Testing Cas9/Cas12a Endonucleases to Improve CRISPR Editing Rates in Rice

Background

- Although CRISPR–Cas9 genome-editing provides precise gene editing in virtually all organisms, finding adequate target sequences that meet the specificities of a given Cas protein on a particular gene of interest is still challenging. This is often the case in plant species, which frequently have large and highly duplicated genomes. Therefore having a suite of Cas proteins helps to identify viable target sequences.

Approach

- We evaluated the efficiency and specificity of the five different Cas9/Cas12a endonucleases (SpCas9, SaCas9, St1Cas9, Mb3Cas12a, and AsCas12a) at 37°C (optimal temperature for most Cas9/Cas12a proteins) and 27°C (optimal temperature for plant tissue culture).
- Editing frequencies to knockout (KO) phytoene desaturase (PDS) were measured by visual screening for a detectable albino phenotype of rice embryogenic callus (Fig.1).

Results

- Among the five Cas enzymes tested, SpCas9 is the most efficient for editing (Fig. 2).
- Editing efficiency for SpCas9 (best performing Cas enzyme) and SaCas9 were similar between 27°C and 37°C while Mb3Cas12a showed relatively low editing efficiency at 27°C compared to at 37°C.
- St1Cas9 and AsCas12a showed no or minimal activity at both 27°C and 37°C.
- The callus visual screen was effective though Illumina-sequencing-based editing rates were generally higher.

Significance

- As SpCas9, SaCas9, and Mb3Cas12a all have a different PAM site to target by guide RNA, their use at the proper temperature provides flexibility when the number of adequate PAM sites is limited, as can happen in large complex plant genomes. This flexibility is needed to avoid off-target editing.
- The rice callus is shown to be effective for rapid screening of new CRISPR-Cas systems in plants.

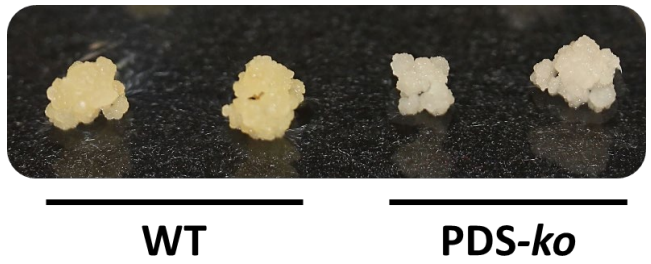

Figure 1. Representative rice embryogenic callus phenotypes. Wild-type (WT) calli are yellow-and PDS-knockout (PDS-ko) calli are white.

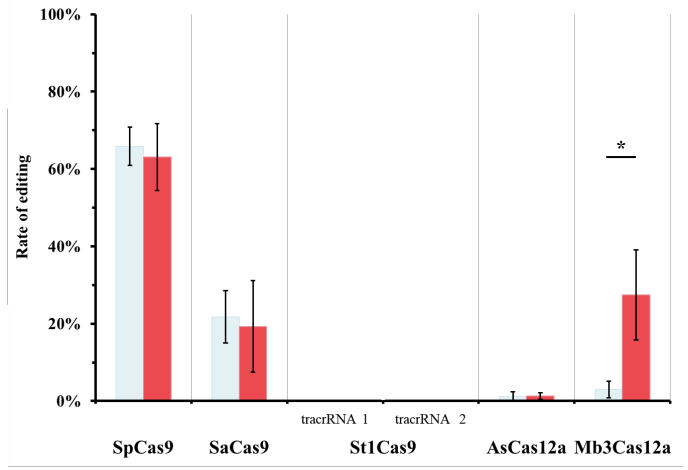

Figure 2. Gene editing efficiency (as percent of gene edits confirmed by sequencing) under different temperatures, 27°C (light blue) and 37°C (red). Significance p-value levels according to t-test are shown as ≤0.05 (*). Error bars from five replicates.

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