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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. : Marie-Béatrice Bogeat-Triboulot

Poplars

➤ scientific interests:

- perennials :
 - cycles of growth, reserves, ...
 - complementary to annual biology
- woody : cambial growth
- clonal propagation
- genome fully sequenced



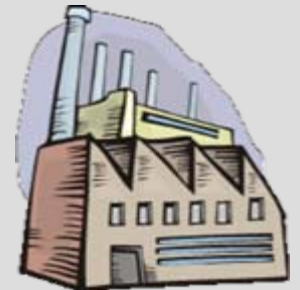
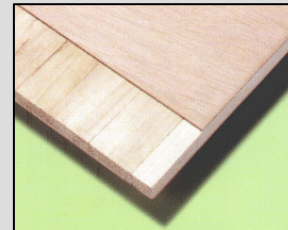
➤ ecological interests:

- soil depollution
- riverside biodiversity



➤ economical interests:

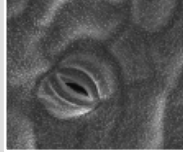
- short rotations: "crop tree"
- 1.3 Mm³ wood in 2008
- crates, paper pulp
- bio-energy / global change



Drought tolerance

stomatal closure
 -> water loss limitation

Stomata



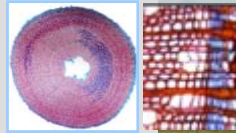
osmotic adjustment
cell detoxication
 -> cell homeostasis

Leaves



cambial growth
 -> productivity

Wood

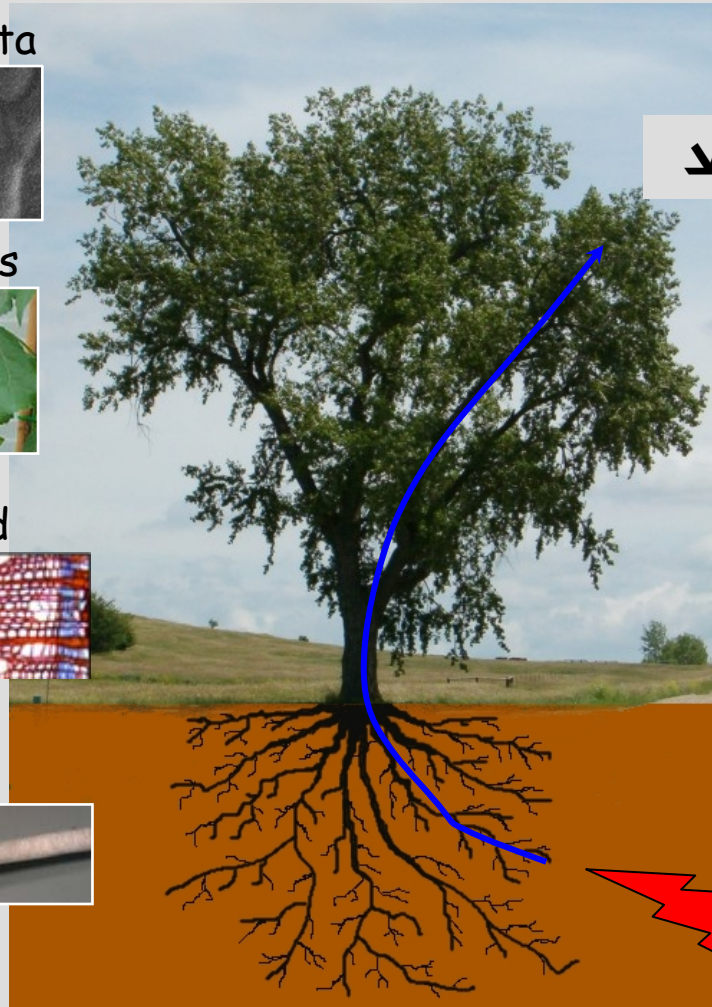


hydraulic K ajustement
 -> water flow
 -> water uptake

Root



root growth
 -> soil prospection

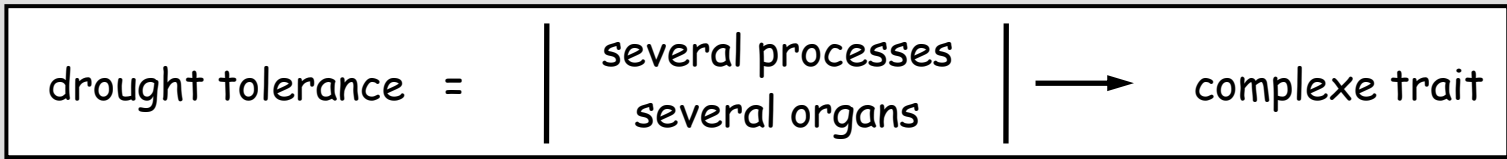


Ψ_{leaf}

↳ leaf growth
 ↳ gas exchange

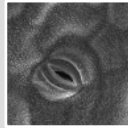
↳ wood growth

↳ productivity



Objectives

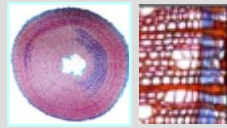
stomatal regulation



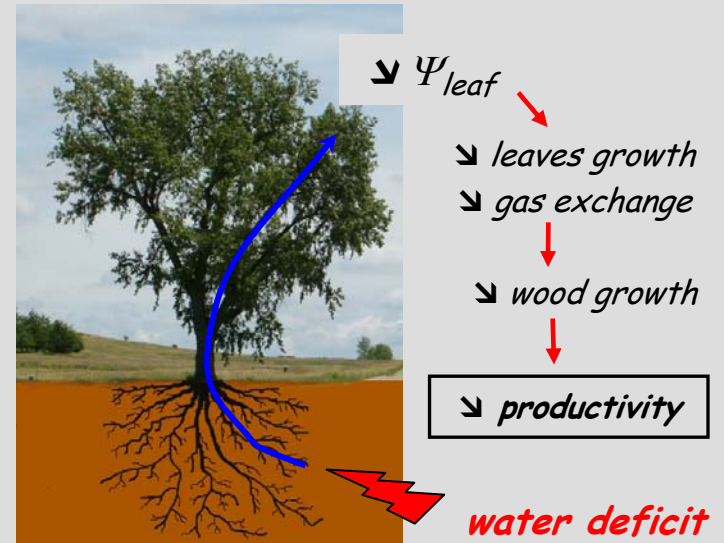
cell homeostasis



cambial growth



root growth



POPSEC Consortium :

- focus on several **processes** involved in drought response
- in several **organs/tissues/cell types**
- at several scales **ecophysiology, transcriptomics and proteomics**

integrative approach

- To better understand the molecular bases of drought tolerance in a **tree**
- Use of **genetic diversity** to assess
 - common bases of drought response
 - specificity of drought tolerant cultivars

