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 s stems have been developed and successfull applied in plants such as homolog -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 (An alone 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 hol 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 b CRISPR/Cas nucleases the predominant repair mechanism in cells is nonhomologous end joining (NHEJ) which usuall results in random indels, as well as the obstacles in deliver of sufficient donor repair template (DRT) into the vicinit 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 b 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 t pes of base substitutions and small indels, and substantiall expands the scope and capabilities of precision genome editing (An alone et al., 2019) (Figure 2A).

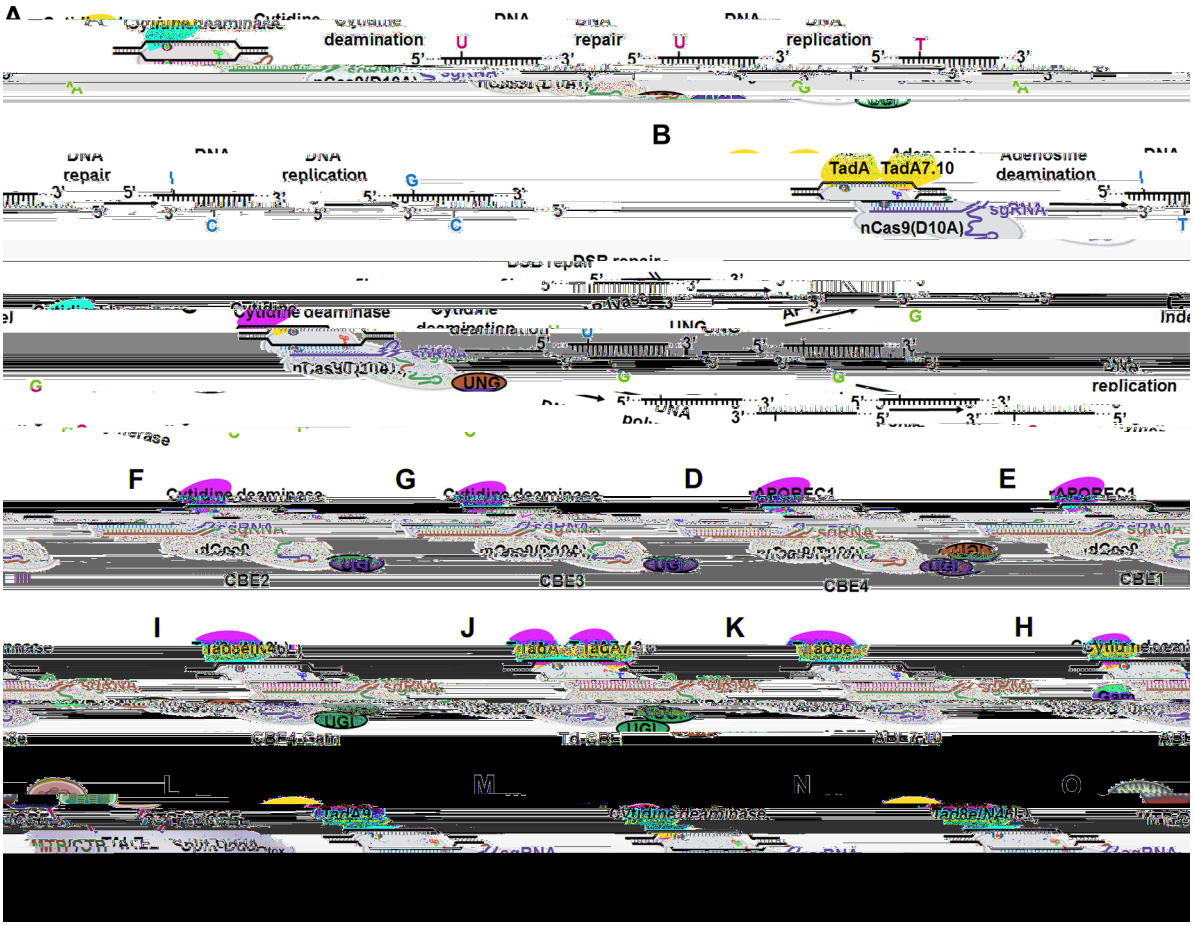
Since the development of the first generation of base editor (BE) and prime editor (PE) for base editing and prime editing in mammalian cells (Komor et al., 2016; Nishida et al., 2016; Gaudelli et al., 2017; An alone et al., 2019), diverse strategies have been exploited to optimi e these two editors in order to improve the precise editing efficienc and specificit , and to expand targeting scopes in plants (Li et al., 2020d; Molla et al., 2021; Hua et al., 2022). Here, we summari e 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 optimi ation of these two editors. We trul 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 customi ed platform for both fundamental biological studies and crop improvement.

