Xu et al., 2019; Gao, 2021; Huang et al., 2021; Li et al., 2021c; Xia et al., 2021; Zhan et al., 2021; Puchta et al., 2022). To date, three major CRISPR/Cas mediated precision genome editing systems have been developed and successfully applied in plants such as homology-directed DNA repair (HDR)-mediated targeted gene replacement or gene targeting (Sun et al., 2016; Li et al., 2019; Li and Xia, 2020; Lu et al., 2020; Chen et al., 2022a; Puchta et al., 2022), base editing (Komor et al., 2016; Nishida et al., 2016; Gaudelli et al., 2017; Li et al., 2017; Lu and Zhu, 2017; Shimatani et al., 2017; Zong et al., 2017; Hua et al., 2018; Wei et al., 2021; Tian et al., 2022) (Figure 1), and prime editing (Anzalone et al., 2019; Butt et al., 2020; Jiang et al., 2020; Hua et al., 2020a; Li et al., 2020c; Lin et al., 2020; Tang et al., 2020; Xu et al., 2020a, 2020c; Lu et al., 2021; Wang et al., 2021b; Perroud et al., 2022) (Figure 2). Among these three precise editing technologies, HDR enables the installation or replacement of all kinds of mutations or various lengths of fragments in a predefined manner, representing the holy grail of genome editing. However, although various strategies have been attempted in the past decade (for review, please check Zhan et al., 2021; Puchta et al., 2022; Chen et al., 2022a), HDR remains challenging in plants due to the facts that once the double-strand breaks (DSBs) are generated by CRISPR/Cas nucleases the predominant repair mechanism in cells is nonhomologous end joining (NHEJ) which usually results in random indels, as well as the obstacles in delivery of sufficient donor repair template (DRT) into the vicinity of the DSB and competition with the original DNA strand/fragment to be replaced in plant cells (Li et al., 2019; Lu et al., 2020; for review, please check Li and Xia, 2020; Zhan et al., 2021; Chen et al., 2022a). In contrast, base editing and prime editing are two alternative promising strategies for precise genome editing without a DSB and a DRT. Whereas base editing has emerged as an alternative and effective tool to HDR-mediated gene replacement for precise single base substitution of an allele with a single SNP, facilitating precise gene editing by transition of one single base to another in a programmable manner (Komor et al., 2016; Nishida et al., 2016; Gaudelli et al., 2017) (Figure 1A–C), prime editing enables the installation of all 12 types of base substitutions and small indels, and substantially expands the scope and capabilities of precision genome editing (Anzalone et al., 2019) (Figure 2A).

Since the development of the first generation of base editor (BE) and prime editor (PE) for base editing and prime editing in mammalian cells (Komor et al., 2016; Nishida et al., 2016; Gaudelli et al., 2017; Anzalone et al., 2019), diverse strategies have been exploited to optimize these two editors in order to improve the precise editing efficiency and specificity, and to expand targeting scopes in plants (Li et al., 2020d; Molla et al., 2021; Hua et al., 2022). Here, we summarize the latest developments of various BEs and PEs, as well as their applications in plants. We also provide recommendations in selection of the proper BEs or PEs in practical applications in plants. Moreover, we propose the perspectives for further optimization of these two editors. We truly believe this review will provide a valuable clue to the readers on how to select the appropriate BEs and PEs, as well as future perspectives to streamline these two editors into the routine and customized platform for both fundamental biological studies and crop improvement.

BASE EDITORS AND THEIR APPLICATIONS IN PLANTS

Base editing is a breakthrough technology that can precisely and efficiently achieve single base transition or transversion at target sites without inducing DSBs and the need for a DRT. Three BEs are currently in use: cytosine base editors (CBEs) for C:G to T:A transition (Figure 1A), adenine base editors (ABEs) for A:T to G:C transition (Figure 1B) and C-to-G base editors (CGBEs) for C:G to G:C transversion (Figure 1C). Precise base editing enables a single nucleotide substitution in a specific target gene to generate either loss-of-function or gain-of-function mutations, thus greatly accelerating functional annotation, crop improvement, de novo domestication or directed evolution of target genes in crop plants (Ren et al., 2018; Bharat et al., 2020; Kuang et al., 2020; Zeng et al., 2020; Xu et al., 2021a; Yan et al., 2021; Tan et al., 2022). Since the report of the first generation of CBE and ABE in 2016 (Komor et al., 2016) and in 2017 (Gaudelli et al., 2017) in mammalian cells, respectively, many efforts have been attempted in order to optimize and upgrade these two BEs in plants.

Cytosine base editors

The first-generation CBE was engineered by fusing a rat cytidine deaminase rAPOBEC1 to the N-terminus of an impaired dead Cas9 (dCas9) (Cas9 with D10A and H840A mutations) to generate rAPOBEC1-dCas9 and designated as CBE1 (Komor et al., 2016) (Figure 1D). The substitution of C to T in DNA is created by deaminating the cytosine (C) into uracil (U) in the exposed non-target DNA strand, and the subsequent DNA repair and replication results in C to T base conversion (Figure 1A). The cellular base excision repair (BER) mechanism enables C:G to T:A transition in vivo, while recognizes any G:U base pair as a mismatch. The BER activity eliminates the uracil with the help of uracil N-glycosylase (UNG), resulting in a low efficiency of the CBE1 system (Komor et al., 2016). To improve base editing efficiency, 202424016 ThTh td1Tf.32140TD(519(as)19