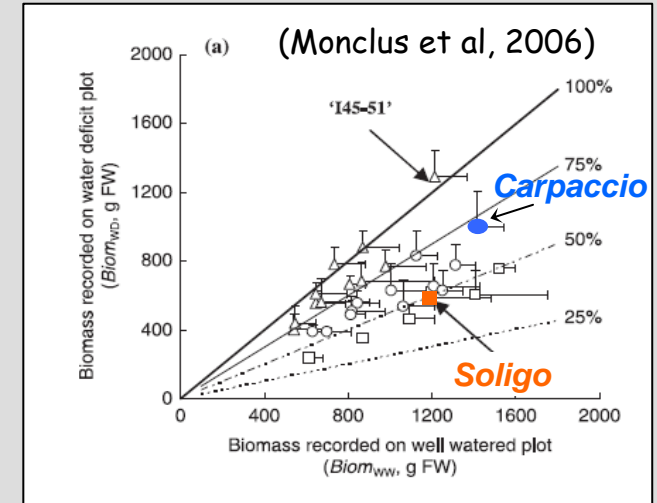
1- Omics analyses in two cultivars

➤ *P. deltoides x nigra*: Carpaccio and Soligo

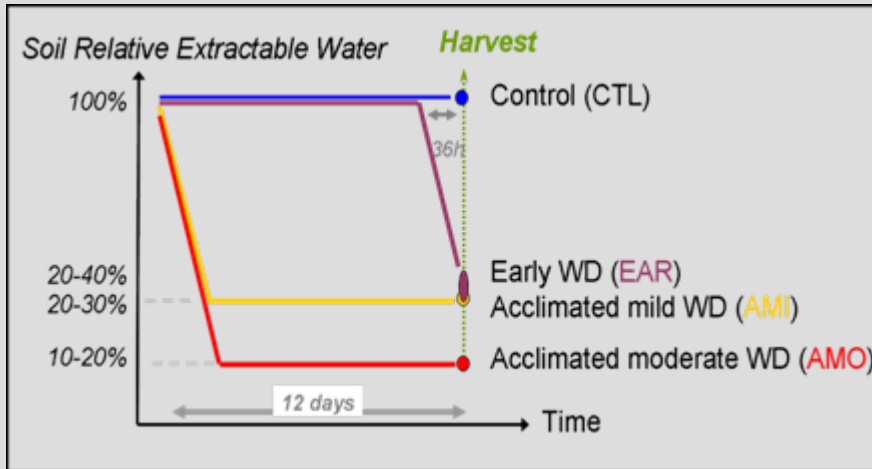
- 2 commercial hybrids

chosen for their

- similar productivity
- similar WUE
- contrasted productivity maintenance under water deficit

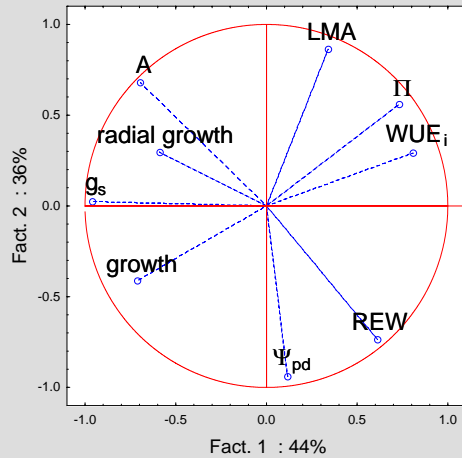


➤ 3 water deficit treatments

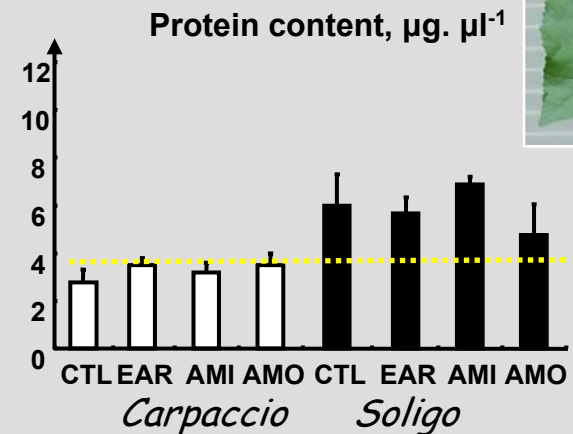
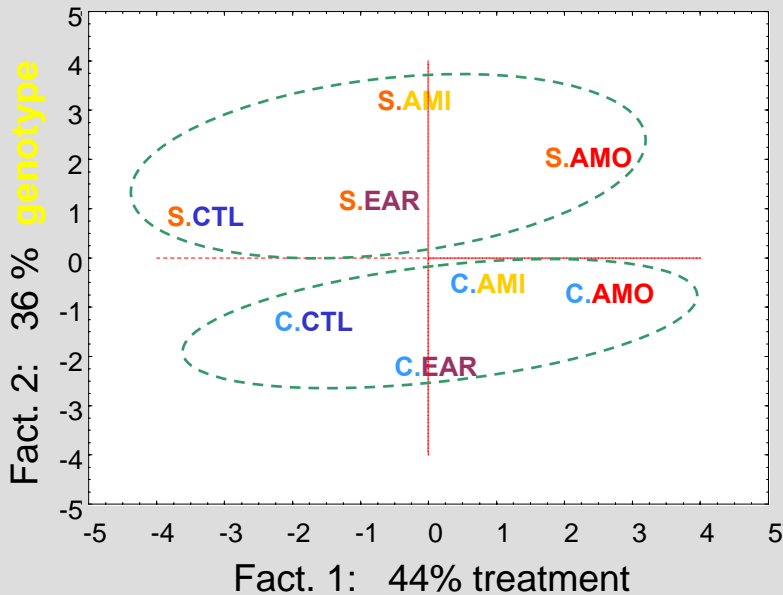
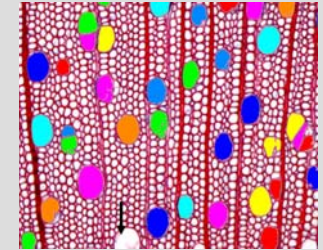


Genotypes drove results as much as drought

PCA on ecophysiological parameters



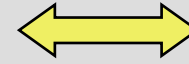
	Vessel lumen area
C_CTL	2836 (± 2)
S_CTL	2399 (± 3)
C_AMO	2102 (± 5)
S_AMO	1864 (± 3)
CTL_C/S	1.18 *
C_AMO/CTL	0,74 *
S_AMO/CTL	0,78 *



