Efficient Mutagenesis and Genotyping of Maize Inbreds Using Biolistics, Multiplex CRISPR/Cas9 Editing, and Indel-Selective PCR

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Background/Objective

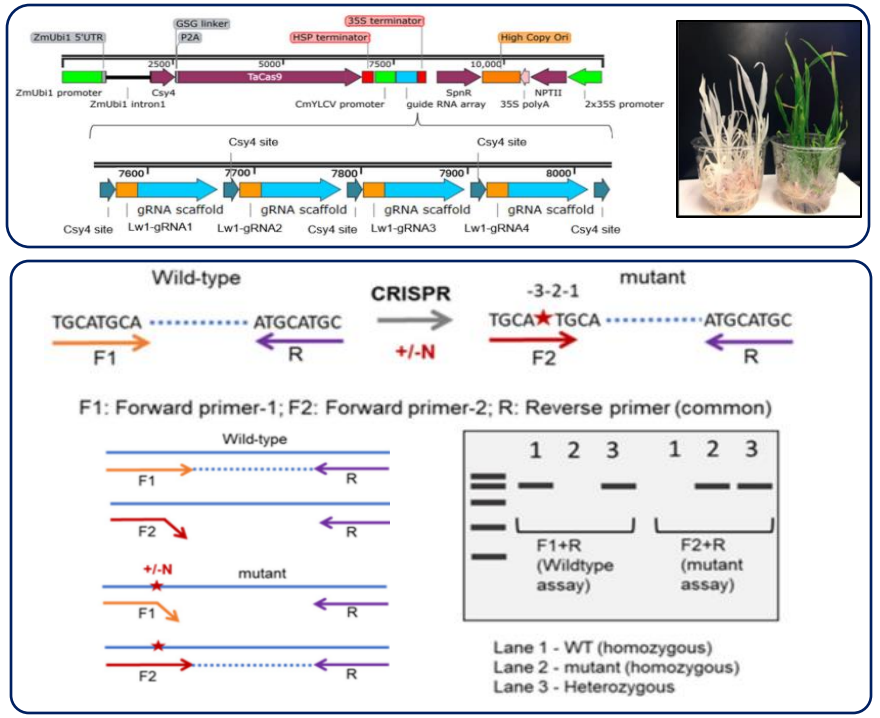
CRISPR/Cas9-based genome editing has advanced our understanding of a myriad of important biological phenomena. Important challenges to multiplex genome editing include assembly of large complex DNA constructs, few genotypes with efficient transformation systems, and cost-/labor-intensive genotyping methods. The current work presents an approach aimed to address each of the above challenges for multiplex genome editing in C_4 grasses.

Approach

Here we present an approach for multiplex CRISPR/Cas9 genome editing system that delivers a single compact DNA construct via biolistics to Type I embryogenic calli. We first demonstrate the creation of heritable mutations at multiple target sites within the same gene. Next, we successfully created individual and stacked mutations for multiple members of a gene family employing a compact Csy4 vector with multiple gRNAs. To design Indel-Selective PCR (IS-PCR) assay, we leveraged the observation that single nucleotide indels, created by CRISPR-Cas9, disrupts base-pairing of PCR primers at adjacent nucleotides and, therefore, disables PCR.

Results

Multiplex genome editing was achieved for both the highly transformable maize inbred line H99 and Illinois Low Protein1 (ILP1), a genotype where transformation has not previously been reported. Genome sequencing found off-target mutations were rare. In addition to screening transformation events for deletion alleles by PCR, we also designed PCR assays that selectively amplify deletion or insertion of a single nucleotide, the most common outcome from DNA repair of CRISPR/Cas9 breaks by non-homologous end-joining.



Multiplex genome editing of LW1 in ILP1 inbred maize line and Indel-Selective PCR assay design for genotyping.

Significance/Impacts

The IS-PCR method enables rapid and high throughput tracking of multiple edited alleles in progeny populations, as well as provides another option for the design of low-cost PCR assays to detect single-base edited alleles with a path for commercialization in the US and other countries that have declared these mutations as exempt from regulatory oversight. This 'end-to-end' pipeline can be applied to accelerate functional genomics of C_4 grasses in a broader diversity of genetic backgrounds. The Csy4 multiplex vectors and indel-selective PCR assays can also be used for genome editing of related grasses, such as sorghum or miscanthus.

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