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# Trees short-time and inter-annual epigenetic somatic memories in response to drought-rewatering cycles

Alexandre DUPLAN<sup>1,2</sup>, Régis FICHOT<sup>1</sup>, Mamadou dia SOW<sup>1</sup>, Alain DELAUNAY<sup>1</sup>, Isabelle LE JAN<sup>1</sup>, Julien VIGNEAUD<sup>1</sup>, Christian DAVIAUD<sup>2</sup>, Odile ROGIER<sup>3</sup>, Patrick POURSAT<sup>3</sup>, Harold DURUFLE<sup>3</sup>, Leopoldo SANCHEZ<sup>3</sup>, Bao-Dong CAI<sup>4</sup>, Hervé COCHARD<sup>5</sup>, Isabel ALLONA<sup>6</sup>, Vincent SEGURA<sup>2</sup>, Yu-Qi FENG<sup>4</sup>, Jörg TOST<sup>2</sup>, Stéphane MAURY<sup>1</sup>

Alexandre.duplan@inrae.fr  
stephane.maury@univ-orleans.fr

<sup>1</sup> Laboratoire de Biologie des Ligneux et des Grandes Cultures, LBLGC, 1207 USC1328, INRAE-Université Orléans, Orléans France

<sup>2</sup> CEA-Institut de Biologie François Jacob, Centre National de Recherche en Génomique Humaine (CNRGH), 2 Rue Gaston Crémieux, 91000 Évry-Courcouronnes, France

<sup>3</sup> BioForA, INRAE, ONF, UMR 0588, F-45075 Orleans, France

<sup>4</sup> Department of Chemistry, Wuhan University, 430072 Wuhan, China

<sup>5</sup> UMR-PIAF, INRAE, Université Clermont-Auvergne, 5 Chemin de Beaulieu, 63113 Clermont-Ferrand, France

<sup>6</sup> Centro de Biotecnología y Genómica de Plantas (CBGP), Campus de Montegancedo, Av. de Montepíncipe, 28223 Madrid, Spain

## Context & Objective

Climate change is characterized by increase in temperatures and decrease in precipitations impacting ecosystems in particular forests with a worldwide decline. Black poplar (*P. nigra*) is present in a large geographic range in Eurasia and is considered as an indicator of the dynamic of the biodiversity. Here, we used European black poplar populations (Fig. 1) to test the effects of drought in this model tree. We focused on their epigenetic response since it has been proposed that stress response is controlled by hormonal balance and chromatin process in meristematic tissues (Fig. 2). Together, these two factors regulate gene expression and the activity of transposable elements allowing plasticity and priming (ability of plants to react better during a second environmental challenge). Here, we analyzed in stressed or not poplars (greenhouse), their cambium: the meristematic tissue producing xylem and phloem vessels. We tested wherever drought-rewatering (WD-RW) cycle is followed by an epigenetic somatic memory in cambium at short time post recovery (7 days) or inter-annual memory.

