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Metabolome analysis of salt stress response in plant cell culture system

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Objectives

To elucidate the coordinating network of adaptations to salt stress, the patterns of the metabolic profiles were monitored over a time course of 72 h after imposition of salinity stress. In addition to presenting the results of our analyses and interpreting their physiological implications, we also describe the application of data-mining tools to the data set obtained.

Materials and Methods

o Plant material and salt stress treatment

Arabidopsis T87 cells were obtained from the RIKEN Bio Resource Center (Tsukuba, Japan). The T87 cells were grown in modified liquid LS medium (30 ml) in a 100 ml flask. The cells were first grown for 3 days and then treated with 100 mM of NaCl. The cells were sampled for analysis after 0.5, 1, 2, 4 h and 0.5, 1, 2, 3 days.

o Metabolites extraction and determination

Extraction, separation, identification, and measurement of SAM, SAH, folate, and amino acids by LC-MS analysis using isotopomers of the target metabolites as internal standards were performed according to Kim *et al.* (2005). For other metabolite analyses, the sample was trimethylsilylated for 30 min at 37°C by adding 100 ml of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA). One microlitre of the derivatized sample was injected into a GC-MS system.

o Principal component analysis (PCA)

In T87 cells, 47 metabolites were identified by GC-MS and LC-MS and the profiled metabolitedata was analyzed using PCA. The PCA was performed for exploratory data analysis according to the usual manner using Pirouette software (Infometrix, Woodinville, WA) with mean-center preprocessing but not transformation.

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o Batch-learning self-organizing map analysis (BL-SOM)

For BL-SOM analysis, relative values of normalized metabolite levels were used. BL-SOM is an improved method over the original SOM with regard to the fact that the initial weight vectors are set by PCA and the learning process is designed to be independent of the order of input of vectors, and hence the result is reproducible.

Results and Discussion

The primary peculiarity of the short-term responses was a drastic accumulation in the ratio of SAM to SAH, suggesting that methylation reaction is induced during the initial phase after salt stress. Also, based on synchronous endogenous changes between SAM/SAH, phenylalanine and tryptophan at the various time points, we suggest that the methylation cycle for supply of methyl groups, phenylpropanoid pathway for lignin production, and tryptophan biosynthesis pathway for the regulation of phenylpropanoid metabolism are strongly coordinated by salt treatment. Correlation analysis of metabolite relationships revealed perturbation of metabolites involved in glycolysis or the sucrose metabolism as long-term responses to salt. The coordinated responses could lead to identification of successive metabolic functions of salt stress response pathways.

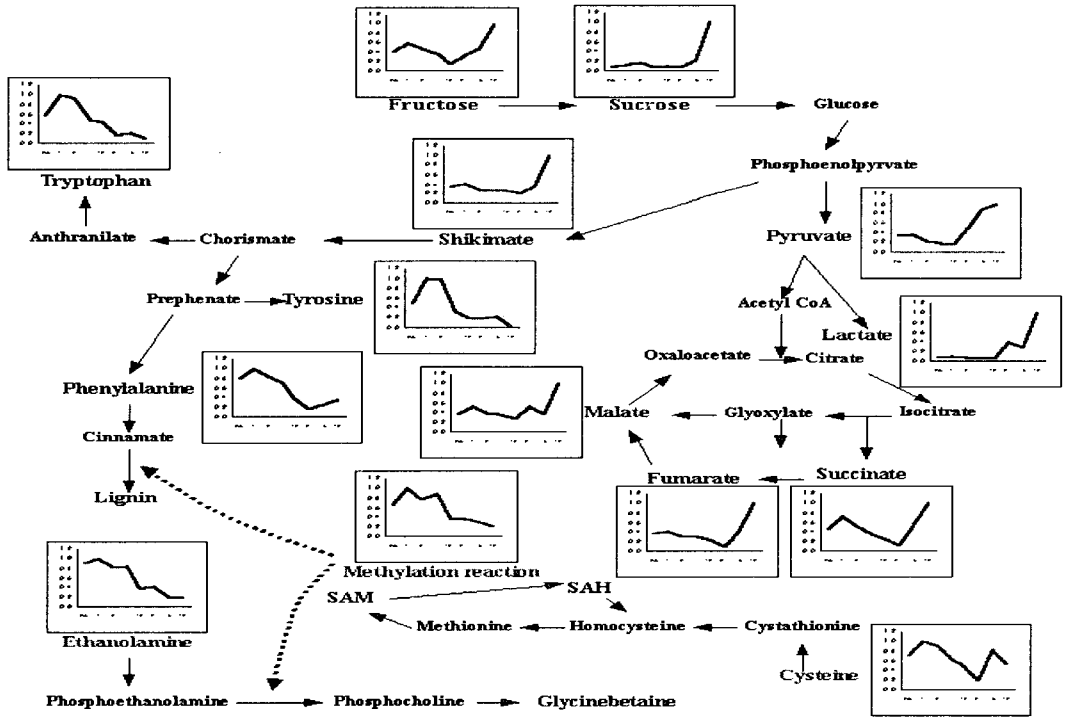

Fig. 1. Mapping of measured metabolite levels onto plant biosynthetic pathways