BASE EDITORS AND THEIR APPLICATIONS IN PLANTS

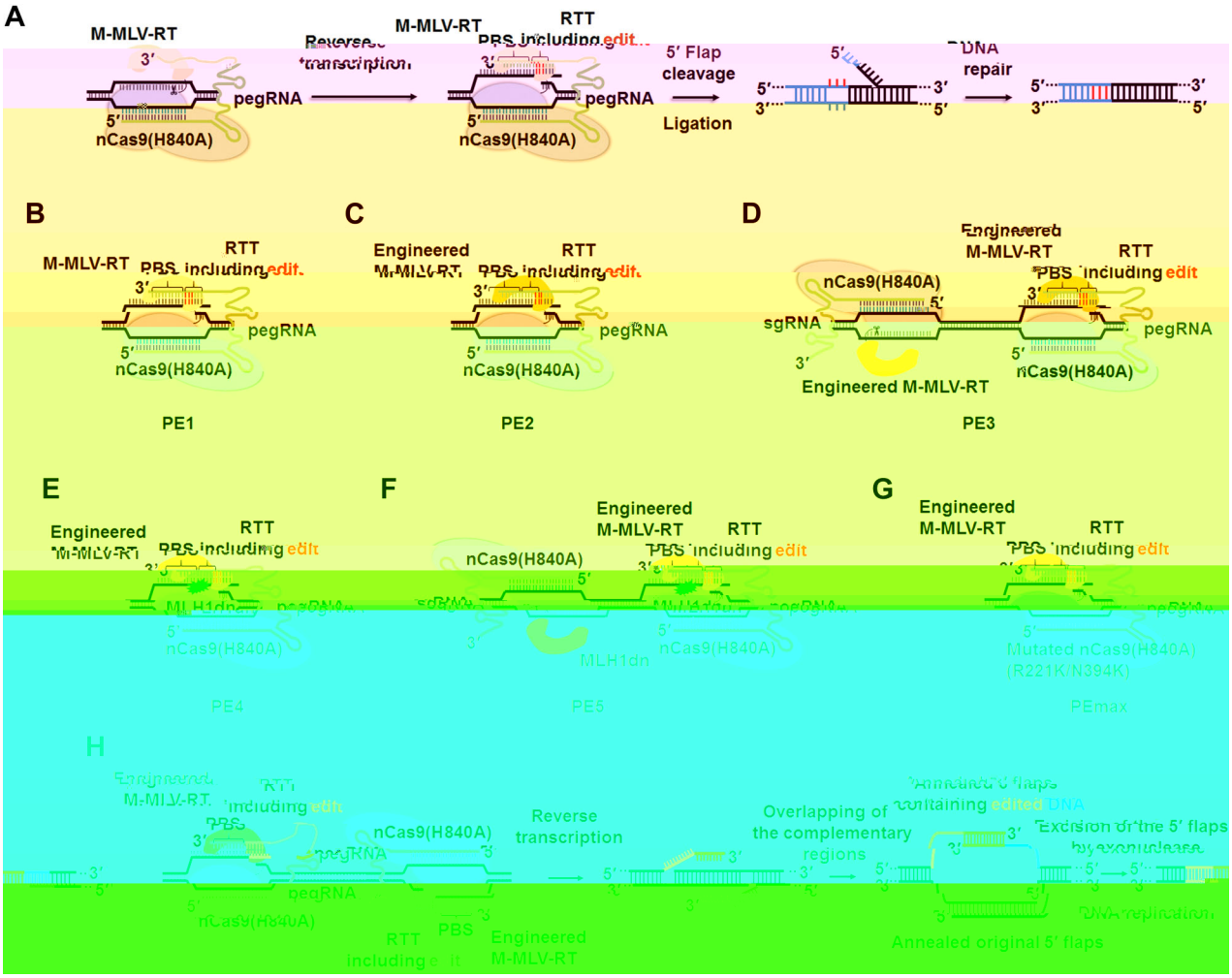
Base editing is a breakthrough technolog that can precisel and efficientl achieve single base transition or transversion at target sites without inducing DSBs and the need for a DRT. Three BEs are currentl in use: c tosine base editors (CBEs) for C:G to T:A transition (Figure 1A), adenine base editors (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 greatl 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, respectivel , man efforts have been attempted in order to optimi e and upgrade these two BEs in plants.

