Poplar cambium and leaf tissues present specific changes in 2D-page patterns in response to drought of heat stress

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POPLAR CAMBIUM AND LEAF TISSUES PRESENT SPECIFIC CHANGES IN 2D-PAGE PATTERNS IN RESPONSE TO DROUGHT OR HEAT STRESS

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Introduction.

Plant stress tolerance is determined by the ability to preserve tissue- and cell- functions. The mechanisms involved in the metabolic defence partly depend on the physiological status, but also on stress sensing and signalling pathways. Accordingly, stress tolerance arises through molecular responses, depending on cell and tissue specific structures.
Thus specific proteomic changes can be expected in leaves and in cambium of *Populus tremula x P. alba* genotype 717-1B4 exposed to stress.

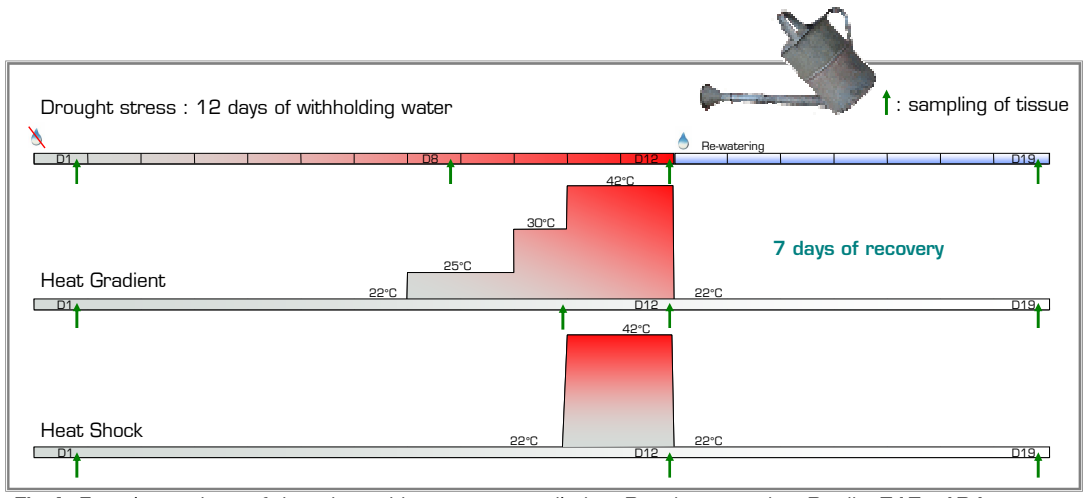


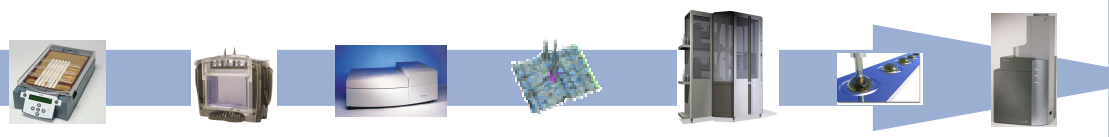
Fig 1: Experimental set of drought and heat stress applied to *Populus tremula x P. alba* 717 - 1B4

Stress characterisation

Young pot-grown poplar plants were submitted to drought or heat stress (with or without a previous temperature gradient) while physiological parameters were monitored (fig. 1). A 7 days return to control conditions period was performed, allowing plants to recover. Tissues were sampled at key steps of the experimental set-up, indicated by (↑) in the figure 1.

Drought decreased H_2O stomatal conductance, CO_2 assimilation, predawn leaf water potential and leaf relative water content. Heat treatments triggered a shorter stress episode, which affected leaf water potential and CO_2 assimilation.

The plants experienced characterised and reversible stresses.



Proteomic analysis

Protein extracts were separated by 2D-DIGE. From 4 biological replicates of each sample, a relative quantification was performed, using DeCyder software.

Leaf tissue analysis showed 169 proteins spots which abundance was significantly ($p<0.05$) altered under the constraints presented in Figure 1, with an absolute value of the average ratio of at least 1.3. MALDI TOF-TOF analysis, still in progress, already allowed the confident identification of 102 proteins.

Cambial zone analysis showed 197 protein spots with differential abundance, among which 155 were confidently identified.

Proteome changes in Leaf and Cambial zone tissues

Specific features appeared between treatments.

In leaf tissue:

➢ **Heat Gradient** had an important inhibitory effect on the Calvin cycle aldolases (◓&◢).

➢ **Heat Shock** induced an increase of 5 Oxygen-evolving enhancer protein isoforms (⬟).

➢ **Water deficit** particularly affected RuBisCO (◕), with an increased abundance of 5 isoforms at the recovery stage.

