Trends in Plant Science

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Spotlight

CRISPR-Cpf1: A New Tool for Plant Genome Editing

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Clustered regularly interspaced palindromic repeats (CRISPR)-CRISPR-associated proteins (CRISPR-Cas), a groundbreaking genome-engineering tool, has facilitated targeted trait improvement in plants. Recently, CRISPR-CRISPR from *Prevotella* and *Francisella* 1 (Cpf1) has emerged as a new tool for efficient genome editing, including DNA-free editing in plants, with higher efficiency, specificity, and potentially wider applications than CRISPR-Cas9.

CRISPR-Cas forms part of the adaptive immune system of prokaryotes, and domestication of this system provided a revolutionary tool for plant genome engineering. Although developed recently, CRISPR-Cas has been established in important model and crop plants, such as rice, wheat, maize, tomato, potato, tobacco, cotton, soybean, and *Arabidopsis thaliana*. Important traits, such as cold tolerance, drought tolerance, herbicide resistance, yield increase, quality improvement, virus disease resistance, and fungal disease resistance, can be introduced and/or improved in economically important crops. Moreover, CRISPR-based approaches have produced DNA-free and/or nongenetically modified (GM) crops [1], which have modified traits but lack exogenous DNA; these crops have been approved for commercial production in the USA [2]. To date, most genome editing with the CRISPR system has used Cas9, a type II nuclease from *Streptococcus pyogenes*. In type II CRISPR-Cas in the prokaryote immune system, RNase III and the Cas9 protein are involved in the processing of the precursor CRISPR RNA (pre-crRNA) in the presence of the *trans*-acting crRNA (tracrRNA). The Cas9/guide RNA ribonucleoprotein complex also recognizes the target site and makes a site-specific double-stranded break (DSB). By contrast, in the type I and type III systems, the recognition and cleavage of target sites involve several Cas proteins. Given the simplicity and efficiency of the type II system, Cas9 proteins are widely used for genome editing. In the simplified system used for genome editing, the tracrRNA and crRNA are replaced by a single-guide RNA (sgRNA) [3].

Cpf1, a recently introduced class II type V endonuclease, has novel, superior features that SpCas9 lacks. First, and the most important from the perspective of gene editing in plants, Cpf1 generates cohesive ends with four- or five-nucleotide (nt) overhangs, compared with SpCas9, which produces blunt ends (Figure 1). These cohesive DNA ends should increase the efficiency of insertion of a desired DNA fragment into the Cpf1-cleaved site using complementary DNA ends through a mechanism known as 'homology-directed repair' (HDR). This should significantly enhance gene insertion at a precise genome location, a feature highly desirable but currently challenging in plants. Second, Cpf1 cleaves the target DNA molecule with a single crRNA that is shorter than the sgRNA for SpCas9 (43 nt versus ~100 nt). Therefore, Cpf1-mediated genome editing with a chemically synthesized crRNA can be achieved at lower cost than editing with SpCas9 and a synthetic sgRNA. Third, Cpf1 recognizes a T-rich protospacer adjacent motif (PAM); it requires 5′-TTTN-3′ (or 5′-TTTV-3′; V = A, C, or G, in some cases) PAM sequences, compared with the G-rich, NGG, PAM sequence in Cas9. In addition, Cpf1 recognizes a PAM that is 5′ instead of 3′ of the target site (Figure 1). Fourth, Cpf1 contains not only DSB-inducing activity, but also RNase III activity for pre-crRNA processing. This activity can be exploited for efficient multiplex genome engineering via tandemly arrayed pre-crRNA-expressing constructs that produce multiple mature crRNAs processed by Cpf1. Finally, one of the major shortcomings of the Cas9 is its high off-target activity, especially in mammalian cells. Although off-target activities are less of a concern in plants, studies have shown that Cpf1 exhibits little to no off-target activities in plant cells, consistent with reports in mammalian cells [4,5]. Among several proteins in the Cpf1 family, LbCpf1 from *Lachnospiraceae bacterium* ND 2006 and AsCpf1 from *Acidaminococcus* sp. BV3L6 function effectively [6].

