

Improved Biofuel Production through Discovery and Engineering of Terpene Metabolism in Switchgrass

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Project Goals:

Of the myriad specialized metabolites that plants form to adapt to environmental challenges, terpenes form the largest group. In many major crops, unique terpene blends serve as key stress defenses that directly impact plant fitness and yield. In addition, select terpenes are used for biofuel manufacture. Thus, engineering of terpene metabolism can provide a versatile resource for advancing biofuel feedstock production, but requires a system-wide knowledge of the diverse biosynthetic machinery and defensive potential of often species-specific terpene blends. This project merges genome-wide enzyme discovery with comparative –omics, protein structural and plant microbiome studies to define the biosynthesis and stress-defensive functions of switchgrass (*Panicum virgatum*) terpene metabolism. These insights would be combined with the development of genome editing tools to design plants with desirable terpene blends for improved biofuel production on marginal lands.

Abstract:

Diterpenoids constitute a diverse class of metabolites with critical functions in plant development, defense, and ecological adaptation. Major monocot crops, such as maize (*Zea mays*) and rice (*Oryza sativa*), deploy diverse blends of specialized diterpenoids as core components of biotic and abiotic stress resilience. This study reports the genome-wide discovery and functional characterization of stress-related diterpene synthases (diTPSs) and cytochrome P450 monooxygenases (P450s) in the bioenergy crop switchgrass (*Panicum virgatum*). Mining of the allotetraploid switchgrass genome identified the largest thus far known diTPS family in plants, comprising 31 members. Biochemical analysis of 11 diTPSs revealed a modular metabolic network producing a diverse array of diterpenoid metabolites. In addition to *ent*-copalyl diphosphate (CPP) and *ent*-kaurene synthases likely involved in gibberellin biosynthesis, we identified *syn*-CPP and *ent*-labda-13-en-8-ol diphosphate (LPP) synthases as well as two diTPSs forming (+)-labda-8,13E-dienyl diphosphate (8,13-CPP) and *ent*-*neo*-*cis*-*trans*-clerodienyl diphosphate (CT-CLPP) scaffolds not previously observed in plants. Structure-guided protein mutagenesis of the (+)-8,13-CPP and *ent*-*neo*-CT-CLPP synthases revealed active site determinants that may resemble neo-functionalization events that occurred during diversification of the switchgrass diTPS family. In addition, we identified an unusual subfamily of P450s that are capable of directly converting the CPP and CLPP predicts into corresponding furanoditerpenoid structures, this bypassing the paradigmatic pathway organization of labdane diterpenoid metabolism.

Formation of several diTPS and P450 products, alongside the elicited expression of the corresponding biosynthetic genes, was induced in switchgrass roots and leaves in response to drought and UV irradiation, indicating their possible roles in abiotic stress adaptation. Together, these findings expand the known chemical space of diterpenoid metabolism in monocot crops toward systematically investigating and ultimately improving stress resilience traits in bioenergy crop species.

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