## Material and methods

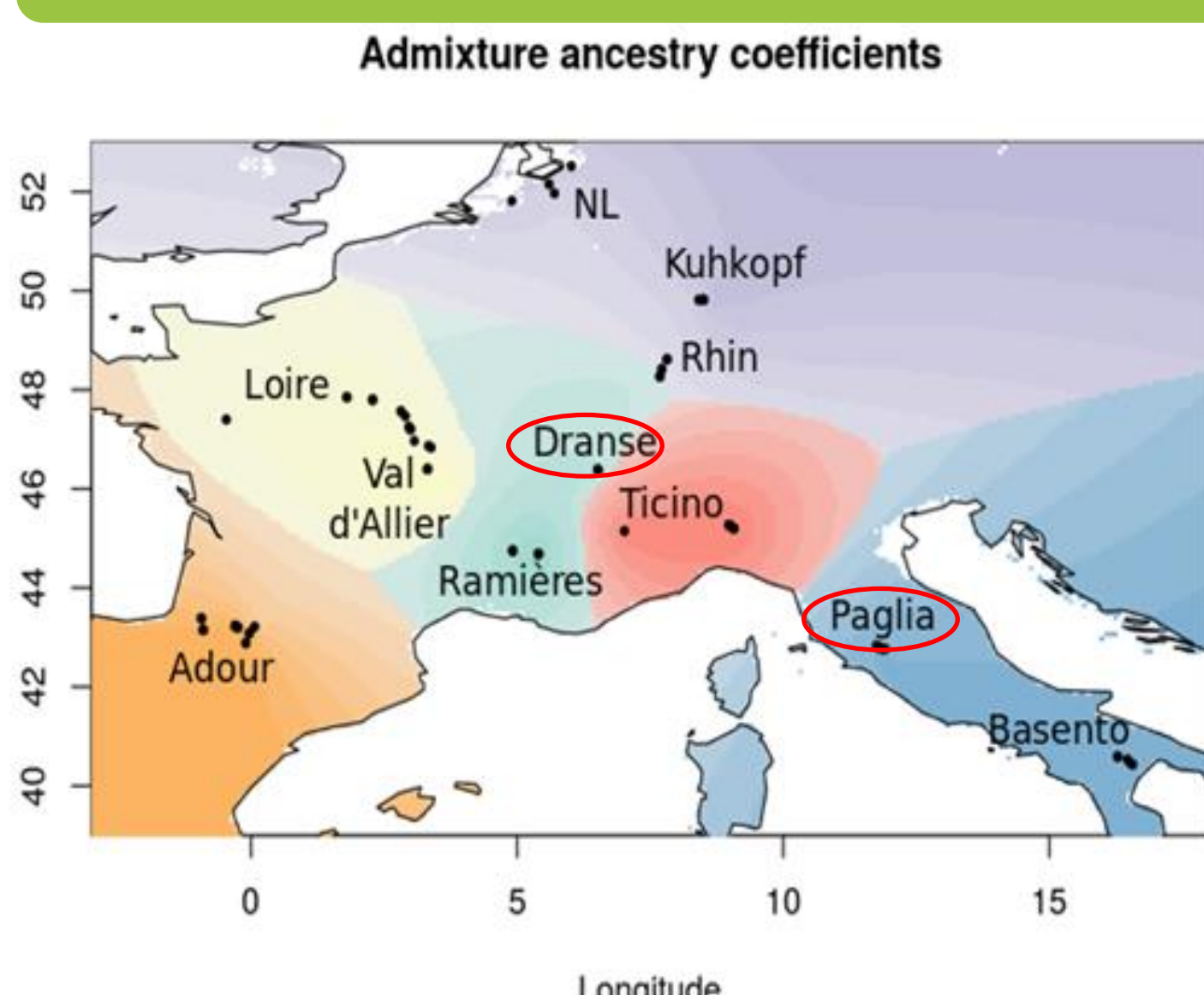


Fig. 1 : Geographical area of the six ancestral European populations of black poplar. The population circled in red are the two contrasted genotypes analyzed in this study.

The **six ancestral European populations of black poplar** are depicted by different colors on the map. A common garden with 241 genotypes from these 6 populations was installed in Orléans. Cuttings were installed later in **greenhouse** for one genotype for each population was grown under **drought rewatering** conditions (Fig. 3). After ecophysiological analysis, **two contrasted genotypes** for drought tolerance (**DRA38** and **PG31**) were selected for multi-omics analysis :

- metabolomic of hormones (LC-MS)
- transcriptomic (RNAseq)
- methylome (WGBS)