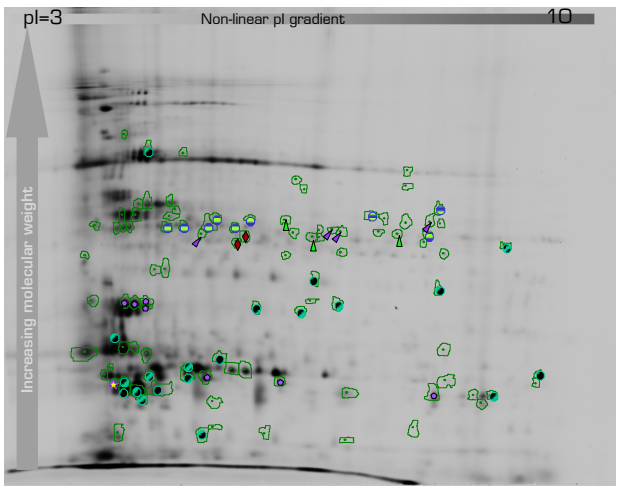


Fig 2. Bidimensional electophoretic separation of proteins from poplar leaves.
The underlined spots were quantitatively different between control and stressed plants.

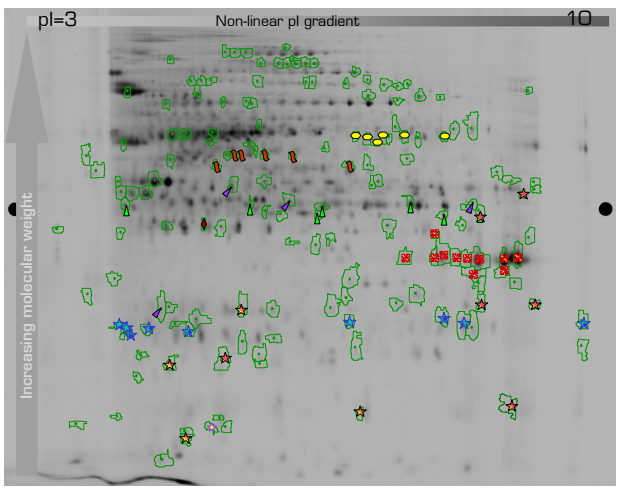


Fig 3. Bidimensional electophoretic separation of proteins isolated from poplar stem cambial tissue.
Underlined spots, differential in abundance between control and stressed plants, were confidently identified.

In Cambial zone tissue:

➢ **Heat Gradient** had a particularly strong impact on **glycolysis**, and among stress-responsive proteins, it affected 9 isoforms of **Osmotin** (★).

➢ **Heat Shock** impacted almost specificaly **SAM Cycle** (◖) & **One-carbon Metabolism** (especially serine hydroxymethyltransferase ⬭),

➢ **Water deficit** induced an important **redox response** (GPX, APX, PRX2★) and a massive decrease of **storage proteins** (✖). These reponses are particularly important on day 12, corresponding to the strongest intensity of the constraint.

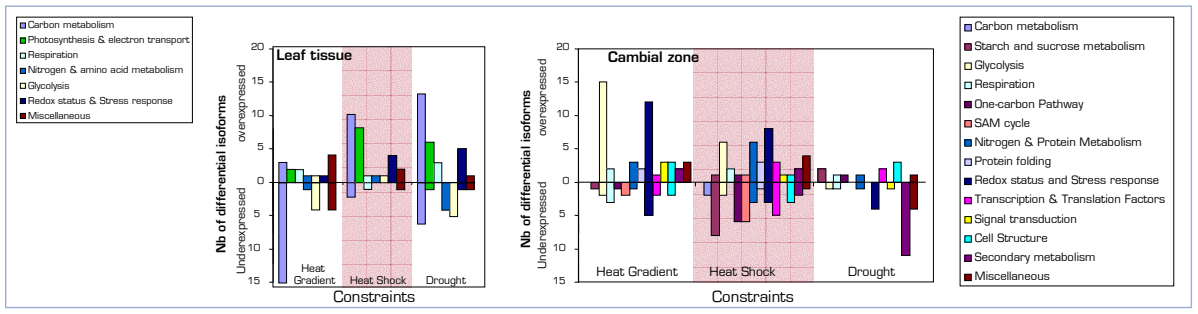


Fig 4. Patterns of the proteomic changes observed in various metabolisms in leaf and cambial zone tissues

The abundance of only 6 proteins (present in 43 protein spots in total) were altered in **both** leaf and cambial proteomes, for instance glyceraldehyde-3-phosphate dehydrogenase (▲) fructose-bisphosphate aldolase (◢), and thioredoxin peroxidase (☆). However, the changes observed in these protein abundances in response to the applied constraints were always dissimilar in the two tissues.
The sole exception was one Quinone oxidoreductase (◆) (cf fig 2 & 3).

Conclusion.

Each kind of abiotic stress endured by the plants triggers partly specific changes in the studied proteomes (fig 2&3). In leaf, 20 out of the 38 identified protein functions present a stress-specificity. They are 44 out of 72 in the cambial tissue. In spite of that relative stress specificity, the same metabolisms are involved in the responses.

When comparing proteome changes between leaf and cambial zone, only 6 protein functions, among the 104 identified, were common between tissues in the responses to the different stresses applied.
The tissue specificity of the response exceeds the stress specificity.

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