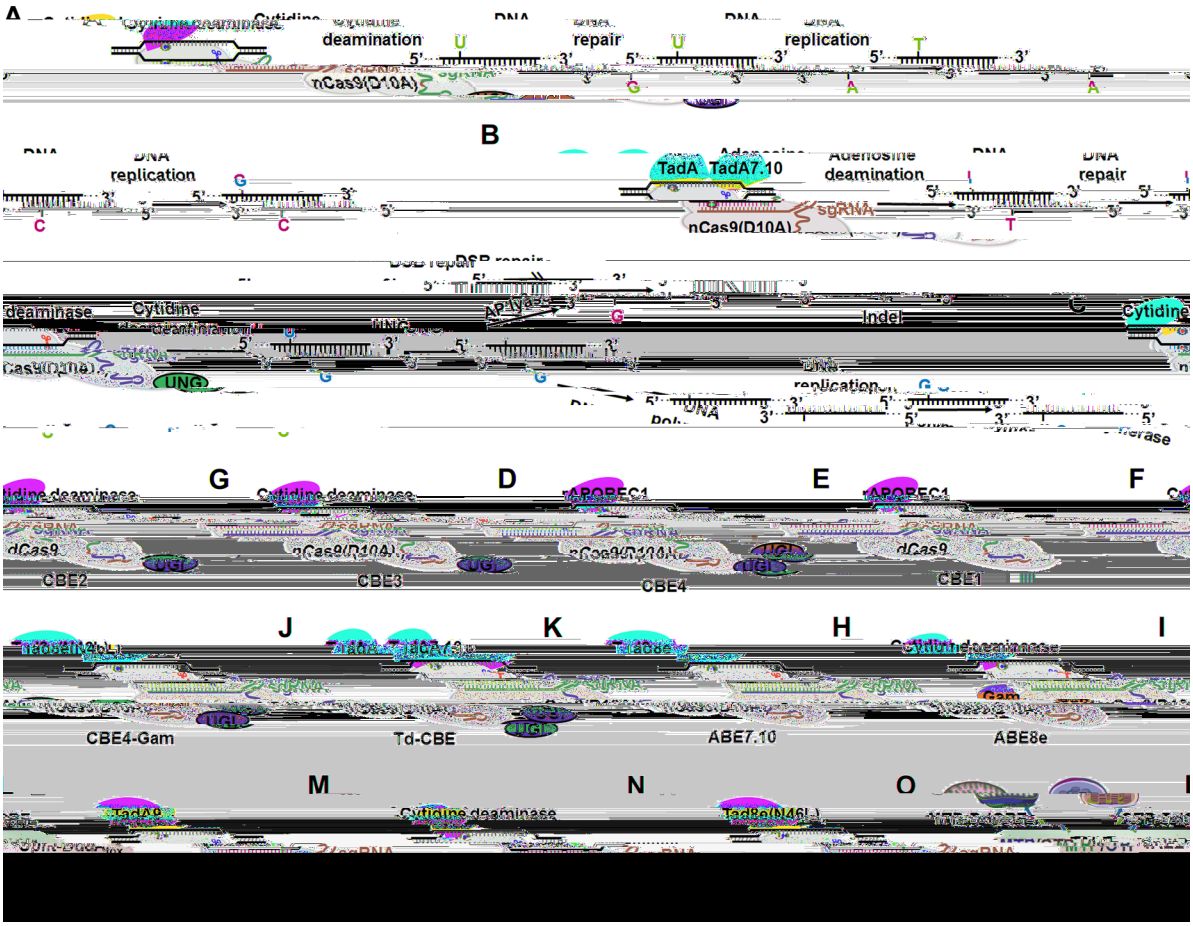

Figure 1. Structural representations of clustered regularly interspaced short palindromic repeats (CRISPR)/nicking CRISPR-associated protein 9 (nCas9)-mediated base editing and the so far developed base editors

(A) CRISPR/nCas9-mediated c‧tosine base editing. A c‧tosine base editor (CBE), which is composed of a catal‧ticall‧ impaired nCas9(D10A) and a c‧tidine deaminase, binds to the target sequence in the genomic DNA in a guide RNA (gRNA)-programmed manner. The c‧tidine deaminase catal‧zes the deamination of c‧tosine (C) in a narrow window of the non-target and makes the base change from C to U (uracil) at a target site. U is recogni‧ed as th‧mine (T) during DNA replication, resulting in a C G to T A transition. (B) CRISPR/nCas9-mediated adenine base editing. An adenine base editor (ABE) is composed of an adenosine deaminase and nCas9(D10A) fusion binding to the target site in a gRNA-programmed manner. The adenosine deaminase catal‧zes an A (adenine) to I (inosine) change at the target site. During replication, the original A is replaced with G (guanine). Finall‧, A T to G C conversion is achieved in the non-target DNA strand. (C) CRISPR/nCas9-mediated C-to-G base editing. The C-to-G base editor (CGBE) is composed of a c‧tidine deaminase, nCas9(D10A), and uracil N-gl‧cos‧lase (UNG), and binds to the target site in a gRNA-programmed manner. The c‧tidine deaminase catal‧zes the deamination of c‧tosine (C) and makes the base change from C to U (uracil). UNG can remove U from the DNA double strands and an error-prone DNA pol‧merase replaces G with C at the target site. The C G to-G C transversion occurs during DNA replication. As nCas9(D10A) nicks the target strand, a DSB is formed when the abasic site on the non-target strand is converted into a nick b‧ an apurinic or ap‧rimidinic site l‧ase (AP l‧ase). The DSB results in indel formation at the target site. (D) The first-generation c‧tosine base editor, CBE1, was engineered b‧ fusing c‧tidine deaminase, rAPOBEC1 to the N-terminus of a dead Cas9 (dCas9, a mutant of Cas9 containing both D10A and H840A mutations). (E) The second-generation base editor, CBE2, was engineered b‧ fusing rAPOBEC1 to the N-terminus of dCas9 and fusing a uracil DNA gl‧cos‧lase inhibitor (UGI) to the C-terminus of dCas9. (F) The third-generation c‧tosine base editor, CBE3, was engineered b‧ fusing different deaminases to the N-terminus of nCas9(D10A), and fusing UGI to the C-terminus of nCas9 (D10A), respectivel‧. The deaminases that have been successfull‧ applied in plants include rAPOBEC1 (Li et al., 2017; Lu and Zhu, 2017; Zong et al., 2017), PmCDA1 (Shimatani et al., 2017; Zhong et al., 2019), hAID (Ren et al., 2018; Wang et al., 2020a), APOBEC3A (Zong et al., 2018), and evoFENRY (Zeng et al., 2020). (G) The fourth-generation c‧tosine base editor, CBE4, was developed b‧ fusing two UGI molecules to the