Transcriptomics in two cultivars

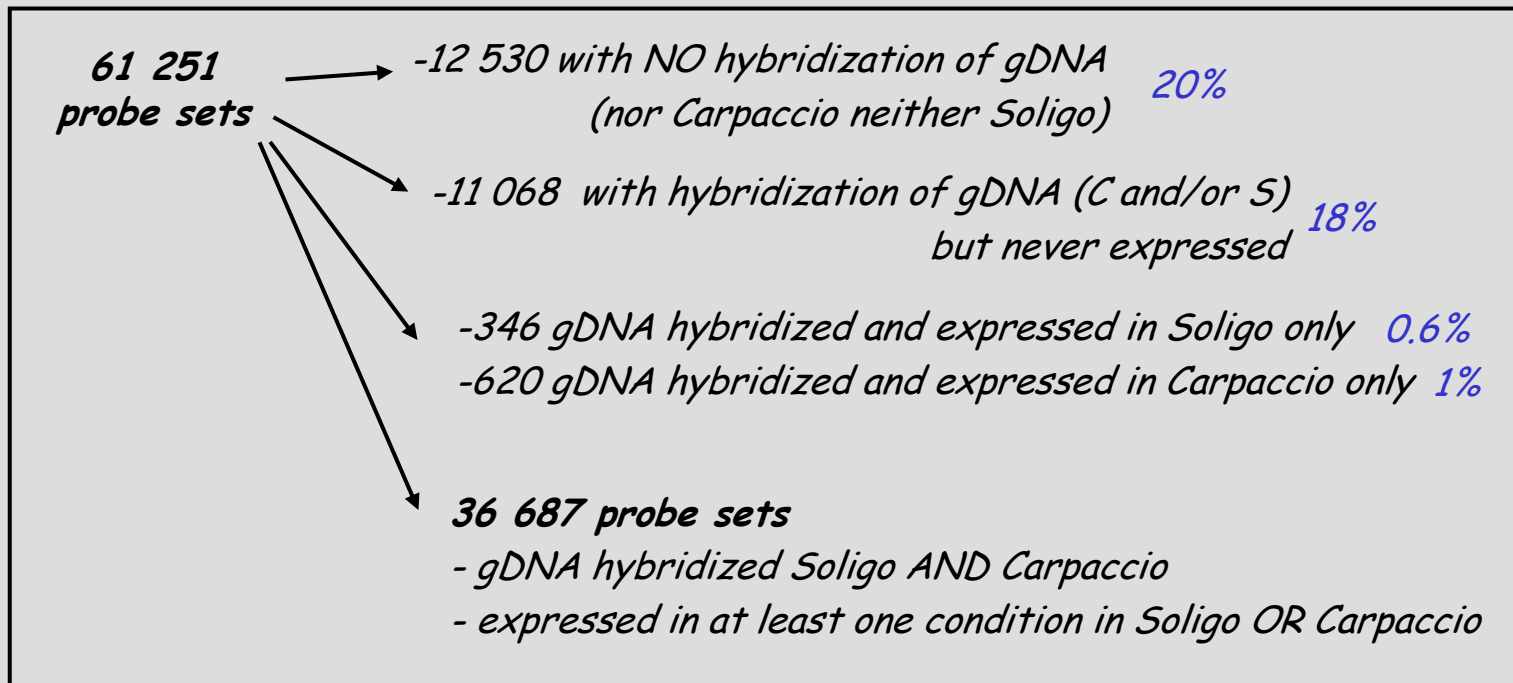
Affymetrix poplar genome array :

- ESTs from 13 species/genotypes :
P. tremula, *P. euphratica*, *P. deltoides x nigra*, ...
- gene models from *P. trichocarpa*



Experiment on 2
P. deltoides x nigra

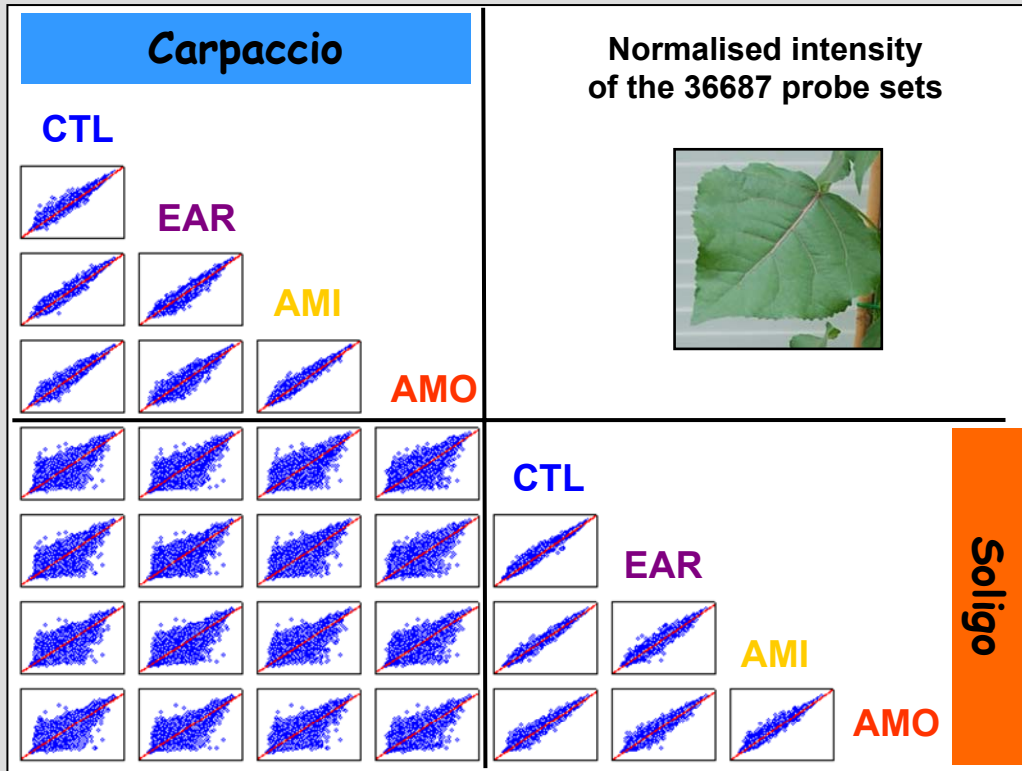
mismatch of hybridization ? → hybridization **genomic DNA** of both cultivars



