

Title: 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, terpenoids form the largest group. In many major crops, unique terpenoid 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 terpenoid 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 terpenoid blends. This project merges genome-wide enzyme discovery with comparative -omics and protein structural studies to define the biosynthesis and stress-defensive functions of switchgrass (*Panicum virgatum*) terpenoid 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 Text: 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 project reports the genome-wide discovery and functional characterization of the stress-related diterpenoid-metabolic network in the bioenergy crop switchgrass (*Panicum virgatum*). Mining of the allotetraploid switchgrass genome identified expansive diterpene synthase (diTPS) and cytochrome P450 monooxygenase (P450) enzyme families critical for the chemical diversity of bioactive diterpenoids. Tissue-specific transcriptome and metabolite analyses of drought-resistant (Alamo) and drought-susceptible (Cave-in-Rock) genotypes showed an earlier onset of transcriptomic changes and significantly more differentially expressed genes in response to drought in Cave-in-Rock. Diterpenoid-biosynthetic genes showed drought-inducible expression in Alamo roots, contrasting largely unaltered triterpenoid and phenylpropanoid pathways. In addition, metabolomic analyses identified common and genotype-specific terpenoids. Consistent with transcriptomic alterations, several root diterpenoids showed significant drought-induced accumulation. Structural analysis of drought-responsive root diterpenoids verified these metabolites as oxygenated furanoditerpenoids

that are perhaps unique to switchgrass. Together, these findings support a role of diterpenoids in switchgrass drought stress tolerance and provide resources for understanding the molecular mechanisms underlying switchgrass environmental resilience.

References/Publications

1. Muchlinski A, Jia M, Tiedge K, Fell JS, Pelot KA, Chew L, Davisson D, Chen Y, Siegel J, Lovell JT, Zerbe P (2021) Cytochrome P450-catalyzed biosynthesis of furanoditerpenoids in the bioenergy crop switchgrass (*Panicum virgatum* L.). *Plant J.* 108:1053-1068.
2. Tiedge K, Muchlinski A, Zerbe P (2020) Genomics-enabled analysis of specialized metabolism in bioenergy crops: current progress and challenges. *Synth Biol (Oxf).* 5:ysaa005.
3. Pelot KA, Chen R, Hagelthorn DM, Young CA, Addison JB, Muchlinski A, Tholl D, Zerbe P (2018) Functional Diversity of Diterpene Synthases in the Biofuel Crop Switchgrass. *Plant Physiol.* 178:54-71.

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