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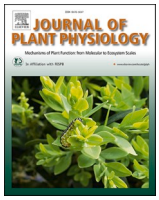

Sophisticated CRISPR/Cas tools for fine-tuning plant performance

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ABSTRACT

Over the last years, the discovery of various natural and the development of a row of engineered CRISPR/Cas nucleases have made almost every site of plant genomes accessible for the induction of specific changes. Newly developed tools open up a wide range of possibilities for the induction of genetic variability, from changing a single bp to Mbps, and thus to fine-tune plant performance. Whereas early approaches focused on targeted mutagenesis, recently developed tools enable the induction of precise and predefined genomic modifications. The use of base editors allows the substitution of single nucleotides, whereas the use of prime editors and gene targeting methods enables the induction of larger sequence modifications from a few bases to several kbp. Recently, through CRISPR/Cas-mediated chromosome engineering, it became possible to induce heritable inversions and translocations in the Mbp range. Thus, a novel way of breaking and fixing genetic linkages has come into reach for breeders. In addition, sequence-specific recruitment of various factors involved in transcriptional and post-transcriptional regulation has been shown to provide an additional class of methods for the fine tuning of plant performance. In this review, we provide an overview of the most recent progress in the field of CRISPR/Cas-based tool development for plant genome engineering and try to evaluate the importance of these developments for breeding and biotechnological applications.

1. Introduction

Since the beginning of plant cultivation, which is now faced with the challenges of feeding an ever growing mankind, efforts were undertaken to improve plant traits through breeding. The main objectives are to increase yield and quality, higher adaptability to abiotic stress factors and resistance to pests, diseases and herbicides. For thousands of years, the obtaining of improved cultivars of existing crops relied solely on the selection of plants with desired traits, following spontaneous mutations and recombination. Later on, new breeding methods such as cross-breeding and hybrid breeding were added which, however, are very time-consuming. In order to increase the frequency of DNA damage and thus to increase genetic variability, plants are treated with chemical or physical genotoxins and then selected according to the desired phenotype or genotype which is referred to as mutagenesis breeding. However, since this undirected mutagenesis leads to mutations in the entire genome, unwanted changes must be compensated for by tedious rounds of backcrossing (Pacher and Puchta, 2017).

From the mid-1990s onwards, the development of programmable sequence-specific nucleases, namely zinc-finger nucleases and transcription activator-like effector nucleases (TALENs), has allowed the site-specific induction of DNA double-strand breaks (DSB), triggering the endogenous repair mechanisms and therefore enabling modifications of the genome at sites of interest (Christian et al., 2010; Wright et al., 2005). These gene editing tools rely mainly on the repair of induced DSB via non-homologous end joining (NHEJ), the predominant repair pathway for DSB in somatic plant cells (Puchta, 2005). NHEJ is highly mutagenic since it involves partial end resection and religation of the free ends without the presence of any template. Therefore, DSB induction in the open reading frame of a gene of interest can be used to generate a knockout mutant, since the resulting repair pattern of small insertions and deletions often leads to frameshift mutations. Thus, sequence-specific synthetic nucleases have revolutionized the development of new traits by enabling programmable targeting of the majority of genomic loci. However, although important advances were made using zinc-finger nucleases and TALENs in plants, both have limitations making their application laborious (Voytas, 2013). This has changed with the characterization of the CRISPR (clustered regularly interspaced

Abbreviations: ABE/CBE, adenine/cytosine base editor; BE, base editing; GT/*ip*GT, gene targeting/ *in planta* GT; PE/PE2/3/3b, prime editing/prime editor 2/3/3b; pegRNA, prime editing guide RNA; RT, reverse transcriptase; UDG, uracil DNA glycosylase; UGI, UDG inhibitor.

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short palindromic repeats)/Cas (CRISPR-associated)-system in 2012 and its adaptation for gene editing purposes (Jinek et al., 2012). Due to its simplicity and precision it overtook the field and gave plant breeders a versatile tool which enabled applications far beyond DSB induction (Zhang et al., 2018).

2. CRISPR/Cas-mediated gene editing

The CRISPR/Cas system is an RNA-mediated adaptive immune system in bacteria and archaea aimed against bacteriophages and mobile genetic elements. It consists of two components, the CRISPR locus, a repeat-spacer array, and the Cas proteins. During the first infection of the cell, a fragment of the invading genome is integrated as a new spacer into the CRISPR locus. A re-infection leads to transcription of the CRISPR locus, resulting in a precursor CRISPR RNA (pre-crRNA) which is further processed into mature crRNAs. These are composed of a constant region and the spacer which is complementary to the foreign DNA. In the third phase called interference, the crRNAs mediate the sequence-specific induction of a DSB into the foreign DNA together with the Cas proteins (Barrangou and Marraffini, 2014; Hille and Charpentier, 2016).

2.1. CRISPR/Cas systems

As is the case with other defense mechanisms, due to constantly changing requirements, a variety of CRISPR/Cas systems has evolved which are divided into two classes with six types and 33 subtypes depending on their effector proteins (Makarova et al., 2020). Whereas in class 1 systems DSB induction is mediated by a protein complex, class 2 CRISPR/Cas systems use a single effector protein and are therefore of particular interest for the application in gene editing. However, class 1 type I CRISPR/Cas systems, which are characterized by the CRISPR-associated complex for antiviral defense (cascade) and the helicase-nuclease Cas3, are the most prevalent systems in nature. Thus, in recent studies the type I E and I C systems were adapted for gene editing in bacteria and human cells (Csörgő et al., 2020; Dolan et al., 2019). Most recently, a type I D CRISPR/Cas system from *Microcystis aeruginosa* was adapted for the targeted induction of short indels and long deletions in tomato (Osakabe et al., 2020).

Class 2 CRISPR/Cas systems are further divided into types II, V and VI (Shmakov et al., 2017). Type II CRISPR/Cas systems are characterized by the nuclease Cas9 (see Fig. 1). In addition to the crRNA, Cas9 requires another short RNA, the trans-activating crRNA (tracrRNA), which hybridizes with the pre-crRNA and is necessary for Cas9 and RNaseIII-dependent processing of the mature crRNA (Deltcheva et al., 2011). The sequence specificity of the DSB induction is dependent on 20 nucleotides at the 5'end of the crRNA which bind to the target via Watson-Crick base pairing. By linking crRNA and tracrRNA via a four-nucleotide tetraloop, a single-guide RNA (sgRNA) was created, resulting in a two-component system that directs Cas9 to the target sequence as efficiently as the dual RNA system (Jinek et al., 2012). Furthermore, the fusion of the two RNAs allows the use of several Cas9 orthologues with different target sequences in the same organism, since they only bind the orthologue-specific sgRNA, while heterologous tracrRNA:crRNA complexes can also be used in the dual RNA system (Chylinski et al., 2013; Fonfara et al., 2014). In addition, Cas9 requires a short orthologue-specific protospacer adjacent motif (PAM) immediately downstream of the target sequence in order to bind to the foreign DNA (Mojica et al., 2009). After binding to the target sequence, DSB induction is mediated by two nuclease domains, RuvC and HNH, which cleave the crRNA complementary and non-complementary strand, respectively 3 bp upstream of the PAM sequence. Inactivation of either of the two nuclease domains of Cas9 by inducing a specific mutation (D10A or H840A), results in a DNA nickase (nCas9) which can be used to induce single-strand breaks into the target or non-target strand, respectively. The mutation of both domains results in a catalytically inactive Cas9 (deadCas9, dCas9) which still binds specifically to the DNA and can be used as a platform to recruit various enzymes to sites of interest (Fauser et al., 2014; Guilinger et al., 2014; Jinek et al., 2012; Tsai et al., 2014). The two predominantly used Cas9 orthologues, Cas9 from *Streptococcus pyogenes* (*Sp*Cas9) and *Staphylococcus aureus* (*Sa*Cas9), recognize a 5'-NGG3' and 5'-NNGRRT3' PAM, respectively (Jiang et al., 2013; Steinert et al., 2015). Sequence specificity of DSB induction, mediated by *Sp*Cas9 and *Sa*Cas9, is provided by protospacers of 20 nt and 24 nt in length, respectively. However, it has been shown that SaCas9 provides robust activity with protospacer lengths between 20 and 24 nt as well (Friedland et al., 2015; Jinek et al., 2012). While those two systems show comparable activity in mammalian cells, *Sa*Cas9 has been shown to provide higher mutagenesis frequencies in plants

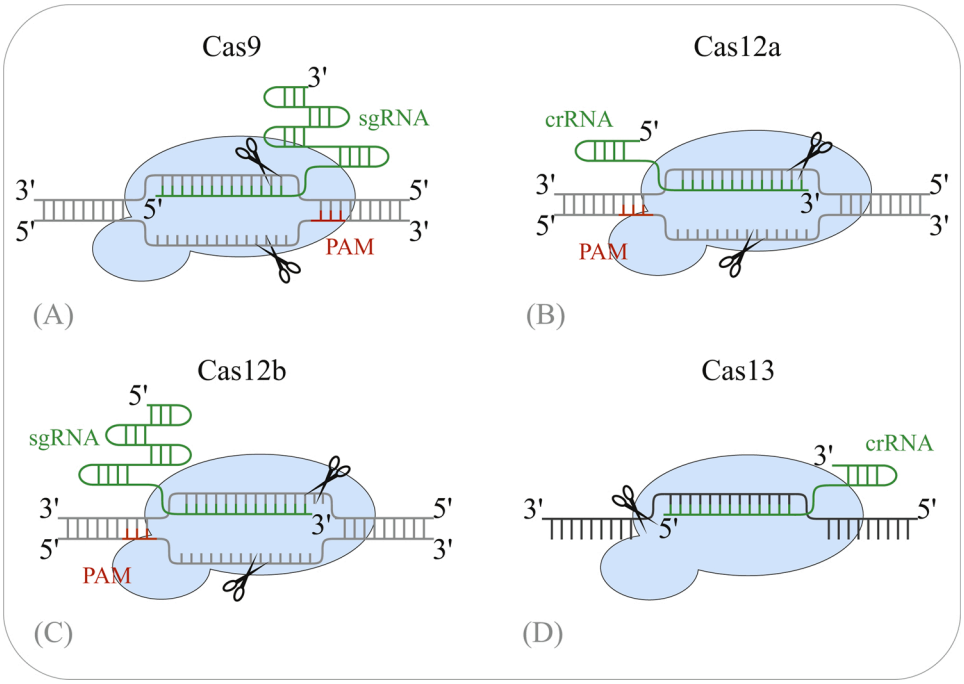

Fig. 1. Schematic structure of Cas proteins. (A) Cas9 is guided to the target site by the single guide RNA (sgRNA) and binds upstream of the protospacer adjacent motif (PAM). Each of the two nuclease domains of Cas9 cleaves one strand, the target and non-target strand, 3 bp upstream of the PAM, leading to a DSB with blunt ends. (B) Cas12a is guided to the target locus by a CRISPR RNA (crRNA) and binds downstream of the PAM. Each of the two nuclease domains of Cas12a induces one SSB, on the target strand and non-target strand, 18 and 23 nt downstream of the PAM sequence, creating sticky ends. (C) Cas12b combines properties of Cas9 and Cas12a. Like Cas9, it is guided by a sgRNA which is obtained from the fusion of a crRNA and tracrRNA. But in contrast to Cas9, like Cas12a, it requires a T-rich PAM and induces a staggered cut at the PAM distal end of the target sequence. (D) Cas13 is guided by a crRNA to its RNA target and induces a SSB mediated by two nuclease domains.

(Steinert et al., 2015). However, the efficiency of *Sp*Cas9-mediated mutagenesis in plants can be strongly enhanced by incubation at 37 °C (LeBlanc et al., 2018). Furthermore, several *Sp*Cas9 variants were engineered providing higher efficiency and lower off-target effects (Chen et al., 2017; Kleinstiver et al., 2016; Liang et al., 2018; Slaymaker et al., 2016). Recently, another Cas9 orthologue, Cas9 from *Streptococcus canis* (*Sc*Cas9), was characterized and shown to mediate efficient gene editing in plants at targets harboring 5'-NAG3' PAMs (Wang et al., 2020a).

In 2015, the class 2 type V—A CRISPR/Cas12a (formerly called Cpf1) system was characterized (see Fig. 1), which, while featuring similar characteristics to the CRISPR/Cas9 system, shows major differences making it a valuable addition to the existing gene editing toolbox (Zetsche et al., 2015). In contrast to Cas9, Cas12a requires a T-rich PAM upstream of the protospacer, which considerably increases the amount of potential target sites. Cas12a has a Nuc and a RuvC nuclease domain which cleave the target strand and non-target strand 18 and 23 nt downstream of the PAM sequence, respectively, resulting in a staggered cut with 5-nucleotide (nt) 5'-overhangs (Yamano et al., 2016). However, cleavage of the non-target strand is essential for cleavage of the target strand. Therefore, inactivation of the Nuc domain results in a Cas12a nickase whereas inactivation of the RuvC domain is sufficient to disable the DNA cleavage activity of Cas12a, resulting in a dCas12a (Yamano et al., 2016). Furthermore, Cas12a uses a shorter crRNA compared to the CRISPR/Cas9 system but requires a longer spacer of at least 22 nt to ensure maximum target specificity (Shmakov et al., 2017; Zetsche et al., 2015). Moreover, it does not require a tracrRNA and is able to process the pre-crRNA by itself (Fonfara et al., 2016). In plants, three Cas12a orthologues from *Francisella novicida U112* (*Fn*Cas12a), *Acidaminococcus spec. BV3L6* (*As*Cas12a) and *Lachnospiraceae bacterium ND2006* (LbCas12a) have been successfully used for targeted mutagenesis. It has been shown that, as in the case of *Sp*Cas9, increasing the temperature to 37 °C further enhances the activity of the latter two (Endo et al., 2016a; Moreno-Mateos et al., 2017; Tang et al., 2017). All three require a 5'-TTTV-3' PAM, although 5'-TTTT-3' PAMs can be recognized with lower efficiency by *Lb*Cas12a and *As*Cas12a (Kim et al., 2017b; Zhong et al., 2018). However, while Cas12a shows robust DNA cleavage activity in mammalian cells, its efficiency in plants varies between species and targets. This is mainly due to its reduced activity at lower temperatures, which are necessary for plant cultivation (Malzahn et al., 2019). To address this limitation, the group of Keith Joung established an enhanced, temperature-insensitive *As*Cas12a variant (en*As*Cas12a) which shows on average a twofold increase in activity at lower temperatures in human cells (Kleinstiver et al., 2019). However, en*As*-Cas12a was still outperformed by wild-type *Lb*Cas12a in *in vitro* assays at 25 °C. In contrast, the recently established temperature-tolerant variant of *Lb*Cas12a (tt*Lb*Cas12a) shows an up to sevenfold higher mutagenesis efficiency compared to wild-type *Lb*Cas12a at 22 °C, providing a powerful tool for gene editing in plants which need to be cultivated at lower temperatures (Schindele and Puchta, 2020).

