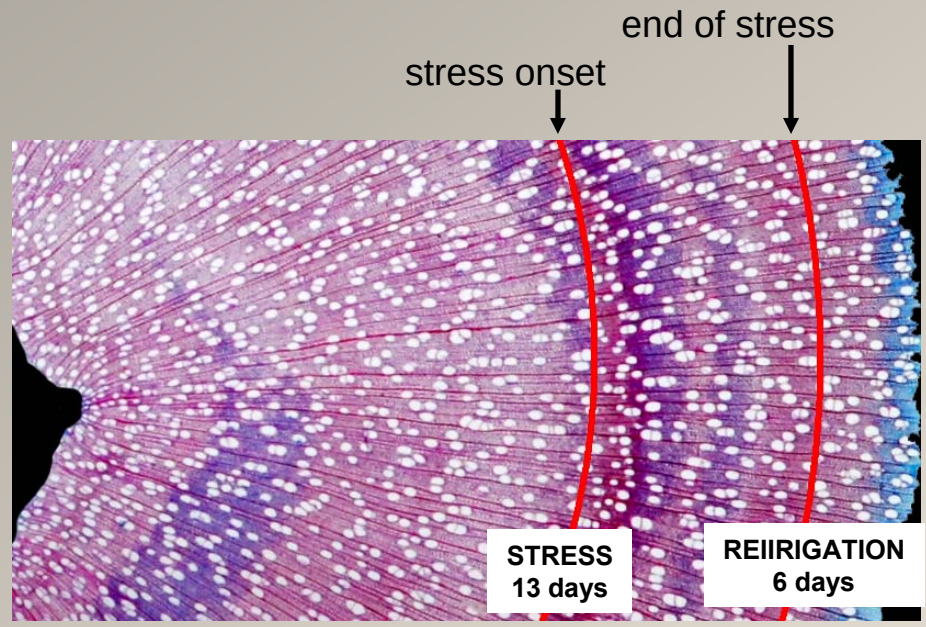
- Rapid and strong induction response in the tolerant genotype
- Strong regulation levels

