

196e High-Throughput Time-Series Metabolomic Analysis to Identify Regulation of *Arabidopsis thaliana* Response to Elevated CO₂ by Sugar Signaling

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Comprehensive understanding of regulation in the primary metabolism of a biological system is a prerequisite for the systematic metabolic engineering of that system. To study regulation of *Arabidopsis thaliana* primary metabolism by sugar signaling and CO₂, we carried out high-throughput, quantitative, dynamic metabolomic analysis of systematically perturbed *A. thaliana* liquid culture system.

Specifically, *A. thaliana* liquid cultures, grown for 12 days under constant light and temperature in B5 Gamborg media were subjected to perturbations for 30 hours of (1) Elevated CO₂ level and (2) Trehalose (sugar) signal - applied individually and in combination. Additionally, the effect of elevated CO₂ was also studied by replacing Sucrose with Glucose in the growth media. Metabolomic profiling using gas chromatography-mass spectrometry was used to monitor the dynamic metabolic response of *A. thaliana* to the perturbations. A new algorithm for metabolomic data normalization and validation was developed to ensure accuracy and reproducibility of the metabolomic data.

The data obtained in the current analysis was used to develop a structured data analysis strategy for studying metabolic regulation of eukaryotic system from the time-series metabolomic data. Further, data analysis methodology for identifying interaction between two simultaneously applied dynamic perturbations was also developed to study the interaction between elevated CO₂ response and sugar signaling. Using these data analysis techniques for the dynamic metabolomic profiles in combination with gene expression analysis, a comprehensive understanding of the regulation of primary metabolism of *A. thaliana* liquid cultures by sugar signaling and CO₂ was obtained. The results were validated in the context of the known *A. thaliana* physiology. Apart from providing information about the response of the of the *A. thaliana* system to perturbations, this analysis is expected to greatly contribute in enhancing the currently available experimental database and in advancing the systems biology computational and experimental toolbox.