## Results

### PCA analysis of 15 hormones (cambium) among natural populations

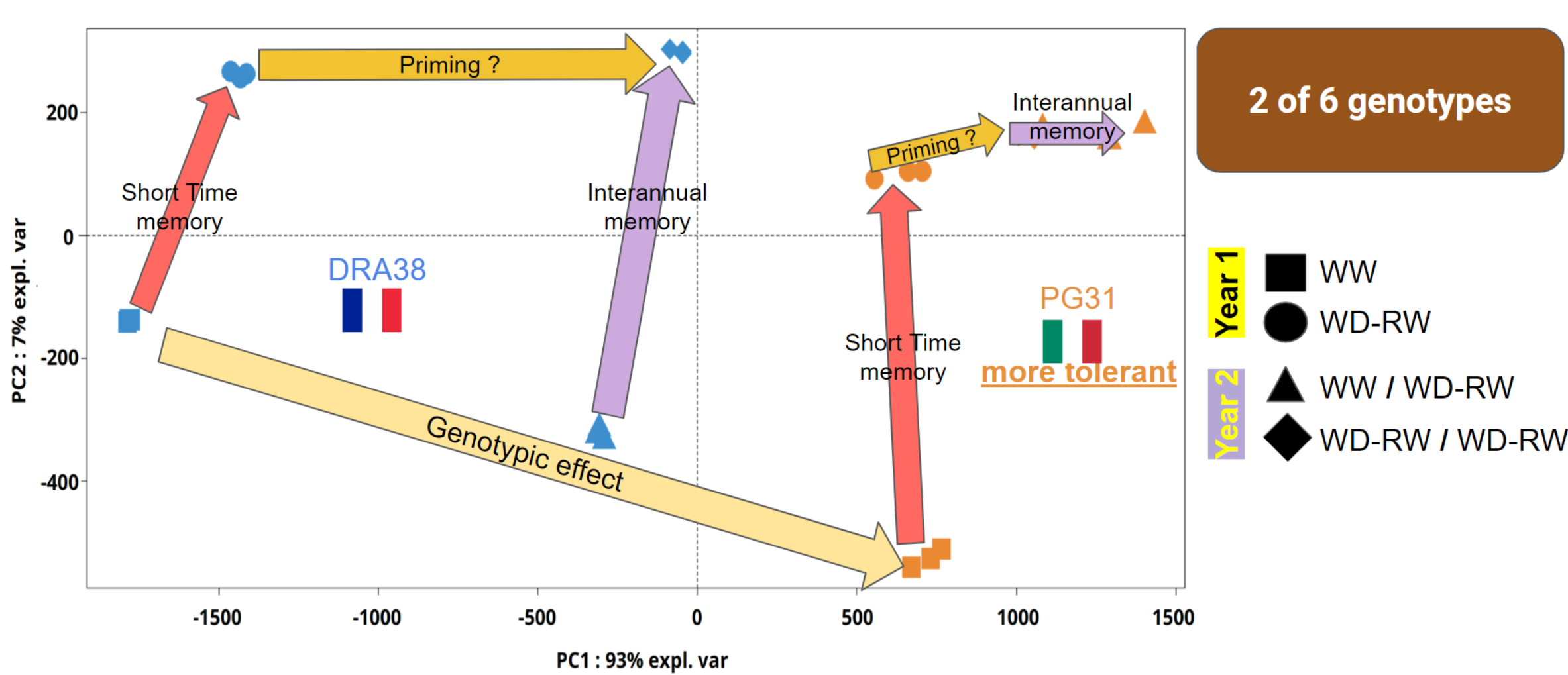


Fig. 4 Principal components analysis of phytohormones quantified on two genotypes of *Populus nigra* : DRA38 et PG31. The samples correspond to short-term and interannual memories after WD-RW cycles.

- Genotypic effects
- Similar short term memory effects
- Interannual memory effects genotype dependent
- Potentially "priming" effect