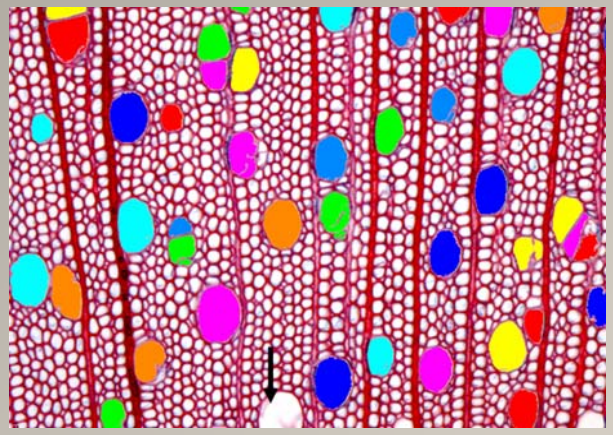
Deeper analysis currently running

Wood histology





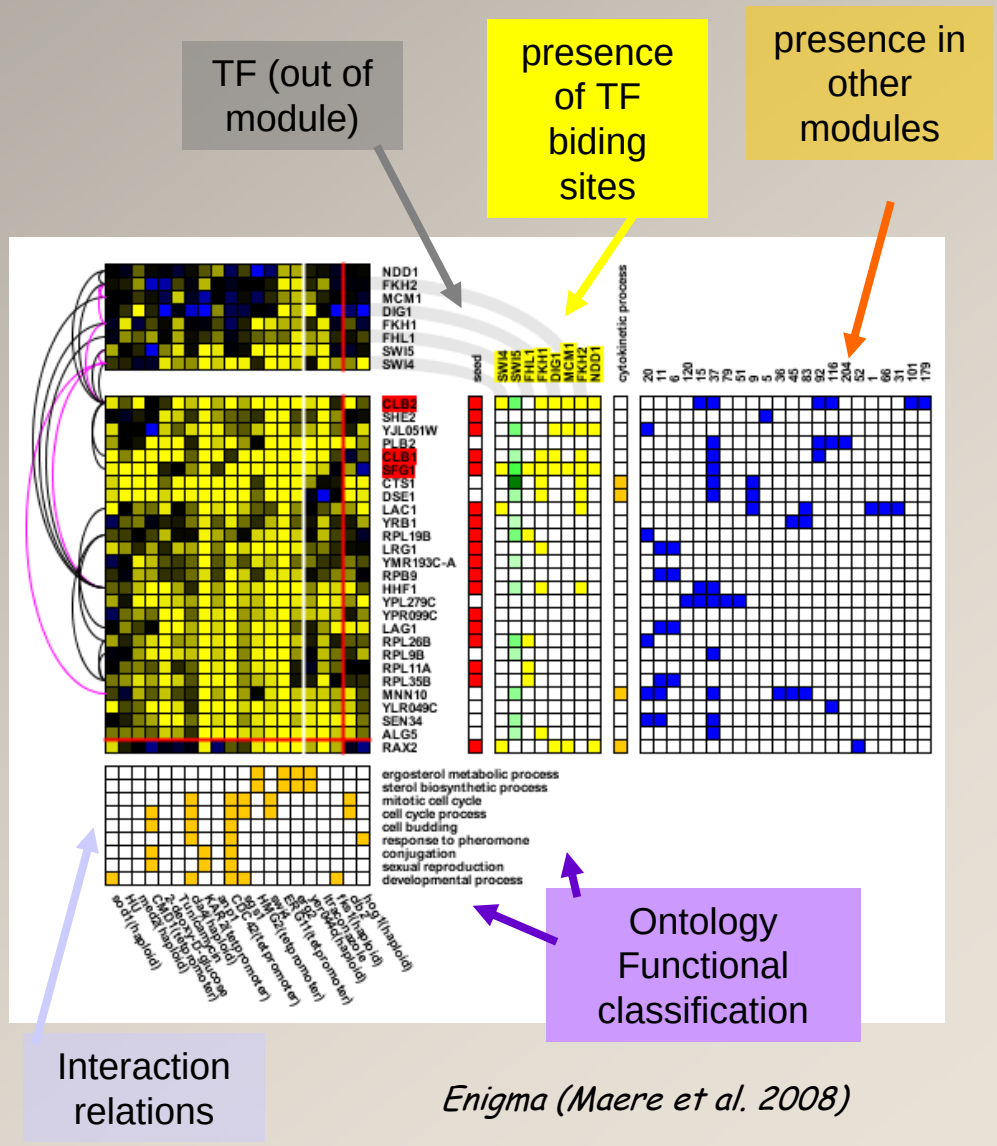




- *Differences in vessel mean CSA and in vessel total CSA fraction between clones*
- *Fiber and vessel mean CSA reduced under WD*
- *Vessel total CSA fraction insensitive to WD*
- *Conservation of genotype differences under WD*

Gene networks in wood

- 484 genes classified in 15 modules
- 7 modules covering 51 % of gene regulation
- Main functional modules :
  - GH17, GH3, GT2, pectine esterase laccase …. Cell wall construction
  - Snare-like, IMP, Aquaporins, ABC transporter : Cell membrane associated proteins



Enigma (Maere et al. 2008)

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Diversity in drought tolerance between clones

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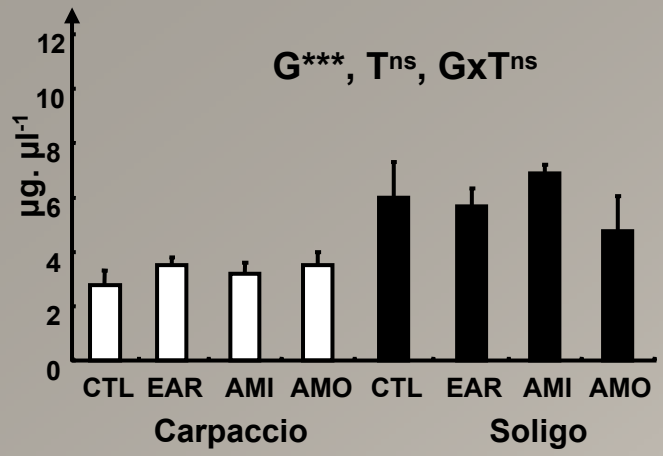
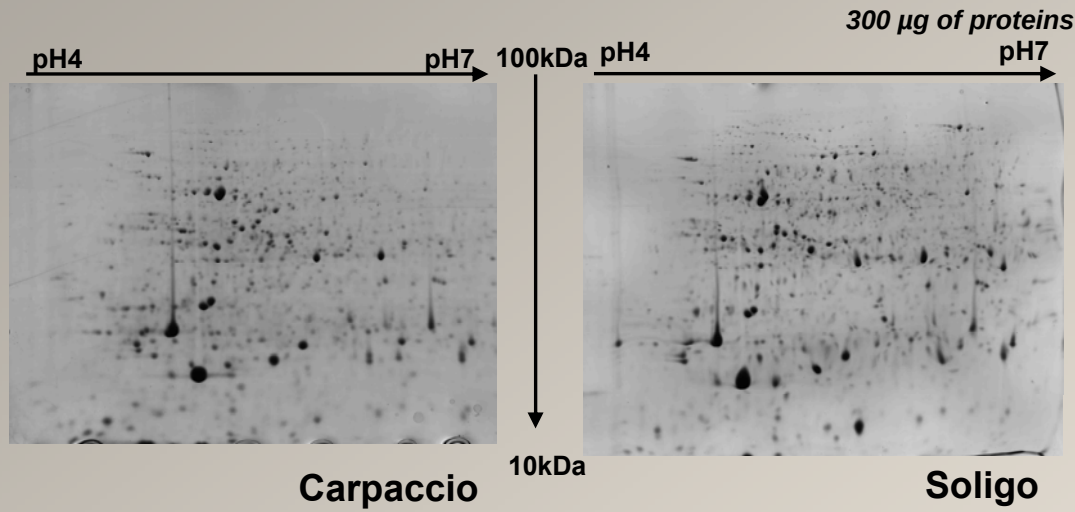