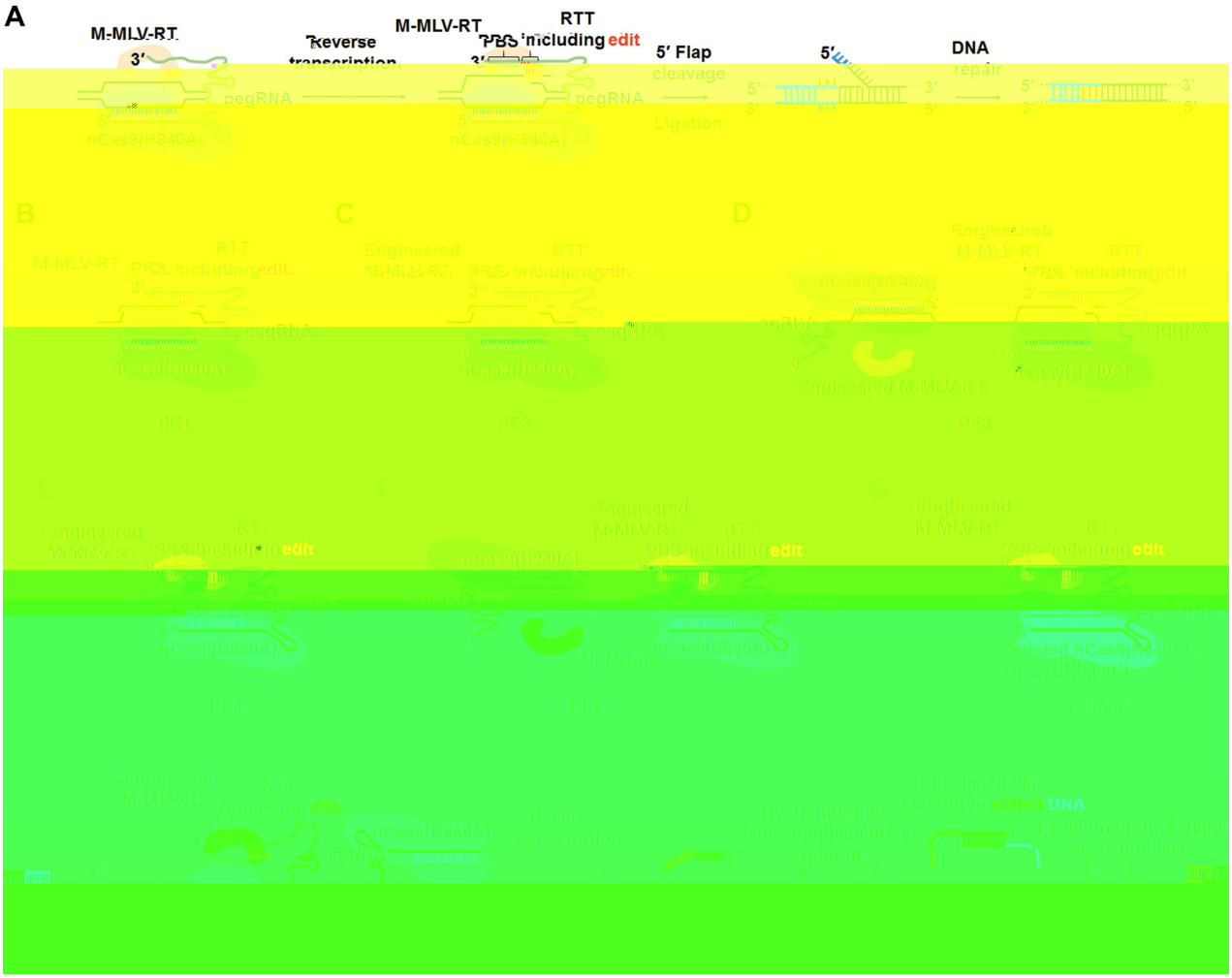

Figure 2. The principle of clustered regularly interspaced short palindromic repeats (CRISPR)/nicking CRISPR-associated protein 9-mediated prime editing and schematic diagrams of the so far developed prime editors

(A) The CRISPR/nCas9-mediated prime editing system. A prime editor mainly consists of a catalytically impaired nCas9(H840A), a M-MLV-RT (Moloney murine leukemia virus reverse transcriptase), and a prime editing guide RNA (pegRNA). pegRNA is composed of three components, including a single-guide RNA (sgRNA) targeting the specific site, a reverse transcription template (RTT) encoding the desired edit, and a primer binding site (PBS) initiating RT. The nCas9(H840A)-M-MLV-RT and pegRNA complex bind to the target sequence in the genomic DNA in a sequence-specific manner. The M-MLV-RT helps the 3′ DNA end from the PBS to prime the reverse transcription of an edit-encoding extension from pegRNA directly into the target site. (B) The first-generation prime editor, PE1, was engineered by fusing a wild M-MLV-RT to the N-terminus of nCas9(H840A). (C) The second-generation prime editor, PE2, was engineered by fusing an engineered M-MLV-RT with six amino acid mutations to the N-terminus of nCas9(H840A). (D) The third-generation prime editor, PE3, was engineered by using an additional sgRNA on the non-targeting strand. (E) The fourth-generation prime editor, PE4, was developed with co-expression of a dominant negative mismatch repair (MMR) protein (MLH1dn) on the basis of PE2. (F) The fifth-generation prime editor, PE5, was developed with transient co-expression of a dominant negative MMR protein (MLH1dn) on the basis of PE3. (G) PEmax was engineered by replacing nCas9(H840A) with a mutated version which harbors R221K and N394K mutations. (H) Overview of the design of twinPE or GRAND editor and the sequence replacement process. The single-strand DNAs (red and blue lines) produced by the paired pegRNAs containing RTTs highlighted in light red and light blue, respectively, bind to each other through their complementary ends highlighted in orange. The original 5′ flaps were replaced by the aannealed 3′ flaps containing the edited DNA following DNA replication and repair.

CBE4, was developed by fusing two UGI molecules to the C-terminal of Cas9 nickase on the basis of CBE3 to enhance the inhibition of UNG (Komor et al., 2017) (Figure 1G). Compared with CBE3, CBE4 not only improves the base editing efficiency but also reduces the frequency of C to A or G transversions by 2.3 times. In addition, bacteriophage Mu Gam protein was added on the basis of CBE4 to construct a BE CBE4-Gam, in order to further improve the product purity and reduce the occurrence of indels (Komor et al., 2017) (Figure 1H).

CBEs, especially CBE3 and CBE4, have been widely used in plants. Initially, a base editing system was developed using a rat APOBEC1 in rice. To validate and test the feasibility of CBEs in plants, by fusing a rat APOBEC1 to the N-terminus of nCas9 (D10A) to form a structure of rAPOBEC1-nCas9 (D10A), two agriculturally important genes of rice, OsNRT1.1B and OsSLR1, were edited at editing efficiencies of 2.7% and 13.3%, respectively (Lu and Zhu, 2017) (Table 1). Simultaneously, three targets in rice, one target (P2)

Table 1. Base editing in plants

Base editors	Structures of various base editors	Species	Target gene	Transformation	Selection	Editing efficiency (%)	Editing window	Trait improvement	References
APOBEC1-CBE2/CBE3	APOBEC1-XTEN-nCas9	Rice	OsNRT1.1B, OsSLR1	Agrobacterium	Hygromycin	2.70–13.30	C4–C8	High nitrogen use efficiency; Dwarf	Lu and Zhu (2017)
	APOBEC1-XTEN-nCas9-UGI	Rice	OsSBEIIb, OsPDS	Bombardment	Hygromycin	20.00	C4–C8	High amylose	Li et al. (2017)
	APOBEC1-XTEN-nCas9/dCas9-UGI	Rice, Wheat Maize	OsCDC48, OsNRT1.1B, OsSPL14, TaLOX2, ZmCENH3	Agrobacterium /Bombardment	Herbicide	0–43.48	C3–C9	High nitrogen use efficiency; High yield	Zong et al. (2017)
	APOBEC1-XTEN-nCas9-UGI	Rice	OsCERK1, OsSERK1, OsSERK2, ipa1, Pi-ta	Agrobacterium	Hygromycin	10.50–38.90	C4–C8	High yield; Blast resistance	Ren et al. (2017)
	APOBEC1-XTEN-nCas9-UGI	Wheat	TaALS-P174	Bombardment	Herbicide	33.00–75.00	C4–C8	Herbicide resistance	Zhang et al. (2019)
	APOBEC1-XTEN-nCas9-UGI	Cotton	GhCLA, GhPEBP	Agrobacterium	Hygromycin	0–57.78	C4–C8	–	Qin et al. (2020)
hAID-CBE3	hAID-XTEN-nCas9	Rice	OsFLS2, OsAOS1, OsJAR1, OsJAR2, OsCOI2, OsPi-D2	Agrobacterium	Hygromycin	8.30–73.30	C3–C8	Blast resistance	Ren et al. (2018)
	hAID-nSpCas9-NG/nCas9-UGI	Rice	OsBZR1, OsSERK2	Agrobacterium	Hygromycin	4.44–27.08	C3–C8	Enhance fruit quality	Ren et al. (2019)
	hAID-nScCas9-UGI hAID-nSpCas9-UGI	Rice	OsMPK9, OsMPK17, OsCPK5, OsMPK15, OsMPK16, OsCPK6, OsCPK7, OsCPK8	Agrobacterium	Hygromycin	2.56–97.92 0–95.83	C3–C8	–	Wang et al. (2020a)
	hAID-XTEN-nSpRY-UGI	Rice	OsCOI2, OsBSR, OsMPK13, OsGS1, OsGSK4	Agrobacterium	Hygromycin	26.00–34.15	C3–C8	–	Xu et al. (2021c)
APOBEC3A-CBE3/CBE4	A3A-XTEN-nCas9-UGI; Gam-XTEN-A3A-nCas9-UGI-UGI	Rice , Wheat Potato	OsAAT, OsCDC48, OsDEP1, OsNRT1, OsOD, TaALS, TaMTL, TaLOX2, StGBSS-T6	Agrobacterium /Bombardment	Herbicide/Hygromycin	0–82.90 1.20–20.00	C1–C17	Herbicide resistance	Zong et al. (2018)
PmCDA1-CBE2/CBE3/CBE4	nCas9/dCas9-PmCDA1	Rice , Tomato	ALS, DELLA, ETR1.	Agrobacterium	Herbicide/Hygromycin	26.20–53.80	C2–C5	Herbicide resistance	Shimatani et al. (2017)
	PmCDA1-xCas9-UGI PmCDA1-nSpCas9-NG-UGI	Rice	OsDEP1, OsCDC48, OsGS3, OsPDS	Agrobacterium	Hygromycin	0–21.10 3.50–56.30	C2–C5	–	Zhong et al. (2019)
	nCas9-NG-PmCDA1-UGI	Tomato potato	SlALS	Agrobacterium	Hygromycin	32.00	C2–C5	Herbicide resistance	Veillet et al. (2020)
	PmCDA1-nScCas9^{+}$^{+}$-UGI-UGI	Rice	OsWaxy, OsEUI1	Agrobacterium	Hygromycin	8.3–86.1	C1–C17	Reduced amylose content	Liu et al. (2021b)
CBE4	FENRY-nCas9-NG-UGI-UGI	Rice	OsCKX2, OsWaxy, OsEUI1, OsSPL4, OsSPL7, OsSPL14,	Agrobacterium	Hygromycin	0–86.30 0–59.40	C4–C12 C2–C5	–	Zeng et al. (2020)

