Doctoral thesis/dissertation Digest Form

Thesis/dissertation Title: Metabolomic Analysis of Primary Metabolic Pathways during Xylem Vessel Cell Differentiation in *Arabidopsis thaliana*

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Xylem vessel cell differentiation is marked by the deposition of secondary cell wall (SCW), made up of cellulose, hemicellulose and lignin. The biosynthesis of SCW is regulated by VASCULAR-RELATED NAC DOMAIN (VND) proteins, a set of NAC transcription factors, the overexpression of which induces ectopic xylem vessel cell differentiation effectively and synchronously. A previous study on the inducible overexpression of VND7 using the GR-DEX inducible system in tobacco BY-2 cells indicated significant changes in primary metabolites during xylem vessel cell differentiation; however, the metabolic flows and regulatory metabolic steps in xylem vessel cell differentiation are not yet well elucidated. In this study, metabolomic analysis was performed using VND7-inducible *A. thaliana* T87 suspension cells to obtain novel insights into primary metabolic regulation important for SCW biosynthesis. This included analysis of metabolic amounts, ^{13}C labeling experiments, and comparisons with transcriptome activity.

Firstly, it was confirmed that xylem vessel cell differentiation is mostly occurring between 24 to 48 hours after induction (HAI). From these differentiating cells, 57 metabolites were quantified by Capillary Electrophoresis Time of Flight Mass Spectrometry (CE-TOF MS). Sparse Partial Least Squares Discriminant Analysis (sPLSDA) of the data indicated significantly changed metabolic profiles during the differentiation. Further data analysis with heatmap and metabolic pathway map indicated that similar metabolic trends occurs for similarly categorized metabolites and that essential precursor metabolites for SCW polymers, such as glucose-6-phosphate, UDP-glucose, erythrose-4-phosphate, and phenylalanine, were transiently increased at 12 to 24 h of DEX treatment.

Next, ^{13}C labeling experiments was carried out to provide insight on metabolic flow during xylem vessel cell differentiation. As a result, time-course CE-TOF MS analysis quantified the ^{13}C labeling ratio for 44 metabolites, of which 18 metabolites showed the increased and decreased ^{13}C labeling ratios after the induction. The increased carbon flow was observed with lignin biosynthesis precursors, including ribose-5-phosphate, shikimate, phenylalanine, and 3-phosphoglycerate. On the other hand, the decreased carbon flow towards pyruvate derivatives, including lactate, was found. These data indicate the metabolic flow for lignin biosynthesis is constantly activated during xylem vessel cell differentiation.

Finally, the comparative analysis of transcriptome and metabolome data was carried. Transcriptome data indicates active regulation of primary metabolic pathways during the differentiation; enzyme genes for UDP glucose biosynthesis and in shikimate pathway for production of phenylalanine were upregulated significantly. In contrast, glycolysis pathway genes (for lactate production), were significantly downregulated. As the expression pattern is partially correlated with observed metabolomic dynamics, active regulation of primary metabolism during the differentiation is expected to be under transcriptional control. Taken together, the study successfully showed active regulation of primary metabolism for SCW polymer biosynthesis. This active regulation would be, at least partly, through transcriptional regulation of enzymatic genes.