Recently, the type V-B CRISPR/Cas12b system was characterized and adapted for gene editing in mammalian cells (Teng et al., 2018). Cas12b combines properties of both, Cas9 and Cas12a, and shows high target specificity with minimal off-target effects. Like Cas9, it requires a tracrRNA that can be fused with the crRNA to form a sgRNA, but similar to Cas12a it recognizes T-rich PAMs at the 5'-end of its target sequence. Furthermore, Cas12b induces staggered cuts distal from the PAM sequence resulting in 6–8 nt long 5'-overhangs (Teng et al., 2018). Most recently, the CRISPR/Cas12b system was engineered for gene editing in plants and was efficiently applied in both, mono and dicotyledons (Ming et al., 2020; Wu et al., 2020).

In contrast to type II and type V CRISPR/Cas systems, the class 2 type VI CRISPR/Cas13 system is characterized by a RNA-dependent RNase, which enables targeted cleavage of single-strand RNA (see Fig. 1). Break induction is mediated by two HEPN domains located on the outer surface of Cas13 and therefore cleave the target outside the bound recognition sequence (East-Seletsky et al., 2016). Although it has been shown that this also causes unspecific cleavage of non-target RNA-molecules *in vitro*, no such off-target activity could be found in eukaryotic cells (Abudayyeh et al., 2017; Liu et al., 2017). Like Cas12a, Cas13 only requires a crRNA which it is able to process from the pre-crRNA by itself. Although Cas13 does not require a PAM sequence, some orthologues prefer targets with a single-base protospacer flanking site (PFS) (Abudayyeh et al., 2016).

2.2. Expanding the targeting range through relaxed PAM requirements

Taken together, the natural diversity of CRISPR/Cas systems offers a wide range of potential target sequences. Nevertheless, PAM restrictions can still prevent access to some sequences. Therefore, several engineered Cas9-proteins have been developed to recognize various PAMs such as 5′-NGA-3′ (VQR-Cas9), 5′-NGMG-3′ (EQR-Cas9, VRER-Cas9), 5'-NG-3' (*Sp*Cas9-NG), 5'-NG-3', 5'-GAA-3' and 5'-GTA-3' (xCas9) or 5'-NNNRRT3' (*Sa*KKH-Cas9) (Hu et al., 2018; Kleinstiver et al., 2015a, b; Nishimasu et al., 2018). However, these variants still favor G-rich PAMs. In order to further broaden the targeting range of Cas9, the group of Yiping Qi therefore exchanged the PAM-interacting (PI) domain of *Sp*Cas9 with the PI domain from *Streptococcus macacae* (*Smac*Cas9), which recognizes 5'-NAA-3' PAMs. The resulting SpMacCas9, and its improved variant iSpMacCas9, show robust editing efficiencies in human cells and were both successfully transferred for the use in plants (Chatterjee et al., 2020; Sretenovic et al., 2020). By combining the mutations of xCas9 and *Sp*Cas9-NG, Niu et al. engineered a new variant called XNG-Cas9, which shows the broadest PAM compatibility in plants so far (Niu et al., 2020). Similarly to Cas9, *Lb*Cas12a and *As*Cas12a variants were engineered, expanding the targeting range to 5'-TYCV-3' and 5'-TATV3' PAMs (Cpf1-RR and Cpf1-RVR, respectively) (Gao et al., 2017). However, relaxed PAM requirements can also lead to more off-target effects due to the presence of more potential target sequences within the genome. Moreover, the presence of those sequences might delay binding of the Cas proteins to their actual target site, which can result in reduced efficiency (Globyte et al., 2019; Wang et al., 2019). Another limitation that has to be taken into account when using variants with almost no PAM restrictions, such as the recently developed *Sp*Cas9 variants SpG and SpRY, is that they might also cleave the sgRNA expression cassette, which could lead to an altered spacer sequence resulting in increased off-target effects (Walton et al., 2020).

2.3. Multiplexing

Another advantage of the CRISPR/Cas system is its particular suitability for multiplexing based applications, where multiple target sequences within one genome are targeted simultaneously (Le et al., 2013). Such an approach can be realized using multiple RNA polymerase III promoters, the increasing size of the construct being a limiting factor for this method (Xing et al., 2014). To overcome this problem and thus allow the simultaneous targeting of a large number of sequences, various systems have been developed to express multiple sgRNAs from a single synthetic gene. For this purpose, the sequences coding for the sgRNA were combined with tRNA sequences or self-cleaving ribozymes to enable processing of the individual sgRNAs by RNases or hammerhead ribozymes (Tang et al., 2016; Xie et al., 2015). However, since Cas12a is able to process crRNAs from the pre-crRNA by itself, it is naturally apt to multiplexing if provided with an artificial CRISPR array (Wang et al., 2017b; Zetsche et al., 2017). In a different approach, *Sp*Cas9 and *Lb*Cas12a were co-expressed with their respective gRNAs under control of a single Pol II-promotor in rice, resulting in efficient multiplex gene editing (Wang et al., 2018).

Harnessing the multiplexing capability of CRISPR/Cas, up to eight different sequences could be successfully targeted and cut simultaneously in rice, providing a broad spectrum of different genotypes and thus offering a large genetic diversity for selection (Shen et al., 2019).

Furthermore, multiplexing can be used for *de novo* domestication of wild plants within only one generation by recreating mutations of quantitative trait loci found in domesticated crops in a suitable genetic background. Thereby, desirable traits, such as stress tolerance, can be combined with agronomically valuable characteristics (Li et al., 2018; Zsögön et al., 2018).

However, targeting of protein coding sequences predominantly results in complete loss-of-function which can be accompanied by unwanted pleiotropic effects. In contrast, targeting of non-coding DNA sequences, containing binding sites for transcription regulating molecules, leads to a variety of alleles with different expression intensity, providing the possibility of fine-tuning gene dosage. Following this approach, Soyk et al. generated new, weakly expressed alleles of two genes in tomato plants resulting in increased yield and larger fruits, while a homozygous knockout of these genes led to a strong branching of the shoot axis and the development of sterile flowers (Soyk et al., 2017).

3. Base editing

CRISPR/Cas-mediated DSB induction has proven to be a valuable tool to increase the genetic variability at specific loci. However, many agronomical important traits are conferred by alleles with only one or a few base changes. Consequently, tools that can mediate single nucleotide changes without indel formation are of special interest for plant breeders.

In 2016, the development of cytosine base editors (CBE) enabled DSB-free base editing (BE) from C to T using a cytidine deaminase fused to a Cas9(D10A) nickase (see Fig. 2). The base substitution is mediated by the cytidine deaminase-dependent C to U conversion. A nick induced into the target strand promotes subsequent DNA repair of the U-G mismatch into a UA base pair that is further resolved to a stable T-A base pair. Initially low efficiencies due to uracil DNA glycosylase (UDG)-mediated uracil removal were overcome by adding an UDG inhibitor (UGI) to the protein complex (Komor et al., 2016). Similar to CBE, adenine base editors (ABE) enable A to G BE, since deamination of adenosine converts it to inosine, which is recognized as G by polymerases. Although there is no known adenine deaminase working on DNA, a tRNA-specific adenine deaminase from *Escherichia coli* (*ec*TadA) was engineered for this purpose (Gaudelli et al., 2017).

In order to increase the amount of potential target sequences, variants of both, CBE and ABE, were developed using Cas variants with relaxed PAM restrictions (Hua et al., 2019b, a; Sretenovic et al., 2020; Wang et al., 2020a). However, ABE with PAM variants showed reduced efficiency compared to the original *Sp*Cas9 or *Sa*Cas9-based ABE (Hua et al., 2019b).

Beside the Cas protein, the deaminase domains were also a target for improvement. In ABE, optimization of the adenine deaminase increased initially low efficiencies (Hua et al., 2020b). Moreover, two recently developed variants, ABE8 and ABE8e, show significantly increased activity in human cells and enhanced compatibility with engineered Cas variants (Gaudelli et al., 2020; Richter et al., 2020). Based on ABE8e, an ABE using the *Sp*Cas9 variant *Sp*RY was successfully used for BE at non-canonical PAM sites in rice (Xu et al., 2020c). Concerning CBE, the initially used rat APOBEC1-based cytidine deaminase showed strongly sequence motif-dependent editing efficiency. Using a human APOBEC3A-based CBE, high BE efficiencies independent of the target motif could be achieved in plants (Zong et al., 2018). However, CBE were shown to induce genome-wide off-target effects in mice and rice (Jin et al., 2019; Lee et al., 2020a). Two recently developed CBE based on the human APOBEC3B cytidine deaminase overcame this limitation and provide high BE efficiency and precision in rice (Jin et al., 2020). Similarly, editors based on the engineered hAPOBEC3G also reduce off-target effects while enabling editing within an enlarged editing window in mammalian cells. Furthermore, those CBE selectively edit the second C in 5'-CC-3' motifs. While their activity has not yet been shown in plants, their unique features may present interesting options for plant

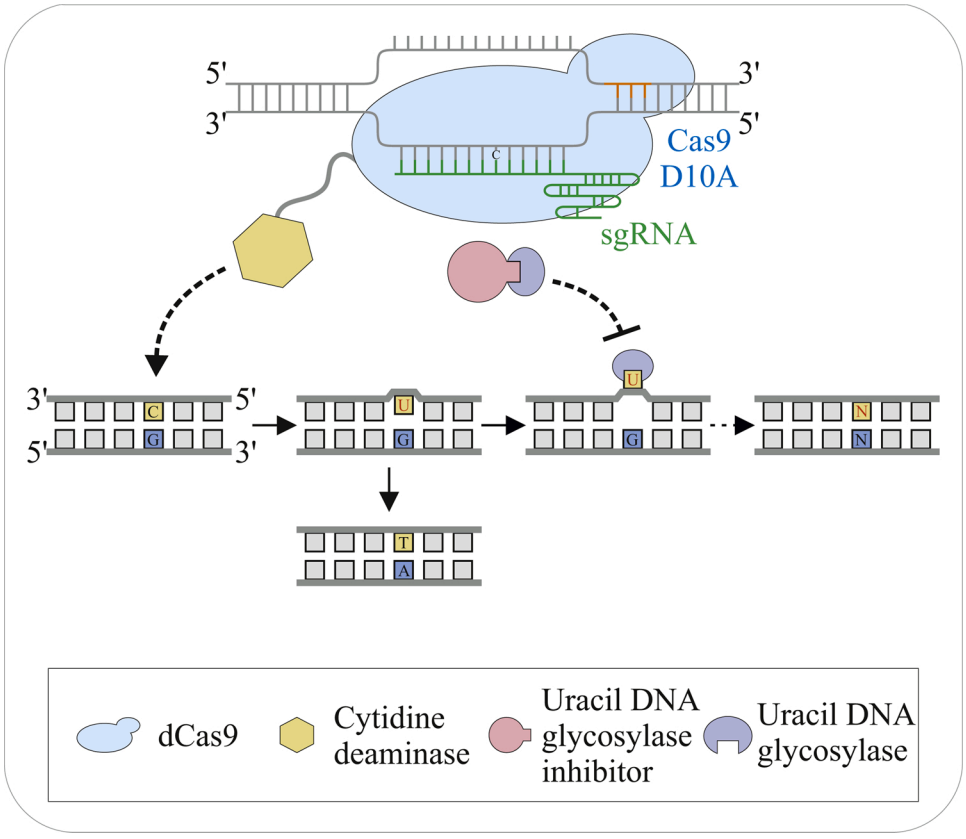

Fig. 2. Schematic illustration of base editing. After the cytidine deaminase has mediated the base substitution (C to U), the Cas9(D10A) nickase induces a SSB within the target strand. This promotes DNA repair of the U-G mismatch to U-A base pair and is further resolved to a T-A base pair. The uracil DNA glycosylase (UDG) inhibits the final step by removing the uracil. This can be prevented by adding a uracil DNA glycosylase inhibitor (UDGI). In contrast, recruiting of the UDG results in an abasic site which can then be nicked by a lyase. Induction of a second nick within the non-target strand can lead to the insertion of any base, with G being preferred for a yet unknown reason.

BE (Lee et al., 2020b; Liu et al., 2020). In contrast, ABE naturally show lower off-target activity as has been demonstrated in rice (Jin et al., 2019).

In order to enable simultaneous C and A BE at different targets, the group of Caixia Gao developed a multiplexing system based on viral RNA aptamers and their corresponding RNA-binding envelope protein (RBP). MS2, PP7, boxB or com RNA aptamers are either fused to the 3'-end of the sgRNA or integrated into the loop of the tetraloop or the stem loops. Through direct fusion of the respective viral envelope protein to a deaminase, the latter can then be recruited to the target sequence (Li et al., 2020b). However, for the purpose of directed evolution of desired loci, dual base editors are more promising (Sakata et al., 2019). In plants, simultaneous C to T and A to G conversions at a specific target site were recently enabled by combining a cytidine deaminase with an adenine deaminase to a dual base editor named STEME (saturated targeted endogenous mutagenesis editor) (Li et al., 2020a). Other CRISPR/Cas based tools for directed evolution include EvolvR, which relies on an error-prone DNA polymerase, and Target-G, which uses an engineered DNA glycosylase (Halperin et al., 2018; Nishida and Kondo, 2015). However, their activity in plants remains to be demonstrated.

The potential of BE for fine-tuning plant performance was recently demonstrated in a remarkable study in which Xing et al. used an APOBEC3A-based CBE to edit the upstream open reading frame of the conserved transcription factor *Fve*bZIPs1.1 in strawberry to generate a broad variety of genotypes resulting in a continuum of sugar content (Xing et al., 2020).