Continued

Table 1. Continued

Base editors	Structures of various base editors	Species	Target gene	Transformation	Selection	Editing efficiency (%)	Editing window	Trait improvement	References
	PmCDA1-nCas9-NG-UGI-UGI APOBEC1-nCas9-NG-UGI-UGI		LF1, OsIAA13, OsMADS57, OsGBSSI			0–66.70	C4–C8		
ABE7.10	TadA-TadA7.10-nCas9(D10A)	Rice , Wheat	OsALS, OsCDC48, OsAAT, OsDEP1, OsACC, OsNRT1.1B, OsEV, OsOD, TaDEP1, TaEPSPS, TaGW2	Agrobacterium /Bombardment	Herbicide/ Hygromycin	3.20–59.10	A4–A8	Herbicide resistance	Li et al. (2018)
	TadA-TadA7.10-nCas9	Rice	OsSERK2, OsMPK6, OsWRKY45, OsMPK13	Agrobacterium	Hygromycin	6.45–62.26	A4–A8	–	Yan et al. (2018)
	TadA-TadA7.10-nSpCas9-NG TadA-TadA7.10-nSaCas9	Rice	OsSPL7, OsSPL14, OsSLR1, OsSPL4, OsSPL16, OsSPL17, OsSPL18	Agrobacterium	Hygromycin	0–26.00 4.80–61.30	A4–A8	High yield	Hua et al. (2018)
	TadA-TadA7.10-nScCas9	Rice	OsMPK14, OsCPK9, OsMPK15, OsCPK10	Agrobacterium	Hygromycin	50.00–94.12	A4–A8	–	Wang et al. (2020a)
ABE-P1S	TadA7.10-nSpCas9 TadA7.10-nSaCas9	Rice	OsSERK2, OsSPL14, SLR1, Tms9-1, OsNRT1.1B, OsACC1, OsDEP1	Agrobacterium	Hygromycin	4.50–96.30 0–61.10	A1–A12	High yield; Herbicide resistance	Hua et al. (2020b)
ABE8e	TadA8e(V106W)-nCas9 TadA8e(V106W)-nCas9-NG	Rice	OsEPSPS, OsALS, OsWaxy	Agrobacterium	Herbicide/ Hygromycin	4.00–100.00 0–100.00	A4–A8	Herbicide resistance	Wei et al. (2021)
	TadA8e-XTEN-nSpRY	Rice	OsCOI2, OsBSR, OsMPK13, OsGS1, OsGSK4	Agrobacterium	Hygromycin	27.79–93.75	A3–A10	Herbicide resistance	Xu et al. (2021c)
	TadA8e-DBD-nCas9-NG TadA8e-DBD-nSpG TadA8e-DBD-nSpRY	Rice	OsSPL14, OsIAA13, OsSPL7, OsLF1, OsGBSSI, OsCKS2, OsEUI1, OsTS	Agrobacterium	Hygromycin	0–90.50 0–92.50 0–100.00	A1–A14 A1–A14 A1–A14	–	Tan et al. (2022)
ABE9	TadA9-XTEN-nSpCas9 TadA9-XTEN-nSpCas9-NG TadA9-XTEN-nSpRY TadA9-XTEN-nScCas9	Rice	OsMPK6, OsMPK13, OsSERK2, OsWRKY45, OsDEP2, OsETR2, OsGSK4, OsJAR1, OsGS1, OsALS1	Agrobacterium	Herbicide/ Hygromycin	0–97.92 0–100.00 0–37.50 0–68.75	A1–A12 A4–A10 A3–A10 A4–A12	–	Yan et al. (2021)
pDuBE1	TadA8e-nCas9-CDA1-UGI	Rice	OsALS, OsBADH2, OsLAZY1, OsPDS	Agrobacterium	Herbicide/ Hygromycin	0.40–87.60	C2–C5 A4–A8	–	Xu et al. (2021a)

in OsPDS, which encodes a phytoene desaturase, and two targets (S3 and S5) in OsSBEIIb, which encodes a starch branching enzyme IIb, were successfully edited in rice by using CBE3 with the efficiencies of 19.2%, 10.5%, and 1.0% at the S5, S3, and P2 targets, respectively (Li et al., 2017) (Table 1). Meanwhile, targeted C G to T A transitions in OsCDC48, OsSPL14, OsNRT1.1B, TaLOX2, ZmCENH3 genes were achieved at frequencies of up to 43.48% from position 3 to 9 within the protospacer in the genomes of rice, wheat and maize by using a nCas9-cytidine deaminase fusion (Zong et al., 2017) (Table 1). Although the editing efficiencies of CBEs has been improved to a certain extent, its application

enables c↑tidine deamination was obtained b↑ phage-assisted continuous evolution (

Selecting the base-edited cells from massive transformed calli for regeneration is time-consuming and labor-intensive during plant tissue culture, especially for the low-efficient ed-

dsDNA, resulting in efficient C:G to T:A conversion in the human mitochondrial genome with high target specificity (Mok et al., 2020). Similar research was performed in lettuce (Lactuca sativa) and rapeseed (Brassica napus) protoplasts with up to 23% efficiencies (Kang et al., 2021). Recently, using the DdCBE linked to a plastid-targeting signal peptide (PTP) of AtRecA1 protein at its N-terminus, three target genes (16s rRNA, rpoC1, psbA) located in the plastid genome were successfully edited without leaving any foreign genes in either the plastid or nuclear genomes in Arabidopsis (Nakazato et al., 2021) (Figure 1O; Table 1). Furthermore, an efficient DdCBE system was constructed by fusing a chloroplast transition peptide (CTP) to its N-terminus. This CTP-DdCBE achieved a conserved chloroplast gene chlorophyll A of photosystem I (psaA), for C to T transitions in rice chloroplasts (Li et al., 2021b) (Figure 1O; Table 1). The edited psaA could potentially improve photosynthetic efficiency and grain yield of crops. The successful implementation of DdCBEs (CRISPR-independent organelle BEs) in plant organelle cells increases the possibility of precise manipulation of organelle genomes for crop improvement (Kang et al., 2021).