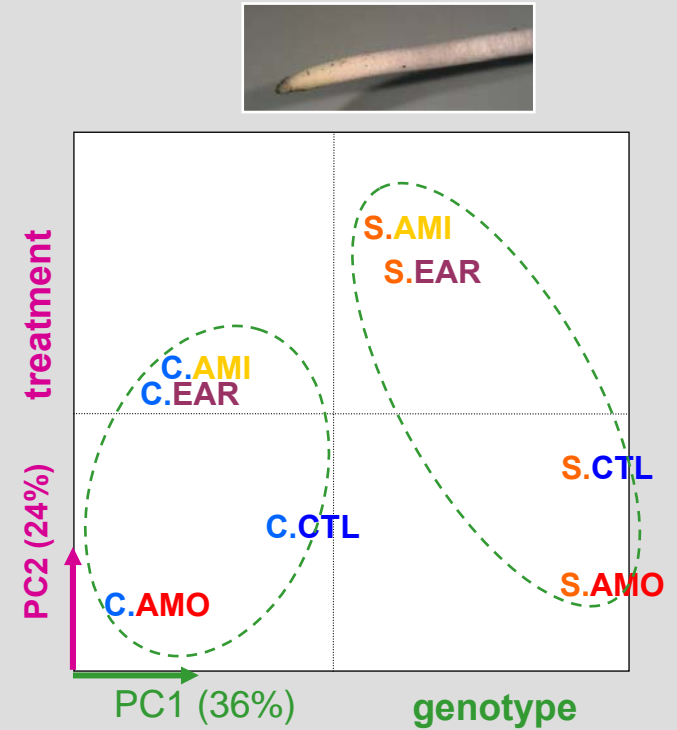
→ *Univocal comparative analysis of two genotypes*

