Engineering Specialized Metabolism in a Single Cell Type

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Project Goals: The long term goal is to significantly increase our understanding of mechanisms that regulate and control specialized metabolism in single cell types of plants and to use that understanding to rationally engineer plants to produce desired metabolite profiles. The glandular trichome secretory cell is our model in vivo cell system. This project is divided into two focus areas. The first focus area involves development and testing of a model of metabolic partitioning between the MEP/terpenoid and shikimate/phenylpropanoid pathways in glandular trichome secretory cells. This model will be used to predict regulatory and control points in the large metabolic network, which then could be targets for modification to achieve desired alteration of metabolism in this single cell type. The second focus area will be the evaluation of the roles of specific protein modifications and of changes in their expression on carbon partitioning and metabolic flux in real world scenarios.

Mathematical Model Development and Data Collection

A number of advances were made to a previously generated kinetic mathematical model of peppermint essential oil biosynthesis. In particular, the Lange laboratory was able to demonstrate that the density and size distribution of glandular trichomes on peppermint leaves was the most important factor determining essential oil yield in various genotypes (wild-type and several transgenic plants) and under different adverse environmental conditions [1]. We considered using an analogous approach to model essential oil biosynthesis in basil, but there are critical gaps in our understanding of the physiology of trichome development and the organization of the terpene/phenylpropanoid pathways. Moreover, results over the course of the past year have supported the contention that significant amounts of non-volatile (ergo non-essential oil) metabolites accumulate in the glandular trichomes of basil. Based on these data, we are assembling a reaction graph representation of the pathways relevant to essential oil formation in sweet basil. We are using a Flux Balance Analysis (FBA) approach with one main difference (and key innovation) compared to existing FBA studies: we do not assume that biomass production is geared toward optimal growth (which works well in microorganisms but there are many problems with more complex organisms) but instead we are focusing our modeling on glandular trichomes. We are assuming the uptake of a transport sugar (stachyose/sucrose) and the maximizing of the production of essential oil and its removal from the network (accumulation in storage cavity of glandular trichome) as the objective function. These modeling activities will provide us with essential information regarding the flux distribution across multiple pathways in glandular trichomes. In addition, this approach does not require as much detailed kinetics data for the network elements as a kinetic model would require.

Advances in metabolite network determination

Work on characterizing the enzymes involved in production of the large array of methoxylated flavones in basil and peppermint has moved ahead very well. Because flavones, mostly highly methoxylated, constitute a considerable carbon sink both in basil and peppermint, which

potentially competes with the isoprenoid pathway for carbon allocation, and the mechanisms of A-ring modifications have been barely studied hitherto, we have worked to characterize the late metabolic steps leading to the formation of these compounds. This information will be required for development and refinement of the model of metabolism in the secretory cells of basil. The functions of a number of enzymes involved in the formation of these compounds have been determined from basil and peppermint. For example, recombinant FOMTs display distinct substrate preferences and product specificities that can account for most detected 7-/6-/4'-methylated, 8-unsubstituted flavones in sweet basil. Apparent K_M values in the low micromolar range and specific gene expression profiles support the involvement of specific FOMTs in the biosynthesis of specific flavones in the different sweet basil lines. Structure homology modeling suggested the involvement of several amino acid residues in defining the proteins' stringent regioselectivities. The roles of these individual residues were confirmed by site-directed mutagenesis. A parallel study of flavone A-ring hydroxylases allowed us to delineate the network from apigenin to salvigenin, gardenin B and nevadensin, the major polymethoxylated flavones that accumulate in sweet basil.

Laser-microdissection of developing glandular trichome secretory cells for functional analysis and model refinement

The goal of this part of the project is to measure gene expression in glandular trichome secretory cells at specific developmental stages. This information is important in order to assign functions to the trichomes and not just leaf cells in general, and also for development and testing of the later mathematical models. We are using the technique of laser-microdissection coupled with RNA-sequencing to isolate specific cell types from sectioned tissue and to profile and quantify transcripts in these cells. Transcript profiles are being compared in peppermint and four basil chemotypes at four defined developmental stages from trichome initiation to fully expanded trichomes.

Reference

1. Rios-Estepa et al. (2010) Mathematical modeling-guided evaluation of biochemical, developmental, environmental and genotypic determinants of essential oil composition and yield in peppermint leaves. Plant Physiol. 152, 2105-2119

This project is supported by the Office of Biological and Environmental Research in the DOE Office of Science.