

Background/Objective

Miscanthus, a C₄ member of Poaceae, is a promising perennial crop for bioenergy, renewable bioproducts, and carbon sequestration. Biotechnology-based procedures to genetically improve *Miscanthus*, to date, only included plant transformation procedures for introduction of exogenous genes into the host genome at random, non-targeted sites. This report demonstrates that the genomes of three *Miscanthus* species can be edited via the CRISPR/Cas9 system.

Approach

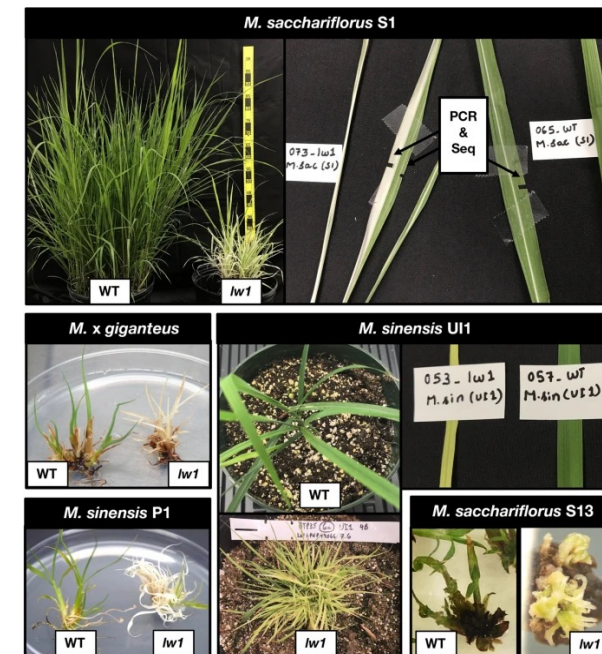
Using sequence information from both *Miscanthus* and sorghum, orthologs of maize *lw1* were identified. Embryogenic calli of *M. sacchariflorus*, *M. sinensis* and *M. x giganteus* were transformed via particle bombardment (biolistics) or *Agrobacterium tumefaciens* introducing the *Cas9* gene and three gRNAs to edit *lw1*.

Results

We developed gene editing procedures for *Miscanthus* using CRISPR/Cas9 that enabled the mutation of a specific (targeted) endogenous gene to knock out its function. Leaves on edited *Miscanthus* plants displayed the same phenotypes noted in *lw1*-edited maize (leaves were chlorotic and/or striped), and Sanger sequencing confirmed editing.

Significance/Impacts

We developed procedures for gene editing via CRISPR/Cas9 in *Miscanthus* and, to the best of our knowledge, are the first to do so. This included five genotypes representing three *Miscanthus* species. The designed gRNAs targeted all copies of *lw1* (homeologous copies and their alleles); results also confirmed *lw1* made a good editing target in species other than *Z. mays*. The ability to target specific loci to enable endogenous gene editing presents a new avenue for genetic improvement of this important biomass crop.



Leaf phenotypes in five *Miscanthus* genotypes transformed with gene editing vector pHA194.