### DEGs among both genotypes and conserved DEGs after each stress cycles

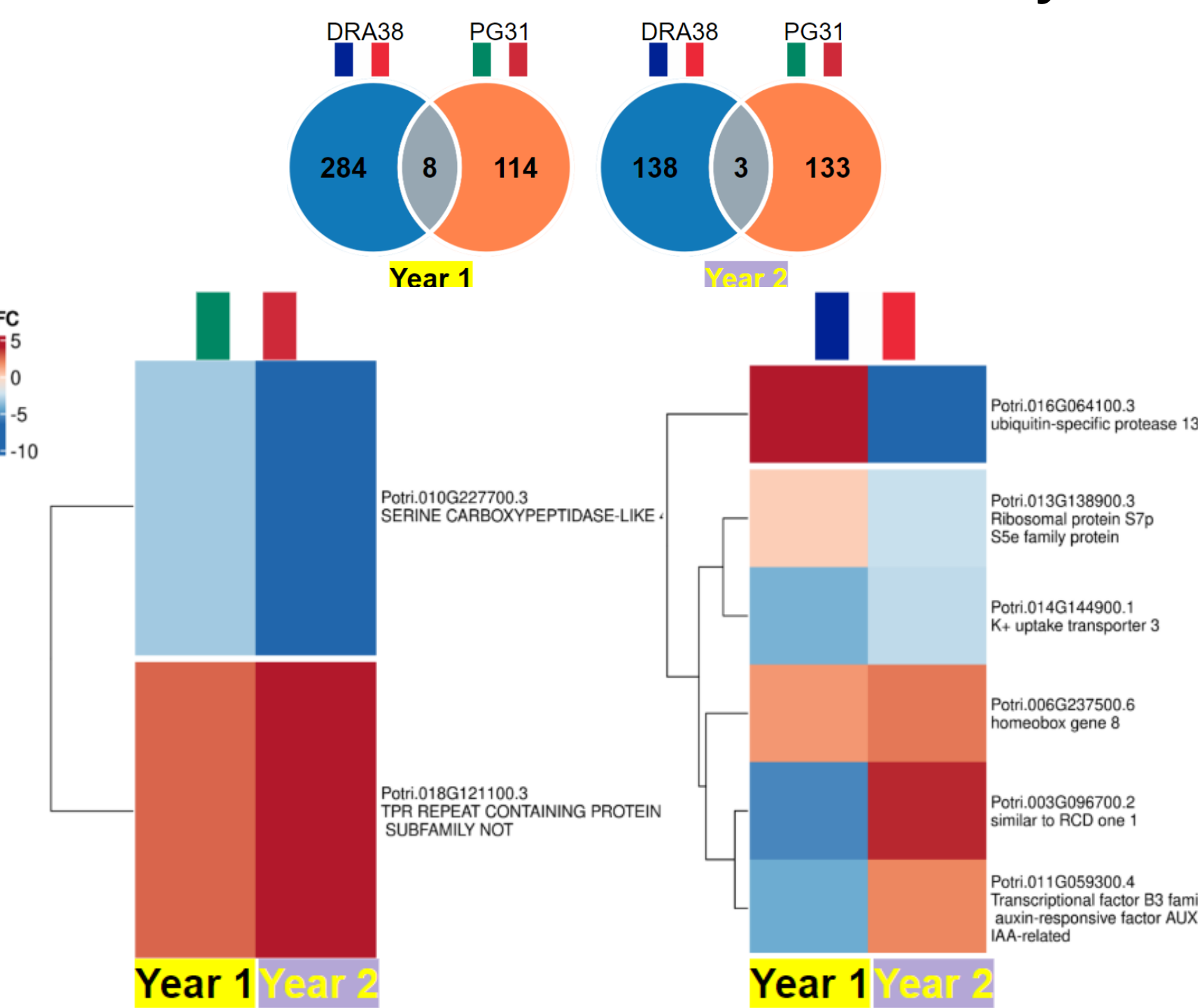


Fig. 5 : Analysis of differentially expressed genes. A) Venn diagram of DEGs detected in short-term and interannual memories in DRA38 and PG31. B) Heatmap representing DEGs detected in both genotypes of *P. nigra* and conserved between both years.

- 114 to 284 DEGs associated to memories
- Distinct DEGs (few common) but conserved GO terms (data not shown)
- Year 1 : DRA38 show more DEG than PG31.
- Year 2 : PG31 and DRA38 show similar number of DEG and GO enrichment reveal common functions. PG31 increasing Year 1 effect and DRA38 shows a transcriptomic reprogramming (with inversion)