Although great advances have been made regarding efficiency, specificity and precision, the small editing window of BE remains a fundamental limiting factor for directed DNA diversification. APOBEC3A-based CBE achieved editing within an enlarged window between position 1 and 17 of the protospacer (counting the PAM-distal end as position 1) and through additional fusion of a single-strand DNA-binding domain the targeting range could be increased up to position 21 (Zhang et al., 2020). However, the more precise APOBEC3B-based CBE are limited to an editing window between position 4 and 8. Similarly, the editing window of most ABE ranges from position 4–8, although editing within an enlarged window, ranging from position 6–14, was achieved using a *Sa*Cas9-based ABE in plants (Hua et al., 2018; Zhu et al., 2020).

While CBE and ABE are only designed to induce base transitions, a remarkable new BE has been shown to efficiently induce C to G transversions in human cells. In contrast to CBE, those CGBE (C to G base editors) rely on the activity of an UDG attached to a CBE without an UGI to mediate the induction of an apyrimidinic (AP) site. Nearby-induction of a nick in the non-target strand leads to template-free repair at this site, which preferably results in the introduction of a G (Kurt et al., 2020). As the authors offered no explanation of the mechanism, we want to suggest a scenario that is able to explain the phenomenon: after deamination of the C, the resulting base, U, is eliminated from the DNA by host proteins, leaving behind an abasic site. Through a template-free reaction a translational polymerase might then incorporate a C opposite to this abasic site during replication. In the next replication cycle, this C serves as template for a G, resulting in the reported transversion. Although application of CGBE in plants has not yet been reported, occasional occurrence of unwanted transversions when using CBE in plants implements that it is only a matter of time (Li et al., 2017; Qin et al., 2020). While a similar approach in *E. coli* predominantly resulted in C to A transversions, repair of AP sites in plants is mediated by the translesion polymerase REV1, which preferably incorporates a C opposite the AP site (Sakamoto, 2019; Takahashi et al., 2007; Zhao et al., 2020). This suggests that C to G transversions can be expected in case of CGBE applications in plants.

Similarly, a recently developed set of tools for the induction of precise deletions, called APOBEC-Cas9 fusion-induced deletion systems (AFIDs), is also based on CBE mediated cytidine deamination and subsequent UDG-dependent creation of an AP site, which can then be nicked by an AP lyase. The resulting nick in combination with a nearby-induced DSB, leads to efficient deletion of the sequence between both strand breaks, as has been demonstrated in rice and wheat (Wang et al., 2020b).

4. Gene targeting by homologous recombination

Apart from NHEJ-based gene editing, HR-based methods are of special interest for genome engineering. While gene editing achieves mainly unpredictable mutations, HR-based methods enable precise and predefined modifications. Hence, gene targeting (GT) has the potential to become an attractive tool for plant breeding (Huang and Puchta, 2019). However, as NHEJ is the preferred DSB repair mechanism in somatic plant cells, the main obstacle is the marginal efficiency of HR (Puchta, 2005). Since GT has been established in plants, the main goal has been to enhance its efficiency to make it more attractive for practical applications in crops (Paszkowski et al., 1988). A major advance was the finding that the induction of a DSB can double HR frequencies at a target site (Puchta et al., 1996).

Various possibilities have been investigated to further enhance GT. The highest GT values were achieved by transforming protoplasts (Townsend et al., 2009; Wright et al., 2005). Unfortunately, the regeneration of protoplasts to fertile plants is only feasible in a few plant species. One promising system to apply GT is therefore the *in planta* GT (ipGT) approach which should be applicable to all transformable plant species independent of their transformation or regeneration efficiencies (Fauser et al., 2012). The ipGT relies on the simultaneous DSB induction at the target site and excision of a donor sequence for HR (see Fig. 3). This can be achieved by using a stably integrated T-DNA, containing a HR matrix and a nuclease expression cassette. The matrix contains sequences homologous to the target locus and the desired modifications. Moreover, it is flanked by recognition sequences required for excision. Recently it has been shown that the ipGT system is not only applicable to dicotyledons but can also be adopted for monocotyledons (Barone et al., 2020). Another innovative option to activate donor sequences for GT is to use geminiviral replicons (Baltes et al., 2014). For this purpose, the geminivirus genome is reduced to a minimum to ensure replication, and, thus, multiplication of the HR matrix. Geminiviruses require binding sites for the replication initiator protein (Rep) within a large and a small intergenic region (LIR, SIR) to initiate rolling-circle replication (see Fig. 3). It has been demonstrated that this setup is applicable to cereals as well as dicots (Atkins and Voytas, 2020; Baltes et al., 2014; Butler et al., 2016; Čermák et al., 2015; Gil-Humanes et al., 2017; Wang et al., 2017a). However, in contrast to tomato, no report has been published yet demonstrating the production of a fertile monocot, indicating that viral replication might interfere with regeneration. Moreover, application of the system in Arabidopsis failed, too (Hahn et al., 2018; de Pater et al., 2018). Only future experiments will show how many plant species are accessible for this system.

A novel kind of strategy relies on the consecutive use of two different DSB repair mechanisms and is referred to as tandem repeat homology-directed repair (TR-HDR) method (Lu et al., 2020). First, a double-stranded oligodeoxyribonucleotide (dsODN), containing homologies to the target locus as well as the desired base substitutions, is integrated into a CRISPR/Cas-induced DSB via NHEJ. Both strands at both ends of the dsODN are modified with phosphorothioate linkages which have been found to drastically enhance integration, due to the fact that nucleolytic degradation is minimized by these modifications. The sequence of the dsODN is designed in such a way that a tandem repeat structure arises at the genomic target after integration. In a second step, another DSB is induced at the newly formed target locus, which triggers single strand annealing between the tandem repeats. This pathway is highly efficient in plants and results in the desired change (Siebert and Puchta, 2002). Thus, by combining two mechanisms that each are more efficient than a classical DSB-induced GT reaction, the authors were able to achieve frequencies in a range attractive for

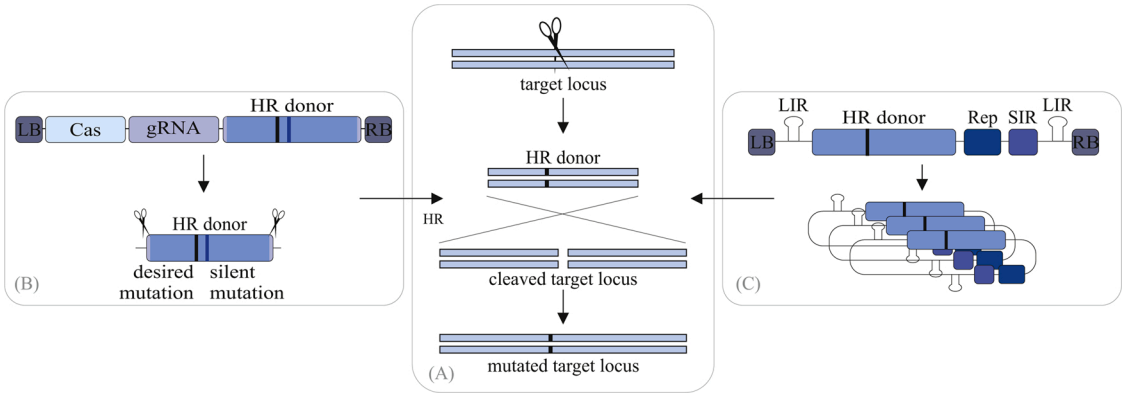

Fig. 3. Design and procedure of Gene Targeting using endogenous templates.
(A) After the DSB induction within the target locus, a donor is needed as a template for DSB repair via HR, leading to a mutated target locus. There are different possibilities for providing an endogenous HR donor. (B) The donor molecule can be provided via the "in planta gene targeting" system. Here, the donor sequence is located on a stably integrated T-DNA next to the nuclease and gRNA expression cassette and is activated by excision. (C) HR donor molecules can also be provided using geminiviral-based replicons. For this purpose, a T-DNA is transformed into plant cells containing the short and large intergenic region which is recognized by Rep to initiate its replication and thus multiplication.

practical applications. However, the strategy comes with a price: on one hand it is more time-consuming and on the other hand, due to the large amount of DNA supplied by particle bombardment in the first step, a lot of vector DNA is integrated elsewhere in the same genome, which might hinder the production of transgene-free mutants in the end. In this respect, the geminiviral as well as *in planta* approaches of GT are superior to TR-HDR. In case of ipGT, only a single copy of the vector is available in the cells, excluding simultaneous ectopic integrations. On the other side, geminiviruses seem to have a mechanism preventing their integration into the host genome, as this could result in virus-resistant plants.

In order to further push GT efficiencies there are different strategies to follow. Focusing on the DSB induction various nuclease were tested. As CRISPR/Cas is a rapidly developing field, new and more efficient nucleases could be characterized and their adoption for ipGT has been proven to be a promising strategy. For example, replacing *Sp*Cas9 (Schiml et al., 2014) with *Sa*Cas9 leads to improved GT efficiencies, as *Sa*Cas9 is more efficient in DSB induction in Arabidopsis (Steinert et al., 2015; Wolter et al., 2018). Recently, it has been shown that, by using *Lb*Cas12a, GT efficiencies can be further increased in Arabidopsis,

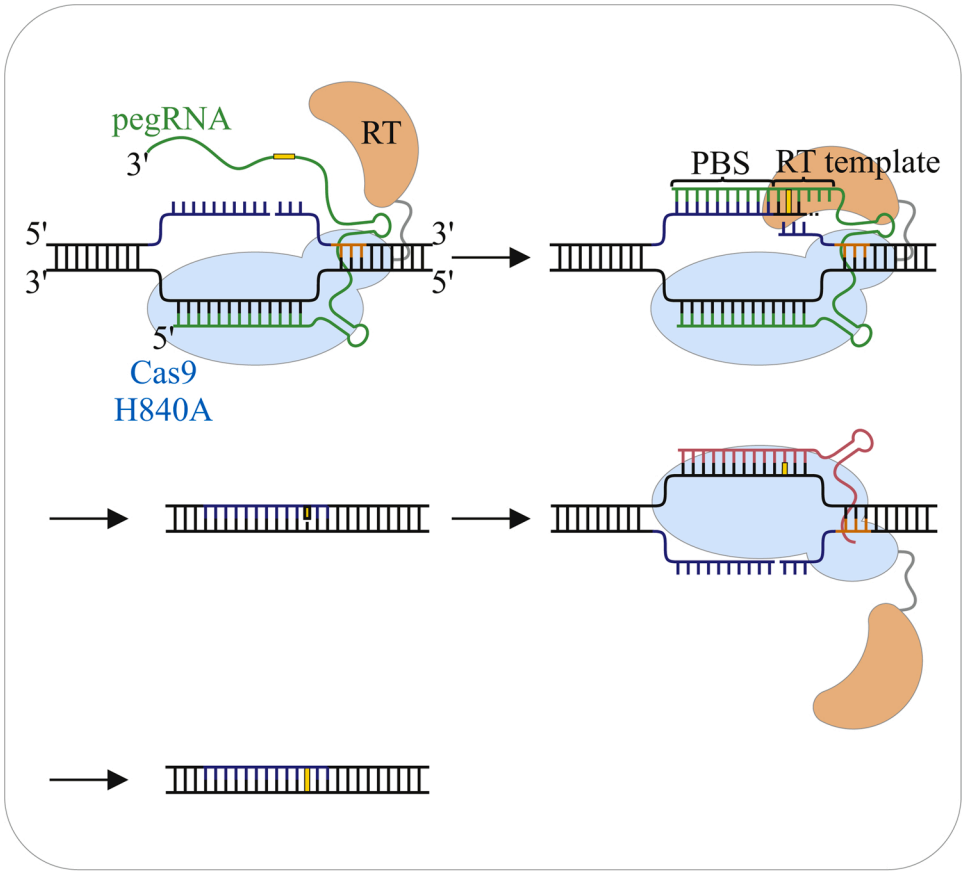

Fig. 4. Schematic illustration of the prime editing 3b system.
The prime editing system consists of a Cas9 (H840A) nickase directly fused to a reverse transcriptase (RT). The fusion protein is guided via the prime editing guide RNA (pegRNA) to the target site and binds to the nicked strand with its primer binding site (PBS). Here, the pegRNA functions as a template for RT, whereby mutations on the pegRNA are transferred to the target locus. A second SSB within the non-target strand is induced as soon as the target strand is edited, promoting the mismatch repair and using the edited strand as a template.

tomato and rice (Li et al., 2020d; van Vu et al., 2020; Wolter and Puchta, 2019). The latest achievement when testing Cas orthologues has been the application of the temperature-tolerant *Lb*Cas12a for ipGT, creating an attractive tool for plants that cannot cope with high temperature (Merker et al., 2020). Heritable GT events can only be achieved if the desired modification is transferred to the germline. A strategy enabling this efficiently in Arabidopsis is the egg cell-specific expression of the Cas nucleases (Miki et al., 2018; Wolter et al., 2018).

Focusing on the manipulation of the DSB repair machinery, GT efficiency could be enhanced by suppressing NHEJ. The aim of blocking NHEJ pathways is to elevate HR frequencies by shifting the imbalance towards HR. This can be done by e.g. knocking out key proteins of NHEJ. It has already been demonstrated that blocking of lig4 and ku70 can increase GT efficiencies (Endo et al., 2016b; Qi et al., 2013).