Single nucleotide variation is the genetic basis for the improvement of important crop traits. Random mutagenesis by physical or chemical methods has long been applied to improve traits in plants, but it is labor-intensive and time-consuming. The base editing system can enable the artificial evolution of agri-

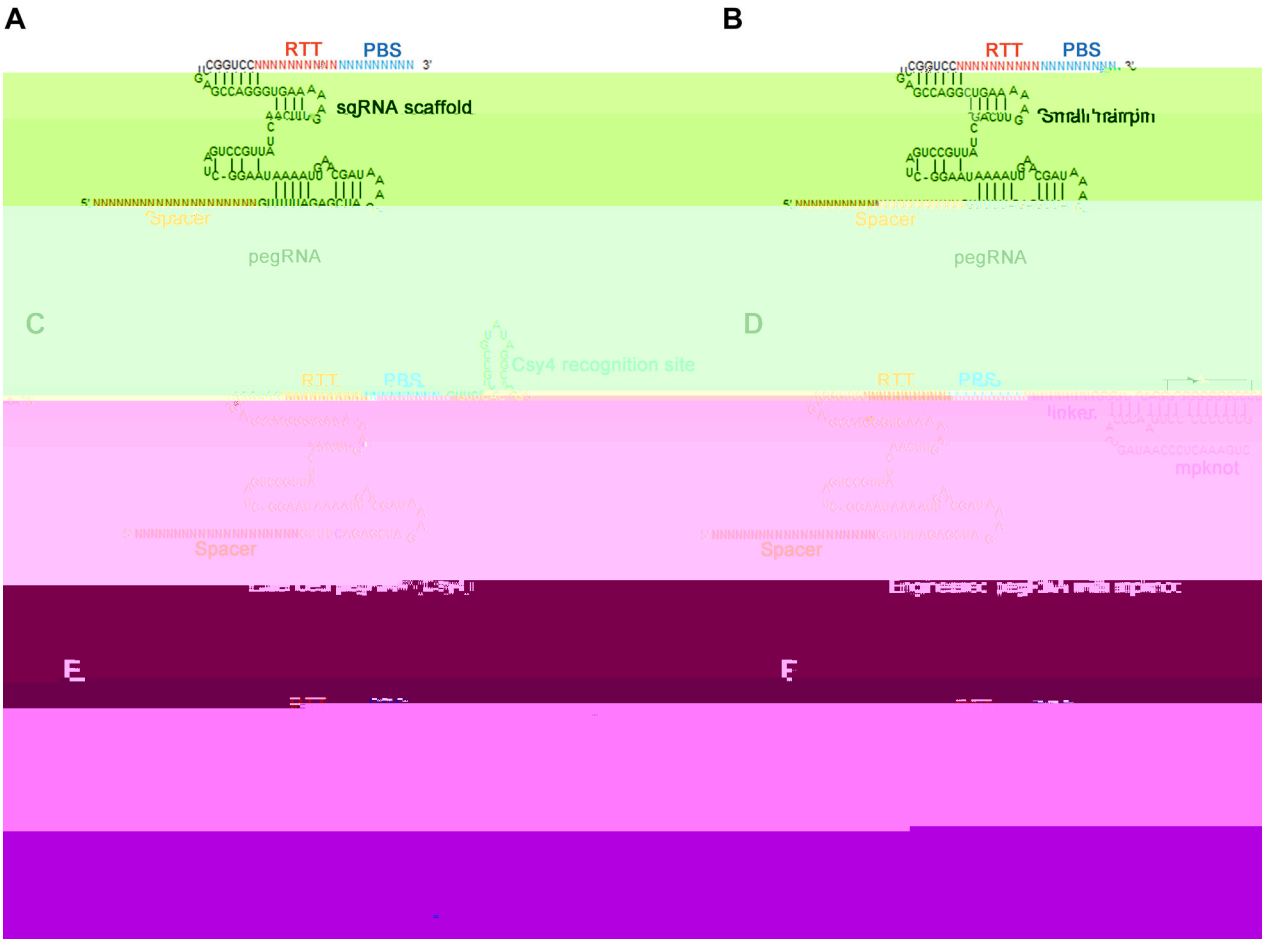

Figure 3. Optimizations of different prime editing guide RNAs (pegRNAs)

(A) The schematic diagram of a canonical pegRNA. A pegRNA is composed of three components, including a single-guide RNA (sgRNA) targeting the specific site, a reverse transcriptase (RT) template (RTT) encoding the desired edit, and a primer binding site (PBS) initiating RT. The RTT sequence is highlighted in red, the PBS sequence is highlighted in blue, and the spacer sequence is highlighted in dark red. (B) The schematic diagram of apegRNA, which has a C/G pair at the bottom of the small hairpin. The C/G base pair is highlighted in purple. (C) The schematic diagram of a Cs4-processed pegRNA, which protects the 3′ extension from degradation b exonucleases. Cs4 is a specialized ribonuclease that selects clustered regularl interspaced short palindromic repeats (CRISPR) transcripts from the cellular milieu for binding and cleavage. With Cs4 processing, the hairpin Cs4 recognition site remains at the 3′ end of the pegRNA as an extension. At the same time, mutation of the fourth one of the consecutive uracils (highlighted in purple) was introduced to the scaffold of pegRNA. The Cs4 recognition site sequence is highlighted in green. (D) An engineered pegRNA with a structured RNA pseudoknot (mpknot), protects its 3′ extension from degradation b exonucleases. The mpknot is a frameshifting pseudoknot from Molone murine leukemia virus (M-MLV), and it is an endogenous template for the M-MLV-RT from which the RT in canonical prime editors was engineered, raising the possibilit that mpknot might help recruit the RT. The mpknot sequence is highlighted in pink. (E) An engineered pegRNA with a structured RNA pseudoknot evopreQ$_1$, which protects the 3′ extension from degradation b exonucleases. evopreQ$_1$, as a modified prequeosine1-1 riboswitch aptamer composed of 42 nucleotides (nt) in length, is one of the smallest naturall derived RNA structural motifs with a defined tertiar structure. The evopreQ$_1$ sequence is highlighted in dark slate. (F) A representative engineered epegRNA with MS2 and f6 RNA aptamers. esgRNA, enhanced sgRNA with modifications highlighted in green. RNA aptamers can recruit their respective effector proteins for efficient gene editing. The MS2 sequence is highlighted in orange. The f6 sequence is highlighted in purple.

degradation of the 3′ extension, and eventuall improving the efficienc of prime editing b 3- to 4-fold in human cells without increasing off-target editing activit (Nelson et al., 2022) (Figure 3D, E). Extension of this strateg in plants significantl enhanced prime editing efficienc (Jiang et al., 2022b; Li et al., 2022b; Zou et al., 2022) (Table 2). In addition, MS2-based PE (MS2-PE) has also been developed to improve the prime editing efficienc b using RNA aptamers (MS2 and f6) in pegRNA and fusion of their binding protein MCP with the PE2 stem (Figure 3F), and achieved up to 10.1-fold increase in editing efficienc at five of six targets in transgenic rice lines (Chai et al., 2021).

