Investigating the Switchgrass Diterpenoid Metabolic Network Toward Improved Stress Tolerance and Biofuel Production

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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 provides 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 would merge 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. Here, we describe the genome-wide identification and functional characterization of stress-related diterpene synthases (diTPSs) 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 observed previously 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. Formation of several specialized diterpenoids, such as 9β-hydroxy-syn-pimar-15-ene, along with the expression of the corresponding diTPS genes was induced in roots and leaves in response to oxidative stress 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.

References

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