The Combined Effect of Abiotic Stresses Reveals Unique Cell Type-Specific Molecular Changes 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 identify stress-responsive genes and proteins in specific cell types of poplar leaves and roots.

Plant responses to abiotic stresses have been primarily studied at the whole plant and tissue levels[1], subjected mainly to a single stress condition. However, these studies provide insufficient information about specific cellular regulations[2] in response to multi-environmental stresses, a scenario that plants naturally cope with. Therefore, it is critical to identify the key stress-responsive regulators at cellular levels to better understand the molecular mechanisms of plant responses to individual and combined abiotic stresses.

In the SyPro project, 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 individual stresses (salinity, heat, and water deficit) and a combination of two or 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 using cryo-sectioning and laser-capture microdissection (LCM) techniques.

Identifying stress-responsive regulatory proteins suffers from technical limitations associated with protein recovery from a low number of isolated cells and mass spectrometry sensitivity. In this work, we employed a novel microfluidic nanodroplet-based sample preparation (nanoPOTS)[3], coupled with laser capture microdissection and ultrasensitive liquid chromatography-mass spectrometry for highly sensitive proteome profiling of a small number of plant cells in a cell-type-specific fashion. We first demonstrated the power of this technology for plant cell-type-specific proteomics by identifying unique poplar leaf- (palisades and vascular) and root- (cortex and vascular) specific proteins in plants grown under normal condition[4]. We then proceeded with the stress-treated samples, in which we examined poplar leaf cell types exposed to individual and combined water deficit, salinity, and heat stresses. Out of 6,172 proteins significantly altered under single and triple stress conditions, 14.2% (palisade) and 33.3% (vascular) of identified proteins were unique to leaf cell types, while 52.4% were abundant in both palisade and vascular cells. Moreover, our biological pathway enrichment analysis revealed triple stress-specific proteins (compared to single stresses) for each cell type.

For example, we identified jasmonic acid biosynthesis and serotonin/melatonin biosynthesis as the most enriched pathways in palisade cells under triple stresses, while acetyl Co-A biosynthesis and chitin degradation pathways were the most active biological processes in leaf vascular cells.

Moreover, a low-input RNA sequencing approach was employed to resolve the plant spatial complexity under the single and simultaneous occurrence of abiotic stresses. Focusing on leaf tissue exposed to individual heat and salinity and a combination of both stresses (salt+heat), 56.3% (palisade) and 26.7% (vascular) of total differentially expressed genes (DEGs) (5899 genes) were regulated in a cell type-specific manner, while 16.9% of total DEGs were active in both cell types. Our functional enrichment analysis identified three categories specifically associated with palisade cells under salt+heat treatment (compared to individual stresses), including photosynthesis, L-methionine, and L-glutamine biosynthesis, while the most enriched biological processes for vascular cells were polysaccharide, glycan, and amino acid metabolism.

The integrative analysis of transcriptomic and proteomic data identified novel cell type-specific stress-responsive regulators highly induced at both transcriptional and translational levels. However, we observed a transcript/protein discordance in specific pathways in both leaf palisade and vascular cell types under heat and triple stress conditions representing a critical layer of cell-type-specific regulatory processes at the post-transcriptional level.

Overall, our findings effectively reveal the underlying molecular mechanisms regulating spatial plasticity under single and multiple stresses, allow future attempts in mapping molecular machinery to the cellular domain, and contribute to the design of poplar trees with enhanced tolerance to abiotic stress combination.

References:

1. Terri A Long, Many needles in a haystack: cell-type specific abiotic stress responses. (2011). *Current Opinion in Plant Biology*, 14, 3, pp. 325-33.
2. Dinneny, J.R., Long, T.A., Wang, J.Y., Jung, J.W., Mace, D., Pointer, S., Barron, C., Brady, S.M., Schiefelbein, J., Benfey, P.N. Cell identity mediates the response of Arabidopsis roots to abiotic stress. (2008). *Science*, 320 (5878), pp. 942-945.
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. Nanodroplet processing platform for deep and quantitative proteome profiling of 10–100 mammalian cells. (2018). *Nature Communications*, 9, 882.
4. Balasubramanian, V. K., Purvine, S. O., Liang, Y., Kelly, R. T., Pasa-Tolic, L., Chrisler, W. B., Blumwald, E., Stewart, C. N., Zhu, Y., & Ahkami, A. H. (2021). Cell-type-specific proteomics analysis of a small number of plant cells by integrating laser capture microdissection with a nanodroplet sample processing platform. Current Protocols, 1, e153. doi: 10.1002/cpz1.153.

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