Genotypes drove results as much as drought

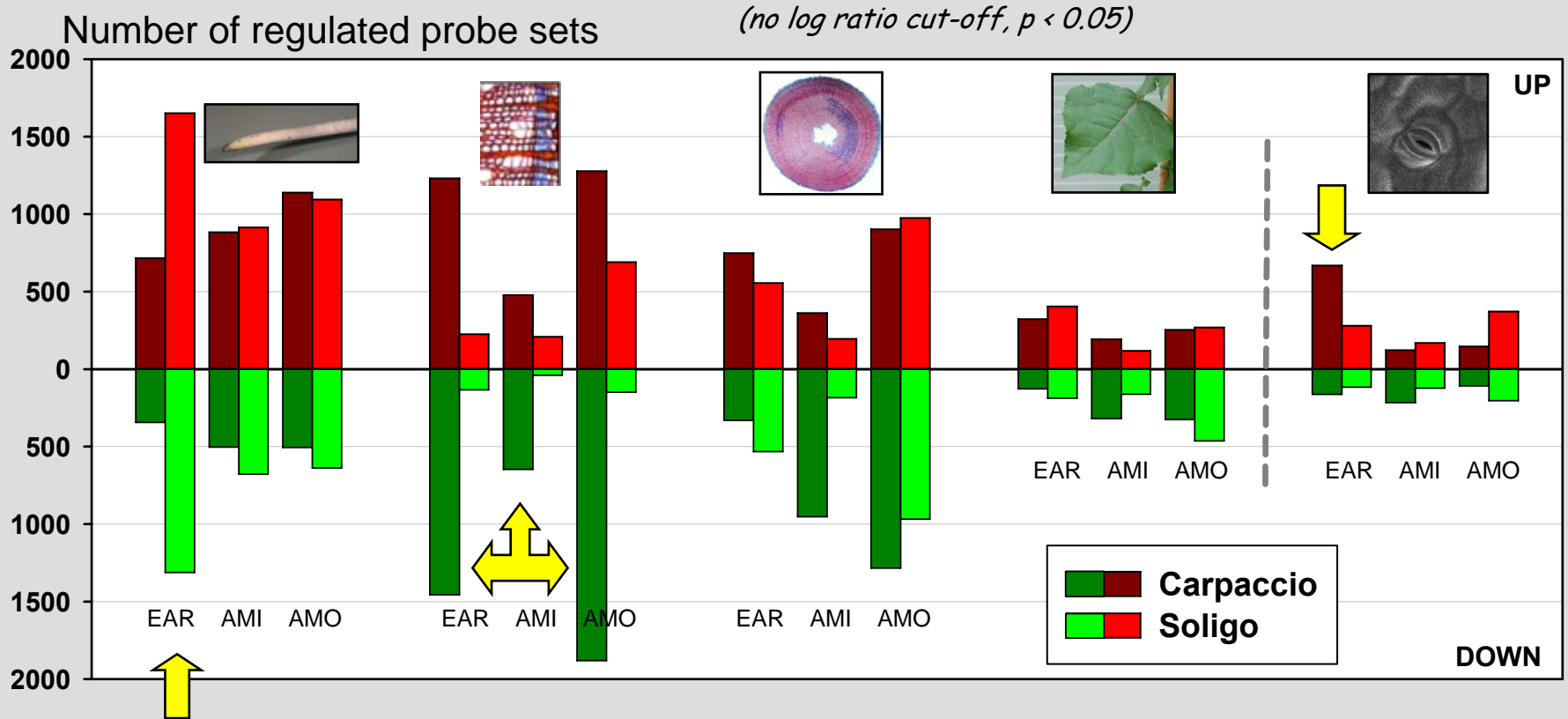
Global analyses of gene expression



PCA on acid proteins



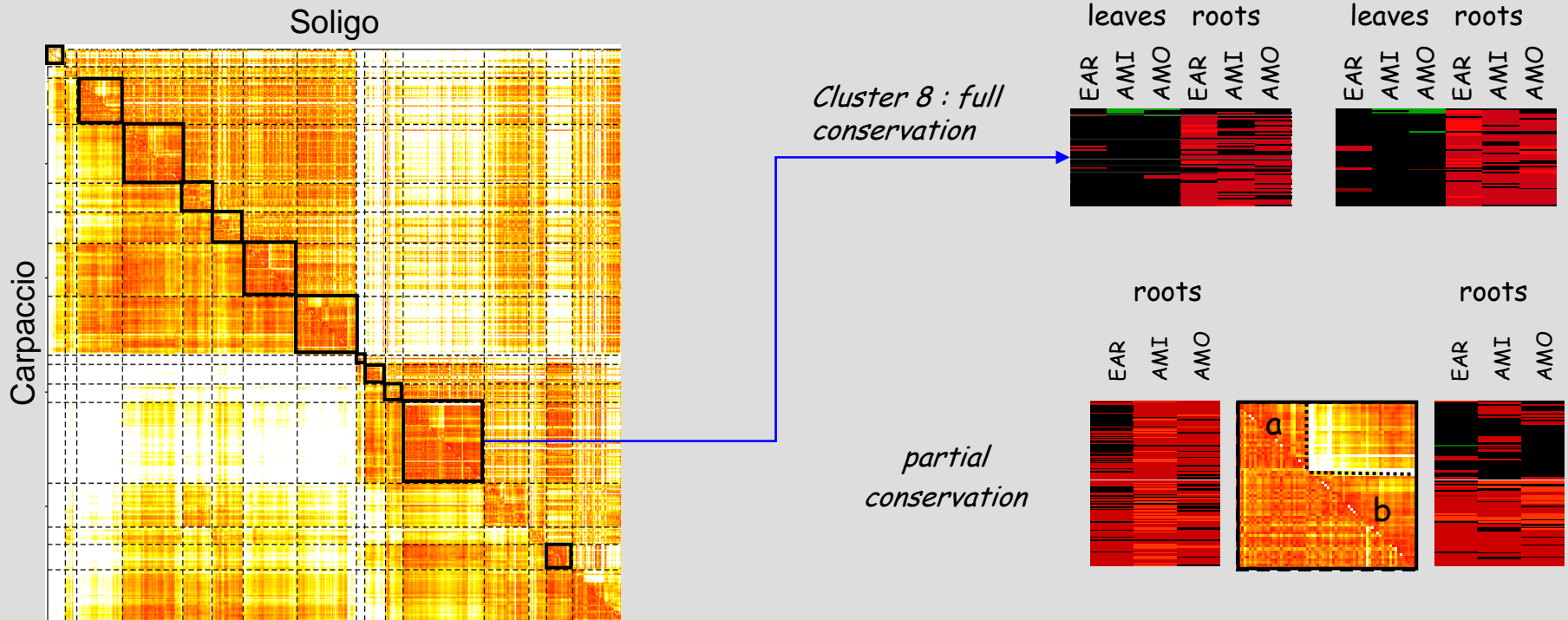
Drought-induced gene regulations



- *Root apices and xylem are highly responsive*
- *Strong cultivar specificity in young xylem (wood growth maintenance?)*
- *Some strong genotype*treatment interactions*
 - *Soligo EAR - root apices*
 - *Carpaccio EAR - stomata*

Search for gene networks

Differential Clustering Algorithm



distance between gene expression profiles -> combined matrix

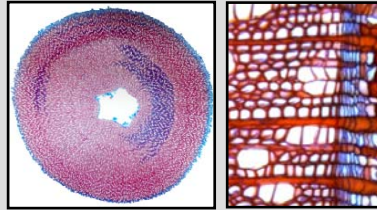
- transcriptional modules are delineated in Carpaccio
- second clustering within each module in Soligo
- test of co-expression relationship within each module in Soligo
- Full conservation : conserved co-regulation of genes across treatments in the two cultivars
- Partial conservation : some co-regulation relationship between genes are lost

→ Identification of similarities and differences of gene regulation between cultivars

Search for gene networks

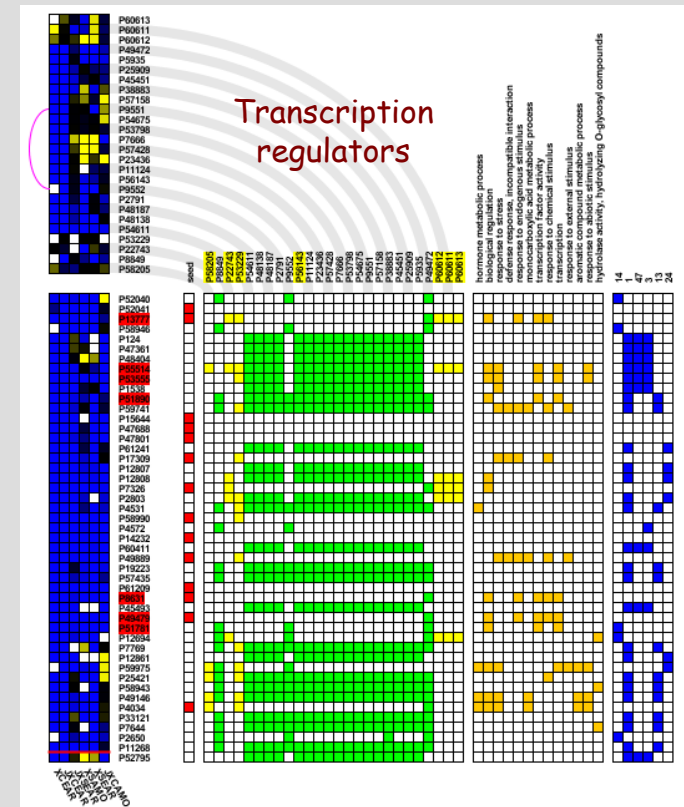
ENIGMA clustering and enrichment method
includes functional information

In wood tissues

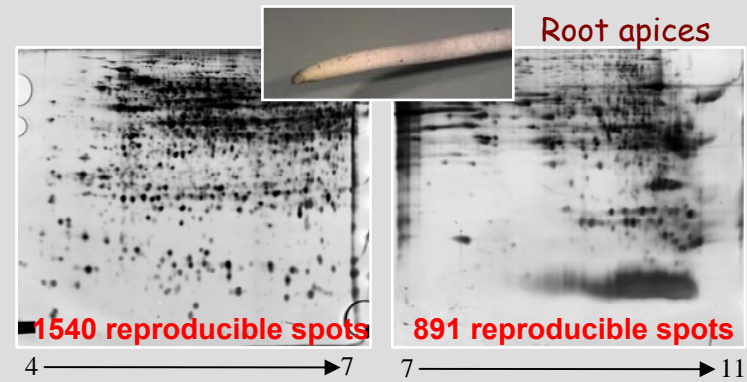
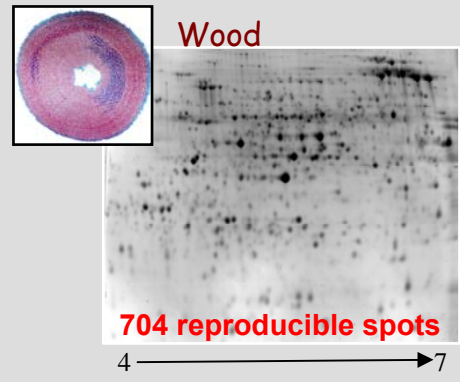
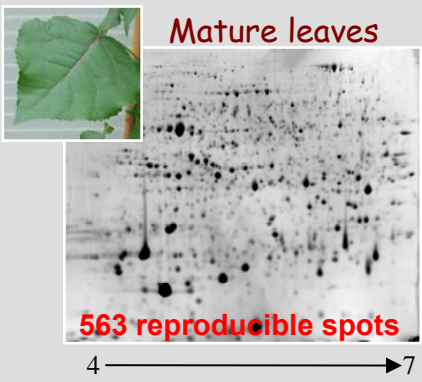


- The network contains **50 modules** and **2776 probe sets** covering the 12 conditions tested
- A few modules propose a **transcription network associated with response to stress**
- Ex : module 42 describes Xylem & Young Xylem in response to EAR and AMO and is enriched in genes involved in :
 - Hormone metabolic process
 - Response to stress
 - Transcription regulation
 - Perception of external stimuli

