

Model-Guided Metabolic Engineering of Increased 2-Phenylethanol Production in Plants

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Project Goals: We seek to employ metabolic modeling for an improved engineering strategy for the overproduction of 2-phenylethanol, a potential biofuel. Our approach is to use a kinetic model to analyze the changes observed in transgenic Arabidopsis plants overexpressing the enzymes catalyzing the multiple steps biosynthesis of 2-phenylethanol from phenylalanine in Arabidopsis. Output from the model identify targets for further metabolic engineering strategies for optimized biofuel production.

2-Phenylethanol (2-PE) is a naturally occurring organic volatile with a characteristic rose scent. Currently, 2-phenylethanol from both natural and artificial sources is utilized for flavoring and fragrance. The physicochemical properties of 2-PE's make it a potential biofuel, which can be used as a substitute for ethanol in petroleum-derived gasoline. However, its use for this purpose is limited by a lack of economically viable large scale production. Over the last decade the biosynthetic pathway of 2-PE formation was established in plants, and its production competes for substrate with lignin biosynthesis, which prevents efficient extraction of cellulose in ethanol production. 2-PE derives from phenylalanine, which is first deaminated and decarboxylated by a single enzyme, phenylacetaldehyde synthase, to form phenylacetaldehyde. Subsequent reduction by phenylacetaldehyde reductase forms 2-PE. While overexpression of these two enzymes, both by ourselves and others, has been successful in obtaining increased production of 2-PE, the *in planta* accumulation observed remains far lower than desired. Here we describe a metabolic model-guided approach to further enhance 2-PE production. Transgenic *Arabidopsis thaliana* were generated that overexpress the phenylacetaldehyde synthase from Arabidopsis (AAS) in tandem with the phenylacetaldehyde reductase (PAR) from tomato. Metabolite analysis revealed an apparent maximum accumulation beyond which higher gene expression was ineffective. Metabolic profiling of emitted volatiles confirmed that this was not due to release of either the 2-PE or its intermediate phenylacetaldehyde to the atmosphere. Moreover, measurements of glycosylated 2-PE indicated that limited 2-PE accumulation in transgenic plants was not due to further metabolic sequestration. Analysis of the results via our dynamic metabolic model of the phenylpropanoid network predicted that 2-PE production in these transgenic plants was substrate limited. This model prediction was further validated by combining overexpression of PAR and PAAS with overexpression a feedback-insensitive DAHP synthase, the latter of which has previously been shown to elevate intracellular phenylalanine content. This model-guided strategy successfully increased 2-PE accumulation by more than an order of magnitude compared to the overexpression of the 2-PE pathway alone. We continue to utilize the kinetic model in combination with metabolic profiling by LC/MS/MS to better refine our strategy and rationalize additional improvements.

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