5. Prime editing

Recently, a new CRISPR/Cas-based tool for DSB-free genome editing, called prime editing (PE), was developed and has been shown to enable the induction of all twelve kinds of transition and transversion mutations, as well as targeted deletions of up to 80 bp and insertions of up to 44 bp in human and mouse cells (Anzalone et al., 2019). Prime editors consist of a *Sp*Cas9-H840A nickase which is fused to the M-MLV reverse transcriptase (RT) (see Fig. 4). A modified 3'-extended guide RNA, the pegRNA (prime editing guide RNA), directs the complex to the target sequence, binds to the nicked DNA strand with a primer binding site (PBS) and serves as template for the RT. Mutations that reside in the pegRNA are hereby transferred into the nicked DNA. Higher PE rates have been obtained using the PE2 system, in which the RT was engineered to improve efficiency. When the 3'-flap, containing the desired base changes, hybridizes to the unedited strand, the resulting heteroduplex can be resolved by DNA repair resulting in a stably edited dsDNA. In the PE3 system, a second nick is induced in the non-edited strand to increase the editing efficiency by promoting mismatch repair, using the edited strand as template. To further enhance the efficiency, the gRNA for the induction of the second nick can be designed to match the edited strand. Therefore, in this PE3b system, the second nick only occurs after a successful editing of the first strand. This makes the PE3b system especially interesting for application in crops, since paired nicks were shown to be highly mutagenic in plants (Schiml et al., 2016). However, first applications in rice, wheat and maize revealed that PE showed much lower efficiencies and higher restrictions concerning RT template and PBS length compared to experiments in mammals (Butt et al., 2020; Hua et al., 2020a; Li et al., 2020c; Lin et al., 2020; Tang et al., 2020; Veillet et al., 2020). While in human cells insertions of up to 44 bp were reported, RT templates of only 10–20 nt were successfully applied in plants. The efficiency of PE significantly decreases with increasing template length. Moreover, unlike previously shown in human cells, induction of a second nick using the PE3 or PE3b system did not increase PE efficiency in rice (Xu et al., 2020a). Attempts in which the M-MLV RT domain was exchanged with the RT domain of plant-specific Cauliflower mosaic virus or a retron derived RT from *E. coli* showed even lower editing efficiency (Lin et al., 2020). Raising the culture temperature from 26 °C to 37 °C has been shown to almost double the efficiency in rice protoplasts, however in another study no difference between incubation at 32 °C and 37 °C could be observed, underlining the strong target-dependent efficiency variability (Lin et al., 2020; Tang et al., 2020). Another limiting factor seems to be the expression level of pegRNAs, since in a recent study higher PE efficiencies could be achieved by enhancing it using a tRNA and ribozyme-flanked pegRNA expression cassette under control of an engineered promotor (Jiang et al., 2020). However, the desired edits were accompanied by a high amount of unwanted pegRNA scaffold-derived edits.

Taken together prime editing is a promising technology which surely will become an important addition to the CRISPR/Cas-based toolbox for gene editing in plants. However, great efforts are required to overcome current limitations and to keep up with longer existing methods, such as BE or GT. At this moment, BE provides similar editing windows with higher efficiency and precision for the induction of single base changes. Concerning sequence changes on a larger scale, no direct comparison between PE and GT has been reported yet, but taking the latest improvements in GT into account, PE at this moment is not able to outcompete GT in plants neither by efficiency nor by length of the inserted sequence.

6. Restructuring genomes

The natural variability within the plant genome forms the basis for classical plant breeding methods. However, the chromosome structure can hinder the transfer of associated traits. Especially inversions are a major obstacle, since they represent the most common large scale chromosomal rearrangements (CR) and were shown to suppress crossovers, thereby promoting reproductive isolation (Lowry and Willis, 2010). Furthermore, genetic linkages between agronomically beneficial and deleterious traits can prevent the achievement of the best possible breeding results. Therefore, the targeted induction of CR of all types is of special interest for breeders in order to reverse naturally occurring CR or induce new ones to stabilize or break trait linkages. A reversion of naturally occurring inversions can make quantitative trait loci (QTL) or resistance markers accessible for recombination and thus enable the transfer of such areas to different crop varieties.

It has already been shown a long time ago that the induction of two DSBs on the same chromosome can lead to the deletion of the sequence in between (Siebert and Puchta, 2002). Using the CRISPR/Cas system, efficient induction of targeted inheritable deletions of up to 120 kb was achieved in plants (Durr et al., 2018; Ordon et al., 2017; Wu et al., 2018). Recently, it was shown that induction of paired DSBs not only leads to targeted deletions, but can also be used to induce inheritable inversions, although at lower frequencies (Schmidt et al., 2019). In this way, a 1.1 Mb natural inversion in Arabidopsis could be reversed. Crosses between the edited line and a cultivar naturally lacking the inversion resulted in the restoration of crossovers within the previously enverted region (Schmidt et al., 2020). This approach should enable reversion of naturally occurring inversions in crop plants, making QTL or resistance markers accessible for recombination and, thus, enable the transfer of such areas to different crop varieties. The more direct approach to influence genetic linkages is the induction of crossovers between homologues. The group of Avi Levy was able to induce somatic HR based exchanges between two homologous chromosomes in tomato (Filler Hayut et al., 2017). While most identified events were gene conversions, a putative crossover event was detected as well. However, it could unfortunately not be transferred to the next generation. Although further work is needed, this study indicates that targeted crossovers may be possible in the future.

Most recently, it was demonstrated that large heritable translocations in the Mb range can be induced between two non-homologous chromosomes in Arabidopsis. Moreover, in some lines perfect translocation events with no additional mutations at the junctions could be identified (Beying et al., 2020). Thus, the CRISPR/Cas system has been shown to not only enable gene editing, but also chromosome engineering and promises to further revolutionize plant breeding by opening up new possibilities of genomic changes (see Fig. 5).

7. Manipulation of the transcriptome and epigenome

Besides protein engineering through the induction of sequence changes, manipulation of gene expression is another powerful approach for fine-tuning plant performance. Most synthetic transcriptional regulators are based on the sequence-specific recruitment of effectors mediated by a dCas protein (see Fig. 6). Early attempts in human and yeast cells used a direct fusion of the repressive KRAB domain and dCas9 to regulate gene expression. A fusion with VP64, a tandem repeat of four

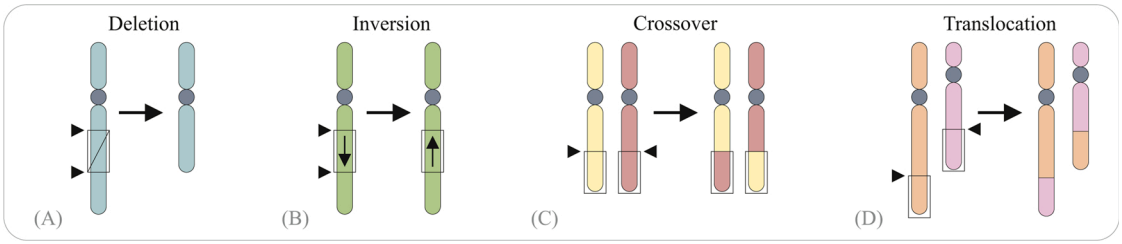

Fig. 5. Schematic overview of possible chromosomal rearrangements.
(A) Deletion: After the induction of two DSBs on the same chromosome the sequence between the DSB can be removed. (B) Inversion: After the induction of two DSBs on the same chromosome the part between can be inverted. (C) Crossover: After the induction of two DSBs on two homologous chromosomes crossovers can be initiated. (D) Translocation: After the induction of two DSBs on two non-homologous chromosomes an exchange of the chromosomal ends can be achieved.

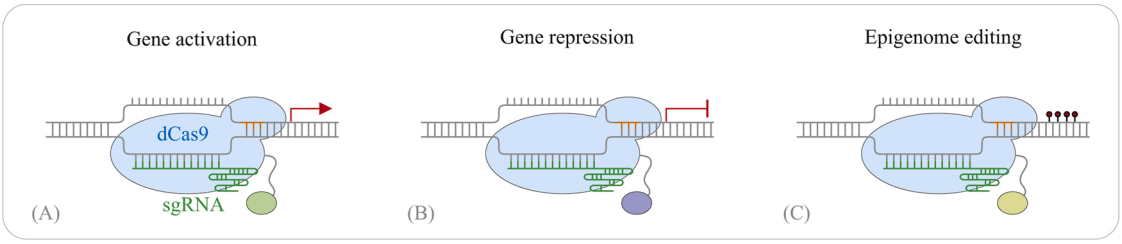

Fig. 6. Overview of applications of Cas-protein fusion-based genome editing.
The catalytically inactive deadCas9 (dCas9) is guided to a target locus by the sgRNA to bring the effector protein in close proximity of the gene of interest. (A) Gene expression can be stimulated by fusing a transcriptional activator to dCas9. (B) The fusion of a transcriptional repressor to dCas9 can be used to suppress expression of a specific gene. (C) Fusion of DNA methyltransferases or histone modifiers can be used to regulate gene expression through epigenetic modifications.

copies of the bacterial transcription enhancer VP16, demonstrated that efficient transcriptional activation is also achievable (Gilbert et al., 2013). This system, along with others using different activator domains such as EDLL or TAL, has been successfully transferred into plants. Transcriptional repression was achieved using a SRDX domain (Lowder et al., 2015; Piatek et al., 2015). However, low activation activity of the dCas9-VP64 system in plant cells has been reported in several studies (Lowder et al., 2018, 2017).

Later approaches used small RNA aptamers that were integrated in stem loops of the sgRNA to indirectly recruit effector domains fused to corresponding specific RNA-binding proteins (RBP) (Mali et al., 2013). This system enables the simultaneous addressing of several targets with different effector domains without the need for different Cas orthologues. Moreover, as two RBP bind to every aptamer, the amount of effector domains is increased at the target locus. This enhances the efficiency of transcriptional regulation since in earlier approaches several sgRNAs targeting the same site were shown to promote synergistic effects (Perez-Pinera et al., 2013; Tak et al., 2017). The integration of more aptamers into the loops or the 3'end of the sgRNA further enhanced transcriptional regulation (Konermann et al., 2015; Shechner et al., 2015). By now, a variety of different RNA-aptamer-RBP systems has been used for protein recruitment, including MS2-MCP, PP7-PCP and boxB-λN22 (Lowder et al., 2018; Ma et al., 2016; Shao et al., 2016). A VP64-based system using a combination of direct and indirect fusion systems has been shown to greatly enhance transcriptional activation in plants (Lowder et al., 2018).

Another approach for an increased dosage of effector domains is the SunTag system (SUperNova tagging system). It consists of a repeat of GCN4 epitopes which are directly fused to dCas9 and a corresponding antibody, scFv, fused to the effector domain (Tanenbaum et al., 2014). Recently, this system was used to drive robust activation of several loci in Arabidopsis by recruiting up to ten copies of the VP64-domain (Papikian et al., 2019). In human cells, a combination of the SunTag-system and the MS2-MCP RNA-aptamer-RBP system was successfully used to further enhance transcriptional activation (Kunii et al., 2018).

In a striking new approach, the group of Rodolphe Barrangou used the CRISPR/Cas class I type I—E specific CRISPR-associated complex for antiviral defense (cascade) to mediate efficient transcriptional activation in maize. The multi-subunit nature of this complex allowed to recruit three copies of the plant-specific transcription activator CBF1 and the authors suggested that more than 12 proteins could be fused to this system (Young et al., 2019).

Apart from transcription factors, the epigenetic state also plays an important role in the regulation of gene expression. Therefore, several CRISPR-based epigenetic modifiers were developed. Similar to synthetic transcriptional regulators, those systems are based on dCas9-mediated recruitment of effector domains, such as DNA methyltransferases or histone acetyltransferases. For example, using the mammalian acetyltransferase p300 or the histone demethylase LSD1, efficient transcriptional activation and repression were achieved, respectively (Hilton et al., 2015; Kearns et al., 2015). In a recent study, a SunTag-based system using the catalytic domain of the *Nicotiana tabacum* DNA methyltransferase DRM achieved site-specific gene silencing (Papikian et al., 2019). Interestingly, a recent study demonstrated that DSB induction-dependent gene editing also leads to altered DNA methylation status (Lee et al., 2019).

Another interesting approach is based on the induction of altered chromatin topology, since chromatin loops were shown to play a role in the regulation of gene expression (Guo et al., 2018; Matthews, 1992; Xu et al., 2020b). In human cells, enhanced gene expression was already achieved through formation of an artificial chromatin loop using a dCas9-based system with two dimerizable proteins (Morgan et al., 2017).

While the approaches mentioned before focus on transcriptional regulation, the CRISPR/Cas13 system enables modifications at the post-transcriptional level. In its native form, the RNA cleavage activity of Cas13 can be harnessed for post-transcriptional repression, and enables the lockdown of specific splicing isoforms (Cox et al., 2017; Mahas et al., 2018). Furthermore, analogous to base editing, a catalytically dead version of Cas13, fused to the adenine deaminase ADAR2dd, can be used for RNA editing. Initially, high amounts of off-target effects were

observed. They were overcome through engineering of the deaminase domain. Furthermore, an engineered version of this deaminase enables C to U BE (Abudayyeh et al., 2019). Since in contrast to DNA base editing RNA editing is not stable but reversible, this system allows delicate fine tuning of the expression of genes of interest (Cox et al., 2017).

A caveat for the application to crop plants of at least most CRISPR/Cas-based approaches for changing the transcriptome or epigenome is the fact that they require the constant presence of a transgene. Thus, in contrast to plants with simple mutations induced by the various CRISPR/Cas system described in the previous section, such plants have to be classified as genetic modified organisms (GMO) and regulated accordingly.

8. Conclusion and perspectives

The discovery of various natural and the establishment engineered CRISPR/Cas nucleases in recent years, and the tools based on them, allows us to make specific changes at almost any location in the genome [for overview of CRISPR/Cas based tools see Pramanik et al., 2020]. From the site-specific increase of genetic variability to the induction of large genomic restructuring, newly developed tools in the fields of base and gene editing, GT and chromosome engineering open up a wide range of possibilities to overcome hurdles in plant breeding quickly and efficiently. Sequence-specific recruitment of various factors for transcriptional and post-transcriptional regulation allows a fine-tuning of plant performance, too.

The constantly increasing set of Cas proteins which have been adapted for biotechnological applications promises great improvements in the field of genome editing. Examples are the small-sized Cas9 orthologue from *Campylobacter jejuni* (*Cj*Cas9) and the recently characterized CRISPR/Cas14 system which is able to target single-stranded DNA efficiently without the requirement of a PAM-sequence (Harrington et al., 2018; Kim et al., 2017a). Most recently, a new type V CRISPR/Cas system, called CRISPR/Casϕ, was characterized and proven to enable site-specific induction of DSB in both, human and plant cells (Pausch et al., 2020). Due to its small size and minimal T-rich PAM (5'-TBN-3') requirement, this bacteriophage-derived system will surely provide a powerful addition to the CRISPR/Cas toolbox.

However, a science-based regulation of genome-edited crops is urgently needed to allow plant breeders to use those powerful tools worldwide to address increasingly challenging environmental conditions due to global warming.

