Gene Targeting and Targeted Mutagenesis for Genetic Improvement of Oilcane

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

Project Goals:

1) Developing and deploying enabling bio-technologies for precision breeding of oil producing sugarcane.
2) Elevating the yield of biomass and vegetative lipids in sugarcane.

Abstract:

Genome editing tools such as CRISPR/Cas9 and other RNA guided nucleases (RGNs) have been employed in several crops. They enable targeted mutagenesis or gene targeting following DNA break repair by Non-Homologues End Joining (NHEJ) or Homology Directed Repair (HDR), respectively. NHEJ generates an abundance of random insertions and deletions (InDels). Frameshift mutations associated with these InDels of unspecified size and sequence might result in loss of function phenotypes of agronomic importance. Gain of function mutations, on the other hand, generally require precise nucleotide editing in the target gene for the replacement of inferior alleles with superior alleles by template mediated HDR. We present data confirming highly efficient HDR mediated precision nucleotide editing in multiple alleles of the acetolactate synthase (ALS) gene in the highly polyploid sugarcane which confer herbicide resistance. Faithful transmission of superior ALS alleles with introduced target mutations at 574 and/or 653 amino acid locations to vegetative progenies was confirmed with Sanger sequencing, PacBio SMRT sequencing and resulted in herbicide resistance.

Creation of knockout phenotypes is challenging in highly polyploid crops like sugarcane. We will present a rapid readout system that accelerates identification of multi-allelic edits by targeting candidate genes in chlorophyll biosynthesis. This system with facilitate the comparison of alternative RGNs and delivery systems.

Following successful demonstration of both, gene targeting and targeted mutagenesis in sugarcane, we are currently generating sugarcane lines with CRISPR/Cas9 mediated genome editing to suppress flowering and hydrolysis of triacyl glycerol (TAG). Extending the vegetative phase in sugarcane is expected to increase the yield of biomass and vegetative lipids. Targeted mutagenesis of the TAG lipase should further elevate lipid accumulation.

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