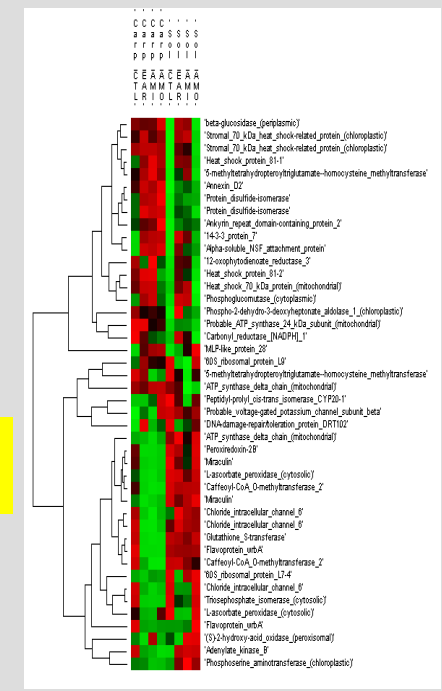
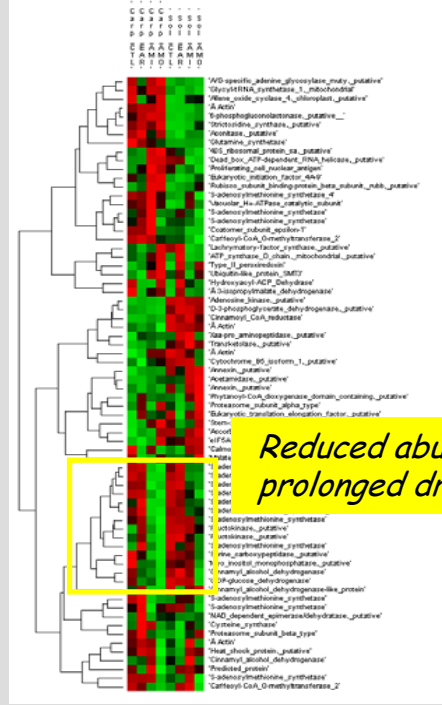
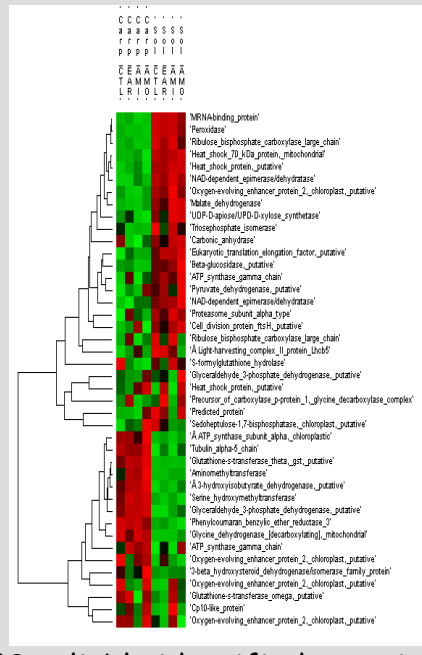
module 42



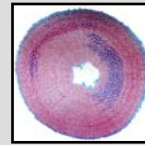
Search for co-regulation of protein abundance



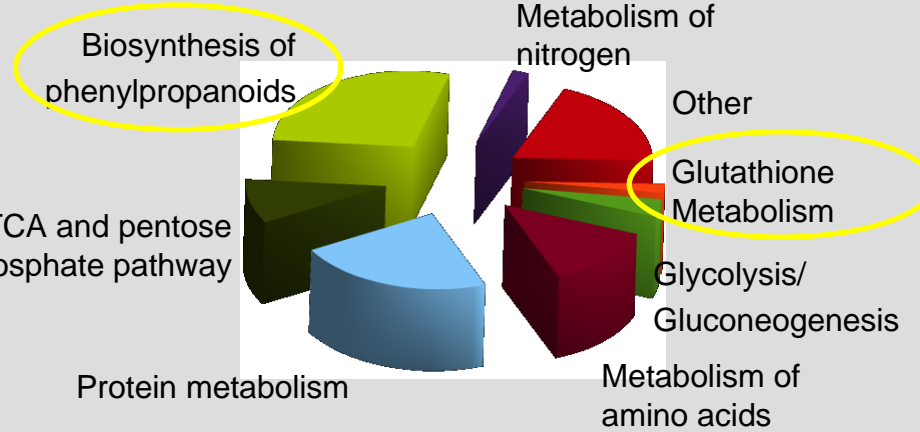
the spots displaying a significant *treatment* effect or *genotype x treatment interaction* were identified by Nano LC-MS/MS



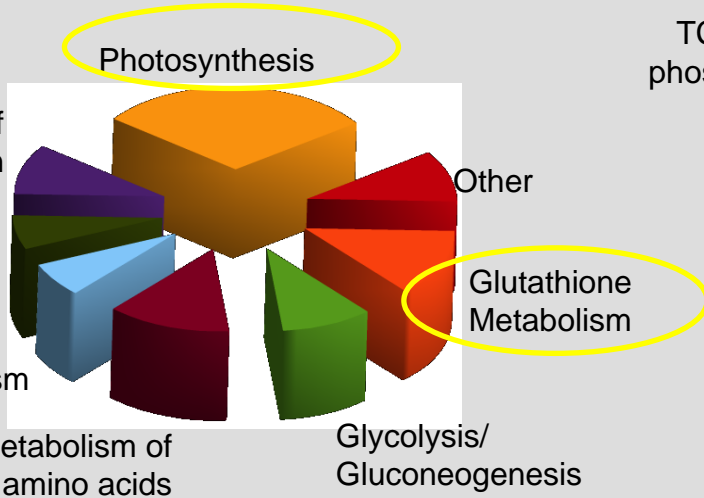
Functional protein groups involved in drought response



