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The Extent of Multiallelic, Co-Editing of LIGULELESS1 in Highly Polyploid Sugarcane Tunes Leaf Inclination Angle and Enables Selection of the Ideotype for Biomass Yield

Background/Objective

- Sugarcane (*Saccharum* spp. hybrid) is a prime feedstock for commercial production of biofuel and table sugar. Optimizing canopy architecture for improved light capture has great potential for elevating biomass yield. *LIGULELESS1* (*LG1*) is involved in leaf ligule and auricle development in grasses. However, confirming the putative sugarcane *LG1* locus and defining the optimal leaf angle in sugarcane is challenging.
- In this study, we demonstrate efficient, multiallelic, targeted mutagenesis of the putative *LG1* gene in sugarcane using CRISPR/Cas9. In contrast to previous *lg1* mutant studies, a range of leaf angle phenotypes was obtained depending on the co-editing frequency of *LG1*, allowing a more in-depth study of the trait.

Approach

Following identification of *LG1* allelic variants and construction of recombinant DNA vectors for targeted mutagenesis by CRISPR/Cas9, 16 gene-edited sugarcane lines were confirmed with co-editing frequencies of 7.4 to 100% of the NGS reads of *LG1*. *Lg1* mutant lines were evaluated in randomized greenhouse and field trials for leaf inclination angle, light penetration into the canopy, biomass accumulation, and biomass related traits.

Results

Greenhouse and field evaluation revealed the ideotype for leaf inclination angle with significantly increased biomass yield. The leaf inclination angle corresponded to light transmission into the canopy and tiller number. Line L35 displaying loss of function in ~12% of the *Lg1* NGS reads exhibited an 18% increase in dry biomass yield supported by a 56% decrease in leaf inclination angle, a 31% increase in tiller number, and a 25% increase in internode number.

Significance/Impacts

To the best of our knowledge, this is the first peer reviewed publication of CRISPR mediated sugarcane improvement under field conditions and the first report confirming the putative sugarcane *LG1* gene as a major determinant of leaf angle. This work also demonstrates efficient multiallelic editing of the *LG1* loci in sugarcane. The tuneable leaf angle phenotypes observed enabled selection of the ideotype for plant canopy architecture in highly polyploid sugarcane. In sugarcane, this trait holds great potential for enhancing crop yield.

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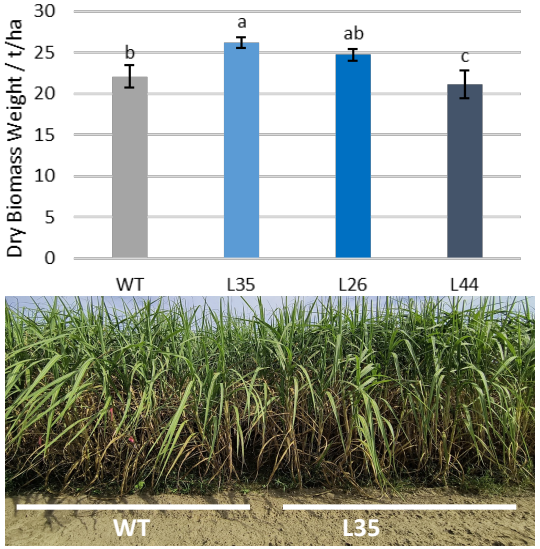

Sugarcane L35 displaying CRISPR-mediated loss of function in ~12% of the *Lg1* NGS reads exhibited a 18% increase in dry biomass yield under field conditions compared to unmodified sugarcane (WT).