Recent studies have shown the efficacy of CRISPR-Cpf1 for efficient and precise genome editing in plants. Endo *et al,* used Cpf1 from *Francisella novicida* (FnCpf1), which recognizes a shorter PAM (TTN) compared with known Cpf1 proteins, for targeted mutagenesis in tobacco and rice [7]. Yin *et al.* knocked out a positive regulator of stomatal development (*EPFL9*) using both Cas9 and Cpf1 in rice and concluded that, 'Despite the fact that low gRNA on-target activity was predicted by DESKGEN in the Cpf1 target site (7% probability for the Cpf1 system, vs. 69% for the Cas9 system), more than double the percentage of edited plants were observed with the LbCpf1 system in T_0' [8]. Wang *et al.* used the Cpf1 system for multiplexed gene editing in rice by editing four *OsBEL* genes [9], and Xu *et al.* also reported an efficient and heritable mutagenesis of *OsPDS* and *OsBEL* genes in rice plants. Hu *et al.* and Begemann *et al.* also reported similar results with CRISPR-Cpf1-mediated targeted mutagenesis in rice [10,11]. Kim *et al.* described the delivery of recombinant Cpf1 proteins with *in vitro* transcribed or chemically synthesized

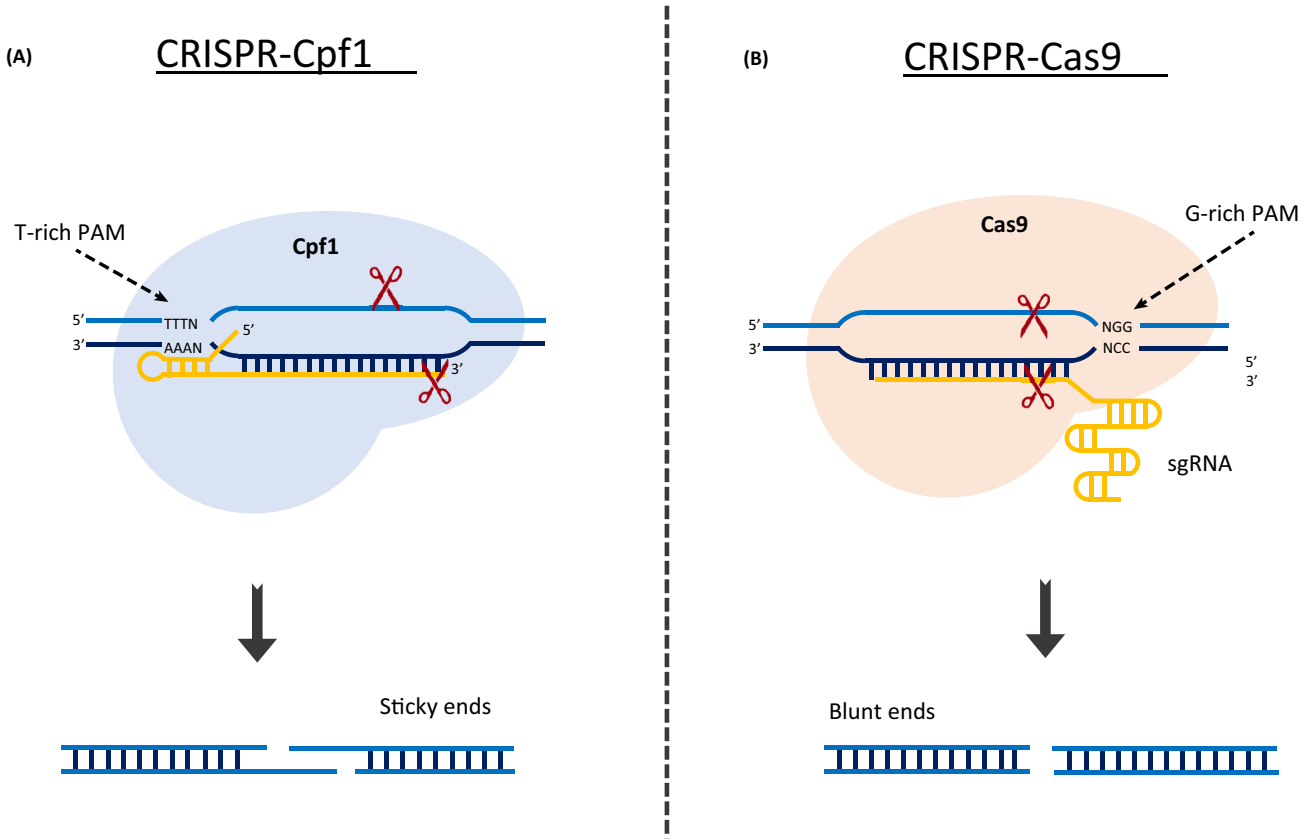

Figure 1. Plant Genome Engineering with Clustered Regularly Interspaced Palindromic Repeats (CRISPR)-CRISPR from *Prevotella* and *Francisella* 1 (Cpf1) Compared with CRISPR-CRISPR-Associated Proteins (Cas9). In a Cpf1-mediated plant genome-editing system (A), a T-rich region (TTTN) acts as protospacer adjacent motif (PAM) and creates double-stranded breaks (DSBs) distal from recognition site, with 5′ staggered ends. In a Cas9-mediated genome-editing system (B), a G-rich region (NGG) acts as PAM, creates DSBs proximal to recognition site (three nucleotides away) and produces blunt ends. In both cases, the DSBs are subsequently repaired by two major cellular mechanisms, nonhomologous end joining (NHEJ) and homology-directed repair (HDR).

target-specific crRNAs into protoplasts isolated from soybean and wild tobacco, and showed successful mutations with no observed off-target effects [12].