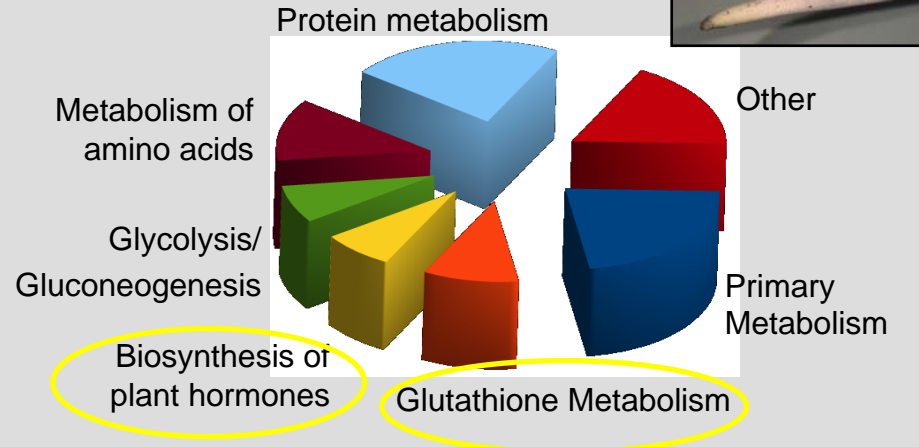
Wood



Mature leaves



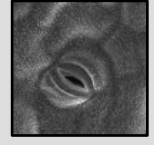


Root apices




Main functional groups of genes involved in drought response

revealed by iterative group analysis

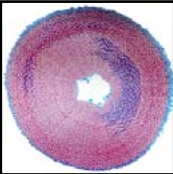
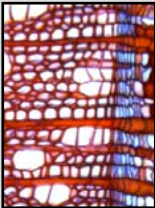
		Carpaccio	Soligo
Root apices 	E A R	Abiotic stress/drought : ABA biosynthesis and signalling (NCED, PP2C, ABA-responsive TF, PYR) Cell rescue / redox homeostasis (DnaJ, HSP, Glutathion-S-transferase, metallothionein) Hypoxia (ADH, Pyruvate decarboxylase, LOB41-like protein)	
	A M I	Growth : Expansion (aquaporins, pectine esterase) Meristem activity (chromosome organisation, DNA replication, constituent of ribosome)	
	A M O		
Mature leaves 	E A R	ABA signalling (NCED, PP2C, ABI5-binding like, PYR like) Water channel	
	A M I	Dead-box helicase like	Detoxication (SOD, Glutaredoxin, metallothionein, germin) Cell rescue (RCI2A-like)
	A M O		Signal transduction (less focused on ABA) Transcription and metabolic processes Photosynthesis
Stomata 	E A R	Abiotic stress/drought ABA signalling Transcription	Water channel (↓) Flavonoid biosynthesis (↓) Transcription
	A M I	Water channel (↑) Detoxication ABA metabolism	Detoxication (galactinol synthase) Transporter Secondary metabolism
	A M O	Cell wall thickening	Response to gibberellin Cell wall (↓)
		Redox homeostasis Cell wall (↓)	ABA signalling ABA response (XERICO-like, PP2C, RCI 2A)

Number of transcription factors



Main functional groups of genes involved in drought response

revealed by topology based method (Topology-Elim, Ontologizer)

		Carpaccio	Soligo
Whole xylem 	E A R	Response to stress (biotic, abiotic, oxydative) Jasmonic acid metabolic process	response to water deprivation (NCED, ABA-responsive TF, Nitrate transporter) Response to cold
	A M I	Auxin transport, Protein amino acid phosphorylation, cell wall, regulation of cell size, cellulose biosynthetic process	photosynthesis, light reaction
	A M O	flavonoid metabolic process response to water deprivation apoplastic response	Cell wall and plasma membrane compartments, microtubule polymerization, response to stress (oxidative, salt, cold)
Young Xylem 	E A R	Response to stress (biotic, abiotic, oxydative) jasmonic acid metabolic process, trehalose biosynthetic process, transcription factor activity	Plasma membrane compartment, response to hormone stimulus (ABA, auxin), trehalose-phosphatase activity, transcription factor activity
	A M I	sesquiterpenoid biosynthetic process, ATP synthesis coupled proton transport, cell wall pectin metabolic process	Chloroplast et apoplastic compartments Response to stress (chemical stimulus) Response to water Hormone stimulus (gibberellin 20-oxidase activity)
	A M O	Plasma membrane and apoplast compartments, Auxin biosynthesis, response to cadmium ion, regulation of ethylene biosynthetic process	Plasmamembrane and apoplast Response to water deprivation, osmosensor activity cellular carbohydrate biosynthetic process proteolysis, transporter

2- Drought response in 8 cultivars

Omics → Transcriptome remodelling analyses and *selection of genes of interest*

Diversity → Test the validity of these genes



Carpaccio



Soligo



I214



Dorskamp



Koster



Flévo



Beaupré



717-1B4 (A)

P. deltooides x nigra

P. trichocarpa *P. tremula x alba*

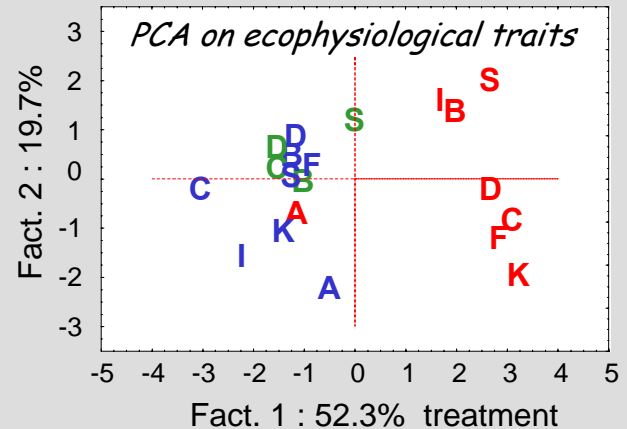
➤ 2008 : independent experiment on 8 cultivars

Prolonged water deficit (10 days at 20% REW)

Control

Water-deficit

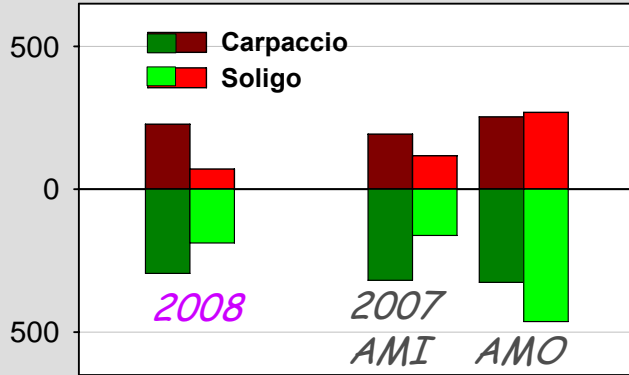
Rei-irrigated



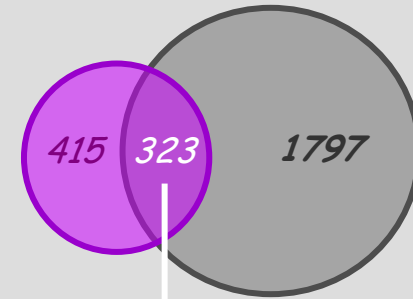
Consistency of drought regulation in mature leaves

Transcriptome of mature leaves of Carpaccio & Soligo

Number of probe sets

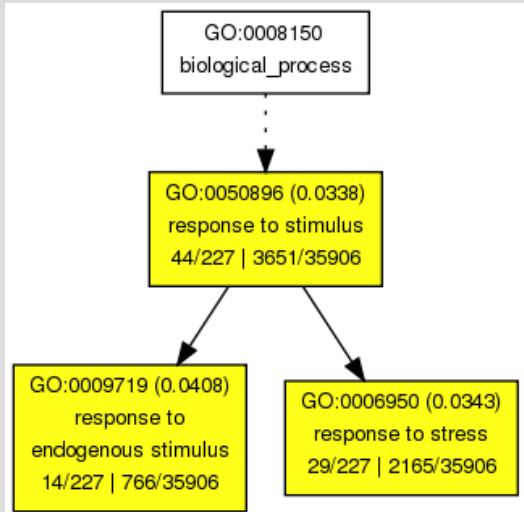
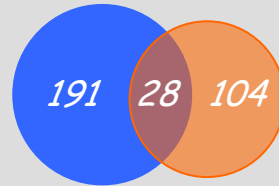


