**Contribution of Serine Biosynthesis and Degradation to Carbon and Nitrogen Metabolism During Salinity stress 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.**

Plant responses to environmental stress are dynamic and involve complex cross-talk between different regulatory pathways[1], including metabolic adjustments and gene/protein expression at the cellular level for physiological and morphological adaptation at the whole-plant level[2]. 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 the study of the cell/molecular properties of specific single cell-types within a tissue to effectively reveal the underlying mechanisms regulating developmental processes and plasticity under suboptimal conditions.

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 salinity stress was 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 using cryo-sectioning and laser-capture microdissection (LCM) techniques. Plants were exposed progressively to 50 mM, 100 mM, and 150 mM NaCl for 10 days (Early Salt Stress, ESS) and maintained at 150 mM NaCl for another 10 days (Late Salt Stress, LSS), followed by a period of recovery from stress where the plants returned to control growth conditions (Recovery, R). Leaf palisade and vascular cell types were collected and processed using nanoPOTS (nanodroplet processing in one-pot for trace samples) platform, which includes a nanoliter-scale liquid handling robotic station in which the cells were lysed for protein extraction[3]. The proteins were then alkylated, digested, and the peptide samples were loaded onto nano-LC coupled to a Tribrid Lumos Mass spectrometer. The collected peptide abundance values were filtered, normalized, and converted to protein abundance values and used to limit reliable detection (LOD) and Z-score calculation.

Proteins detected at least in either control or stress conditions were used to calculate total Palisade and vascular identified proteins. The relative abundance values (salt vs. control) of

identified proteins were used for Z-score assessment analysis, and proteins altered under stress (+2<Z-SCORE<-2) were used to calculate %cell type unique and shared proteins. A higher percentage of proteins significantly altered under stress were found to be explicitly unique to both Palisade (ESS-44.5%, LSS-34.6%, R-34.2%) and vascular (ESS-49.4%, LSS-55.2%, R-60.2%), suggesting that cell type unique proteins might regulate the leaf proteomic responses to salt stress and recovery.

Our results showed that a higher number of proteins associated with the 'phosphorylated pathway' for Serine (Ser) biosynthesis and Ser degradation accumulated in the vascular tissue at LSS. The production of Ser from 3-P-glycerate in plastids, together with glycine (Gly) catabolism in the mitochondria, provide cytosol with Ser and generating intermediaries that play important roles in pathways associated with energy production, carbon/nitrogen (C/N) balance, and lignification. Under stress conditions, 2-OXG may be used in the TCA cycle, providing energy supply in the vascular tissue for the increasing demand brought about the stress and contributing to the transport and reallocation of resources to sink tissues. 2-OXG is associated with ammonia re-assimilation, supplying the carbon skeleton needed by the GS/GOGAT cycle. Glycine Decarboxylase (GDC) carries Gly catabolism in the mitochondria yielding Ser. This reaction also produces high amounts of ammonia that are re-assimilated to amino acids (via Asparagine synthetase (AS) and Carbamoyl phosphate synthase (CPS)), to avoid toxicity. Ser catabolism to Gly, mediated by Ser hydroxymethyl transferase (SHMT) and the recycling of Gly to Ser in the mitochondria by GDC, are the main Carbon source of C-1 metabolism in plants. Thus, Ser is crucial for tetrahydrofolate (THF) metabolism, providing methyl group donors through S-adenosylmethionine (SAM) cycle, an important process for synthesizing lignin in vascular tissues.

Phosphorylated and non-phosphorylated pathways of Ser biosynthesis are important processes linking Carbon and Nitrogen metabolism, maintaining energy levels under stress conditions[4]. Salinity stress-induced changes in protein levels of these pathways in vascular and Palisade tissues will be presented and discussed.

**References:**


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