Author contribution

All authors wrote and edited the manuscript. L.M. and N.C. created the figures.

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CRediT authorship contribution statement

Niklas Capdeville: Writing - original draft, Visualization. **Laura Merker:** Writing - original draft, Visualization. **Patrick Schindele:** Writing - review & editing. **Holger Puchta:** Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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References

Abudayyeh, O.O., Gootenberg, J.S., Konermann, S., Joung, J., Slaymaker, I.M., Cox, D.B. T., Shmakov, S., Makarova, K.S., Semenova, E., Minakhin, L., Severinov, K., Regev, A., Lander, E.S., Koonin, E.V., Zhang, F., 2016. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science (New York, N. Y.) 353 (6299). https://doi.org/10.1126/science.aaf5573 aaf5573.

Abudayyeh, O.O., Gootenberg, J.S., Essletzbichler, P., Han, S., Joung, J., Belanto, J.J., Verdine, V., Cox, D.B.T., Kellner, M.J., Regev, A., Lander, E.S., Voytas, D.F., Ting, A. Y., Zhang, F., 2017. RNA targeting with CRISPR-Cas13. Nature 550 (7675), 280–284. https://doi.org/10.1038/nature24049.

Abudayyeh, O.O., Gootenberg, J.S., Franklin, B., Koob, J., Kellner, M.J., Ladha, A., Joung, J., Kirchgatterer, P., Cox, D.B.T., Zhang, F., 2019. A cytosine deaminase for programmable single-base RNA editing. Science (New York, N.Y.) 365 (6451), 382–386. https://doi.org/10.1126/science.aax7063.

Anzalone, A.V., Randolph, P.B., Davis, J.R., Sousa, A.A., Koblan, L.W., Levy, J.M., Chen, P.J., Wilson, C., Newby, G.A., Raguram, A., Liu, D.R., 2019. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 576 (7785), 149–157. https://doi.org/10.1038/s41586-019-1711-4.

Atkins, P.A., Voytas, D.F., 2020. Overcoming bottlenecks in plant gene editing. Curr. Opin. Plant Biol. 54, 79–84. https://doi.org/10.1016/j.pbi.2020.01.002.

Baltes, N.J., Gil-Humanes, J., Cermak, T., Atkins, P.A., Voytas, D.F., 2014. DNA replicons for plant genome engineering. Plant Cell 26 (1), 151–163. https://doi.org/10.1105/tpc.113.119792.

Barone, P., Wu, E., Lenderts, B., Anand, A., Gordon-Kamm, W., Svitashev, S., Kumar, S., 2020. Efficient gene targeting in maize using inducible CRISPR-Cas9 and marker-free donor template. Mol. Plant 13 (8), 1219–1227. https://doi.org/10.1016/j.molp.2020.06.008.

Barrangou, R., Marraffini, L.A., 2014. CRISPR-Cas systems: prokaryotes upgrade to adaptive immunity. Mol. Cell 54 (2), 234–244. https://doi.org/10.1016/j.molcel.2014.03.011.

Beying, N., Schmidt, C., Pacher, M., Houben, A., Puchta, H., 2020. CRISPR-Cas9-mediated induction of heritable chromosomal translocations in Arabidopsis. Nat. Plants 6 (6), 638–645. https://doi.org/10.1038/s41477-020-0663-x.

Butler, N.M., Baltes, N.J., Voytas, D.F., Douches, D.S., 2016. Geminivirus-mediated genome editing in potato (Solanum tuberosum L.) using sequence-specific nucleases. Front. Plant Sci. 7, 1045. https://doi.org/10.3389/fpls.2016.01045.

Butt, H., Rao, G.S., Sedeek, K., Aman, R., Kamel, R., Mahfouz, M., 2020. Engineering herbicide resistance via prime editing in rice. Plant Biotechnol. J. https://doi.org/10.1111/pbi.13399.

Čermák, T., Baltes, N.J., Čegan, R., Zhang, Y., Voytas, D.F., 2015. High-frequency, precise modification of the tomato genome. Genome Biol. 16, 232. https://doi.org/10.1186/s13059-015-0796-9.

Chatterjee, P., Lee, J., Nip, L., Koseki, S.R.T., Tysinger, E., Sontheimer, E.J., Jacobson, J. M., Jakimo, N., 2020. A Cas9 with PAM recognition for adenine dinucleotides. Nat. Commun. 11 (1), 2474 https://doi.org/10.1038/s41467-020-16117-8.

Chen, J.S., Dagdas, Y.S., Kleinstiver, B.P., Welch, M.M., Sousa, A.A., Harrington, L.B., Sternberg, S.H., Joung, J.K., Yildiz, A., Doudna, J.A., 2017. Enhanced proofreading governs CRISPR-Cas9 targeting accuracy. Nature 550 (7676), 407–410. https://doi.org/10.1038/nature24268.

Christian, M., Cermak, T., Doyle, E.L., Schmidt, C., Zhang, F., Hummel, A., Bogdanove, A. J., Voytas, D.F., 2010. Targeting DNA double-strand breaks with TAL effector nucleases. Genetics 186 (2), 757–761. https://doi.org/10.1534/genetics.110.120717.

Chylinski, K., Le Rhun, A., Charpentier, E., 2013. The tracrRNA and Cas9 families of type II CRISPR-Cas immunity systems. RNA Biol. 10 (5), 726–737. https://doi.org/10.4161/rna.24321.

Cox, D.B.T., Gootenberg, J.S., Abudayyeh, O.O., Franklin, B., Kellner, M.J., Joung, J., Zhang, F., 2017. RNA editing with CRISPR-Cas13. Science (New York, N.Y.) 358 (6366), 1019–1027. https://doi.org/10.1126/science.aaq0180.

Csörgő, B., León, L.M., Chau-Ly, I.J., Vasquez-Rifo, A., Berry, J.D., Mahendra, C., Crawford, E.D., Lewis, J.D., Bondy-Denomy, J., 2020. A compact Cascade-Cas3 system for targeted genome engineering. Nat. Methods. https://doi.org/10.1038/s41592-020-00980-w.

de Pater, S., Klemann, B.J.P.M., Hooykaas, P.J.J., 2018. True gene-targeting events by CRISPR/Cas-induced DSB repair of the PPO locus with an ectopically integrated repair template. Sci. Rep. 8 (1), 3338 https://doi.org/10.1038/s41598-018-21697-z.

Deltcheva, E., Chylinski, K., Sharma, C.M., Gonzales, K., Chao, Y., Pirzada, Z.A., Eckert, M.R., Vogel, J., Charpentier, E., 2011. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. Nature 471 (7340), 602–607. https://doi.org/10.1038/nature09886.

Dolan, A.E., Hou, Z., Xiao, Y., Gramelspacher, M.J., Heo, J., Howden, S.E., Freddolino, P. L., Ke, A., Zhang, Y., 2019. Introducing a Spectrum of long-range genomic deletions in human embryonic stem cells using type I CRISPR-Cas. Mol. Cell 74 (5), 936–950. https://doi.org/10.1016/j.molcel.2019.03.014 e5.

Durr, J., Papareddy, R., Nakajima, K., Gutierrez-Marcos, J., 2018. Highly efficient heritable targeted deletions of gene clusters and non-coding regulatory regions in Arabidopsis using CRISPR/Cas9. Sci. Rep. 8 (1), 4443. https://doi.org/10.1038/s41598-018-22667-1.

East-Seletsky, A., O'Connell, M.R., Knight, S.C., Burstein, D., Cate, J.H.D., Tjian, R., Doudna, J.A., 2016. Two distinct RNase activities of CRISPR-C2c2 enable guide-RNA processing and RNA detection. Nature 538 (7624), 270–273. https://doi.org/10.1038/nature19802.

Endo, A., Masafumi, M., Kaya, H., Toki, S., 2016a. Efficient targeted mutagenesis of rice and tobacco genomes using Cpf1 from Francisella novicida. Sci. Rep. 6, 38169. https://doi.org/10.1038/srep38169.

Endo, M., Mikami, M., Toki, S., 2016b. Biallelic gene targeting in rice. Plant Physiol. 170 (2), 667–677. https://doi.org/10.1104/pp.15.01663.

Fauser, F., Roth, N., Pacher, M., Ilg, G., Sánchez-Fernández, R., Biesgen, C., Puchta, H., 2012. In planta gene targeting. Proc. Natl. Acad. Sci. U. S. A. 109 (19), 7535–7540. https://doi.org/10.1073/pnas.1202191109.

Fauser, F., Schiml, S., Puchta, H., 2014. Both CRISPR/Cas-based nucleases and nickases can be used efficiently for genome engineering in Arabidopsis thaliana. Plant J. 79 (2), 348–359. https://doi.org/10.1111/tpj.12554.

Filler Hayut, S., Melamed Bessudo, C., Levy, A.A., 2017. Targeted recombination between homologous chromosomes for precise breeding in tomato. Nat. Commun. 8, 15605. https://doi.org/10.1038/ncomms15605.

Fonfara, I., Le Rhun, A., Chylinski, K., Makarova, K.S., Lécrivain, A.-L., Bzdrenga, J., Koonin, E.V., Charpentier, E., 2014. Phylogeny of Cas9 determines functional exchangeability of dual-RNA and Cas9 among orthologous type II CRISPR-Cas systems. Nucleic Acids Res. 42 (4), 2577–2590. https://doi.org/10.1093/nar/gkt1074.

Fonfara, I., Richter, H., Bratovič, M., Le Rhun, A., Charpentier, E., 2016. The CRISPR-associated DNA-cleaving enzyme Cpf1 also processes precursor CRISPR RNA. Nature 532 (7600), 517–521. https://doi.org/10.1038/nature17945.

Friedland, A.E., Baral, R., Singhal, P., Loveluck, K., Shen, S., Sanchez, M., Marco, E., Gotta, G.M., Maeder, M.L., Kennedy, E.M., Kornepati, A.V.R., Sousa, A., Collins, M. A., Jayaram, H., Cullen, B.R., Bumcrot, D., 2015. Characterization of Staphylococcus aureus Cas9: a smaller Cas9 for all-in-one adeno-associated virus delivery and paired nickase applications. Genome Biol. 16 (1), 257. https://doi.org/10.1186/s13059-015-0817-8.

Gao, L., Cox, D.B.T., Yan, W.X., Manteiga, J.C., Schneider, M.W., Yamano, T., Nishimasu, H., Nureki, O., Crosetto, N., Zhang, F., 2017. Engineered Cpf1 variants with altered PAM specificities. Nat. Biotechnol. 35 (8), 789–792. https://doi.org/10.1038/nbt.3900.

Gaudelli, N.M., Komor, A.C., Rees, H.A., Packer, M.S., Badran, A.H., Bryson, D.I., Liu, D. R., 2017. Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. Nature 551 (7681), 464–471. https://doi.org/10.1038/nature24644.

Gaudelli, N.M., Lam, D.K., Rees, H.A., Solá-Esteves, N.M., Barrera, L.A., Born, D.A., Edwards, A., Gehrke, J.M., Lee, S.-J., Liquori, A.J., Murray, R., Packer, M.S., Rinaldi, C., Slaymaker, I.M., Yen, J., Young, L.E., Ciaramella, G., 2020. Directed evolution of adenine base editors with increased activity and therapeutic application. Nat. Biotechnol. 38 (7), 892–900. https://doi.org/10.1038/s41587-020-0491-6.

Gilbert, L.A., Larson, M.H., Morsut, L., Liu, Z., Brar, G.A., Torres, S.E., Stern-Ginossar, N., Brandman, O., Whitehead, E.H., Doudna, J.A., Lim, W.A., Weissman, J.S., Qi, L.S., 2013. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. Cell 154 (2), 442–451. https://doi.org/10.1016/j.cell.2013.06.044.

Gil-Humanes, J., Wang, Y., Liang, Z., Shan, Q., Ozuna, C.V., Sánchez-León, S., Baltes, N. J., Starker, C., Barro, F., Gao, C., Voytas, D.F., 2017. High-efficiency gene targeting in hexaploid wheat using DNA replicons and CRISPR/Cas9. Plant J. 89 (6), 1251–1262. https://doi.org/10.1111/tpj.13446.

Globyte, V., Lee, S.H., Bae, T., Kim, J.-S., Joo, C., 2019. CRISPR/Cas9 searches for a protospacer adjacent motif by lateral diffusion. EMBO J. 38 (4) https://doi.org/10.15252/embj.201899466.

Guilinger, J.P., Thompson, D.B., Liu, D.R., 2014. Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification. Nat. Biotechnol. 32 (6), 577–582. https://doi.org/10.1038/nbt.2909.

Guo, L., Cao, X., Liu, Y., Li, J., Li, Y., Li, D., Zhang, K., Gao, C., Dong, A., Liu, X., 2018. A chromatin loop represses WUSCHEL expression in Arabidopsis. Plant J. 94 (6), 1083–1097. https://doi.org/10.1111/tpj.13921.

Hahn, F., Eisenhut, M., Mantegazza, O., Weber, A.P.M., 2018. Homology-directed repair of a defective glabrous gene in Arabidopsis with Cas9-based gene targeting. Front. Plant Sci. 9, 424. https://doi.org/10.3389/fpls.2018.00424.

Halperin, S.O., Tou, C.J., Wong, E.B., Modavi, C., Schaffer, D.V., Dueber, J.E., 2018. CRISPR-guided DNA polymerases enable diversification of all nucleotides in a tunable window. Nature 560 (7717), 248–252. https://doi.org/10.1038/s41586-018-0384-8.

Harrington, L.B., Burstein, D., Chen, J.S., Paez-Espino, D., Ma, E., Witte, I.P., Cofsky, J.C., Kyrpides, N.C., Banfield, J.F., Doudna, J.A., 2018. Programmed DNA destruction by miniature CRISPR-Cas14 enzymes. Science (New York, N.Y.) 362 (6416), 839–842. https://doi.org/10.1126/science.aav4294.

Hille, F., Charpentier, E., 2016. CRISPR-Cas: biology, mechanisms and relevance. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 371 (1707) https://doi.org/10.1098/rstb.2015.0496.

Hilton, I.B., D'Ippolito, A.M., Vockley, C.M., Thakore, P.I., Crawford, G.E., Reddy, T.E., Gersbach, C.A., 2015. Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. Nat. Biotechnol. 33 (5), 510–517. https://doi.org/10.1038/nbt.3199.

