

Targeted Precision Nucleotide Substitutions in Sugarcane Following CRISPR/Cas9 and Template Mediated Genome Editing Confer “Gain of Function”

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

Project Goals: We employ the “plants-as-factories” approach, in which biofuels, bioproducts, high-value molecules, and foundation molecules for conversion are synthesized directly in plant stems. This approach circumvents the challenges of developing efficient lignocellulose deconstruction methods, while still retaining residual biomass for deconstruction by traditional or emerging methods. The main thrust of this specific project within the Center for Advanced Bioenergy and Bioproduct Innovation (CABBI) is on genome editing and metabolic engineering of sugarcane. Sugarcane is the world’s highest biomass producer with demonstrated potential for accumulation of oil in vegetative biomass after metabolic engineering (Zale et al. 2016).

Abstract: Genome editing tools such as CRISPR/Cas9 and TALEN have been employed in several crop genomes, including sugarcane in the PI’s laboratory (Jung and Altpeter 2016; Kannan et al. 2018). They enable precise targeting and introduction of double stranded DNA breaks in vivo. Subsequent cellular repair mechanisms, predominantly non-homologous end joining (NHEJ), act as critical steps to endogenous gene editing or correction. However, there is very limited control over NHEJ, which generates an abundance of random insertions and deletions (indels) near the target site. Frameshift mutations associated with these indels of unspecified size and sequence might result in “loss of function” phenotypes of agronomic importance including improved feedstock quality (Kannan et al. 2018). “Gain of function” mutations, on the other hand, generally require precise nucleotide substitutions in the target locus. This can be accomplished with the aid of a homologous repair template and involves the cellular homology directed repair (HDR) mechanism. We are presenting an efficient HDR mediated genome editing approach conferring herbicide resistance in the highly polyploid sugarcane as an example for “gain of function”. This is not only the first report of targeted precision nucleotide substitution in the complex sugarcane genome but also represents a critical enabling technology for multiplexed genome editing to address cane improvement objectives in CABBI.

References

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Funding statement: This material is based upon work supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Pending Award for CABBI (Center for Advanced Bioenergy and Bioproduct Innovation) Proposal Number 0000227119.