Proposal 507320 - CSP Functional Genomics Proposal (FG 2020 Q4)

Title: Elucidation of the Gene Regulatory Networks Governing Terpenoid-Mediated Plant-Environment Interactions to Enable Advanced Bioenergy Crops

Proposal id: 507320

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Status: Proposal In Progress (Mar 12 2021, 02:33PM)

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Sequencing and Metabolomics Request (Products)

Estimated number of samples to be sent for each product:
Plant RNA-seq: 48
Metabolomics nonpolar analysis: 96
DAP-seq: 45

Samples

Estimated total number of samples to be sent: 189

Utilizes samples collected outside of the United States? No

Samples regulatory compliance comment:
The development and production of optimized bioenergy crops must be environmentally and socially sustainable. Therefore, knowledge, gene resources, and protocols associated with this project are strictly used in non-profit research and will be openly shared with the research community through presentations at professional meetings, peer-reviewed publications, and public data repositories. Gene constructs and metabolites will be shared directly by the PIs within standard regulations for material transfer agreements between the PI's institutions, DOE JGI and the interested third parties. Any sharing of genetic and plant material will be coordinated in accordance with the respective national and international transfer and import regulations. Other possible long-term implications of current or planned research (especially pertaining to the modification of perennial grasses for bioenergy production) include loss of biodiversity due to dedicated bioenergy crop production (e.g. switchgrass), and land use conflicts for multi-purpose plants (e.g. maize). In particular, bioenergy production from maize and sorghum must be carefully monitored, due to its resource-intensive cultivation and importance for global food security. To minimize the risk of these and other contentious issues, crop performance should be optimized for climate change by tailoring specific metabolic systems and ecological interactions

to utilize degraded land, and optimize carbon storage and use of water resources. We believe the deeper knowledge of mechanistic principles coordinating plant-environment relationships developed in this project will be instrumental in future research, engineering, breeding and associated policy-making. As a proof-of-concept from existing greenhouse studies, the resulting knowledge and resources can be applied to modify select terpenoid defenses in transgenic maize [1; see Supporting File 1 for References].

Organisms

Taxonomy	Characterization
Plant Panicum virgatum	Genome size: 1230 MB
Plant Zea mays	Genome size: 2400 MB
Plant Sorghum bicolor	Genome size: 732 MB

Synthesis Request

Total request 400 kb

Number of constructs for each product	
Product	**Number of Constructs**
Constructs <5kb	175
Constructs 5kb to 10kb	
Constructs >10kb	
Combinatorial/Libraries	60
SgRNA libraries	
Strain Engineering/CRAGE	
Total Constructs	235

Sequence Data Mining Requested

See item 5 on the CSP Functional Genomics Call for a description of Sequence Data Mining.

If answered Yes, a description in detail what data mining capabilities are requested from the JGI, including the types of sequences to be identified, the source data sets to be mined, and the search/identification/prioritization methods to be used.

None

Strain Engineering/CRAGE strains

None

Sequence Certification

I certify that to the best of my knowledge, for all the DNA sequences that will be provided in the context of this project, I have or have obtained through my collaborators the necessary rights or permissions to request the synthesis of these sequences.

To the best of my knowledge, the sequences:

1. Will not infringe any existing intellectual property;
2. Have not been misappropriated;
3. Are not export controlled;
4. Do not require registration or other action under the Federal Select Agent Program regulations or other biosecurity requirements;
5. Will be used for RESEARCH ONLY.

Name: **Philipp Zerbe** Date: **2020-07-30**

Are any of the sequences to be synthesized:

6. Related to the pathogenicity of an organism? **false**
7. Known to or shows a potential to encode any form of infectious agent or viral life-cycle component? **false**
8. Known to have any toxicity or the likelihood that this project might increase toxicity? **false**
9. Intended for use in creating a vaccine? **false**

Sequence certification comments:

Biosecurity, Biosafety, Biocontainment, and Environmental screening

(pre-4/2021, combined format):

As detailed below, extensive precautions will be taken to mitigate any possible risk for handling recombinant DNA, microbial strains, plant material, and metabolites associated with the proposed work.