Further studies will explore the advantages and disadvantages of Cpf1 in plant genome-engineering applications. For example, although Cpf1 enables genome editing with the least off-target activity, its stringent PAM sequence limits the range of target sequences. Most of the Cpf1-mediated mutations observed in rice were relatively long deletions, compared with the common short indels [one to two base pairs (bp)] generated by Cas9. Moreover, instead of biallelic mutations usually carried out by Cas9, the mutated rice lines in which Cpf1 was used were either chimeric or heterozygous [5]. This suggests that Cpf1-induced mutants are largely somatic and are produced as a result of non-homologous end-joining repair on the 4–5-nt 5′ overhangs resulting from the staggered cutting of Cpf1 (Figure 1). However, further work should lead to plausable answers based on experimental evidence. The expression of Cpf1 in plants can be enhanced with codon optimization and the addition of a nuclear localization signal, translational enhancer, transcriptional terminator, and strong constitutive promoters; it is likely that a combination of a stable expression system and these additional tweaks will contribute to improving the genome-editing activity of Cpf1 in plant cells. In addition to Cpf1, at least 53 other class II CRISPR-Cas candidates have been characterized. Among them, C2c2 nucleases have the unique property of targeting single-stranded RNA. This offers the possibility of enabling gene-knockdown applications by targeting mRNAs. In addition, C2c2 also serves dual nuclease activity, similar to Cpf1, although the application of C2c2 in eukaryotes, including plants, remains to be explored.

Applications of genome editing in plants can broadly expand using CRISPR-Cpf1, facilitating approaches such as genome-wide functional screening based on gene knockouts, transcriptional repression using catalytically inactivated Cpf1 (dCpf1), or transcriptional activation using

dCpf1 fused with a transcription activator domain, analysis of chromatin dynamics using dCpf1 fused with fluorescent proteins, epigenome editing with dCpf1 fused to epigenetic modifiers, and the tracking of cell lineages with DNA-barcoding techniques. These advances expand the molecular toolbox of plant genome engineering and the possibilities for the targeted improvement of crop traits, such as yield, quality enhancement of produce, and input use efficiency, for ensuring sustainable food security.

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