Molecular Regulation of Cell-type Specific Responses to Abiotic Stresses in Poplar

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Project Goals: The main goal of the SyPro project is the development of transgenic trees with sustained photosynthetic activity and increased biomass production under the simultaneous occurrence of water deficit, increased soil salinity and elevated temperatures. To achieve that, we intend to (1) identify stress-responsive genes and proteins in specific cell-types of poplar leaves and roots; (2) discover novel *cis*-regulatory elements; (3) construct stress-responsive synthetic promoters; and (4) use these promoter-gene fusions to develop abiotic stress-tolerant poplar. The transgenic poplar trees will be evaluated under both controlled and field conditions.

Plants react to abiotic stress with a combination of physiological, biochemical, and developmental changes. These responses include alterations in signaling components, gene transcription, synthesis of proteins and metabolites which occur in a cell-type and tissue-specific manner [1]. However, each cell-type in plant tissues is defined by specific transcriptional, protein, and metabolic profiles that determine its function and response(s) to stress [2]. Thus, determining the plant responses to environmental changes requires an understanding of the cell/molecular properties of specific single cell-types within a tissue.

In this work, clones of *Populus tremula x alba* (INRA 717 1-B4) were rooted for at least 25 days, grown in the greenhouse for 45 days and the plant response(s) to water deficit, salinity, heat, and the combination of all three stresses were monitored. Leaf and root tissues were collected at different time points, fixed and embedded for cell-type specific omics analyses. We targeted distinct poplar cell types and tissues including leaf mesophyll, xylem/phloem, root epidermis and cortex cells using cryo-sectioning and laser-capture microdissection (LCM) techniques. Samples from same plants were also collected for whole tissue omics (RNA-seq, metabolite) analysis.

To decode the structural and regulatory gene networks that mediate spatiotemporal specialization, we employed a cell-type specific gene expression profiling approach. To this end, RNA was extracted from 400-500 cells per cell-type. Full length cDNA and template libraries were generated, quantified and sequenced. Our data revealed that under all investigated water-deficit stress conditions including Early Water-Stress (EWS), Late Water-Stress (LWS), and Recovery (R), 585 and 138 transcripts were significantly regulated in leaf palisade and vascular cell types, respectively. The observed transcriptional changes were further assessed at the protein translation level. For proteomics analysis, total protein was extracted from 300-800 cells per cell-type, and samples were processed within ultrasmall-volume “nanowells” (nanoPOTS technology)

[3]. In response to water-deficit stress and water recovery conditions, a total of 2,384 and 3,490 proteins were identified as leaf palisade mesophyll and vascular-specific proteins, respectively. Among these, 498 (in palisade cells) and 270 (in vascular cells), 184 (in palisade cells) and 336 (in vascular cells), and 392 (in palisade cells) and 428 (in vascular cells) proteins were exclusively identified at EWS, LWS, and R phases, respectively, as unique candidate cell-type specific drought-responsive proteins. Our pathway enrichment analysis revealed a number of proteins involved in amino acid and carbohydrate metabolism, photosynthesis, sucrose biosynthesis/degradation and antioxidant metabolism as responsive proteins exclusively in palisade mesophyll or vascular cells in water-deficit stress and post-recovery phases. These findings are being correlated with whole tissue metabolite profiles and linked to the spatiotemporal accumulation of selected metabolites using a MALDI-MS imaging approach[4]. These results will be presented and discussed.

Overall, our results indicate that specific cell-types in poplar tissues are defined by distinctive transcriptional and protein profiles under abiotic stress. This information is being used for motif discovery, the results of which will be used for engineering of cell-, tissue- and stress-specific promoters. Subsequent poplar transformation with constructs of stress-mitigating genes driven by these promoters will be performed. These efforts will contribute to the design of stress-tolerant poplar trees, a strategic bioenergy crop, and to a better understanding of the roles of different poplar cell types on the response to abiotic stress.

References:

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