Project personnel will be trained in lab safety, plant biosafety (containment, handling and disposal of materials), and handling of hazardous materials according to the safety regulations of the participating institutions. All participating labs are equipped with biological safety cabinets and other laboratory equipment required for safe experimental work, and are further authorized for Biosafety Level 1 (BSL1) for handling recombinant DNA and microbial hosts. Access to laboratories is restricted to authorized personnel only.

Recombinant DNA: We anticipate that the targeted synthetic genes do not form toxic or otherwise harmful compounds. However, expression of these genes in heterologous hosts could form unintended byproducts. All microbial hosts (E. coli, A. tumefaciens, S. cerevisiae), vectors and genes used here meet BSL1 requirements, involving well-characterized agents not known to cause disease in healthy humans, and of minimal potential hazard to personnel and the environment.

Metabolites: This project will produce various terpenoid small molecules. While some terpenoids are known to be toxic, the terpenoids produced during this project are not known to pose a risk to human, plant or environmental health. All samples containing unknown enzyme products or plant metabolites will be handled in fume hoods and stored in explosion-proof freezers. Where required, research materials will be shipped as hazardous materials according to federal guidelines.

Transgenic plants: All transgenic N. benthamiana plants will be labeled with the transformed genes and date. All transgenic plants will be moved between locations in closed plastic trays, and grown in contained growth chambers only. No seed or progeny of transgenic plants will be generated.

All waste containing recombinant DNA, microbial and transgenic plant material will be stored in containment (separate disposal bags) and devitalized by autoclaving (121 C°, 15 psi, 30 minutes) in autoclave bags with indicator tape.

Furthermore, resources associated with the proposed project present no known risk regarding vaccine effectiveness, antibiotic or antiviral resistance, bioweapon development, or pathogen virulence, transmissibility, host range and detectability. At this stage in the research, no mitigating activities are deemed necessary beyond the requisite biosafety requirements for safe handling, containment and disposal of related materials, and our ethical mandate of continuous scientific progress monitoring and responsible innovation.

Lay Description:

General Background: Plant biomass production from agricultural crops can contribute significantly to global energy supplies, while addressing key environmental targets such as climate adaptation and carbon neutrality. To realize this goal, sustainable bioenergy crop production must balance arable land and water needed for food and feed to support a rapidly growing population. However, current trends in global crop yields are projected to fall short of fully meeting future demand, due to shifting climate conditions and associated environmental stress factors that already cause severe harvest losses. A deeper knowledge of the mechanisms

plants employ to adapt to and interact with their environment will be instrumental in addressing these challenges. To survive in changing environments, plants use a vast range of small molecule chemicals with various biological activities to coordinate their interaction with other species (e.g. insects and microorganisms). Terpenoids comprise a diverse group of such chemicals and also have broad human uses such as high-energy biofuels, food additives, and therapeutics.

Research Goal: Our aim is to generate a deeper knowledge of how plants regulate the formation of common and species-specific metabolites that mediate environmental interactions in three major bioenergy crops switchgrass, maize, and sorghum. We will combine a customizable discovery pipeline with functional genomics and biochemical studies to gain a comprehensive understanding of the network of transcription factors that plants employ to form bioactive terpenoids, as a major group of small molecules for plant interaction with the environment. JGI's genomics, metabolomics, and DNA synthesis capacity will help to leverage existing knowledge and gene resources of terpenoid-biosynthetic pathways to accelerate the identification of the regulatory processes driving terpenoid production in the focal crops.

Research Utility: Collaborating with the JGI at the interface of functional genomics and experimental biology studies, the proposed project will generate much-needed knowledge about the molecular machinery of stress resistance in bioenergy crops. The research team brings the full suite of complementary expertise to explore the complex mechanisms of terpenoid metabolism and offers the necessary skillset for application to DOE priorities of sustainable bioenergy production.