2008 :
783 drought-responsive probe sets



2007 :
2120 drought-responsive probe sets

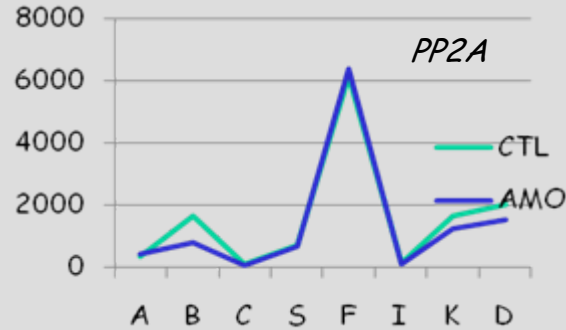
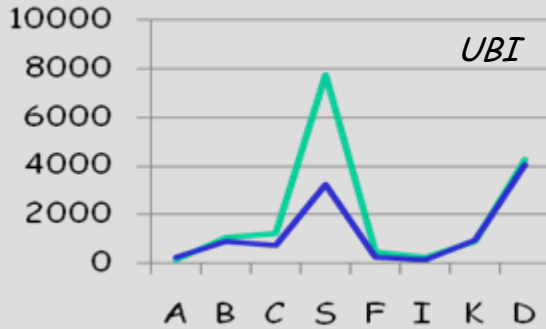
(Carpaccio, 219) (Soligo, 132)



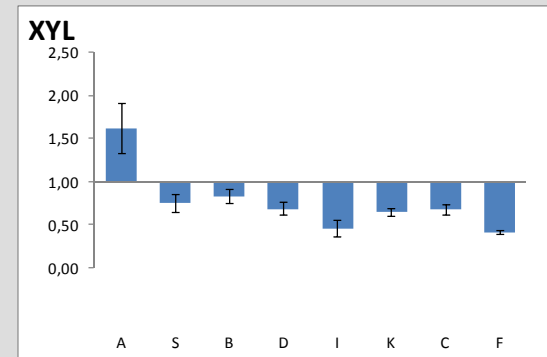
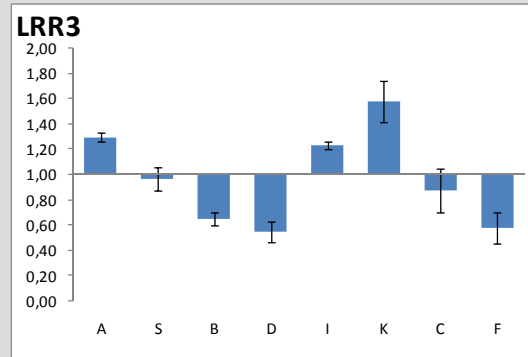
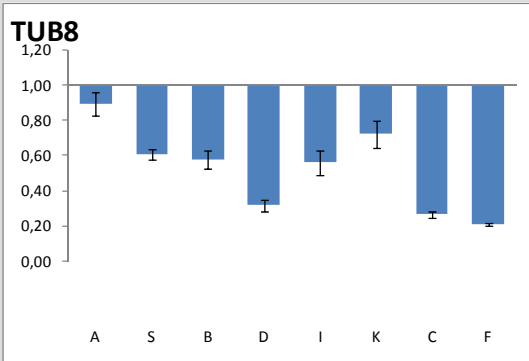
- Strong environmental effect
- 276 genes robustly responsive to prolonged drought
- Sense of regulation and cultivar specificity conserved

Selected gene expression in 8 cultivars

➤ Reference genes



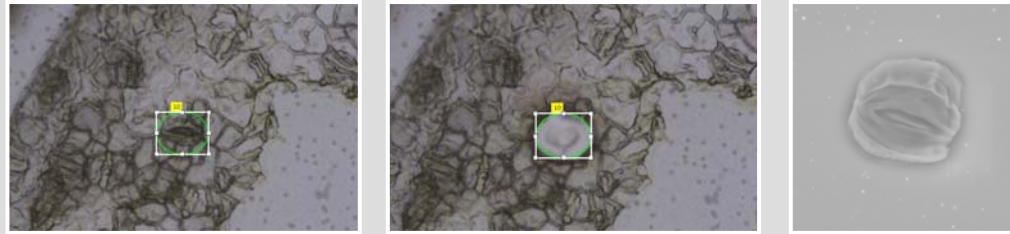
➤ Expression measured by RT-qPCR in Young Xylem



- Sense of regulation conserved between 2007 and 2008 (for Carpaccio and Soligo)
- Homogeneous or diverse expression depending on tested genes

Technical issues

- Hybridization of **genomic DNA** when using a multispecies chip
- **Protocols** to get access to the **proteome of root apices** (small amount of material)
to the **transcriptome of stomata** (lyophilisation, laser microdissection)

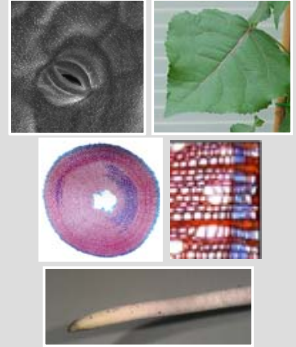


- Interconnected **transcriptome databases** dedicated to poplar (ForOrthology, ForExpress)
- Implementation of **proteome database** (ProticDB)
- Improvement of **statistical procedures** for Affymetrix analyses (normalisation)
- New insights in **gene and protein functions in poplar**

Emerging perspectives

➤ Continue with **integrative approaches** & improve **quantitative phenotyping** of the response

- Multiscale approach gave an integrative view of processes involved in drought tolerance
 - Difference of responsiveness of organs/tissues
 - Tissue dependency/independency of enriched functional groups
- Meta-analysis of the 5 "organs/tissues/cell types"



➤ Better take **diversity** into account

- Genotypes drove results as much as drought (physiological traits, histology, transcripts and proteins)
- **High throughput RNA sequencing** should become an important tool

➤ Continue the **search for the molecular bases of drought tolerance**

- Focus the questions on highlighted cultivar-contrasted processes :
 - **rapid and water deficit-specific vs delayed and generic stress response**
 - **responsiveness of cambial tissue**
- Track the **recovery phase** in addition to drought acclimation

➔ **Stepping stone for studies of other woody / perennial species**

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- Nathalie NINGRE
- Erwin DREYER

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- Jean-Pierre RENO

➤ **INRA Nancy (UMR IAM)**

- Emilie TISSERANT

➤ **Ecogenomic platform of INRA Nancy**



Thank you for your attention