- higher protein content in Soligo
- higher spots number in Soligo (1200) than in Carpaccio (600)
  - → comparison of 563 reproducible spots

	ANOVA *p* -value ≤ 0.05	FDR *q* -value ≤ 0.05
G	400	400
T	40	0
G x T	43	0

	ANOVA *p* -value ≤ 0.05	FDR *q* -value ≤ 0.05
G	361	361
T	59	0
G x T	55	0

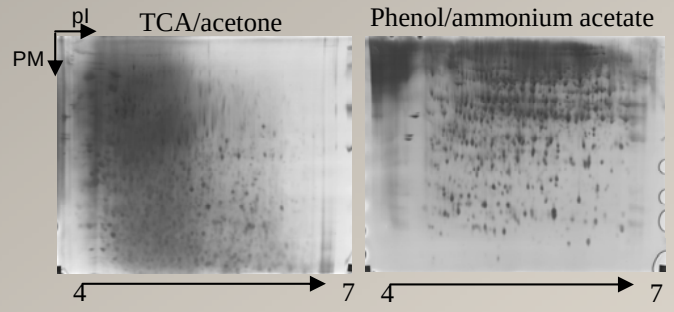
	ANOVA *p* -value ≤ 0.05	FDR *q* -value ≤ 0.05
T	27	0

	ANOVA *p* -value ≤ 0.05	FDR *q* -value ≤ 0.05
T	53	1

- strong genotype effect
- after FDR, no significantly WD-regulated protein was discovered