Broader Implications: In the future, we will struggle to produce sufficient food, feed and energy to support the world's population while sustaining a healthy ecosystem. A shifting climate already threatens agricultural production. Adapting plants to grow in more extreme environments can contribute to alleviating this issue. Knowing which of the plant's machinery is involved in complex environmental responses can help spearhead collaborative partnerships to develop agricultural crops that meet growing bioenergy needs through improved plant breeding, engineering and production.

Supporting files

Download bundle of all file attachments for the proposal: proposal_507320_bundle.zip

	Filename	Description	Type
Download	Proposal__507320_-_PI___Co-PI_CVs.pdf	Proposal #507320 - PI & Co-PI CVs	CV
Download	Proposal__507320_-_Figures.pdf	Proposal #507320 - Figures	Supporting
Download	Proposal__507320_-_Supporting_File_1_-_Cited_Literature.pdf	Proposal #507320 - Supporting File 1 - Cited Literature	Supporting
Download	Proposal__507320_-_Table_1.xlsx	Proposal #507320 - Table 1	Supporting

Summary

Description:

Many monocot bioenergy and food crops deploy complex blends of terpenoid metabolites as core mediators of chemical defense and interorganismal interactions that directly impact crop fitness [2,3]. The proposed project aims to elucidate the gene-regulatory networks (GRNs) underlying terpenoid metabolism in three major bioenergy crops, switchgrass (Panicum virgatum), maize (Zea mays), and sorghum (Sorghum bicolor), to aid crop development for enhanced stress resilience and biofuel production from biomass feedstock. Prior studies supported by a DOE JGI DNA Synthesis Award (CSP#2568) identified more than 50 terpene synthases (TPS) and cytochrome P450 monooxygenases (P450), as key enzymes in generating bioactive terpenoids, in several bioenergy plants including switchgrass and maize [1-2,4-9] (Fig. 1). This knowledge now enables a precise investigation of the GRNs that govern common and species-specific terpenoid pathways. We will integrate transcription factor (TF) functional studies, TF binding site mapping, transcript and metabolite profiling, and terpenoid pathway engineering to identify the terpenoid-metabolic TF networks mediating stress responses in switchgrass, maize and sorghum (Fig. 2).

As preliminary data, we identified 50 maize TFs with significant correlation to terpenoid metabolism by analyzing patterns of tissue-specific co-expression with core TPS/P450 genes in large in-house and public transcriptome and GRN datasets [1,10] and TF binding sites for select TPS/P450 promoters using public ChIPseq resources [11] (Table 1). Several of these TF candidates have previously been implicated with functions in monocot stress responses [3,12,13]. Identification of terpenoid-metabolic switchgrass and sorghum TFs will then be achieved by using the maize TFs to mine existing genome and transcriptome resources [14,15], followed by gene synteny and gene co-expression studies to assess probable roles in terpenoid metabolism. Applying this approach to sorghum already revealed TF orthologs with significant correlation to sorghum terpenoid and other specialized pathways (Fig. 3).

To examine the activity of TFs in regulating terpenoid metabolism, DNA synthesis is requested for 80-90 TFs for functional analysis using established Yeast-One-Hybrid (Y1H) assays [16] with the promoters of 20-30 TPSs and P450s representing key pathway nodes in switchgrass, maize and sorghum terpenoid metabolism. TFs of interest will further be analyzed in vivo via over-expression of TFs with key TPS/P450 genes under their native promoters using Nicotiana benthamiana as a host system [1,17,18]. Combined Y1H and co-expression assays will provide insight into TF functions in controlling distinct terpenoid pathway branch points and identify high-priority TF targets for long-term transgenic studies to empirically examine TF functions and their impact on crop stress resilience in planta.