Hu, J.H., Miller, S.M., Geurts, M.H., Tang, W., Chen, L., Sun, N., Zeina, C.M., Gao, X., Rees, H.A., Lin, Z., Liu, D.R., 2018. Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. Nature 556 (7699), 57–63. https://doi.org/10.1038/nature26155.

Hua, K., Tao, X., Yuan, F., Wang, D., Zhu, J.-K., 2018. Precise A•T to G•C base editing in the rice genome. Mol. Plant 11 (4), 627–630. https://doi.org/10.1016/j.molp.2018.02.007.

Hua, K., Tao, X., Han, P., Wang, R., Zhu, J.-K., 2019a. Genome engineering in rice using Cas9 variants that recognize NG PAM sequences. Mol. Plant 12 (7), 1003–1014. https://doi.org/10.1016/j.molp.2019.03.009.

Hua, K., Tao, X., Zhu, J.-K., 2019b. Expanding the base editing scope in rice by using Cas9 variants. Plant Biotechnol. J. 17 (2), 499–504. https://doi.org/10.1111/pbi.12993.

Hua, K., Jiang, Y., Tao, X., Zhu, J.-K., 2020a. Precision genome engineering in rice using prime editing system. Plant Biotechnol. J. https://doi.org/10.1111/pbi.13395.

Hua, K., Tao, X., Liang, W., Zhang, Z., Gou, R., Zhu, J.-K., 2020b. Simplified adenine base editors improve adenine base editing efficiency in rice. Plant Biotechnol. J. 18 (3), 770–778. https://doi.org/10.1111/pbi.13244.

Huang, T.-K., Puchta, H., 2019. CRISPR/Cas-mediated gene targeting in plants: finally a turn for the better for homologous recombination. Plant Cell Rep. 38 (4), 443–453. https://doi.org/10.1007/s00299-019-02379-0.

Jiang, W., Bikard, D., Cox, D., Zhang, F., Marraffini, L.A., 2013. RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Nat. Biotechnol. 31 (3), 233–239. https://doi.org/10.1038/nbt.2508.

Jiang, Y.-Y., Chai, Y.-P., Lu, M.-H., Han, X.-L., Lin, Q., Zhang, Y., Zhang, Q., Zhou, Y., Wang, X.-C., Gao, C., Chen, Q.-J., 2020. Prime Editing Efficiently Generates W542L and S621I Double Mutations in Two ALS Genes in Maize.

Jin, S., Zong, Y., Gao, Q., Zhu, Z., Wang, Y., Qin, P., Liang, C., Wang, D., Qiu, J.-L., Zhang, F., Gao, C., 2019. Cytosine, but not adenine, base editors induce genome-wide off-target mutations in rice. Science (New York, N.Y.) 364 (6437), 292–295. https://doi.org/10.1126/science.aaw7166.

Jin, S., Fei, H., Zhu, Z., Luo, Y., Liu, J., Gao, S., Zhang, F., Chen, Y.-H., Wang, Y., Gao, C., 2020. Rationally designed APOBEC3B cytosine base editors with improved specificity. Mol. Cell 79 (5), 728–740. https://doi.org/10.1016/j.molcel.2020.07.005 e6.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., Charpentier, E., 2012. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science (New York, N.Y.) 337 (6096), 816–821. https://doi.org/10.1126/science.1225829.

Kearns, N.A., Pham, H., Tabak, B., Genga, R.M., Silverstein, N.J., Garber, M., Maehr, R., 2015. Functional annotation of native enhancers with a Cas9-histone demethylase fusion. Nat. Methods 12 (5), 401–403. https://doi.org/10.1038/nmeth.3325.

Kim, E., Koo, T., Park, S.W., Kim, D., Kim, K., Cho, H.-Y., Song, D.W., Lee, K.J., Jung, M. H., Kim, S., Kim, J.H., Kim, J.H., Kim, J.-S., 2017a. In vivo genome editing with a small Cas9 orthologue derived from Campylobacter jejuni. Nat. Commun. 8, 14500 https://doi.org/10.1038/ncomms14500.

Kim, H.K., Song, M., Lee, J., Menon, A.V., Jung, S., Kang, Y.-M., Choi, J.W., Woo, E., Koh, H.C., Nam, J.-W., Kim, H., 2017b. In vivo high-throughput profiling of CRISPR-Cpf1 activity. Nat. Methods 14 (2), 153–159. https://doi.org/10.1038/nmeth.4104.

Kleinstiver, B.P., Prew, M.S., Tsai, S.Q., Nguyen, N.T., Topkar, V.V., Zheng, Z., Joung, J. K., 2015a. Broadening the targeting range of Staphylococcus aureus CRISPR-Cas9 by modifying PAM recognition. Nat. Biotechnol. 33 (12), 1293–1298. https://doi.org/10.1038/nbt.3404.

Kleinstiver, B.P., Prew, M.S., Tsai, S.Q., Topkar, V.V., Nguyen, N.T., Zheng, Z., Gonzales, A.P.W., Li, Z., Peterson, R.T., Yeh, J.-R.J., Aryee, M.J., Joung, J.K., 2015b. Engineered CRISPR-Cas9 nucleases with altered PAM specificities. Nature 523 (7561), 481–485. https://doi.org/10.1038/nature14592.

Kleinstiver, B.P., Pattanayak, V., Prew, M.S., Tsai, S.Q., Nguyen, N.T., Zheng, Z., Joung, J.K., 2016. High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. Nature 529 (7587), 490–495. https://doi.org/10.1038/nature16526.

Kleinstiver, B.P., Sousa, A.A., Walton, R.T., Tak, Y.E., Hsu, J.Y., Clement, K., Welch, M. M., Horng, J.E., Malagon-Lopez, J., Scarfò, I., Maus, M.V., Pinello, L., Aryee, M.J., Joung, J.K., 2019. Engineered CRISPR-Cas12a variants with increased activities and improved targeting ranges for gene, epigenetic and base editing. Nat. Biotechnol. 37 (3), 276–282. https://doi.org/10.1038/s41587-018-0011-0.

Komor, A.C., Kim, Y.B., Packer, M.S., Zuris, J.A., Liu, D.R., 2016. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature 533 (7603), 420–424. https://doi.org/10.1038/nature17946.

Konermann, S., Brigham, M.D., Trevino, A.E., Joung, J., Abudayyeh, O.O., Barcena, C., Hsu, P.D., Habib, N., Gootenberg, J.S., Nishimasu, H., Nureki, O., Zhang, F., 2015. Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. Nature 517 (7536), 583–588. https://doi.org/10.1038/nature14136.

Kunii, A., Hara, Y., Takenaga, M., Hattori, N., Fukazawa, T., Ushijima, T., Yamamoto, T., Sakuma, T., 2018. Three-component repurposed technology for enhanced expression: highly accumulable transcriptional activators via branched tag arrays. CRISPR J. 1, 337–347. https://doi.org/10.1089/crispr.2018.0009.

Kurt, I.C., Zhou, R., Iyer, S., Garcia, S.P., Miller, B.R., Langner, L.M., Grünewald, J., Joung, J.K., 2020. CRISPR C-to-G base editors for inducing targeted DNA transversions in human cells. Nat. Biotechnol. https://doi.org/10.1038/s41587-020-0609-x.

Le, Cong, Ran, F.A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P.D., Wu, X., Jiang, W., Marraffini, L.A., Zhang, F., 2013. Multiplex genome engineering using CRISPR/Cas systems. Science (New York, N.Y.) 339 (6121), 819–823. https://doi.org/10.1126/science.1231143.

LeBlanc, C., Zhang, F., Mendez, J., Lozano, Y., Chatpar, K., Irish, V.F., Jacob, Y., 2018. Increased efficiency of targeted mutagenesis by CRISPR/Cas9 in plants using heat stress. Plant J. 93 (2), 377–386. https://doi.org/10.1111/tpj.13782.

Lee, J.H., Mazarei, M., Pfotenhauer, A.C., Dorrough, A.B., Poindexter, M.R., Hewezi, T., Lenaghan, S.C., Graham, D.E., Stewart, C.N., 2019. Epigenetic footprints of CRISPR/

