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ABSTRACT

In order to analyze root proteins involved in drought tolerance in poplar, a proteomic study was conducted on the roots of two *Populus deltoïdes* × *Populus nigra* cultivars, Carpaccio and Soligo. These cultivars were selected based on contrasted physiological responses under water stress. Plants were exposed to different water stress levels (Control, Early, Medium and Severe), and root tips collected. Technical optimization included protein extraction and resolubilization, as well as the fine-tuning of electrophoretic conditions. The best results were obtained upon using phenol extraction, a resolubilization solution containing two reduction reagents, DTT and 2-ME, and 100 µg protein load on both acidic (pH 4-7) and basic (pH 7-11NL) IPG strips. A total of 48 2-D gels were then produced and quantitatively analyzed using SameSpot Progenesis software. Statistical analyses were completed using normalized spot volumes. Multivariate analyses (correlation matrix, PCA and HCA) and two-way ANOVAs showed that protein expression was first regulated by genotype effect and second by treatment effect. Differentially regulated proteins are being identified based on tandem mass spectrometry. This functional information should shed some lights on the molecular players involved in drought-stress response and potentially underlying adaptation to this abiotic constraint.

1/ INTRODUCTION

Drought is one of the most important constraints limiting the growth of plants and ecosystem productivity around the world. Several recent studies have dealt with molecular responses to water shortage, however they have focused mainly on short-term responses to acute stress rather than on long-term acclimation processes to moderate and gradually increasing water deficits. While short-term studies provide useful information about water deficit sensing and signaling pathways, long-term studies may shed light on proteins involved in long-term responses to water deficit and in potential acclimation to low water availability.

Poplars are known to be drought sensitive so their natural distribution area is mainly restricted to riparian zones. However, some diversity occurs among species and clones with respect to water use efficiency and drought tolerance. The genus Populus is an obvious choice for analyzing the responses and acclimation processes occurring during soil water depletion in a tree species, due to the recent sequencing of its genome (Tuskan *et al*., 2004). Only two studies have been published so far on poplar responses to water stress at the proteomic level (Plomion *et al*., 2006; Bogeat-Triboulot *et al*., 2007).

In this follow-up study, two poplar cultivars displaying contrasting drought tolerance were subjected to several water deficit stresses varying in intensity and duration. Expression profiles from both acidic and alkaline proteins were obtained from roots, the organs initially sensing the water constraint and triggering adaptative responses.

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2/ MATERIALS & METHODS

Experimental design

-2 poplar cultivars: *Populus deltoïdes* × *Populus nigra* cv. Carpaccio (**C**) and Soligo (**S**) grown into potted soil under greenhouse monitored conditions.

-4 treatments: **Con.** Full watering for the whole duration of the experiment, **Ear.** Short mild water deficit (watering was stopped 30h before harvest and soil relative extractable water (REW) was reduced to 20-35% of field capacity), **Med.** Extended mild water deficit (soil relative extractable water (REW) was reduced and maintained in the range 20-30 % of field capacity for 12 days) and **Sev.** Extended moderate water deficit (soil relative extractable water (REW) was reduced and maintained in the range 10-20 % of field capacity for 12 days).

Plant materials

-Young roots visible at the soil/pot interface from both Carpaccio and Soligo cultivars (16 samples including 3 biological replicates).

Proteomic analysis

-Protein extraction using phenol/ammonium acetate method.

-Protein assay using 2-D Quant Kit (GE Healthcare).

-Protein separation (100µg) using two-dimensional electrophoresis (2-DE) along 4-7 and 7-11NL pH ranges during immobilized pH gradient-isoelectric focusing (IPG-IEF) and 11% acrylamide SDS-PAGE (2 technical replicates).

-Protein staining using silver nitrate protocol.

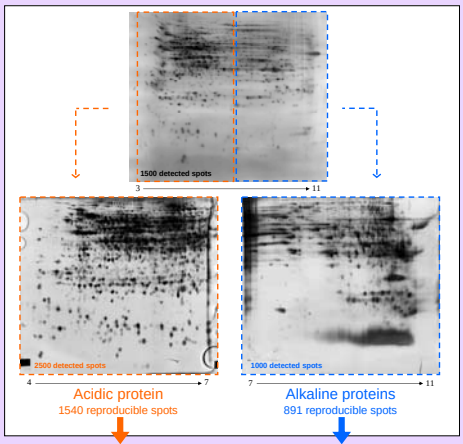
-Image analysis using SameSpot Progenesis (NonLinear)

Statistical analyses

-Multivariate analyses: Principal Component Analyses using R software were performed to unravel the main sources of protein quantity variation.

-Univariate analyses: a two-way Analyses of Variance (ANOVA) model with FDR multiple testing adjustment was used to isolate water deficit-responsive proteins.

3/ RESULTS



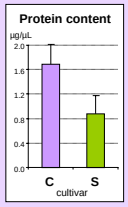


Figure 1. Carpaccio roots contain more proteins than Soligo.

Figure 2. The number of spots and their resolution on 2-D profiles is greatly improved upon using narrow pH ranges. Acidic proteins are twice more numerous than alkaline ones.

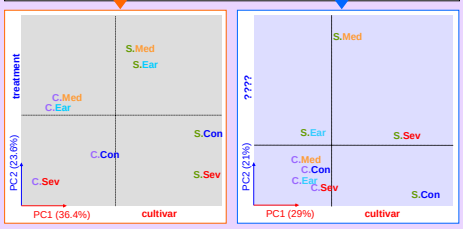


Figure 3. PCA indicates that protein quantities are mainly affected by genotype effect, then by treatment effect, with Ear-Med opposing Con-Sev.

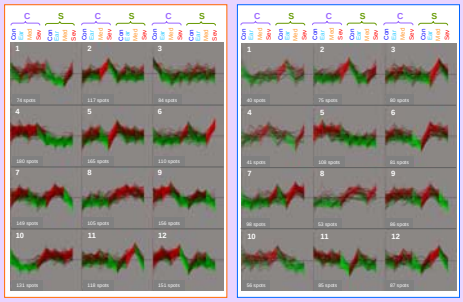


Figure 4. The 12 main expression profiles confirm PCA results for both acidic and alkaline proteins.

Two-way ANOVA isolated 88 acidic proteins whose expression profiles varied significantly (5%) under water deficit treatments.

Two-way ANOVA isolated 51 alkaline proteins whose expression profiles varied significantly (5%) under water deficit treatments.

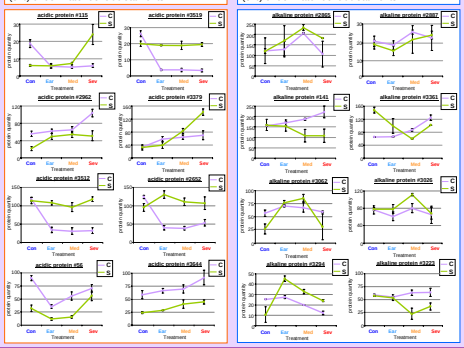


Figure 5. The expression profiles of acidic and alkaline proteins responding to water deficit follow different trends.

4/ CONCLUSIONS & PERSPECTIVES

Alkaline proteins are seldom considered in plant proteomic analyses. In this study, we were able to detect 1500 acidic proteins and 900 alkaline proteins on 2-D patterns from poplar roots. Genotype is the main source of variation of protein abundances. Protein expression profiles varied under the increasing water deficit intensity as well. The identification of water stress responsive proteins by mass spectrometry will highlight the metabolic processes involved in poplar drought adaptation.