To gain deeper insight into the relevance of identified TFs to terpenoid-mediated and broader stress responses, RNA-seq capacity is requested to complement existing transcriptome resources for studying tissue-specific, drought- and/or pathogen-induced co-expression patterns of TFs and terpenoid-metabolic genes in select crop varieties. Pairing transcriptomics with JGI-supported untargeted metabolomics and in-house terpenoid profiling will enable the correlation of transcript and metabolite abundance. In parallel, DAP-seq resources for mapping of transcription binding sites for 45 terpenoid-metabolic TFs will be used to identify the GRNs driving common and species-specific pathways. Finally, JGI support for generating 60 combinatorial pathway libraries will leverage our large TPS/P450 catalog [1,2,4,6-8,19-21] and an established E. coli

platform for terpenoid production [17,22] to develop a modular metabolic engineering tool that can serve as a resource for accelerating the discovery of terpenoid-metabolic and regulatory genes in a broader range of bioenergy crops.

Justification:

Crop improvement increasingly depends on knowledge of gene functions that contribute to desired crop traits and offer resources for precision crop engineering or breeding [23]. Particularly, knowledge of the molecular processes that enable plants to adapt to environmental pressures is needed to address imminent challenges of exacerbating crop losses caused by climate shifts and associated pest and pathogen damage [23-25]. Terpenoid metabolites are important for plant vigor and stress resilience due to their functions as pest and pathogen defenses and as modulators of various ecological interactions [2,3]. For example, in maize, distinct terpenoids that confer antifungal and antifeedant activity against major pathogenic fungi and insect pests, and mediate drought tolerance in below-ground tissues, can inform plant engineering and breeding [1,6,26,27]. In addition, terpenes, such as farnesene and bisabolene, are used for engineering of renewable biofuels [28,29]. Essential to advancing a broader use of terpenoids for bioenergy feedstock production and biofuel engineering is a system-wide knowledge of the diverse biosynthetic and regulatory circuitry of species-specific terpenoid networks. Advanced DNA synthesis and functional analysis capacities have accelerated the discovery of terpenoid-metabolic pathways in several monocot crops, providing detailed mechanistic insights into the enzymatic machinery underlying the production of species-specific terpenoid blends [2,3,30]. By contrast, the regulatory components controlling terpenoid metabolism have remained elusive. To date, only a few TFs have been empirically shown to regulate terpenoid metabolism in monocot crops [3]. For example, maize ZmWRKY79 activates genes in the stress-elicited formation of defensive terpenoids [12], and the bHLH TF, DPF, regulates defensive diterpenoid pathways in rice [31]. Understanding the complex TF networks that plants employ to coordinate pathway branches en route to terpenoids of different bioactivity is needed to address limitations in crop trait optimization and metabolic engineering.

Pairing the genome-wide discovery of terpenoid-metabolic TFs with comparative functional genomics, TF functional studies, and pathway engineering will identify the core GRNs driving terpenoid-metabolism. Applying this approach to switchgrass, maize, and sorghum as three bioenergy crops with sequenced genomes and greater than 50% ortholog synteny across genes with high annotation confidence will provide comparative insights into the regulation of metabolite-guided plant-environment interactions that, ultimately, can be expanded to a broader range of bioenergy crops.

JGI DNA synthesis support will accelerate the functional analysis of the large catalog of TF candidates. TF activity studies via established Y1H [16] and N. benthamiana co-expression [1,17] assays will expedite the translation of genomics data into a network of TF functions. Combining these insights with metabolomics, transcriptomics, and transcription binding site mapping in collaboration with the JGI will facilitate continued research toward decoding the regulatory mechanisms underlying the ecological interactions of terrestrial biomes among plants, microbes and animals. JGI support in pathway engineering will further help develop modular microbial platforms as a community resource to accelerate gene function, metabolite and related studies, and enable synthesis of a broader spectrum of terpenoid bioproducts.

The project team (Philipp Zerbe, UC Davis, PI; Eric Schmelz, UC San Diego, CoPI; Alisa

Huffaker, UC San Diego, CoPI) already developed effective workflows through ongoing, collaboration and joint publications to be well-positioned to undertake the proposed studies. Resources and data will be shared throughout the project, and research will be coordinated through ongoing monthly video conferences and biannual in person meetings as possible within current and anticipated travel limitations.

Utilization:

JGI contributions proposed for this project are (i) DNA synthesis and construct assembly for TF functional analyses, (ii) Transcriptomic and metabolomic data across select crop genotypes, (iii) DAP-seq resources for TF binding site mapping, and (iv) Synthesis of combinatorial pathway libraries for metabolic engineering.