Cas9-mediated genome editing in plants. Front. Plant Sci. 10, 1720. https://doi.org/10.3389/fpls.2019.01720.
Lee, H.K., Smith, H.E., Liu, C., Willi, M., Hennighausen, L., 2020a. Cytosine base editor 4 but not adenine base editor generates off-target mutations in mouse embryos. Commun. Biol. 3 (1), 19. https://doi.org/10.1038/s42003-019-0745-3.
Lee, S., Ding, N., Sun, Y., Yuan, T., Li, J., Yuan, Q., Liu, L., Yang, J., Wang, Q., Kolomeisky, A.B., Hilton, I.B., Zuo, E., Gao, X., 2020b. Single C-to-T substitution using engineered APOBEC3G-nCas9 base editors with minimum genome- and transcriptome-wide off-target effects. Sci. Adv. 6 (29) https://doi.org/10.1126/sciadv.aba1773 eaba1773.
Li, J., Sun, Y., Du, J., Zhao, Y., Xia, L., 2017. Generation of targeted point mutations in rice by a modified CRISPR/Cas9 system. Mol. Plant 10 (3), 526–529. https://doi.org/10.1016/j.molp.2016.12.001.
Li, T., Yang, X., Yu, Y., Si, X., Zhai, X., Zhang, H., Dong, W., Gao, C., Xu, C., 2018. Domestication of wild tomato is accelerated by genome editing. Nat. Biotechnol. 36 (12), 1160–1163. https://doi.org/10.1038/nbt.4273.
Li, C., Zhang, R., Meng, X., Chen, S., Zong, Y., Lu, C., Qiu, J.-L., Chen, Y.-H., Li, J., Gao, C., 2020a. Targeted, random mutagenesis of plant genes with dual cytosine and adenine base editors. Nat. Biotechnol. 38 (7), 875–882. https://doi.org/10.1038/s41587-019-0393-7.
Li, C., Zong, Y., Jin, S., Zhu, H., Lin, D., Li, S., Qiu, J.-L., Wang, Y., Gao, C., 2020b. SWISS: multiplexed orthogonal genome editing in plants with a Cas9 nickase and engineered CRISPR RNA scaffolds. Genome Biol. 21 (1), 141. https://doi.org/10.1186/s13059-020-02051-x.
Li, H., Li, J., Chen, J., Yan, L., Xia, L., 2020c. Precise modifications of both exogenous and endogenous genes in rice by prime editing. Mol. Plant 13 (5), 671–674. https://doi.org/10.1016/j.molp.2020.03.011.
Li, S., Zhang, Y., Xia, L., Qi, Y., 2020d. CRISPR-Cas12a enables efficient biallelic gene targeting in rice. Plant Biotechnol. J. 18 (6), 1351–1353. https://doi.org/10.1111/pbi.13295.
Liang, Z., Chen, K., Yan, Y., Zhang, Y., Gao, C., 2018. Genotyping genome-edited mutations in plants using CRISPR ribonucleoprotein complexes. Plant Biotechnol. J. 16 (12), 2053–2062. https://doi.org/10.1111/pbi.12938.
Lin, Q., Zong, Y., Xue, C., Wang, S., Jin, S., Zhu, Z., Wang, Y., Anzalone, A.V., Raguram, A., Doman, J.L., Liu, D.R., Gao, C., 2020. Prime genome editing in rice and wheat. Nat. Biotechnol. 38 (5), 582–585. https://doi.org/10.1038/s41587-020-0455-x.
Liu, L., Li, X., Ma, J., Li, Z., You, L., Wang, J., Wang, M., Zhang, X., Wang, Y., 2017. The molecular architecture for RNA-Guided RNA cleavage by Cas13a. Cell 170 (4), 714–726. https://doi.org/10.1016/j.cell.2017.06.050 e10.
Liu, Z., Chen, S., Shan, H., Jia, Y., Chen, M., Song, Y., Lai, L., Li, Z., 2020. Precise base editing with CC context-specificity using engineered human APOBEC3G-nCas9 fusions. BMC Biol. 18 (1), 111. https://doi.org/10.1186/s12915-020-00849-6.
Lowder, L.G., Zhang, D., Baltes, N.J., Paul, J.W., Tang, X., Zheng, X., Voytas, D.F., Hsieh, T.-F., Zhang, Y., Qi, Y., 2015. A CRISPR/Cas9 toolbox for multiplexed plant genome editing and transcriptional regulation. Plant Physiol. 169 (2), 971–985. https://doi.org/10.1104/pp.15.00636.
Lowder, L.G., Paul, J.W., Qi, Y., 2017. Multiplexed transcriptional activation or repression in plants using CRISPR-dCas9-Based systems. Methods Mol. Biol. (Clifton, N.J.) 1629, 167–184. https://doi.org/10.1007/978-1-4939-7125-1_12.
Lowder, L.G., Zhou, J., Zhang, Y., Malzahn, A., Zhong, Z., Hsieh, T.-F., Voytas, D.F., Zhang, Y., Qi, Y., 2018. Robust transcriptional activation in plants using multiplexed CRISPR-Act2.0 and mTALE-Act systems. Mol. Plant 11 (2), 245–256. https://doi.org/10.1016/j.molp.2017.11.010.
Lowry, D.B., Willis, J.H., 2010. A widespread chromosomal inversion polymorphism contributes to a major life-history transition, local adaptation, and reproductive isolation. PLoS Biol. 8 (9) https://doi.org/10.1371/journal.pbio.1000500.
Lu, Y., Tian, Y., Shen, R., Yao, Q., Wang, M., Chen, M., Dong, J., Zhang, T., Li, F., Lei, M., Zhu, J.-K., 2020. Targeted, efficient sequence insertion and replacement in rice. Nat. Biotechnol. https://doi.org/10.1038/s41587-020-0581-5.
Ma, H., Tu, L.-C., Naseri, A., Huisman, M., Zhang, S., Grunwald, D., Pederson, T., 2016. Multiplexed labeling of genomic loci with dCas9 and engineered sgRNAs using CRISPRainbow. Nat. Biotechnol. 34 (5), 528–530. https://doi.org/10.1038/nbt.3526.
Mahas, A., Neal Stewart, C., Mahfouz, M.M., 2018. Harnessing CRISPR/Cas systems for programmable transcriptional and post-transcriptional regulation. Biotechnol. Adv. 36 (1), 295–310. https://doi.org/10.1016/j.biotechadv.2017.11.008.
Makarova, K.S., Wolf, Y.I., Iranzo, J., Shmakov, S.A., Alkhnbashi, O.S., Brouns, S.J.J., Charpentier, E., Cheng, D., Haft, D.H., Horvath, P., Moineau, S., Mojica, F.J.M., Scott, D., Shah, S.A., Siksnys, V., Terns, M.P., Venclovas, Č., White, M.F., Yakunin, A. F., Yan, W., Zhang, F., Garrett, R.A., Backofen, R., van der Oost, J., Barrangou, R., Koonin, E.V., 2020. Evolutionary classification of CRISPR-Cas systems: a burst of class 2 and derived variants. Nat. Rev. Microbiol. 18 (2), 67–83. https://doi.org/10.1038/s41579-019-0299-x.
Mali, P., Aach, J., Stranges, P.B., Esvelt, K.M., Moosburner, M., Kosuri, S., Yang, L., Church, G.M., 2013. CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. Nat. Biotechnol. 31 (9), 833–838. https://doi.org/10.1038/nbt.2675.
Malzahn, A.A., Tang, X., Lee, K., Ren, Q., Sretenovic, S., Zhang, Y., Chen, H., Kang, M., Bao, Y., Zheng, X., Deng, K., Zhang, T., Salcedo, V., Wang, K., Zhang, Y., Qi, Y., 2019. Application of CRISPR-Cas12a temperature sensitivity for improved genome editing in rice, maize, and Arabidopsis. BMC Biol. 17 (1), 9. https://doi.org/10.1186/s12915-019-0629-5.
Matthews, K.S., 1992. DNA looping. Microbiol. Rev. 56 (1), 123–136.
Merker, L., Schindele, P., Huang, T.-K., Wolter, F., Puchta, H., 2020. Enhancing in planta gene targeting efficiencies in Arabidopsis using temperature-tolerant CRISPR/LbCas12a. Plant Biotechnol. J. https://doi.org/10.1111/pbi.13426.
Miki, D., Zhang, W., Zeng, W., Feng, Z., Zhu, J.-K., 2018. CRISPR/Cas9-mediated gene targeting in Arabidopsis using sequential transformation. Nat. Commun. 9 (1), 1967. https://doi.org/10.1038/s41467-018-04416-0.
Ming, M., Ren, Q., Pan, C., He, Y., Zhang, Y., Liu, S., Zhong, Z., Wang, J., Malzahn, A.A., Wu, J., Zheng, X., Zhang, Y., Qi, Y., 2020. CRISPR-Cas12b enables efficient plant genome engineering. Nat. Plants 6 (3), 202–208. https://doi.org/10.1038/s41477-020-0614-6.
Mojica, F.J.M., Díez-Villaseñor, C., García-Martínez, J., Almendros, C., 2009. Short motif sequences determine the targets of the prokaryotic CRISPR defence system. Microbiology (Reading, England) 155 (Pt 3), 733–740. https://doi.org/10.1099/mic.0.023960-0.
Moreno-Mateos, M.A., Fernandez, J.P., Rouet, R., Vejnar, C.E., Lane, M.A., Mis, E., Khokha, M.K., Doudna, J.A., Giraldez, A.J., 2017. CRISPR-Cpf1 mediates efficient homology-directed repair and temperature-controlled genome editing. Nat. Commun. 8 (1), 2024. https://doi.org/10.1038/s41467-017-01836-2.
Morgan, S.L., Mariano, N.C., Bermudez, A., Arruda, N.L., Wu, F., Luo, Y., Shankar, G., Jia, L., Chen, H., Hu, J.-F., Hoffman, A.R., Huang, C.-C., Pitteri, S.J., Wang, K.C., 2017. Manipulation of nuclear architecture through CRISPR-mediated chromosomal looping. Nat. Commun. 8, 15993. https://doi.org/10.1038/ncomms15993.
Nishida, K., Kondo, A., 2015. Method for Modifying Genome Sequence to Introduce Specific Mutation to Targeted DNA Sequence by Base-removal Reaction, and Molecular Complex Used Therein C12N15/10; C12N15/11; C12N9/22; C12N9/24.
Nishimasu, H., Shi, X., Ishiguro, S., Gao, L., Hirano, S., Okazaki, S., Noda, T., Abudayyeh, O.O., Gootenberg, J.S., Mori, H., Oura, S., Holmes, B., Tanaka, M., Seki, M., Hirano, H., Aburatani, H., Ishitani, R., Ikawa, M., Yachie, N., Zhang, F., Nureki, O., 2018. Engineered CRISPR-Cas9 nuclease with expanded targeting space. Science (New York, N.Y.) 361 (6408), 1259–1262. https://doi.org/10.1126/science.aas9129.
Niu, Q., Wu, S., Li, Y., Yang, X., Liu, P., Xu, Y., Lang, Z., 2020. Expanding the scope of CRISPR/Cas9-mediated genome editing in plants using an xCas9 and Cas9-NG hybrid. J. Integr. Plant Biol. 62 (4), 398–402. https://doi.org/10.1111/jipb.12886.
Ordon, J., Gantner, J., Kemna, J., Schwalgun, L., Reschke, M., Streubel, J., Boch, J., Stuttmann, J., 2017. Generation of chromosomal deletions in dicotyledonous plants employing a user-friendly genome editing toolkit. Plant J. 89 (1), 155–168. https://doi.org/10.1111/tpj.13319.
Osakabe, K., Wada, N., Miyaji, T., Murakami, E., Marui, K., Ueta, R., Hashimoto, R., Abe-Hara, C., Kong, B., Yano, K., Osakabe, Y., 2020. Genome editing in plants using CRISPR type I-D nuclease. Commun. Biol. 3 (1), 648. https://doi.org/10.1038/s42003-020-01366-6.
Pacher, M., Puchta, H., 2017. From classical mutagenesis to nuclease-based breeding - directing natural DNA repair for a natural end-product. Plant J. 90 (4), 819–833. https://doi.org/10.1111/tpj.13469.
Papikian, A., Liu, W., Gallego-Bartolomé, J., Jacobsen, S.E., 2019. Site-specific manipulation of Arabidopsis loci using CRISPR-Cas9 SunTag systems. Nat. Commun. 10 (1), 729. https://doi.org/10.1038/s41467-019-08736-7.
Paszkowski, J., Baur, M., Bogucki, A., Potrykus, I., 1988. Gene targeting in plants. EMBO J. 7 (13), 4021–4026.
Pausch, P., Al-Shayeb, B., Bisom-Rapp, E., Tsuchida, C.A., Li, Z., Cress, B.F., Knott, G.J., Jacobsen, S.E., Banfield, J.F., Doudna, J.A., 2020. CRISPR-CasΦ from huge phages is a hypercompact genome editor. Science (New York, N.Y.) 369 (6501), 333–337. https://doi.org/10.1126/science.abb1400.
Perez-Pinera, P., Kocak, D.D., Vockley, C.M., Adler, A.F., Kabadi, A.M., Polstein, L.R., Thakore, P.I., Glass, K.A., Ousterout, D.G., Leong, K.W., Guilak, F., Crawford, G.E., Reddy, T.E., Gersbach, C.A., 2013. RNA-guided gene activation by CRISPR-Cas9-based transcription factors. Nat. Methods 10 (10), 973–976. https://doi.org/10.1038/nmeth.2600.
Piatek, A., Ali, Z., Baazim, H., Li, L., Abulfaraj, A., Al-Shareef, S., Aouida, M., Mahfouz, M.M., 2015. RNA-guided transcriptional regulation in planta via synthetic dCas9-based transcription factors. Plant Biotechnol. J. 13 (4), 578–589. https://doi.org/10.1111/pbi.12284.
Pramanik, D., Shelake, R.M., Kim, M.J., Kim, J.-Y., 2020. CRISPR-mediated engineering across the central dogma in plant biology for basic research and crop improvement. Mol. Plant. https://doi.org/10.1016/j.molp.2020.11.002.
Puchta, H., 2005. The repair of double-strand breaks in plants: mechanisms and consequences for genome evolution. J. Exp. Bot. 56 (409), 1–14. https://doi.org/10.1093/jxb/eri025.
Puchta, H., Dujon, B., Hohn, B., 1996. Two different but related mechanisms are used in plants for the repair of genomic double-strand breaks by homologous recombination. Proc. Natl. Acad. Sci. U. S. A. 93 (10), 5055–5060. https://doi.org/10.1073/pnas.93.10.5055.
Qi, Y., Zhang, Y., Zhang, F., Baller, J.A., Cleland, S.C., Ryu, Y., Starker, C.G., Voytas, D.F., 2013. Increasing frequencies of site-specific mutagenesis and gene targeting in Arabidopsis by manipulating DNA repair pathways. Genome Res. 23 (3), 547–554. https://doi.org/10.1101/gr.145557.112.
Qin, L., Li, J., Wang, Q., Xu, Z., Sun, L., Alariqi, M., Manghwar, H., Wang, G., Li, B., Ding, X., Rui, H., Huang, H., Lu, T., Lindsey, K., Daniell, H., Zhang, X., Jin, S., 2020. High-efficient and precise base editing of C•G to T•A in the allotetraploid cotton (Gossypium hirsutum) genome using a modified CRISPR/Cas9 system. Plant Biotechnol. J. 18 (1), 45–56. https://doi.org/10.1111/pbi.13168.
Richter, M.F., Zhao, K.T., Eton, E., Lapinaite, A., Newby, G.A., Thuronyi, B.W., Wilson, C., Koblan, L.W., Zeng, J., Bauer, D.E., Doudna, J.A., Liu, D.R., 2020. Phage-assisted evolution of an adenine base editor with improved Cas domain compatibility

