The use of natural and engineered CRISPR variants in plants

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Cas9 nucleases from *Streptococcus pyogenes* (Sp) is commonly used. Recently, we reported that Cas9 from *Staphylococcus aureus* (Sa) can be used for targeted mutagenesis in rice and tobacco. SaCas9 has higher sequence recognition capacity than SpCas9 and is useful for reducing off-target mutations in crop. The protein size of SaCas9 is smaller than that of SpCas9. This property lowers the size of the gene cassette required to express SaCas9. Furthermore, we show that SaCas9 can be split, and that split-SaCas9 can induce targeted mutagenesis in *Nicotiana benthamiana*. We are currently applying split-SaCas9 for the virus vector mediated targeted mutagenesis using two kinds of the virus vector system in tobacco.

Cpf1 is a newly characterized RNA-guided endonuclease that has two distinct features as compared to Cas9. Cpf1 utilizes a thymidine-rich PAM while Cas9 prefers a guanidine-rich PAM. Cpf1 generates DNA ends with a 5' overhang, whereas Cas9 creates blunt DNA ends after cleavage. Sticky DNA ends could increase the efficiency of insertion and substitution of a desired DNA fragments into the Cpf1-cleaved site using complementary DNA ends. We show that Cpf1 from *Francisella novicida* (FnCpf1) can be applied to targeted mutagenesis in rice and tobacco. We also show that FnCpf1 can be used targeted insertion of a short DNA fragment at specific site using PEG mediated protoplast transformation system.

CRISPR/Cas9-mediated genome editing systems require an appropriate protospacer adjacent motif (PAM) sequence at the target site. SpCas9 recognizes the shortest known PAM, NGG, but we show here that an engineered SpCas9 (SpCas9-NGv1) recognizing only NG as the PAM sequence can efficiently mutagenize the rice and Arabidopsis genomes. SpCas9-NGv1 nickase fused to the cytidine deaminase enabled C to T substitutions near 5' end of the 20-nt target sequence.