Towards an Efficient Multiallelic Gene Editing Platform for Highly Polyploid Sugarcane

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https://cabbi.bio/research/feedstocks-theme/

Project Goals

The goal of this project is to develop strategies to achieve high levels of multiallelic targeted mutagenesis in sugarcane using CRISPR/Cas9 and other RNA guided nucleases. An efficient genome editing platform for sugarcane will support metabolic engineering approaches to convert sugarcane into oilcane. Oilcane is a genetically modified sugarcane that hyperaccumulates lipids in its vegetative biomass (Zale et al. 2016; Parajuli et al. 2020). This project goal supports CABBI's "plants-as-factories" paradigm, in which biofuels, bioproducts, high-value molecules, and foundation molecules for conversion are synthesized directly in plant stems.

Abstract

Site-specific nucleases (SSNs) such as CRISPR/Cas9 have revolutionized crop improvement by allowing targeted and precise gene editing. Targeted mutagenesis by CRISPR/Cas9 relies on DNA targeting by a single guide RNA molecule (sgRNA) and DNA cleavage by Cas9 nuclease. This ribonucleoprotein (RNP) complex identifies the target DNA through complementarity to the sgRNA molecule only when located upstream of a protospacer adjacent motif (PAM) that is recognized by the Cas9 moiety. Cas9 has demonstrated high gene editing levels at a canonical PAM sequence of NGG. Alternative RNA-guided nucleases have different or less stringent PAM site limitations. Reducing the PAM site limitations of Cas9 will increase the targeting possibilities, which is particularly important for complex, highly polyploid genomes like the sugarcane genome (2n=10–13x=100–130). For instance, the Cas9 variant NG (NgCas9) has been engineered to require fewer contacts with the PAM sequence and use NG instead of NGG. Following the cleavage, the DNA double-strand breaks (DSBs) are repaired by cellular repair pathways including Non-Homologues End Joining (NHEJ) or Homology Directed Repair (HDR). NHEJ is an error-prone pathway that frequently results in indels, allowing us to construct knockouts with loss of gene function. In contrast, HDR depends on a repair template which can be co-delivered with editing reagents to introduce targeted nucleotide substitutions for gain of function.

Construction of loss-of-function phenotypes by targeted mutagenesis is more challenging in highly polyploid crops than in diploid crops. The large number of homeologs and homologs in sugarcane causes functional redundancy. However, this also offers the opportunity to generate a range of phenotypes depending on the number of co-mutated copies/alleles, similar to RNAi. Alternative approaches are needed to optimize and reevaluate gene editing reagents or their delivery to address the genetic complexity and redundancy in sugarcane. Therefore, we developed an approach that allows the generation of a rapidly scorable phenotype following multiallelic mutagenesis of magnesium chelatase (MgCh), a key gene in chlorophyll

biosynthesis. Through Sanger sequencing, we were able to detect more than 50 copies of MgCh in sugarcane. The disruption of the majority of copies of this gene by CRISPR/Cas9-mediated targeted mutagenesis resulted in yellow plants that display severe depletion of green pigment already when regenerating from tissue culture. In contrast to phytoene desaturase gene (PDS) mutants which display a dwarf and albino phenotype, MgCh mutants show yellow leaf color and no obvious growth retardation. This facilitates sampling of tissues for molecular analysis and allows us to exclude analysis of somaclonal variants that may resemble the albino phenotype of PDS mutants. Sequencing analysis of yellow MgCh mutants revealed co-editing frequencies of more than 70% of the MgCh copies/alleles. Differences in different editing outcomes and phenotypes using two sgRNAs and different tissue culture treatments will be discussed.

This efficient approach to monitor events with multiallelic co-editing will accelerate further optimizations to overcome PAM site limitations and improve delivery of reagents. We are currently using this approach to compare gene-editing efficiency of NgCas9 and Cas9 using the same sgRNAs. Efficient genome editing tools will support ongoing metabolic engineering approaches to convert sugarcane into oilcane with hyperaccumulation of lipids in its vegetative biomass.

References

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Funding Statement

This research was funded by the DOE Center for Advanced Bioenergy and Bioproducts Innovation (U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research) under award number DE-SC0018420. Any opinions, findings, and conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the U.S. Department of Energy.