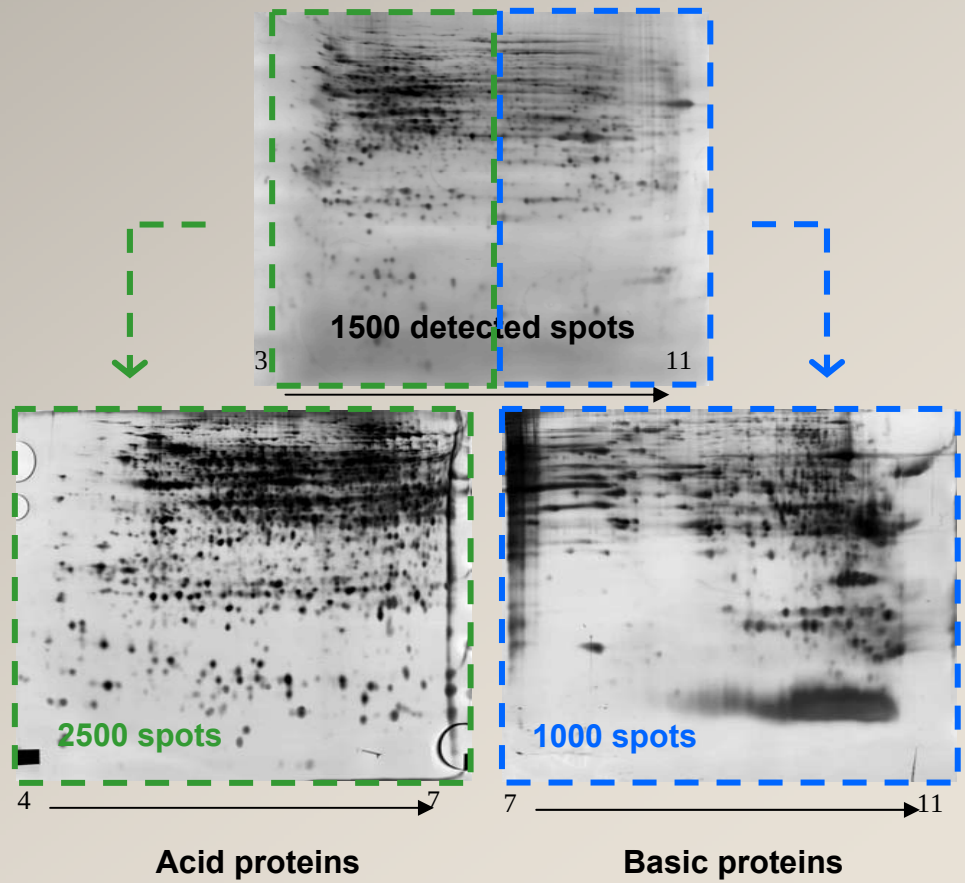
Root apex proteome

- 3*40 root apices, i.e. about 500 mg fresh weight
- Required protocol optimisation of several steps :
  - extraction
  - electrophoresis conditions : small pH range (4-7 & 7-11)



- Acid and basic proteins

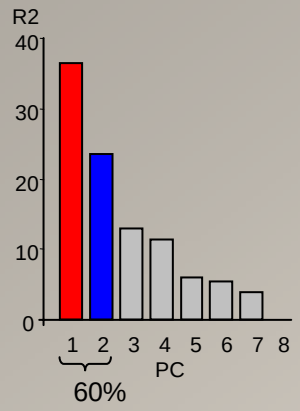
- Statistical analysis
  - PCA
  - Hierarchical clustering

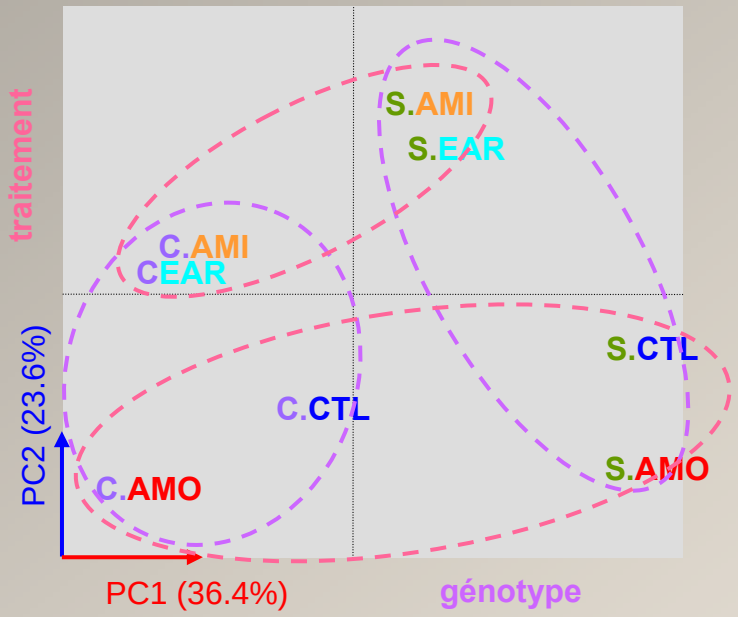


Root apex proteome

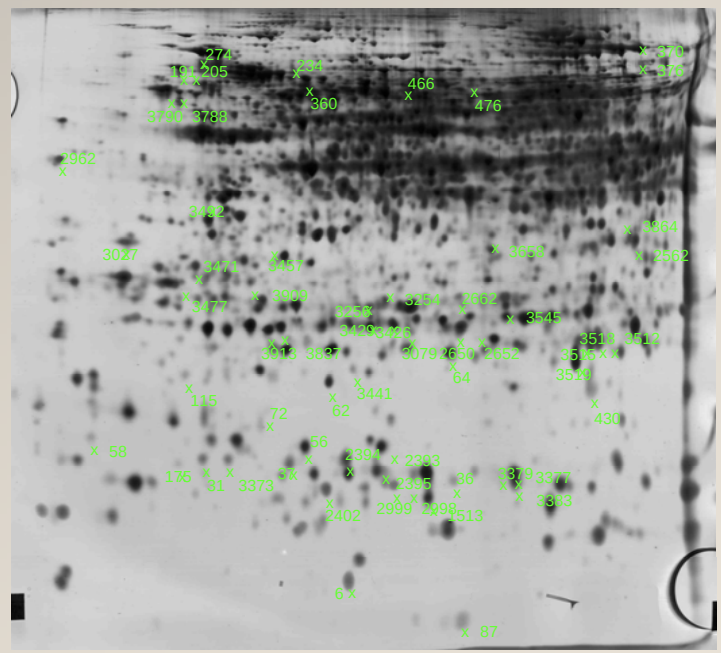
Acid proteins
1540 reproducible spots

- 1st source of variation of protein quantities: genotype
- 2nd source of variation of protein quantities: treatment





