Comprehensive metabolic analysis of the cinnnamate/monolignol and lignan pathways

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The cinnamate/monolignol pathway supplies precursors for various phenylpropanoid compounds such as lignins, lignans, and norlignans, which are involved in cell-wall and heartwood formation of herbaceous and woody plants. The lignan pathway which follows the cinnamate/monolignol pathway is involved in heartwood formation and production of bioactive compounds. Thus, these pathways play central roles in plant secondary metabolism and are of importance in secondary xylem formation, which is composed of cell wall formation and heartwood formation.

During the last decade, substantial progress has been made in studies of these pathways, mostly due to the development of molecular biological techniques. For example, a novel pathway towards syringyl lignin via 5-hydroxyconiferylaldehyde was elucidated, and a transgenic aspen with less lignin and greater cellulose contents was reported. However, the comprehensive mechanisms controlling these pathways remain to be elucidated.

In the post-genomic era, to understand the mechanisms, comprehensive analyses such as transcriptomics, proteomics and metabolomics are becoming key strategies. However, accurate and comprehensive quantitation methods for whole metabolites, i.e. true metabolomics, have not yet been established.

As a first step, we focused on the cinnamate/monolignol and lignan pathways and established a comprehensive quantitation system for metabolic intermediates of the pathways. For this purpose, we employed a stable-isotope-dilution method because this is the most reliable quantitation method.

First, we synthesized deuterium-labeled and unlabeled standards for each of more than 30 metabolites in these pathways, and established standard calibration curves for the target compounds. Next, the system was successfully applied to characterization of the pathways in *Carthamus tinctorius* (safflower) seeds where biosynthesis of both lignins and lignans increases rapidly during maturation. In addition, ^{13}C-labeled precursors were administered to the seeds, and ^{13}C incorporation into the downstream metabolites was measured comprehensively. These metabolic profiling data during seed maturation were analyzed together with time-dependent gene expression data obtained by real-time PCR, resulting in identification of several cDNA clones involved in lignin and lignan biosynthesis. Furthermore, the presence of a biosynthetic route specific to lignan biosynthesis among many possible routes in the cinnamate/monolignol pathway was strongly suggested.

We are now trying to apply the metabolic analysis system to evaluating the effects of various factors on the growth of tropical fast growing trees, such as *Acacia* spp.