BRC Science Highlight
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CRISPR/Cas9-Mediated Multi-Allelic Gene Targeting in Sugarcane Confers Herbicide Tolerance

Background/objective

Sugarcane (*Saccharum* spp. hybrid) is the source of 80% of the sugar and 26% of the bioethanol produced globally. However, its complex, highly polyploid genome (2n = 100 – 120) impedes crop improvement. Genome editing with sequence-specific nucleases (including CRISPR/Cas9) is revolutionizing crop breeding and has promising applications for sugarcane and other vegetatively propagated polyploid crops with complex genomes. This study explored homology-directed repair (HDR)-mediated gene targeting (GT) in sugarcane, which allows for the introduction of precise genetic modifications, including single-nucleotide substitutions, gene replacements, and large insertions.

Approach

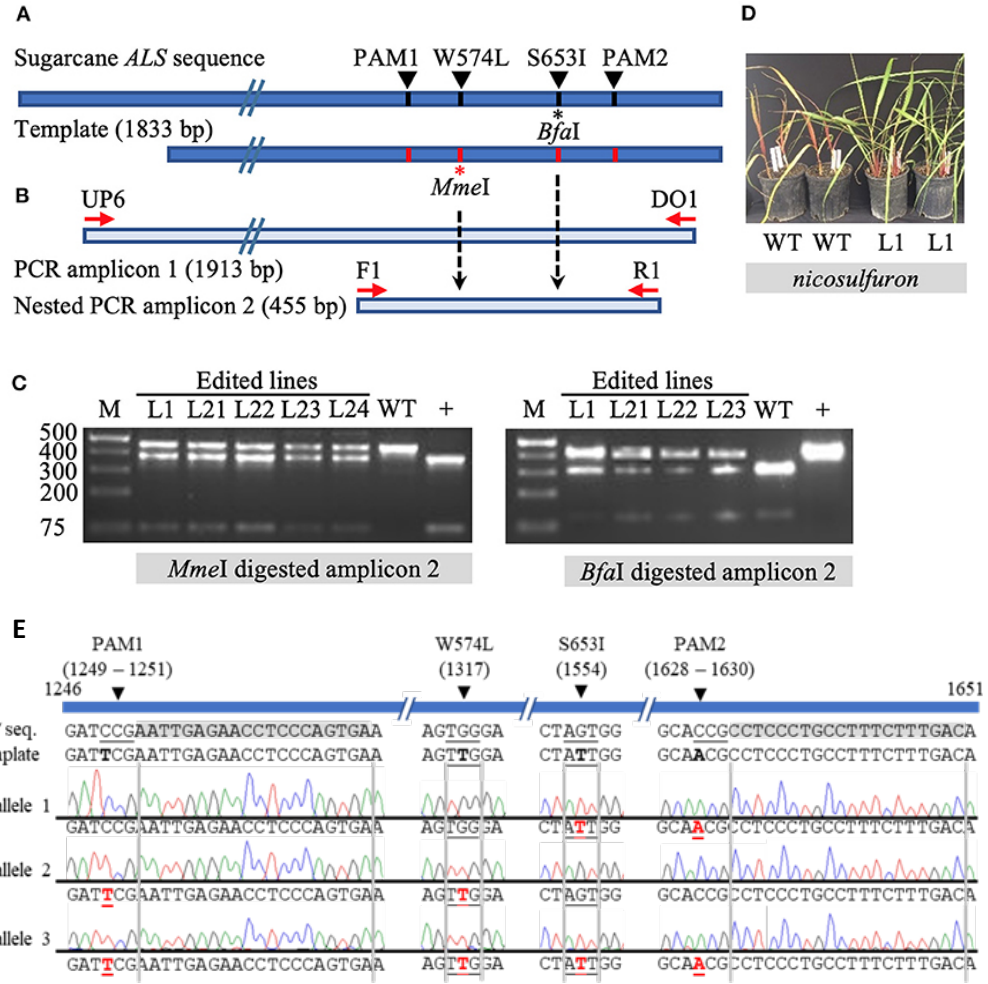
- Plasmids carrying expression cassettes and repair template were constructed and introduced into sugarcane callus through biolistic gene transfer for indirect embryogenesis.
- The initial screening of target mutations was completed via restriction enzyme (RE) digestion, followed by Taqman based SNP genotyping, SANGER sequencing of cloned target gene amplicons and herbicide application.

Results

- The evaluation of 146 independently transformed plants from five different experiments revealed 54 lines with a targeted nucleotide replacement that resulted in one or two targeted amino acid substitutions W574L and/or S653I in the acetolactate synthase gene.
- Co-editing of up to three acetolactate synthase copies/alleles that confer herbicide tolerance was confirmed by Sanger sequencing of cloned long PCR amplicons.

Significance

This study reported for the first time efficient and reproducible GT in sugarcane. This work will enable crop improvement by modifying inferior alleles to superior alleles through multiplexed gene targeting and without the linkage drag associated with conventional breeding.



Identification of gene targeting in sugarcane by restriction endonuclease assays, Sanger sequencing and herbicide application.

Oz, M.T., et al. 2021."CRISPR/Cas9-Mediated Multi-Allelic Gene Targeting in Sugarcane Confers Herbicide Tolerance." Front. Genome Ed. 3:67356. DOI: https://doi.org/10.3389/fgeed.2021.673566.

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