

## **Integration of Physiological Phenotyping and Cell-Type-Specific Omics Approaches to Study Individual and Abiotic Stress Combinations in Poplar**

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**Project Goals: The main goal of 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.**

Plant responses to environmental perturbations are dynamic and involve complex cross-talk between different regulatory pathways<sup>1</sup>, including metabolic adjustments and gene/protein expression at cellular level for physiological and morphological adaptation at the whole-plant level<sup>2</sup>. Therefore, a single-cell-type analysis approach is needed to effectively reveal the underlying molecular mechanisms regulating developmental processes and plasticity when grown under suboptimal conditions.

In this work, clones of *Populus tremula x alba* (INRA 717 1-B4) were rooted for at least 25 days. Following rooting, plant response(s) to water deficit, salinity, heat, and the combination of all three stresses were monitored. Total biomass (root and shoot) was measured at the end of the experiment, with a reduction of 35%, 70%, 50% and 70% in dry weight of shoots and 40%, 60%, 40% and 70% depletion in dry weight of roots under salinity, water-deficit, heat and combination of three stresses, respectively. While photosynthesis levels and stomatal conductance were reduced under all stresses, the most significant effect was observed under drought and combined stress conditions.

At three sampling-time points (including recovery time), leaf and root tissues were collected, fixed and embedded for cell-type specific transcriptome and proteome 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. For transcriptomics, RNA was extracted from 100-200 cells per cell-type. Full length cDNA and template libraries were generated, and template quantification and preparation for sequencing is in progress. For proteomics, total protein was extracted from 300-800 cells per cell-type, and samples were processed within ultrasmall-volume “nanowells” (nanoPOTS technology)

<sup>3</sup>. Focusing on leaf tissue, a total of 7,515 and 6,023 proteins were identified as palisade mesophyll and vascular-specific proteins, respectively, under all investigated conditions. Among these, 273 (in palisade cells) and 332 (in vascular cells) proteins were identified as candidate cell-type specific abiotic stress related-proteins. For example, several proteins involved in photosynthesis, chloroplast division, carbon allocation to sink tissues, chaperone binding and chromatin organization were identified as drought-, salt-, or combined stress-responsive proteins exclusively in palisade or vascular cells. Our results provide information that is missing in whole tissue-based analyses, including cell population-specific protein profiles that are unique for the distinct cellular layers of poplar leaves and roots. The obtained information (sequences) is being used for motif discovery using bioinformatics approaches and then for promoter engineering for subsequent poplar transformation.

### **References:**

1. L. Shaar-Moshe, E. Blumwald and Z. Peleg. 2017. Unique physiological and transcriptional shifts under combinations of salinity, drought, and heat. *Plant Physiology*, 174 (1) 421-434.
2. Julia Krasensky and Claudia Jonak. 2012. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *Journal of Experimental Botany*, 63 (4) 1593–1608.
3. Zhu, Y.; Piehowski, P. D.; Zhao, R.; Chen, J.; Shen, Y.; Moore, R. J.; Shukla, A. K.; Petyuk, V. A.; Campbell-Thompson, M.; Mathews, C. E. 2018. Nanodroplet processing platform for deep and quantitative proteome profiling of 10–100 mammalian cells. en. *Nature Communications*, 9, 882.

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