and activity. Nat. Biotechnol. 38 (7), 883–891. https://doi.org/10.1038/s41587-020-0453-z.
Sakamoto, A.N., 2019. Translesion synthesis in plants: ultraviolet resistance and beyond. Front. Plant Sci. 10, 1208. https://doi.org/10.3389/fpls.2019.01208.
Sakata, R.C., Ishiguro, S., Mori, H., Tanaka, M., Seki, M., Masuyama, N., Nishida, K., Nishimasu, H., Kondo, A., Nureki, O., Tomita, M., Aburatani, H., Yachie, N., 2019. A Single CRISPR Base Editor to Induce Simultaneous C-to-T and A-to-G Mutations.
Schiml, S., Fauser, F., Puchta, H., 2014. The CRISPR/Cas system can be used as nuclease for in planta gene targeting and as paired nickases for directed mutagenesis in Arabidopsis resulting in heritable progeny. Plant J. 80 (6), 1139–1150. https://doi.org/10.1111/tpj.12704.
Schiml, S., Fauser, F., Puchta, H., 2016. Repair of adjacent single-strand breaks is often accompanied by the formation of tandem sequence duplications in plant genomes. Proc. Natl. Acad. Sci. U. S. A. 113 (26), 7266–7271. https://doi.org/10.1073/pnas.1603823113.
Schindele, P., Puchta, H., 2020. Engineering CRISPR/LbCas12a for highly efficient, temperature-tolerant plant gene editing. Plant Biotechnol. J. 18 (5), 1118–1120. https://doi.org/10.1111/pbi.13275.
Schmidt, C., Pacher, M., Puchta, H., 2019. Efficient induction of heritable inversions in plant genomes using the CRISPR/Cas system. Plant J. 98 (4), 577–589. https://doi.org/10.1111/tpj.14322.
Schmidt, C., Fransz, P., Rönspies, M., Dreissig, S., Fuchs, J., Heckmann, S., Houben, A., Puchta, H., 2020. Changing local recombination patterns in Arabidopsis by CRISPR/Cas mediated chromosome engineering. Nat. Commun. 11 (1), 4418 https://doi.org/10.1038/s41467-020-18277-z.
Shao, S., Zhang, W., Hu, H., Xue, B., Qin, J., Sun, C., Sun, Y., Wei, W., Sun, Y., 2016. Long-term dual-color tracking of genomic loci by modified sgRNAs of the CRISPR/Cas9 system. Nucleic Acids Res. 44 (9), e86. https://doi.org/10.1093/nar/gkw066.
Shechner, D.M., Hacisuleyman, E., Younger, S.T., Rinn, J.L., 2015. Multiplexable, locus-specific targeting of long RNAs with CRISPR-display. Nat. Methods 12 (7), 664–670. https://doi.org/10.1038/nmeth.3433.
Shen, L., Hua, Y., Fu, Y., Li, J., Liu, Q., Jiao, X., Xin, G., Wang, J., Wang, X., Yan, C., Wang, K., 2019. Erratum to: rapid generation of genetic diversity by multiplex CRISPR/Cas9 genome editing in rice. Sci. China Life Sci. 62 (9), 1270. https://doi.org/10.1007/s11427-019-9599-5.
Shmakov, S., Smargon, A., Scott, D., Cox, D., Pyzocha, N., Yan, W., Abudayyeh, O.O., Gootenberg, J.S., Makarova, K.S., Wolf, Y.I., Severinov, K., Zhang, F., Koonin, E.V., 2017. Diversity and evolution of class 2 CRISPR-Cas systems. Nat. Rev. Microbiol. 15 (3), 169–182. https://doi.org/10.1038/nrmicro.2016.184.
Siebert, R., Puchta, H., 2002. Efficient repair of genomic double-strand breaks by homologous recombination between directly repeated sequences in the plant genome. Plant Cell 14 (5), 1121–1131. https://doi.org/10.1105/tpc.001727.
Slaymaker, I.M., Gao, L., Zetsche, B., Scott, D.A., Yan, W.X., Zhang, F., 2016. Rationally engineered Cas9 nucleases with improved specificity. Science (New York, N.Y.) 351 (6268), 84–88. https://doi.org/10.1126/science.aad5227.
Soyk, S., Lemmon, Z.H., Oved, M., Fisher, J., Liberatore, K.L., Park, S.J., Goren, A., Jiang, K., Ramos, A., van der Knaap, E., van Eck, J., Zamir, D., Eshed, Y., Lippman, Z. B., 2017. Bypassing negative epistasis on yield in tomato imposed by a domestication gene. Cell 169 (6), 1142–1155. https://doi.org/10.1016/j.cell.2017.04.032 e12.
Sretenovic, S., Yin, D., Levav, A., Selengut, J.D., Mount, S.M., Qi, Y., 2020. Expanding Plant Genome-Editing Scope by an Engineered iSpyMacCas9 System That Targets A-Rich PAM Sequences. Plant Communications, 100101. Plant Communications, 100101. https://doi.org/10.1016/J.XPLC.2020.100101.
Steinert, J., Schiml, S., Fauser, F., Puchta, H., 2015. Highly efficient heritable plant genome engineering using Cas9 orthologues from Streptococcus thermophilus and Staphylococcus aureus. Plant J. 84 (6), 1295–1305. https://doi.org/10.1111/tpj.13078.
Tak, Y.E., Kleinstiver, B.P., Nuñez, J.K., Hsu, J.Y., Horng, J.E., Gong, J., Weissman, J.S., Joung, J.K., 2017. Inducible and multiplex gene regulation using CRISPR-Cpf1-based transcription factors. Nat. Methods 14 (12), 1163–1166. https://doi.org/10.1038/nmeth.4483.
Takahashi, S., Sakamoto, A.N., Tanaka, A., Shimizu, K., 2007. AtREV1, a Y-family DNA polymerase in Arabidopsis, has deoxynucleotidyl transferase activity in vitro. Plant Physiol. 145 (3), 1052–1060. https://doi.org/10.1104/pp.107.101980.
Tanenbaum, M.E., Gilbert, L.A., Qi, L.S., Weissman, J.S., Vale, R.D., 2014. A protein-tagging system for signal amplification in gene expression and fluorescence imaging. Cell 159 (3), 635–646. https://doi.org/10.1016/j.cell.2014.09.039.
Tang, X., Zheng, X., Qi, Y., Zhang, D., Cheng, Y., Tang, A., Voytas, D.F., Zhang, Y., 2016. A single transcript CRISPR-Cas9 system for efficient genome editing in plants. Mol. Plant 9 (7), 1088–1091. https://doi.org/10.1016/j.molp.2016.05.001.
Tang, X., Lowder, L.G., Zhang, T., Malzahn, A.A., Zheng, X., Voytas, D.F., Zhong, Z., Chen, Y., Ren, Q., Li, Q., Kirkland, E.R., Zhang, Y., Qi, Y., 2017. A CRISPR-Cpf1 system for efficient genome editing and transcriptional repression in plants. Nat. Plants 3, 17018. https://doi.org/10.1038/nplants.2017.18.
Tang, X., Sretenovic, S., Ren, Q., Jia, X., Li, M., Fan, T., Yin, D., Xiang, S., Guo, Y., Li, Liu, Zheng, X., Qi, Y., Zhang, Y., 2020. Plant prime editors enable precise gene editing in rice cells. Mol. Plant 13 (5), 667–670. https://doi.org/10.1016/j.molp.2020.03.010.
Teng, F., Cui, T., Feng, G., Guo, L., Xu, K., Gao, Q., Li, T., Li, J., Zhou, Q., Li, W., 2018. Repurposing CRISPR-Cas12b for mammalian genome engineering. Cell Discov. 4, 63. https://doi.org/10.1038/s41421-018-0069-3.
Townsend, J.A., Wright, D.A., Winfrey, R.J., Fu, F., Maeder, M.L., Joung, J.K., Voytas, D. F., 2009. High-frequency modification of plant genes using engineered zinc-finger nucleases. Nature 459 (7245), 442–445. https://doi.org/10.1038/nature07845.
Tsai, S.Q., Wyvekens, N., Khayter, C., Foden, J.A., Thapar, V., Reyon, D., Goodwin, M.J., Aryee, M.J., Joung, J.K., 2014. Dimeric CRISPR RNA-guided FokI nucleases for highly specific genome editing. Nat. Biotechnol. 32 (6), 569–576. https://doi.org/10.1038/nbt.2908.
van Vu, T., Sivankalyani, V., Kim, E.-J., Doan, D.T.H., Tran, M.T., Kim, J., Sung, Y.W., Park, M., Kang, Y.J., Kim, J.-Y., 2020. Highly efficient homology-directed repair using CRISPR/Cpf1-geminiviral replicon in tomato. Plant Biotechnol. J. https://doi.org/10.1111/pbi.13373.
Veillet, F., Kermarrec, M.-P., Chauvin, L., Guyon-Debast, A., Chauvin, J.-E., Gallois, J.-L., Nogué, F., 2020. Prime Editing Is Achievable in the Tetraploid Potato, but Needs Improvement.
Voytas, D.F., 2013. Plant genome engineering with sequence-specific nucleases. Annu. Rev. Plant Biol. 64, 327–350. https://doi.org/10.1146/annurev-arplant-042811-105552.
Walton, R.T., Christie, K.A., Whittaker, M.N., Kleinstiver, B.P., 2020. Unconstrained genome targeting with near-PAMless engineered CRISPR-Cas9 variants. Science (New York, N.Y.) 368 (6488), 290–296. https://doi.org/10.1126/science.aba8853.
Wang, M., Lu, Y., Botella, J.R., Mao, Y., Hua, K., Zhu, J.-K., 2017a. Gene targeting by homology-directed repair in rice using a geminivirus-based CRISPR/Cas9 system. Mol. Plant 10 (7), 1007–1010. https://doi.org/10.1016/j.molp.2017.03.002.
Wang, M., Mao, Y., Lu, Y., Tao, X., Zhu, J.-K., 2017b. Multiplex gene editing in rice using the CRISPR-Cpf1 system. Mol. Plant 10 (7), 1011–1013. https://doi.org/10.1016/j.molp.2017.03.001.
Wang, M., Mao, Y., Lu, Y., Wang, Z., Tao, X., Zhu, J.-K., 2018. Multiplex gene editing in rice with simplified CRISPR-Cpf1 and CRISPR-Cas9 systems. J. Integr. Plant Biol. 60 (8), 626–631. https://doi.org/10.1111/jipb.12667.
Wang, J., Meng, X., Hu, X., Sun, T., Li, J., Wang, K., Yu, H., 2019. xCas9 expands the scope of genome editing with reduced efficiency in rice. Plant Biotechnol. J. 17 (4), 709–711. https://doi.org/10.1111/pbi.13053.
Wang, M., Xu, Z., Gosavi, G., Ren, B., Cao, Y., Kuang, Y., Zhou, C., Spetz, C., Yan, F., Zhou, X., Zhou, H., 2020a. Targeted base editing in rice with CRISPR/ScCas9 system. Plant Biotechnol. J. https://doi.org/10.1111/pbi.13330.
Wang, S., Zong, Y., Lin, Q., Zhang, H., Chai, Z., Zhang, D., Chen, K., Qiu, J.-L., Gao, C., 2020b. Precise, predictable multi-nucleotide deletions in rice and wheat using APOBEC-Cas9. Nat. Biotechnol. https://doi.org/10.1038/s41587-020-0566-4.
Wolter, F., Puchta, H., 2019. In planta gene targeting can be enhanced by the use of CRISPR/Cas12a. Plant J. 100 (5), 1083–1094. https://doi.org/10.1111/tpj.14488.
Wolter, F., Klemm, J., Puchta, H., 2018. Efficient in planta gene targeting in Arabidopsis using egg cell-specific expression of the Cas9 nuclease of Staphylococcus aureus. Plant J. 94 (4), 735–746. https://doi.org/10.1111/tpj.13893.
Wright, D.A., Townsend, J.A., Winfrey, R.J., Irwin, P.A., Rajagopal, J., Lonosky, P.M., Hall, B.D., Jondle, M.D., Voytas, D.F., 2005. High-frequency homologous recombination in plants mediated by zinc-finger nucleases. Plant J. 44 (4), 693–705. https://doi.org/10.1111/j.1365-313x.2005.02551.x.
Wu, R., Lucke, M., Jang, Y.-T., Zhu, W., Symeonidi, E., Wang, C., Fitz, J., Xi, W., Schwab, R., Weigel, D., 2018. An efficient CRISPR vector toolbox for engineering large deletions in Arabidopsis thaliana. Plant Methods 14, 65. https://doi.org/10.1186/s13007-018-0330-7.
Wu, F., Qiao, X., Zhao, Y., Zhang, Z., Gao, Y., Shi, L., Du, H., Wang, L., Zhang, Y.-J., Zhang, Y., Liu, L., Wang, Q., Kong, D., 2020. Targeted mutagenesis in Arabidopsis thaliana using CRISPR-Cas12b/C2c1. J. Integr. Plant Biol. https://doi.org/10.1111/jipb.12944.
Xie, K., Minkenberg, B., Yang, Y., 2015. Boosting CRISPR/Cas9 multiplex editing capability with the endogenous tRNA-processing system. Proc. Natl. Acad. Sci. U. S. A. 112 (11), 3570–3575. https://doi.org/10.1073/pnas.1420294112.
Xing, H.-L., Dong, L., Wang, Z.-P., Zhang, H.-Y., Han, C.-Y., Liu, B., Wang, X.-C., Chen, Q.-J., 2014. A CRISPR/Cas9 toolkit for multiplex genome editing in plants. BMC Plant Biol. 14, 327. https://doi.org/10.1186/s12870-014-0327-y.
Xing, S., Chen, K., Zhu, H., Zhang, R., Zhang, H., Li, B., Gao, C., 2020. Fine-tuning sugar content in strawberry. Genome Biol. 21 (1), 230. https://doi.org/10.1186/s13059-020-02146-5.
Xu, R., Li, J., Liu, X., Shan, T., Qin, R., Wei, P., 2020a. Development of plant prime-editing systems for precise genome editing. Plant Commun. 1 (3), 100043 https://doi.org/10.1016/J.XPLC.2020.100043. Plant Communications 1 (3), 100043.
Xu, W., Li, K., Li, S., Hou, Q., Zhang, Y., Liu, K., Sun, Q., 2020b. The R-Loop atlas of Arabidopsis development and responses to environmental stimuli. Plant Cell 32 (4), 888–903. https://doi.org/10.1105/tpc.19.00802.
Xu, Z., Kuang, Y., Ren, B., Yan, D., Yan, F., Spetz, C., Sun, W., Wang, G., Zhou, X., Zhou, H., 2020c. SpRY Greatly Expands the Genome Editing Scope in Rice With Highly Flexible PAM Recognition.
Yamano, T., Nishimasu, H., Zetsche, B., Hirano, H., Slaymaker, I.M., Li, Y., Fedorova, I., Nakane, T., Makarova, K.S., Koonin, E.V., Ishitani, R., Zhang, F., Nureki, O., 2016. Crystal structure of Cpf1 in complex with guide RNA and target DNA. Cell 165 (4), 949–962. https://doi.org/10.1016/j.cell.2016.04.003.
Young, J.K., Gasior, S.L., Jones, S., Wang, L., Navarro, P., Vickroy, B., Barrangou, R., 2019. The repurposing of type I-E CRISPR-Cascade for gene activation in plants. Commun. Biol. 2, 383. https://doi.org/10.1038/s42003-019-0637-6.
Zetsche, B., Gootenberg, J.S., Abudayyeh, O.O., Slaymaker, I.M., Makarova, K.S., Essletzbichler, P., Volz, S.E., Joung, J., van der Oost, J., Regev, A., Koonin, E.V., Zhang, F., 2015. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell 163 (3), 759–771. https://doi.org/10.1016/j.cell.2015.09.038.
Zetsche, B., Heidenreich, M., Mohanraju, P., Fedorova, I., Kneppers, J., DeGennaro, E.M., Winblad, N., Choudhury, S.R., Abudayyeh, O.O., Gootenberg, J.S., Wu, W.Y., Scott, D.A., Severinov, K., van der Oost, J., Zhang, F., 2017. Multiplex gene editing by CRISPR-Cpf1 using a single crRNA array. Nat. Biotechnol. 35 (1), 31–34. https://doi.org/10.1038/nbt.3737.

Zhang, Y., Massel, K., Godwin, I.D., Gao, C., 2018. Applications and potential of genome editing in crop improvement. Genome Biol. 19 (1), 210. https://doi.org/10.1186/s13059-018-1586-y.

Zhang, X., Chen, L., Zhu, B., Wang, L., Chen, C., Hong, M., Huang, Y., Li, H., Han, H., Cai, B., Yu, W., Yin, S., Yang, L., Yang, Z., Liu, M., Zhang, Y., Mao, Z., Wu, Y., Liu, M., Li, D., 2020. Increasing the efficiency and targeting range of cytidine base editors through fusion of a single-stranded DNA-binding protein domain. Nat. Cell Biol. 22 (6), 740–750. https://doi.org/10.1038/s41556-020-0518-8.

Zhao, D., Li, J., Li, S., Xin, X., Hu, M., Price, M.A., Rosser, S.J., Bi, C., Zhang, X., 2020. Glycosylase base editors enable C-to-A and C-to-G base changes. Nat. Biotechnol. https://doi.org/10.1038/s41587-020-0592-2.

Zhong, Z., Zhang, Y., You, Q., Tang, X., Ren, Q., Liu, S., Yang, L., Wang, Y., Liu, X., Liu, B., Zhang, T., Zheng, X., Le, Y., Zhang, Y., Qi, Y., 2018. Plant genome editing using FnCpf1 and LbCpf1 nucleases at redefined and altered PAM sites. Mol. Plant 11 (7), 999–1002. https://doi.org/10.1016/j.molp.2018.03.008.

Zhu, H., Li, C., Gao, C., 2020. Applications of CRISPR-Cas in agriculture and plant biotechnology. Nat. Rev. Mol. Cell Biol. https://doi.org/10.1038/s41580-020-00288-9.

Zong, Y., Song, Q., Li, C., Jin, S., Zhang, D., Wang, Y., Qiu, J.-L., Gao, C., 2018. Efficient C-to-T base editing in plants using a fusion of nCas9 and human APOBEC3A. Nat. Biotechnol. https://doi.org/10.1038/nbt.4261.

Zsögön, A., Čermák, T., Naves, E.R., Notini, M.M., Edel, K.H., Weinl, S., Freschi, L., Voytas, D.F., Kudla, J., Peres, L.E.P., 2018. De novo domestication of wild tomato using genome editing. Nat. Biotechnol. 36 (12), 1211–1216. https://doi.org/10.1038/nbt.4272.