BRC Science Highlight
June 2021

Multiallelic, Targeted Mutagenesis of Magnesium Chelatase With CRISPR/Cas9 Provides a Rapidly Scorable Phenotype in Highly Polyploid Sugarcane

Background/objective

Sugarcane is a prime bioenergy feedstock candidate due to its productivity, but developing efficient genome-editing protocols for highly polyploid crops remains challenging due to the high level of genetic redundancy. Here, we describe CRISPR/Cas9-mediated targeted mutagenesis of the magnesium chelatase gene (MgCh), which is a high-copy gene in sugarcane and a key enzyme for chlorophyll biosynthesis. Specifically, this study explored whether targeted mutagenesis of magnesium chelatase subunit I with CRISPR/Cas9 provides a rapidly scorable phenotype for predicting the extent of multiallelic editing in highly polyploid sugarcane.

Approach

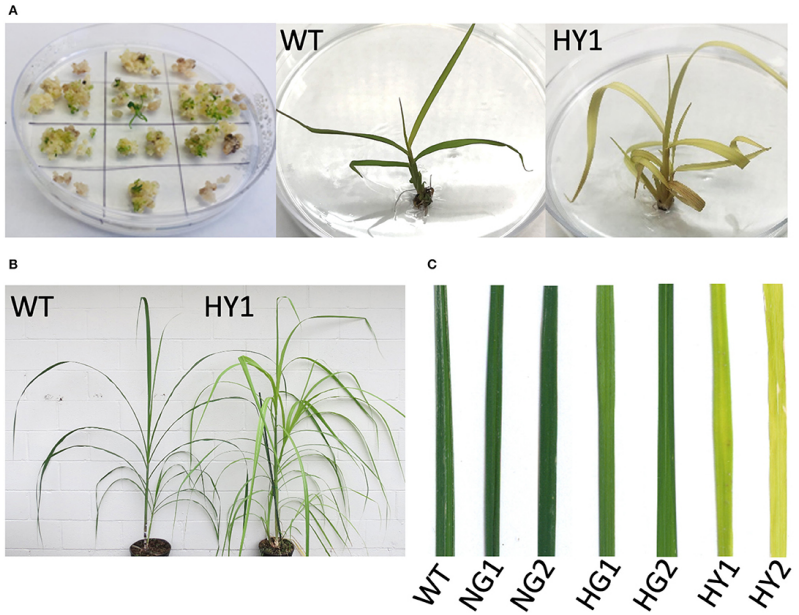
- Magnesium chelatase was used as a target due to potential for chlorophyll content as a scorable phenotype at the time of plant regeneration.
- CRISPR/Cas9-mediated targeted co-mutagenesis of 49 copies/alleles of magnesium chelatase was confirmed via Sanger sequencing of cloned PCR amplicons.

Results

- Heat treatment following the delivery of genome editing reagents elevated the editing frequency two-fold and drastically promoted co-editing of multiple alleles, which proved necessary to create a phenotype that was visibly distinguishable from the wild type.
- Despite their yellow leaf color, the edited plants were established well in the soil and did not show noticeable growth retardation.

Significance

- This study demonstrated an approach that will facilitate the establishment of genome-editing protocols for recalcitrant crops and will support important optimizations for the elevation of gene-editing efficiencies, including the evaluation of alternative tissue culture protocols, genome editing reagents, and their delivery.
- To the best of our knowledge, this report is the first to describe the targeted mutagenesis of MgCh in plants.



Detection of chlorophyll depletion phenotype during *in vitro* propagation. (A) Development of chlorophyll depletion phenotype compared to the WT. (B) Comparison of line HY1 (right) to WT (left) after establishment in soil under greenhouse conditions. (C) Close-up of leaves from NG1, NG2, HG1, HG2, HY1, and HY2 compared to WT.

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