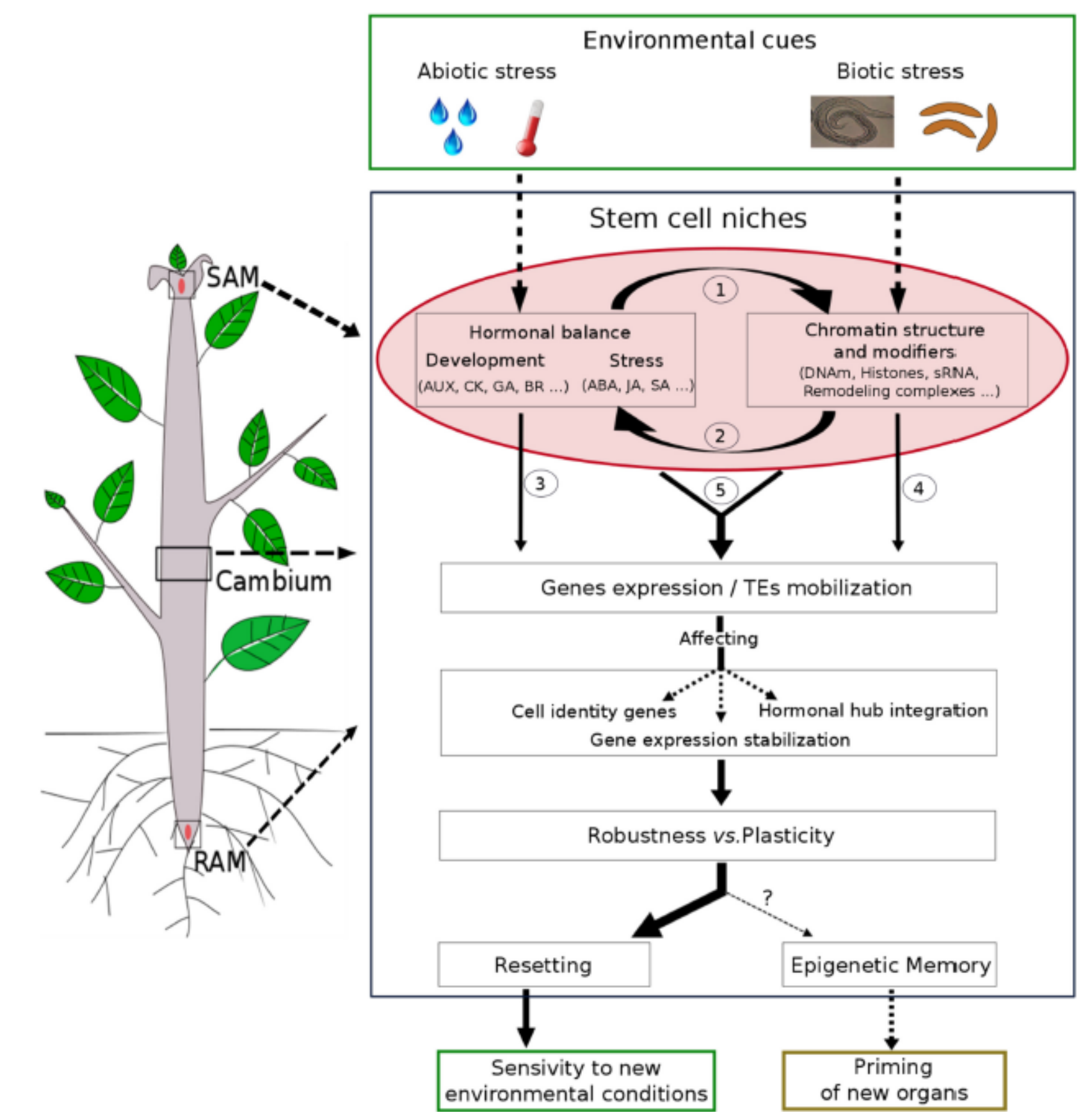


Fig. 2 : Schematic model of phytohormones and chromatin crosstalk during plant developmental plasticity and priming, according to S. Maury et al. (2019).