(i) JGI's DNA synthesis capacity will support TF functional analysis via Y1H and N. benthamiana co-expression assays. DNA synthesis will mitigate limitations of gene cloning from plant material and accelerate the analysis of the expansive catalog of TFs with predicted roles in terpenoid metabolism. A total of 80-90 monocot TF genes will be synthesized as codon-optimized genes for use in Y1H assays [16]. A set of 20 maize TPS and P450 promoters representing key pathway nodes has been generated for this purpose, and cloning of select switchgrass and sorghum promoters is underway. In addition, ~40 TFs prioritized based on Y1H assays will be synthesized for optimized expression in N. benthamiana under strong constitutive promoters. N. benthamiana co-expression of these TFs with combinations of select TPSs and P450s under control of the native promoters will be used to identify TF functions by measuring pathway metabolite production [2,17]. Significant metabolite production in N. benthamiana will provide sufficient dynamic range to capture meaningful activation signatures and ultimately confidence in TF targets for the pursuit of transgenic approaches in switchgrass, maize, and sorghum beyond the scope of this proposal.

(ii) Transcriptome data (48 samples) will be used to investigate the role of focal TFs in driving stress-elicited terpenoid-metabolic networks. Defined maize and switchgrass genotypes will be used for transcript profiling of roots and leaves after pathogen and/or drought treatment to complement non-elicited, tissue-specific switchgrass, maize and sorghum transcriptome data generated by the PI's labs. To demonstrate links between TF activity, terpenoid abundance and plant stress tolerance, transcriptomics will be paired with metabolite profiling of the same tissues (96 samples) using a combined approach of JGI's non-polar metabolomics platform and quantitative terpenoid profiling established in the PIs labs. As a foundational dataset, the potential for continued collaborative studies will provide a long-term community resource. Specifically, TF functions will guide detailed transgenic gene function studies in planta, and the transcriptome and metabolite data will benefit research groups studying gene and metabolite abundance in diverse monocot lines beyond the information on terpenoid metabolism captured in this project.

(iii) DAP-seq resources for 45 TFs (including synthesis of affinity-tagged genes) selected based on TF functions (i) and gene-metabolite correlations (ii) will enable mapping of TF binding sites to characterize GRNs controlling common and species-specific terpenoid pathways. Paired with

transcript/metabolite abundance (ii), these insights will elucidate the functional connectivity of TFs shown to regulate terpenoid metabolism.

(iv) Using an established E. coli platform for terpenoid engineering [32,33], the Zerbe lab routinely uses co-expression of >50 functionally distinct TPSs and P450s for enzyme functional studies and terpenoid production [34]. Utilizing JGI's capacity for combinatorial pathway library synthesis, development of 60 libraries with our large TPS/P450 catalog and different promoter and terminator variations will enable the development of customizable pathway modules to serve as a community resource for the scalable production of diverse terpenoids for use as bioproducts and numerous analytical approaches such as gene function and metabolite bioactivity studies.

Continued research utilizing the generated resources is supported by ongoing DOE Early Career (grant #DE-SC0019178 to PZ) and NSF-Plant Biotic Interactions (grant #1758976, to ES and PZ) awards.

Community interest:

A deeper understanding of the role that specialized metabolites play in complex plant-microbe interactions and environmental adaptation is increasingly being recognized as an important factor for improving agricultural productivity and sustainability. Knowledge, gene resources and tools from this project will help spearhead academic and industry partnerships to develop agricultural crops for meeting future bioenergy needs through improved plant breeding, engineering and production. For example, enhancements to drought and disease tolerance promise significant environmental and economic benefits, including improved biomass yields and more sustainable water and land management. Socially, expanding bioenergy feedstock production can stabilize employment in bioenergy farming communities, and foster sustainable agricultural practices regionally, nationally and globally. The collective data produced spans genome-wide TF gene discovery, TF gene functional annotation, and transcriptomic regulation of GRNs driving common and species-specific specialized pathways involved in interorganismal and environmental interactions (Fig. 2). In addition, platforms for the modular assembly of terpenoid-metabolic genes will provide resources for gene discovery and the engineering of a broad spectrum of bioactive terpenoids. These deliverables can promote collaborative research at the interface of metabolite-guided plant-environment interactions, crop optimization and synthetic biology to help translate natural defensive mechanisms into strategies for improving crop resilience, bioenergy production and bioproduct manufacture. Given the diverse roles terpenoid metabolites play in shaping overall plant health, the community that will use the generated resources is likely to include academic, AgBiotech industry, government researchers, and affiliated groups across the broad areas of plant science/breeding, microbiology, and synthetic biology as they overlap at the aim of advancing bioenergy and natural product production. Aligned with the focus of DOE Bioenergy Center programs (e.g. Great Lakes Bioenergy Center [GLBC] and Joint Bioenergy Institute [JBEI]) on large-scale breeding, genomic selection and genetic mapping of complex field traits for developing improved bioenergy feedstock, findings of the current proposal on the specialized metabolic pathways driving environmental interactions and crop resilience traits will be complementary with research toward elucidating defined metabolic processes that can be applied to crop improvement. In addition, partnership with the JGI to use our expansive gene catalog for developing modular

constructs and microbial systems for terpenoid production would provide a community resource for the customizable production of biofuels and other commodity chemicals. Beyond bioproduct manufacture, the ability to efficiently produce a broad range of terpenoids and other plant metabolites enables numerous further approaches, including empirical gene function studies in planta and analysis of individual plant-microbe interactions, which will result in a deeper understanding of how dynamic interorganismal interactions contribute to crop productivity. In this context, project findings will be disseminated to the community via appropriate conferences and publications, and gene sequences, constructs, and metabolites made available in public repositories upon publication (per journal policy) or directly by the PIs.

DOE mission:

Agricultural biomass production can make a major contribution to satisfying future energy demand of a rapidly growing global population, while addressing key sustainability targets of climate adaptation, agroecosystem management, and carbon neutrality. However, current trends in agricultural productivity are projected to fall substantially short of this goal, due to intensifying harvest losses caused by shifting climate pressures and associated pest damage and crop diseases [24,25]. A precise knowledge of how plants regulate specialized metabolite networks that are fundamental to immunity and interorganismal interactions is needed to develop crops capable of withstanding current and future environmental pressures. To arrive at this impact, this project will leverage prior collaborative research on the functional characterization and engineering of plant terpenoid-metabolic networks and crop functional genomics with the diverse panel of DOE JGI capacities in DNA synthesis, transcriptomics, metabolomics, and synthetic biology. Applying this approach to three major bioenergy crops, switchgrass, maize, and sorghum, will help determine the universal and species-specific GRNs that enable plants to precisely coordinate dynamic interactions with their biotic and abiotic environment. Expansive gene catalogs and knowledge of gene functions in environmental adaptation will promote further collaborative research aligned with the DOE mission of developing innovative solutions for sustainable bioenergy resources. Faced with severe climate-driven harvest losses across the U.S. and globally, elevating stress resilience and crop efficiency is crucial to expand the economic competitiveness of high-energy biomass feedstock without jeopardizing existing industries, products and uses. To this end, comparative investigation of common and unique adaptive metabolic strategies across three monocot crops will advance our ability to transfer these processes into other established and emerging bioenergy crops. Among the focal crops for the proposed project, elevating stress tolerance is crucial for maize and sorghum in light of the dramatic climate-driven harvest losses in recent years [24,35]. Furthermore, investigating the largely unknown terpenoid-regulatory mechanisms in switchgrass will establish the fundamental knowledge needed to manage threatening plant environmental pressures and improve bioenergy crop production. Building a broadly applicable platform around this approach would offer new bioenergy solutions to address imminent challenges of harvest losses and agricultural water and land utilization for a balanced production of bioenergy, food and feed plants. To realize this vision, it is imperative to instigate innovative and interdisciplinary collaborations that successfully combine insights from foundational and translational plant research. In this capacity, gene, transcriptome, and metabolite resources gleaned from this project will be distributed to the user community through the respective JGI and other public depositories. In addition, the research team would share project findings and resources at regular JGI and other

relevant international meetings, including the annual American Society of Plant Biologists (ASPB) and TERPNET meetings, to foster knowledge exchange across the larger community for accelerating research and application toward an increasingly bio-based future.