While canonical PEs mainl enable base conversions and installation of small indels (Anzalone et al., 2019), development of PE capable of knock-in or replacement of large DNA fragments is highl desirable either for gene therap or crop improvement. Recentl, several powerful strategies have been developed to precisel replace, insert, and delete large DNA fragments in human cells, including twinPE (Anzalone et al., 2022) (Figure 2H), GRAND editing (Wang et al., 2022c) (Figure 2H), PRIME-Del (a prime editing-based method, which induces a deletion using a pair of pegRNAs that target opposite DNA strands) (Choi et al., 2022), and PEDAR (PE-Cas9-based

Table 2. Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associate protein 9 (Cas9) mediated prime editing in plants

PEs	PE features (PBS length (nt) and RT template length (nt))	Target gene	Mutation type	Editing effienc		Transformation	Selection	Trait improvement	References
				Desired (%)	Undesired (%)				
PE2									
PE2	13, 15-20	OsALS, OsIPA1, OsTB1	2–4 bp Subs, 2 bp Ins	0.00–2.04	NR	Agrobacterium	Hygromycin, Bispribac sodium	Herbicide resistance, High yield	Butt et al. (2020)
Sp-PE2	13, 13	GFP	2 bp Subs	15.60	NR	Agrobacterium	Hygromycin	-	Hua et al. (2020a)
pPE2	10–13, 10–34	HPTII, OsPDS, OsACC, OsWx	1–3 bp Ins, 1 bp Subs	0.00–59.90	NR	Agrobacterium	Hygromycin	-	Xu et al. (2020a)
PE2	13,16; 13, 14–23	OsALS, OsACC	1–3 bp Subs	1.00–7.60	0.00	Agrobacterium	Hygromycin	Herbicide resistance	Jiang et al. (2022b)
pPE2	10–13, 11–18	OsPDS, OsACC, OsALS, OsCDC48	1 bp Ins, 1–2 bp Subs	0.00–29.17	NR	Agrobacterium	Hygromycin	Herbicide resistance	Li et al. (2022b)
PPE	8, 17	OsALS	2 bp Subs	2.10	NR	Agrobacterium	Hygromycin	Herbicide resistance	Zong et al. (2022)
pPE2 (an engineered pegRNA with mpknot)	10–13, 13–18	OsPDS, OsALS, OsCDC48	1 bp Ins, 1–2 bp Subs	10.42–25.00	NR	Agrobacterium	Hygromycin	Herbicide resistance	Li et al. (2022b)
pPE2 (an engineered pegRNA with evopreQ_1)	10–13, 11–18	OsPDS, OsACC, OsALS, OsCDC48	1 bp Ins, 1–2 bp Subs	2.08–50.00	NR	Agrobacterium	Hygromycin	Herbicide resistance	Li et al. (2022b)
ePPE(replac-ing M-MLV-RT with M-MLV-RT-ΔRNaseH)	8, 17	OsALS	2 bp Subs	11.30	NR	Agrobacterium	Hygromycin	Herbicide resistance	Zong et al. (2022)
pZ1WS (drived by the CaMV35S-CmYLCV-U6 composite promoter)	13, 16	ZmALS1, ZmALS2	2–3 bp Subs	4.80–53.20	NR	Agrobacterium	Glufosinate ammonium	Herbicide resistance	Jiang et al. (2020)
PE3									
Sp-PE3	13, 13–16	APO1, GFP, OsALS	1–2 bp Subs	0.00–17.10	NR	Agrobacterium	Hygromycin, Bispribac sodium	Herbicide resistance	Hua et al. (2020a)
PE3	13, 28–59	HPTII, OsEPSPS	3–7 bp Subs	2.22–9.38	NR	Bombardment	Hygromycin	Herbicide resistance	Li et al. (2020c)

Continued

Table 2. Continued

PEs	PE features (PBS length (nt) and RT template length (nt))	Target gene	Mutation type	Editing effienc		Transformation	Selection	Trait improvement	References
				Desired (%)	Undesired (%)				
PPE3	10–12, 9–17	OsCDC48, OsALS	1–3 bp Subs, 6 bp Del	2.60–21.80	NR	Bombardment	Hygromycin	Herbicide resistance	Lin et al. (2020)
pPE3	13, 10	OsWx, OsACC	1 bp Subs	0.00–16.70	NR	Agrobacterium	Hygromycin	Herbicide resistance	Xu et al. (2020a)
PE-P1	11–14, 14–23	OsDEP1, OsALS, OsACC	1–4 bp Subs	0.00–1.40	0.00	Agrobacterium	Hygromycin	Herbicide resistance	Xu et al. (2020c)
PE3	9–13, 9–28	OsSPL14, OsDHDPS, OsNR2	2–3 bp Subs	0.00–1.00	NR	Bombardment	Hygromycin	-	Li et al. (2022a)
PE3	13,16; 13, 14–23	OsALS, OsACC, OsEPSPS	1–3 bp Subs	1.30–70.30	9.00–37.90	Agrobacterium	Hygromycin	Herbicide resistance	Jiang et al. (2022b)
PPE3-unmodified	11–14, 11–18	OsROC, OsALS, OsCDC48, OsDEP1	1–3 bp Subs, 3 bp Ins	0.00–2.90 (Normal) 0.00–15.20 (HT)	NR	Agrobacterium	Hygromycin	Herbicide resistance	Zou et al. (2022)
pPE3b	13, 10	OsACC	1 bp Subs	6.25	NR	Agrobacterium	Hygromycin	Herbicide resistance	Xu et al. (2020a)
PE-P2(nCas9 (H840A)-M-MLV-T2A-hpt)	11–14, 14–23	OsDEP1, OsALS, OsACC	1–4 bp Subs	1.70–26.00	0.00–8.00	Agrobacterium	Hygromycin	Herbicide resistance	Xu et al. (2020c)
pCXPE03 (drived by the RPS5A promoter)	14, 17–18	SlGAI, SlALS, SlPDS	2 bp Subs, 2 bp Ins	0.00–6.70	NR	Agrobacterium	Hygromycin	Herbicide resistance	Lu et al. (2021)
PPE3-evopreQ$_1$	11–14, 11–18	OsROC, OsALS, OsCDC48, OsDEP1	1–3 bp Subs, 3 bp Ins	2.60–47.50 (Normal) 5.00–60.50 (HT)	NR	Agrobacterium	Hygromycin	Herbicide resistance	Zou et al. (2022)
PPE3-mpknot	11–14, 11–18	OsROC, OsALS, OsCDC48, OsDEP1	1–3 bp Subs, 3 bp Ins	0.00–4.20 (Normal) 0.00–6.30 (HT)	NR	Agrobacterium	Hygromycin	Herbicide resistance	Zou et al. (2022)
PE-P2-RT-S (N-terminal M-MLV + a single desired mutation in RTT)	8–14, 13–23	OsGS3, OsALS, OsACC, OsChalk5, OsDEP1, OsWaxy, OsGRF4, OsSD1, OsEPSPS, OsCold1, OsPSR1	1 bp Subs	0.00–61.40	0.00–15.00	Agrobacterium	Hygromycin	-	Xu et al. (2022)
PE-P3-RT-M (C-terminal M-MLV +, multiple synonymous base mutations in RTT)	8–14, 13–23	OsGS3, OsALS, OsACC, OsChalk5, OsDEP1, OsWaxy, OsGRF4, OsSD1, OsEPSPS, OsCold1, OsPSR1	3–4 bp Subs	0.00–82.60	0.00–18.00	Agrobacterium	Hygromycin	-	Xu et al. (2022)

