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Spatially Resolved Proteome Profiling Reveals Novel Drought-Responsive Proteins in Root Tips of Switchgrass Plants

Switchgrass (*Panicum virgatum*) is a bioenergy crop with strong adaptability to environmental stress. Root development and function depend on specialized cell types in the root tip, which respond differently to abiotic stress conditions. In this study, we identified cell type-specific proteomes in switchgrass root tips using laser capture microdissection (LCM) coupled with Nano POTS proteomics. Five distinct cell types were analyzed: (1) quiescent center and stem cell niche (QuC), (2) protoderm epidermal cells (PEC) in the meristem zone, (3) elongated epidermal cells (Epi) in the elongation zone, (4) peripheral root cap cells (PRC), and (5) columella root cap cells (Col). Experimental plants were sourced from field-grown switchgrass plots at Tennessee State University under well-watered and naturally drought-stressed conditions.

From approximately 1,000 cells per sample, with five replicates per cell type, we identified 6,336–6,640 proteins per proteome. Principal component analysis (PCA) revealed distinct clustering of cell types, with Epi and PEC forming separate clusters despite belonging to the same developmental lineage. QuC and Col were closely associated, while PRC clustered closer to Col. Functional enrichment analysis showed that QuC was enriched in proteins related to chromosomal behavior and mitosis, PEC was associated with cell division, and Epi displayed enrichment in cell wall remodeling proteins.

Drought stress induced global proteomic changes, including decreased abundance of ribosomal proteins, spliceosome components, and enzymes involved in sucrose metabolism and glycolysis, while proteins related to cell wall remodeling, lipid biosynthesis, and secondary metabolism were upregulated. Additionally, cell type-specific responses were evident, particularly in QuC and meristematic cells, which exhibited alterations in phytohormone signaling, metabolic pathways, and DNA repair mechanisms.

In conclusion, this study provides a high-resolution map of the switchgrass root tip proteome and reveals cell-type-specific proteomic responses to drought stress. These findings offer valuable insights into the molecular mechanisms underlying root adaptation and resilience in bioenergy crops. This project was supported by DOE-EMSL User Project 60294, USDA NIFA grant Proposal Number: 2021-12803, and Award Number #: 2020-69008-3276.