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Relative Quantification of Sugar Compounds in Wild-type and Transgenic *Arabidopsis thaliana* Using ^{1}H NMR Spectroscopy

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Objectives

Fourier transformed NMR (FT-NMR) spectroscopy, in combination with chemometrics, was performed to calibrate sugar compounds in transformed Arabidopsis plants.

Materials and Methods

1. Plant materials

Arabidopsis thaliana (L.) Heynh (Ecotype Columbia 0) and its trehalase knockout mutant

2. Methods

The pure standard sugar compounds (e.g. glucose, fructose, trehalose, sucrose) and powder sample of whole plants (pure: 0.1 to 5mgl^{-1}, plant: 18mg^{-1}) with adding D_2O+CD_3OD(4:1, 0.005% w/v TSP-*d*4:sodium salt trimethylsilylpropionic acid) were subjected to FT-NMR.

Results and Discussion

Metabolic profiling by ^{1}H-NMR was tested for absolute quantification of sugars. The metabolite responsible for each peak of spectra was identified from spectra of pure compound. The relative concentration of sugar compounds from sample was estimated through the calibration curves derived from each pure compound. Trehalose content from tre-3 mutant (0.033mg/g dry wt) was higher (about 10 times) than those of wild type plant (0.003mg/g dry wt). However, sucrose content was the highest in wild type plant. In this study, we found that ^{1}H-NMR metabolic profiling without complex extraction could be applied to determination of individual metabolite concentration from plant materials. Therefore using these results, the discriminant model to predict concentration of metabolite from unknown samples can be built up with statistical tools such as PLS-DA (Partial Least Square Discriminant Analysis) or PCR (Principle Component Regression).

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