Table 2. Continued

PEs	PE features (PBS length (nt) and RT template length (nt))	Target gene	Mutation type	Editing effienc		Transformation	Selection	Trait improvement	References
				Desired (%)	Undesired (%)				
PE-P3-RT-S (C-terminal M-MLV + a single desired mutation in RTT)	8–14, 14–23	OsGS3, OsALS, OsACC, OsChalk5, OsDEP1, OsWaxy	1 bp Subs	0.00–22.70	0.00–15.00	Agrobacterium	Hygromycin	-	Xu et al. (2022)
PE-P2-RT-M (N-terminal M-MLV + multiple synonymous base mutations in RTT)	8–14, 14–23	OsGS3, OsALS, OsACC, OsChalk5, OsDEP1, OsWaxy	3 bp Subs	0.00–26.00	0.00–8.00	Agrobacterium	Hygromycin	-	Xu et al. (2022)
PE3-HS (hygromycinY46*-based)	9–13, 9–28; 13, 19	OsSPL14+mhptII, OsDHDPS+mhptII, OsNR2+mhptII	2–3 bp Subs, 1 bp Subs	1.30–2.10	NR	Bombardment	Hygromycin	-	Li et al. (2022a)
PE3-AS (OsALS-S627I-based)	9–13, 9–28; 13, 12	OsSPL14+OsALS, OsDHDPS+OsALS, OsNR2+OsALS, OsSPL14+OsALS +OsDHDPS	2–3 bp Subs, 3 bp Subs	2.40–14.30	NR	Bombardment	Hygromycin, Bispyribac sodium	-	Li et al. (2022a)
PE3-DS (PE3-HS +PE3-AS)	9–13, 9–28; 13, 12; 13, 19	OsSPL14+OsALS +mhptII, OsDHDPS +OsALS+mhptII, OsNR2+OsALS+ mhptII, OsSPL14 +OsALS+ OsDHDPS+mhptII, OsSPL14+OsALS+ OsEPSPS+mhptII	2–7 bp Subs, 3 bp Subs, 1 bp Subs	3.20–54.20	NR	Bombardment	Hygromycin, Bispyribac sodium	-	Li et al. (2022a)
PE4									
PE4	13,16; 13, 14–23;	OsALS, OsACC	1–3 bp Subs	5.20–27.10	0.00–2.10	Agrobacterium	Hygromycin	Herbicide resistance	Jiang et al. (2022b)
PE5									
PE5	13,16; 13, 14–23;	OsALS, OsACC, OsEPSPS	1–3 bp Subs	1.60–64.10	6.40–18.30	Agrobacterium	Hygromycin	Herbicide resistance	Jiang et al. (2022b)
PEmax									
ePE3max (the PEmax protein +an engineered pegRNA with evopreQ$_1$)	13, 22	OsEPSPS	3 bp Subs	37.20–39.80	18.40–21.00	Agrobacterium	Hygromycin	Herbicide resistance	Jiang et al. (2022b)

Continued

Table 2. Continued

PEs	PE features (PBS length (nt) and RT template length (nt))	Target gene	Mutation type	Editing efficiency		Transformation	Selection	Trait improvement	References
				Desired (%)	Undesired (%)				
PEmax ePE5max (ePE3max+ OsMLH1dn) pPE2max-evopreQ$_1$ (PEmax+an	13, 22	OsEPSPS	3 bp Subs	38.20–39.50	20.80–20.90	Agrobacterium	Hygromycin	Herbicide resistance	Jiang et al. (2022b)

deletion and repair) method (Jiang et al., 2022a). These prime editing systems were developed by employing similar strategies such as using a pair of designed pegRNAs that target the opposite DNA strands, and the RTTs from the two respective pegRNAs were nonhomologous to the target sites but partially complementary to each other (Anzalone et al., 2022; Choi et al., 2022; Jiang et al., 2022a; Wang et al., 2022c). Although the above systems for replacement or knock-in of large DNA fragments through prime editing had been successfully applied in mammalian cells, the feasibilities of twinPE, GRAND editing, PRIME-Del, and PEDAR in plant prime editing remain to be investigated in the near future.

Applications of diverse PEs in plants

Prime editing system substantially expands the scope and capabilities of precision genome editing and holds great promise to introduce precise genome modifications such as SNP and/or small indels into plant genomes to improve agriculturally important traits in crops (Li et al., 2020d). Since the first report of prime editing in mammalian cells in 2019 (Anzalone et al., 2019), the feasibilities and efficacies of PE2 and PE3 for precise genome editing had soon been investigated in rice (Butt et al., 2020; Hua et al., 2020a; Jiang et al., 2020; Li et al., 2020c; Lin et al., 2020; Tang et al., 2020; Xu et al., 2020a, 2020c), other plant species (Jiang et al., 2020; Lin et al., 2020; Lu et al., 2021), and followed by further optimization to improve their prime editing efficiencies thereafter (Jiang et al., 2020; Li et al., 2020c; Lu et al., 2021; Xu et al., 2022; Li et al., 2022a; Jiang et al., 2022b; Xu et al., 2020a, 2020c) (Table 2). Applications of diverse PEs in plants, their features and editing efficiencies and so forth, are summarized in Table 2.

The feasibility and efficacy of a series of plant codons optimized from PE2 and PE3 were first validated and investigated in rice and wheat protoplasts (Lin et al., 2020; Tang et al., 2020) or stable rice plants (Li et al., 2020c; Xu et al., 2020a, 2020c) almost simultaneously in five laboratories. Except for the intrinsic nature of target genes, various parameters such as PBS length, RT template length, and the position of nicking sgRNA significantly affected the precise editing efficiency of PE2 and PE3 in rice and wheat protoplasts (Lin et al., 2020). The PE2 system could also induce programmable editing at different genome sites at a frequency of 0% to 31.3% in rice stable lines, suggesting that the efficiency of pPE2 varied greatly at different genomic sites and with pegRNAs of diverse structures (Xu et al., 2020a). By using the polII promoter Actin to drive the expression of the tandem repeats of polycistronic transfer RNAs to simultaneously produce pegRNA and nicking sgRNA in a PE3, 28 bp and a 59 bp fragments with desired edits were precisely installed into an exogenous inactive hygromycin phosphotransferase (HPT) gene hptII to restore its function, and an endogenous gene OsEPSPS, which encodes a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), to generate a novel allele with TAP-IVS mutations (T173I, A174V, and P177S) which confers rice glyphosate resistance in rice stable lines, respectively (Li et al., 2020c).

Furthermore, development of a plant PE by fusion of HPT to the C-terminus of nCas9-M-MLV with a self-cleaving 2A peptide (P2A) linker and paired with an enhanced sgRNA (esgRNA) improved the prime editing efficiency and achieved versatile nucleotide substitutions in rice stable lines (Xu et al., 2020c). Compared with normal PE, this strategy could increase the editing efficiency up to 22-fold at the OsALS-1 site (from 1.20% to 26.00%) (Xu et al., 2020c). Later on, a PE2-based plant PE with a pegRNA of 13-nt PBS and 15-nt RTT was transformed into rice to alter the target codon TGG for Trp548 of the ALS gene generated herbicide resistant rice plants (Butt et al., 2020). At the same time, prime editing of OsIPA1 in rice reduced the number of unproductive tillers and improved rice yield (Butt et al., 2020). An inactive eGFP gene was used as a transgenic reporter. After prime editing, it was restored into a wild-type EGFP sequence with two precise base conversions (T-G and G-C) at efficiencies of 15.60% and 17.10% for PE2 and PE3, respectively (Hua et al., 2020a). Simultaneously, by using a pegRNA designed to introduce a S627N mutation in OsALS, PE3 was also employed to gen-

ALS2 and PDS1 were obtained at the efficiencies of 6.70% and 3.40%, respectively (Lu et al., 2021) (Table 2).