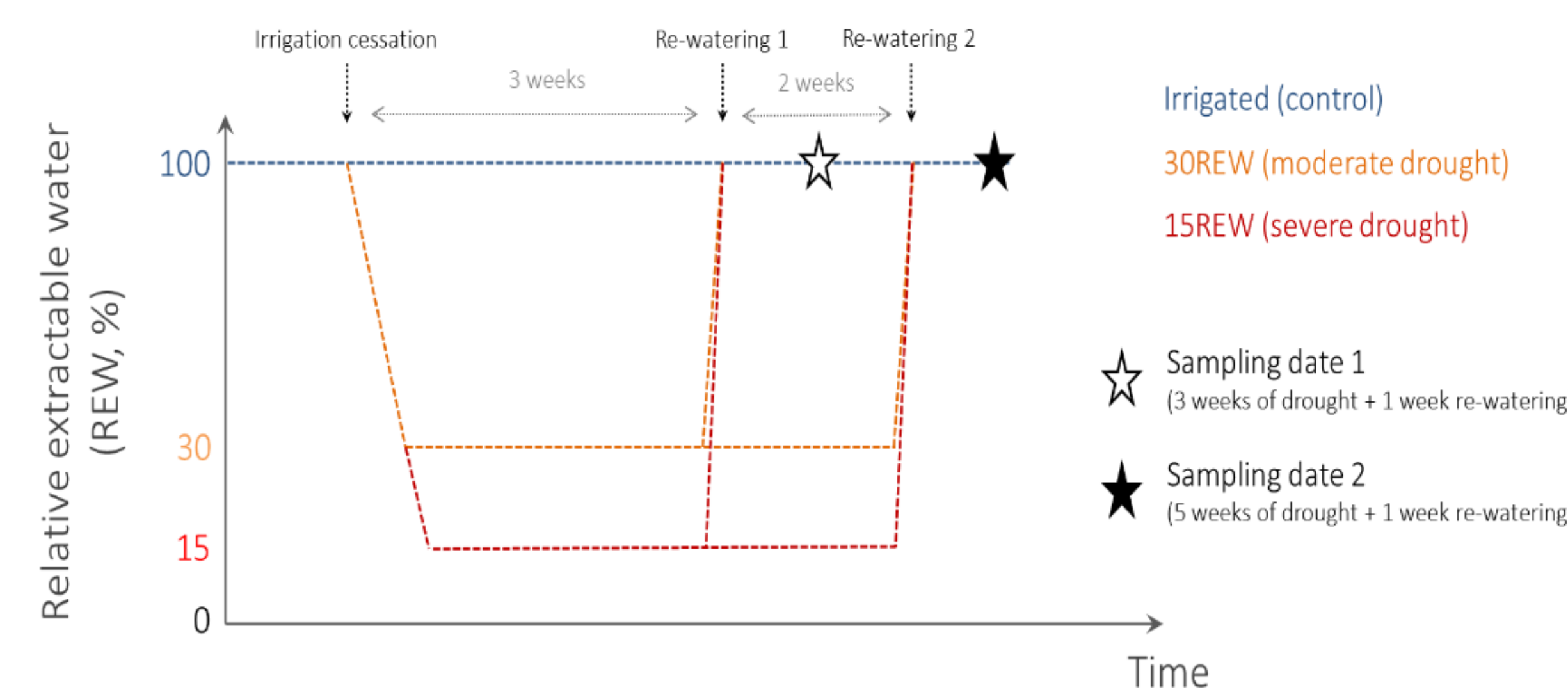


Fig. 3 : Experimental setup for drought rewatering experiments. Variations of the relative extractable water (REW) content over weeks for drought treatment on poplar in greenhouse.

