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Selection is required for efficient Cas9-mediated genome editing in *Fusarium graminearum*

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Selection is required for efficient Cas9-mediated genome editing in *Fusarium graminearum*

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Abstract

Genome engineering using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated nucleases, such as Cas9 (CRISPR-associated protein 9), are revolutionising molecular biology. In this study, we established a Cas9-based genome editing system in *Fusarium graminearum*, a highly destructive fungal pathogen of cereal crops. Although the molecular toolkit of *F. graminearum* is well developed compared to other fungi, Cas9-mediated engineering offers a number of potential benefits, such as the ability to create marker free mutants in this species. Here we have used a codon-optimised Cas9 nuclease and dual ribozyme-based expression of a single guide RNA (sgRNA) to induce mutations. Cas9-mediated mutations were identified through a fungicide resistance-based phenotypic screen, which selects for null mutations in the *FgOs1* gene encoding an osmosensor histidine kinase. In the absence of selection, however, mutations were identified at very low frequency. Examination of the mutant alleles identified suggests that, a microhomology-mediated end joining (MMEJ) DNA repair pathway is likely to be the predominant process involved in erroneous repairing of Cas9-induced double-stranded breaks in *F. graminearum*.

Highlights

- Cas9-based genome editing is established in *F. graminearum*.
- In the absence of selection, mutation of target site is relatively rare.
- Microhomology-mediated end joining is the primary mode of erroneous DNA repair.

Keywords

CRISPR; Os1; Fusarium head blight; MMEJ