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dCas9-3xSRDX-Mediated Transcriptional Repression in Sugarcane

Background/Objective

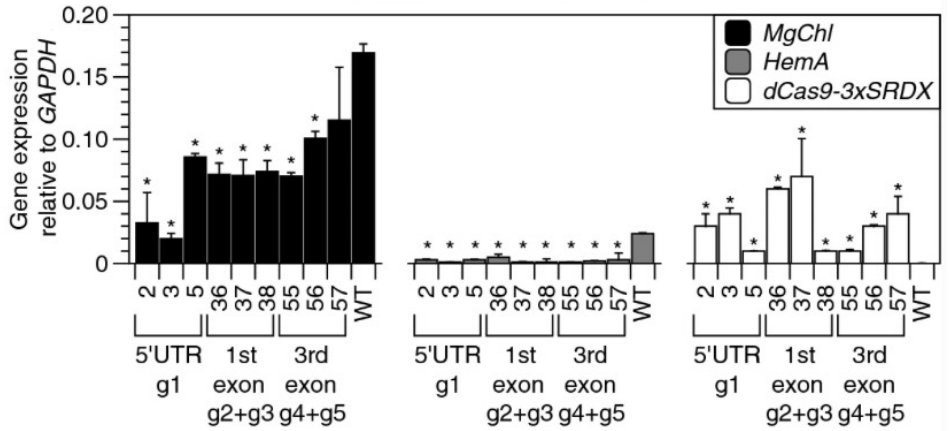
Sugarcane has the most complex genome of all crops and provides 80% of the world's sugar and 26% of the global bioethanol. CRISPRi is an approach that facilitates multiplexed targeted gene suppression, representing a very valuable tool for metabolic engineering. CRISPRi relies on reversible transcriptional repression by targeting the catalytically inactive Cas9 (dCas9) fused to a transcription repressor to a specific locus with the help of a sgRNA. CRISPRi has not yet been reported for sugarcane. Here we compare the response of dCas9-3xSRDX vectors targeted to different locations of the magnesium chelatase (MgChl) in stably transformed sugarcane.

Approach

We deployed CRISPRi for transcriptional repression of MgChl by using a C-terminal fusion of three copies of the SRDX repression domain from the ERF transcriptional factor with dCas9 to obtain dCas9-3xSRDX. Following gene transfer and selection on geneticin containing culture media, 88 independent transgenic events were regenerated to plants and confirmed by PCR.

Results

Targeting the 5′ UTR resulted in stronger MgChl suppression in our study than targeting the exons. Highly polyploid crops like sugarcane typically display greater sequence conservation in the 5′ UTR than in promoter regions, which facilitates co-suppression of multiple copies/alleles when targeting the 5′UTR for CRISPRi.



MgChl, HemA, and dCas9-3xSRDX expression (RT-qPCR) from the analyzed lines relative to GAPDH using the 2−ΔΔCT method.

Significance/Impacts

dCas9-mediated synthetic transcription repression is reported for the first time in sugarcane. Repression with CRISPRi leads to similar phenotypes as RNAi, but with higher specificity and multiplexing potential. This approach will benefit future metabolic engineering strategies.

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