### Epigenetic reprogramming

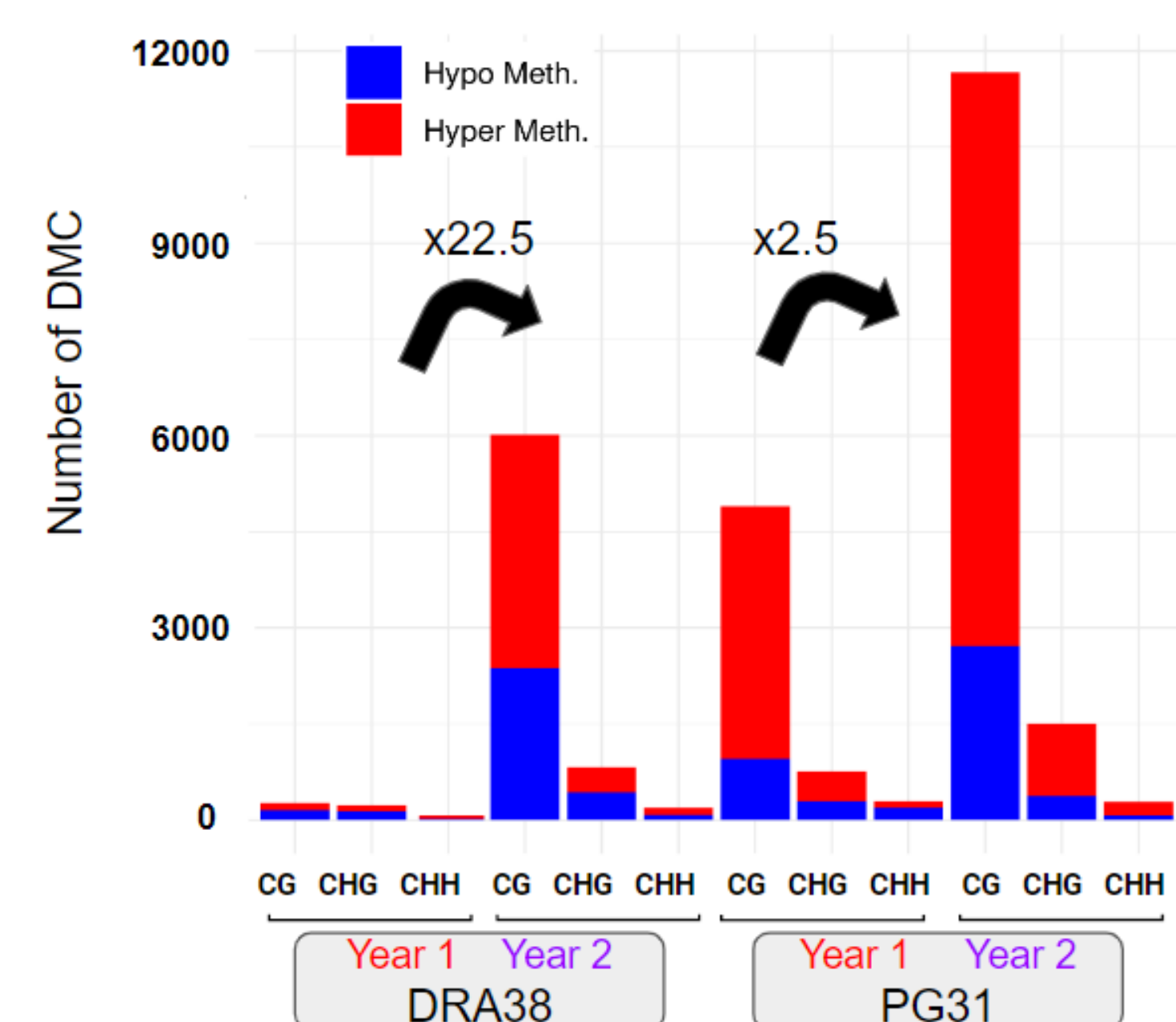


Fig. 6: Histograms of differentially methylated cytosine (DMC) detected in the 3 contexts (CG, CHG, CHH) in short-term and interannual memories. Blue color defines hypomethylation and red color defines hypermethylation.

- Huge genotypic effects
- Increase of DMC at the second stress cycle.
- Total epigenetic reprogramming for the less tolerant genotype (with some gene differentially methylated genes related to signaling of water stress, auxin, DNA repair and chromatin organization)

## Conclusions

Our results showed hormonal and epigenetic signatures (genotype dependant) associated to short-term and inter-annual somatic memories in response to drought rewatering cycles in cambium. These signatures are associated to variations in gene expression (direct or indirect, silencing or differential of expression) related to development or signaling genes (hormones...). Altogether, we proposed that trees display short-term and long-term somatic memory for abiotic stress in agreement with previous results about plasticity and inter-seasonal memory in shoot apical meristem (Le Gac et al., 2018; Sow et al., 2021). The ability of these somatic memories to induce priming effects remain to be evaluated.