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mTREX2 Co-expression Promotes Large Deletions in CRISPR/Cas12a Editing of the Highly Polyploid Sugarcane Genome

Background/Objective

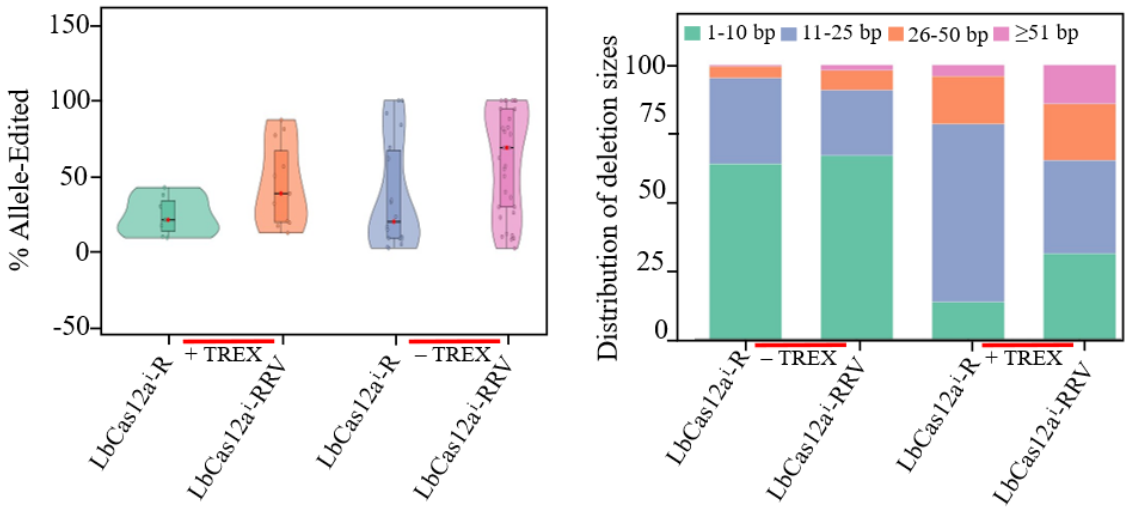
Sugarcane (*Saccharum spp.* hybrids) is a major feedstock for biofuel production and the primary source of global table sugar. However, its highly polyploid and complex genome constrains the effective deployment of functional genomics and precision breeding strategies. While CRISPR/Cas9-mediated genome editing has been reported in sugarcane, Cas12a-based editing remains unexplored despite its advantages in targeting AT-rich genomic regions.

Approach

This study reports the efficient CRISPR/Cas12a-mediated targeted mutagenesis of the *lemon white1* (*lw1*) gene in sugarcane. *lw1* is a key regulator of chlorophyll and carotenoid biosynthesis, and targeted *lw1* mutagenesis results in a visible phenotype. The researchers compared the editing performance of intron-containing LbCas12a-R (LbCas12a^{i}-R) and LbCas12a-RRV (LbCas12a^{i}-RRV) variants and assessed the impact of *Mus musculus* Three Prime Repair Exonuclease 2 (mTREX2) co-expression on editing efficiency and deletion profiles.

Results

Editing efficiencies ranged from 31 to 85%, and LbCas12a^{i}-RRV consistently outperformed LbCas12a^{i}-R in both overall editing efficiency and allelic editing efficiency. Co-expression of the mTREX2 shifted the mutation spectrum toward larger deletions, increasing the frequency of moderate-to-large deletions. The ≥ 51 bp deletion class increased ~7-fold in both Cas12a variants. Capillary electrophoresis-based genotyping provided a cost-effective, high-throughput alternative to next-generation sequencing and accurately predicted allelic editing efficiency and indel size.



Allelic editing efficiencies (left panel) and deletion profiles (right panel) when comparing Cas12a variants (R and RRV) with or without co-expression of Three Prime Repair Exonuclease 2 (mTREX2)

Significance/Impacts

This work demonstrates the first example of CRISPR/Cas12a-mediated genome editing in sugarcane and provides a framework for future engineering of AT-rich cis-regulatory elements in the polyploid sugarcane genome.

Ali et al. 2026. "mTREX2 Co-expression Promotes Large Deletions in CRISPR/Cas12a Editing of the Highly Polyploid Sugarcane Genome." *Plant Cell Reports.* DOI: 10.1007/s00299-026-03935-1.