Directed evolution (DE) is a technology of making random mutation(s) in a target gene to generate novel germplasms and enrich genetic diversity (Zhang and Qi, 2019). Currently, base editing can enable artificial evolution of agriculturally important genes in crops to explore novel gene resources and germplasms (Kuang et al., 2020; Li et al., 2020a; Liu et al., 2020; Xu et al., 2021a; Wang et al., 2022b). Compared with base editing, prime editing has greater potential for evolving plant genes, because it can install all types of small genetic modifications that can be harnessed for producing all possible substitutions for key amino acids with improved agronomic performance when combined with a well-designed pegRNA library. For example, a prime editing library-mediated saturation mutagenesis (PLSM) method had been developed to identify 16 types of herbicide resistance-conferring mutations at six different target residues in OsACC1 using a pegRNA library with all possible combinations of substitutions (64 types), which enabled a more comprehensive screening than that achieved by base editing (Xu et al., 2021b). Among the 16 kinds of mutations, three types of mutations were first reported in plants. The PLSM system is an alternative approach to create novel germplasms for crop breeding.

RECOMMENDATION OF PROPER BE AND PE FOR BOTH BASIC RESEARCH IN PLANTS AND CROP IMPROVEMENT

According to optimizations and applications of the current BEs and PEs in plants as described above, we recommend to select appropriate BEs and PEs for precise gene editing for both basic biological research in plants and crop improvement. For base editing, we recommend using evoFERNY for CBE (Zeng et al., 2020), and TadA9 or TadA8e-DBD (hTadA8e) for ABE (Yan et al., 2021; Tan et al., 2022) in base editing. In addition, it would be good to select the appropriate Cas protein capable of targeting the region near the desired editing site due to the limitations of PAM sites and the editing windows of various BEs. At present, the suitable choice is to select the editors constructed from these three Cas proteins with broad PAM sites, including Cas9-NG (NGN PAM), ScCas9^{++} (NNG PAM), and SpRY (NNN prefer NRN PAM) (Hua et al., 2019; Ren et al., 2019; Zhong et al., 2019; Wang et al., 2020a; Liu et al., 2021b). Moreover, using a surrogate system to restore the defective genes into the functional ones encoding antibiotics or herbicides could be more cost-effective and improve the base editing efficiency (Xu et al., 2020b).

For prime editing, we recommend using PEmax or a PE with M-MLV-RT fused to the N-terminus of nCas9(H840A) (Chen et al., 2021; Jiang et al., 2022b; Li et al., 2022b; Xu et al., 2022) (Figure 2). Further, except the intended base substitutions, introduction of additional multiple-nucleotide synonymous substitutions in RTT could stimulate prime editing efficiency (Chen et al., 2021; Li et al., 2022c; Xu et al., 2022). For example, synonymous substitutions could be introduced at +1 | +6 positions (counting 3′-base of RTT as position +1), in order to avoid the repeat nicking of edited targets (Xu et al., 2022) (Figure 3). Furthermore, additional structured RNA sequences, such as evopreQ$_1$ appended to the 3′-end of pegRNA will stabilize the pegRNAs and thus improve the prime editing efficacy (Li et al., 2022b; Nelson et al., 2022; Zou et al., 2022) (Figure 3). Moreover, a strong composite promoter, such as CaMV 35S enhancer+CmYLCV promoter+U6 promoter, could be used to enhance the expression of the pegRNA and thus improve the prime editing efficiency (Jiang et al., 2020). Lastly, using the reporter genes such as antibiotics or herbicides as surrogates to enrich the lines with desired edits improved the prime editing efficiency in a cost-effective and labor-saving way, especially for multiplex prime editing in plants (Li et al., 2022a) (Table 2).

FUTURE PERSPECTIVES FOR FURTHER OPTIMIZATION OF BE AND PE IN PLANTS

Although impressive progresses have been made during the last several years, the following aspects such as optimization of the existing BEs, exploitation of novel BEs, and optimization of PEs to further improve their precise editing efficiencies as well as developing novel PEs capable of installation of larger indels in plants, would be highly desirable in the next few years.

Optimization of the existing BEs and exploitation of novel BEs

To date, CBE and ABE for base transition have been well optimized in terms of improving editing efficiency, expanding the target scope and reducing off-targets. However, for base transversion, such as CGBE for C to G and C to A, the editing efficiency is relatively lower in comparison with other BEs in plants (Koblan et al., 2021; Tian et al., 2022). Thus, it is still necessary to increase the efficiency of CGBE. Most importantly, in order to increase the flexibility of BEs, exploitation of other types of BEs for transversion of A to C (T to G) or A to T (T to A) will certainly be very beneficial in substitution of any base pair into the desired one within the editing window in a target gene of interest in plants. In addition, concerning the base editing window, two aspects are worthy of further optimization. (i) Narrow the editing window of BE to a single base, reduce the by-products of unintended editing, for example, a more precise adenine base editor ABE9 (Figure 1L), which was developed recently by introducing two mutations L145T and N108Q in ABE8e, maintained the editing activity and minimized the editing window to position 5–6 in mammalian cells (Chen et al., 2022b). Furthermore, by combining with PAM-less Cas proteins, it will be possible to

achieve accurate single base editing at an target sites in the genome. (ii) Widen the width of the editing window of BE for saturation mutation studies such as de novo domestication or DE to generate novel gene resources or germplasm in plants. For example, fusion of T7 RNA polymerase with different deaminases (cytidine and adenosine deaminase), substantially widens the mutational spectrum in mammalian cells (Cravens et al., 2021). In addition, engineering BEs fused with additional chromatin modulating peptides, such as pioneer factor SOX2 (SRY-box transcription factor 2), to initiate chromatin unfolding and stimulate transcription, could be a promising strategy to further increase base editing efficacy (Yang et al., 2022).

Optimization of PEs

A series of parameters such as stable and properly folded pegRNAs, effective assembly of the PE-pegRNA complex, and more active reverse transcriptase are essential for efficient prime editing. In PE, the canonical pegRNA consists of a sgRNA, a RTT and a PBS (Figure 2A). PBS and RTT at the 3′-terminal of pegRNA are easy to partially degrade by exoribonucleases inside the cells, resulting in truncated pegRNAs (Feng et al., 2022; Nelson et al., 2022). The truncated pegRNAs can still search and recognize the target sites, but not be able to complete the correct editing due to loss of the PBS or RTT-PBS (Nelson et al., 2022). Adding a

PEs, especially PE, are not widely used or even impossible in the polyploid species and agriculturally important food crops such as common wheat due to its complex hexaploid genome, gene redundancy, as well as relatively lower transformation efficiency (Li et al., 2021c). Third, for base editing and prime editing in different plant species, we suggest using the aforementioned optimized strategies in combination with a stronger promoter to drive the expression of both nCas-deaminase and the sgRNA for BE, or nCas-M-MLV-RT and pegRNA for PE, respectively (Li et al., 2022b). Finally, it is worth noting that the innate nature of target genes may affect the editing outcomes of both BEs and PEs in plants; for example, some genes or targets could only be edited at a very lower efficiency or even not be accessible (Hua et al., 2022). Understanding the potential mechanism underlying this phenomenon will certainly benefit the precision genome editing of any targets at will in a user-defined manner in plants. Nevertheless, following the continuous endeavors on optimization of BE and PE as well as engineering a novel generation of BE and PE, we envision that both BEs and PEs will become the routine and customized precise gene editing tools for both plant fundamental research and crop improvement in the near future.

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