- Excision of selected proteins
  - 61 acid proteins
  - 33 basic proteins



- LC-MS/MS identification

  Heat Shock Proteins (HSP70, HSP82)

  14-3-3 protein

  2 APX, PRX2B, GST

  etc…

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Selection of some candidate genes by:
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qPCR on other clones of different drought tolerance

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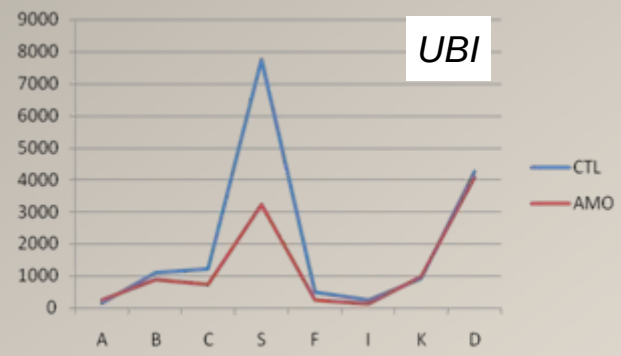
Transformation?

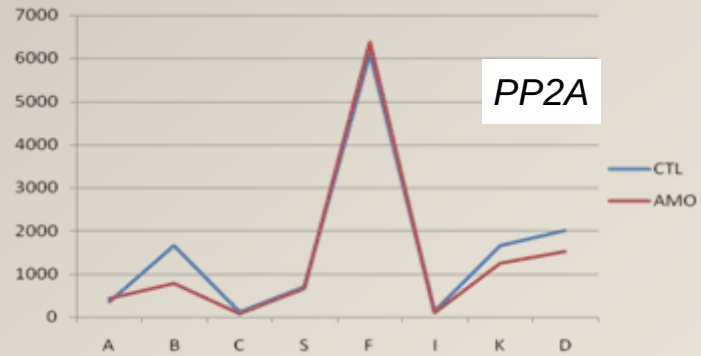
Expression in other clones

- Independent experiment on 8 genotypes:
  - Carpaccio, Soligo
  - 4 others P. deltoides x nigra :
    I214, Dorskamp, Koster, Flévo
  - P. trichocarpa Beaupré
  - P. tremula x alba 717-1B4

- long-term water deficit (10 days at 20% REW)

- RT-qPCR analyses
  - housekeepers genes
  - currently running





Conclusions from this integrative study

- Validated water deficit "markers"
  - previously identified in other species or poplar genotypes
- Identified candidates genes for drought tolerance in poplar
- Focused on cell types and specialised tissues :
  - stomata vs whole leaf
  - growing xylem vs whole wood
- Between genotype differences higher than drought response
  - for physiological traits, histology, transcripts and proteins
  - also pointed out by Wilkins et al (2009, mature leaves)
    - this point has to be addressed by diversity analyses
- Root apices more "responsive" than mature leaves
  - seen at the scale of the whole genome as well as at the scale of one multigene family
  - seen at the transcript level as well as at the protein level
    - growing vs mature tissue ?
    - root : closer to the constraint ?
    - organ specificity ?

Collective work :

- INRA NANCY (UMR EEF)
  - Irène Hummel
  - David Cohen
  - Rémy Merret
  - Didier Le Thiec
  - Nathalie Ningre
  - Erwin Dreyer
- INRA ORLEANS (UAGPF)
  - Jean-Charles Leplé
  - Philippe Label
  - Gilles Pilate
  - Annabelle Déjardin
  - Isabelle Bourgait
- ORLEANS UNIVERSITY (LBLGC)
  - Franck Brignolas
  - Domenico Morabito
  - Ludovic Bonhomme
- INRA BORDEAUX (UMR Biogeco)
  - Delphine Vincent
  - Christophe Plomion
- URGV Evry
  - Sandrine Balzergue
  - Marie-Laure Martin-Magniette
  - Jean-Pierre Renou
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  - Emilie Tisserant
- Ecogenomic plateform of INRA Nancy

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Thank you for your attention