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Comparison of Genotyping Assays for Detection of Targeted CRISPR/Cas Mutagenesis in Highly Polyploid Sugarcane

Background

Genome editing with sequence-specific nucleases holds tremendous promise for sugarcane breeding, an important biofuel feedstock. While DNA sequencing provides direct evidence of targeted mutagenesis, it is cost-prohibitive as a primary screening method. Most other methods of identifying mutant lines have not been optimized for use in highly polyploid species.

Objective/Approach

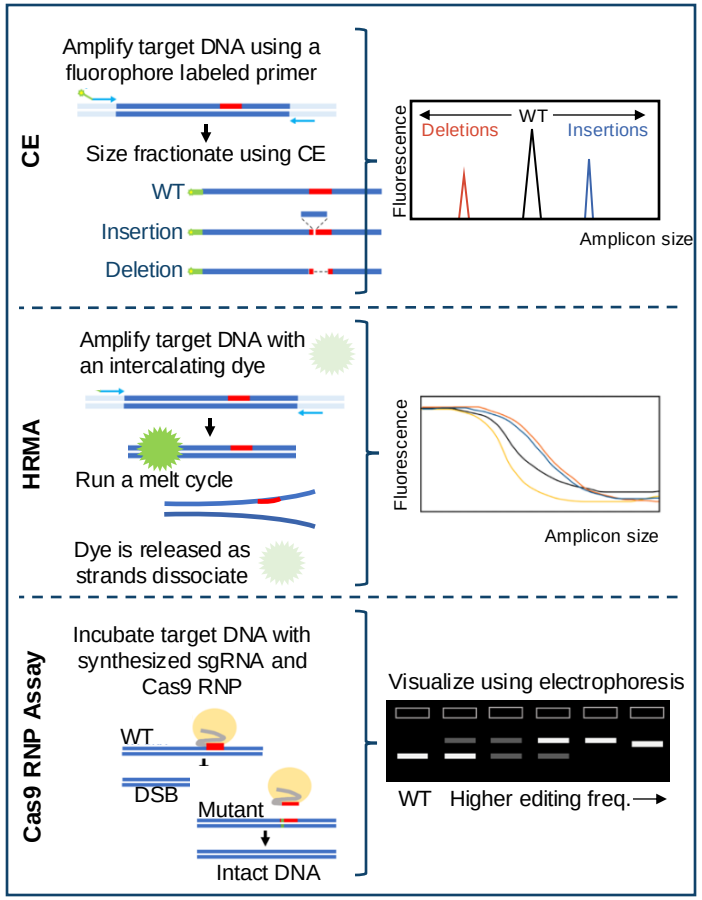
In this study, capillary electrophoresis (CE), Cas9 RNP assays, high-resolution melt analysis (HRMA), and next-generation sequencing were explored for their potential application in CRISPR/Cas9-mediated mutation screening at six sgRNA target sites.

Results

All three methods distinguished edited lines from wild type, with co-mutation frequencies ranging from 2% to 100%. Cas9 RNP assays were able to identify mutant sugarcane lines with as low as 3.2% co-mutation frequency. CE was highlighted as the most comprehensive assay, delivering precise information on both mutagenesis frequency and indel size to a 1 bp resolution across all six targets.

Significance/Impacts

This work represents an economical and comprehensive alternative to sequencing-based genotyping methods which could be applied in other polyploid species. While all three methods discussed are capable of screening mutant sugarcane lines, CE represents the most comprehensive, cost-effective method, providing information on individual indel frequency and size, with low labor requirements.



Methods of mutant screening investigated within the study, including capillary electrophoresis (CE), high resolution melt analysis (HRMA), and Cas9 RNP assay.

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