

CRISPR/Cas9 Mediated Targeted Mutagenesis of *LIGULELESS-1* in Sorghum Provides a Rapidly Scorable Phenotype by Altering Leaf Inclination Angle

Background/objective

Sorghum (*Sorghum bicolor* L. Moench) is a versatile cereal crop used for food, feed, syrup, and as biofuel feedstock. Biotechnology approaches have great potential to complement traditional crop improvement. Earlier studies in rice and maize revealed that *LIGULELESS-1* (*LG1*) is responsible for formation of the ligule and auricle, which determine the leaf inclination angle. This study describes CRISPR/Cas9 mediated targeted mutagenesis of *LG1* in sorghum and phenotypic changes in mono- and bi-allelic *lg1* mutants. This research highlights *lg1* knockout as both a potential rapidly scorable phenotype and a promising target for sorghum crop improvement.

Approach

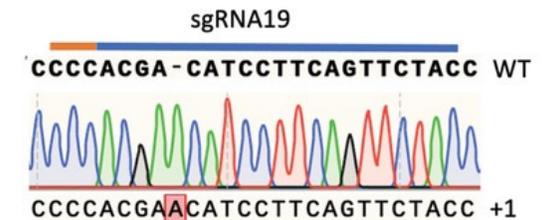
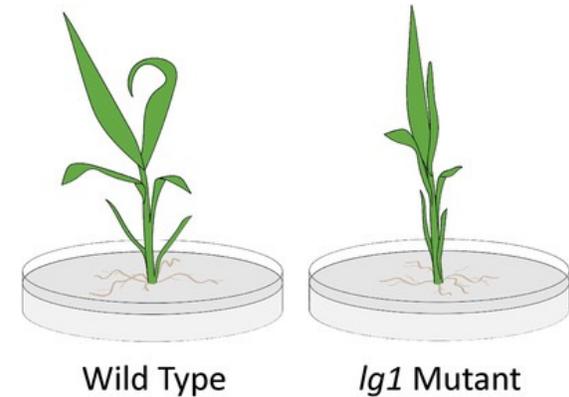
- ❖ *LG1* was retrieved as Accession ABXC03000000 in Phytozome v12.1. Two sgRNAs, sgRNA19 and sgRNA25, were designed to cleave within *LG1* Exon1.
- ❖ Tissue culture, selection, and regeneration of transgenic plants were carried out using biolistic gene transfer. Transgenic plants were grown to maturity in a greenhouse and self-pollinated for production of T1 generation seeds.
- ❖ Leaf inclination angles were measured using a protractor, with origin placed where leaf midrib meets stem.

Results

- ❖ Two sgRNAs, designed to target exon 1 of *LG1*, successfully cleaved the target DNA in an in vitro cleavage assay.
- ❖ Among the sequenced plants, both the monoallelic and biallelic *lg1* mutant plants exhibited distinct upright leaf phenotypes, with average leaf inclination angles ranging from 7.0°–12.9° and 2.0°–5.4°, respectively.
- ❖ These results confirm that targeted mutagenesis of *LG1* generates a rapidly scorable phenotype in sorghum, allowing phenotyping for edits within 6–7 weeks of bombardment.

Significance

- ❖ The present study demonstrates the first successful *lg1* knockout in sorghum using CRISPR/Cas9, producing a visually distinct phenotype for both monoallelic and biallelic edits.
- ❖ Targeted mutagenesis of *LG1* provides a rapidly scorable phenotype in tissue culture and will facilitate optimization of genome editing protocols. Altering leaf inclination angle also has the potential to elevate yield in high-density plantings.



CRISPR/Cas9 mediated targeted mutagenesis of the *LG1* gene provides a phenotype with more upright leaves, which is rapidly scorable in tissue culture and facilitates optimization of genome editing protocols.