Application of CRISPR-Cas12a temperature sensitivity for improved genome editing in rice, maize, and Arabidopsis

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Abstract

Background

CRISPR-Cas12a (formerly Cpf1) is an RNA-guided endonuclease with distinct features that have expanded genome editing capabilities. Cas12a-mediated genome editing is temperature sensitive in plants, but a lack of a comprehensive understanding on Cas12a temperature sensitivity in plant cells has hampered effective application of Cas12a nucleases in plant genome editing.

Results

We compared AsCas12a, FnCas12a, and LbCas12a for their editing efficiencies and non-homologous end joining (NHEJ) repair profiles at four different temperatures in rice. We found that AsCas12a is more sensitive to temperature and that it requires a temperature of over 28 °C for high activity. Each Cas12a nuclease exhibited distinct indel mutation profiles which were not affected by temperatures. For the first time, we successfully applied AsCas12a for generating rice mutants with high frequencies up to 93% among T0 lines. We next pursued editing in the dicot model plant *Arabidopsis*, for which Cas12a-based genome editing has not been previously demonstrated. While LbCas12a barely showed any editing activity at 22 °C, its editing activity was rescued by growing the transgenic plants at 29 °C. With an early high-temperature treatment regime, we successfully achieved germline editing at the two target genes, GL2 and TT4, in *Arabidopsis* transgenic lines. We then used high-temperature treatment to improve Cas12a-mediated genome editing in maize. By growing LbCas12a T0 maize lines at 28 °C, we obtained Cas12a-edited mutants at frequencies up to 100% in the T1 generation. Finally, we demonstrated DNA binding of Cas12a was not abolished at lower temperatures by using a dCas12a-SRDX-based transcriptional repression system in *Arabidopsis*.

Conclusion

Our study demonstrates the use of high-temperature regimes to achieve high editing efficiencies with Cas12a systems in rice, *Arabidopsis*, and maize and sheds light on the mechanism of temperature sensitivity for Cas12a in plants.

Electronic supplementary material

The online version of this article (10.1186/s12915-019-0629-5) contains supplementary material, which is available to authorized users.

Keywords: CRISPR-Cas12a, Temperature, Genome editing, Rice, *Arabidopsis*, Maize