Sample preparation:

(i) A total of ~200 Kbp of DNA synthesis is requested for generating ~130 TF genes for functional analysis. Target sequences will be submitted in an excel sheet conforming to JGI requirements, including information on cDNA, protein, and vector sequences, codon optimization, and other modifications such as synthesis of TFs with fused HA-tags to monitor protein levels in N. benthamiana assays. The PIs will provide any necessary vectors for Y1H assays in Saccharomyces cerevisiae (pDEST-AD-2μ, D-TOPO)[16] and for co-expression assays in N. benthamiana (pEAQ-HT [36] and as derived vectors carrying targeted TPS/P450 promoters). Maize TF sequences for DNA synthesis will be provided upon start of the project, followed by switchgrass and sorghum TF sequences once these have been identified approximately within the first 2-3 month of the project.

(ii) For transcriptomics studies, root and leaf tissue of switchgrass (Alamo AP13, Cave-in-Rock) and maize (B73, Mo17) varieties with contrasting terpenoid contents will be analyzed after stress treatment. Pathogen treatment will follow established protocols using Fusarium graminearum as a major pathogen with a broad host range across monocot crops [1,26]. Abiotic stress will be applied for 2-4 weeks of continuous drought following established approaches for maize and switchgrass [27,37]. Total RNA will be prepared from plant tissue following JGI guidelines using established, commercial extraction kits. To ensure high RNA integrity, samples will be assessed using Bioanalyzer 2100 RNA Nano-chip assays according to JGI QC protocols. Samples not meeting the quality threshold would be re-extracted. Sample metadata will be completed as required and RNA of the required 3 μg quantity and 40-1,000 ng/μg concentration will be prepared in RNase-free water and barcoded tubes or plates provided by JGI, and shipped on dry ice after receipt of approval. We estimate to ship RNA samples within 3-4 months upon start of the project to allow for sufficient time for plant treatment experiments and sample preparation.
For nonpolar metabolite profiling using the JGI LC-MS/MS platform, metabolites will be extracted from plant material using methanol and dried for shipment to the JGI and analysis using MS and MS/MS fragmentation patterns. Untargeted metabolite analysis will be conducted using in-house mass spectral databases and JGI's Pactolus platform for prediction of MS/MS fragments. In addition, linkage of metabolite and transcriptome data will be analyzed using JGI's MAGI (Metabolites, Annotations, and Genes Integrated) pipeline for integration of transcriptomic and metabolomic data.

(iii) DAP-seq resources are requested for mapping of binding sites for 45 TF genes. To facilitate these assays DNA synthesis of ~70 Kbp is needed to generate the required affinity-tagged TF constructs as specified for the JGI platform. Genomic DNA libraries will be prepared in collaboration with the JGI using established protocols for library construction, genomic DNA fragmentation, and ligation with Illumina-based sequencing adaptors.

(iv) A total of ~130 Kbp of DNA synthesis is requested for generating combinatorial libraries for terpenoid pathway engineering. Optimal expression of targeted TPS and P450 genes will require codon optimization and, where applicable, removal of plastidial transit peptides and/or optimization of GC/AT composition. Optimized sequences as outlined above (ii) and established synthesized TPS and P450 genes for E. coli expression will be provided to the JGI upon start of the project to establish pathway libraries. Golden Gate construct assembly and E. coli strain engineering will be performed in collaboration with JGI using chassis-independent recombinase assisted genome engineering (CRAGE). Additional required resources and infrastructure for TPS/P450 co-expression and analytical capacity for product identification (GC/MS, LC/MS and NMR) are established in the PIs laboratories.