Cytosine base editors

The first-generation CBE was engineered b fusing a rat c tidine 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 b deaminating the c tosine (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 recogni es an G:U base pair as a mismatch. The BER activit eliminates the uracil with the help of uracil N-gl cos lase (UNG), resulting in a low efficienc of the CBE1 s stem (Komor et al., 2016). To improve base editing efficienc , 202424016 ThTh td1Tf.32140TD(519(as)19



( ) 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 es 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. ( ) 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 es 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. ( ) 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 es 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. ( ) 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). ( ) 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. ( ) 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). ( ) The fourth-



( ) The CRISPR/nCas9-mediated prime editing s stem. A prime editor mainl consists of a catal ticall impaired nCas9(H840A), a M-MLV-RT (Molone 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 directl into the target site. ( ) The first-generation prime editor, PE1, was engineered b fusing a wild M-MLV-RT to the N-terminus of nCas9(H840A). ( ) The second-generation prime editor, PE2, was engineered b fusing an engineered M-MLV-RT with six amino acid mutations to the N-terminus of nCas9(H840A). ( ) The third-generation prime editor, PE3, was engineered b using an additional sgRNA on the non-targeting strand. ( ) 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. ( ) The fifth-generation prime editor, PE5, was developed with transient co-expression of a dominant negative MMR protein (MLH1dn) on the basis of PE3. ( ) PEmax was engineered b replacing nCas9(H840A) with a mutated version which harbors R221K and N394K mutations. ( ) Overview of the design of twinPE or GRAND editor and the sequence replacement process. The single-strand DNAs (red and blue lines) produced b the paired pegRNAs containing RTTs highlighted in light red and light blue, respectivel , bind to each other through their complementar ends highlighted in orange. The original 5 flaps were replaced b the aannealed 3 flaps containing the edited DNA following DNA replication and repair.

CBE4, was developed b 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 onl improves the base editing efficienc but also reduces the frequenc of C to A or G transversions b 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 purit and reduce the occurrence of indels (Komor et al., 2017) (Figure 1H).

CBEs, especiall CBE3 and CBE4, have been widel used in plants. Initiall , a base editing s stem was developed using a rat APOBEC1 in rice. To validate and test the feasibilit of CBEs in plants, b fusing a rat APOBEC1 to the N-terminus of nCas9 (D10A) to form a structure of rAPOBEC1-nCas9 (D10A), two agriculturall important genes of rice, OsNRT1.1B and OsSLR1, were edited at editing efficiencies of 2.7% and 13.3%, respectivel (Lu and Zhu, 2017) (Table 1). Simultaneousl , 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 efficienc (%)	Editing window	Trait improvement	References
APOBEC1-CBE2/CBE3	APOBEC1-XTEN-nCas9	Rice	OsNRT1.1B, OsSLR1	Agrobacterium	H grom cin	2.70–13.30	C4–C8	High nitrogen use efficienc ; Dwarf	Lu and Zhu (2017)
	APOBEC1-XTEN-nCas9-UGI	Rice	OsSBEIIb, OsPDS	Bombardment	H grom cin	20.00	C4–C8	High am lose	Li et al. (2017)
	APOBEC1-XTEN-nCas9/dCas9-UGI	Rice, Wheat Mai e	OsCDC48, OsNRT1.1B, OsSPL14, TaLOX2, ZmCENH3	Agrobacterium /Bombardment	Herbicide	0–43.48	C3–C9	High nitrogen use efficienc ; High ield	Zong et al. (2017)
	APOBEC1-XTEN-nCas9-UGI	Rice	OsCERK1, OsSERK1, OsSERK2, ipa1, Pi-ta	Agrobacterium	H grom cin	10.50–38.90	C4–C8	High ield; 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	H grom cin	0–57.78	C4–C8	–	Qin et al. (2020)
hAID-CBE3	hAID-XTEN-nCas9	Rice	OsFLS2, OsAOS1, OsJAR1, OsJAR2, OsCOI2, OsPi-D2	Agrobacterium	H grom cin	8.30–73.30	C3–C8	Blast resistance	Ren et al. (2018)
	hAID-nSpCas9-NG/nCas9-UGI	Rice	OsBZR1, OsSERK2	Agrobacterium	H grom cin	4.44–27.08	C3–C8	Enhance fruit qualit	Ren et al. (2019)
	hAID-nScCas9-UGI hAID-nSpCas9-UGI	Rice	OsMPK9, OsMPK17, OsCPK5, OsMPK15, OsMPK16, OsCPK6, OsCPK7, OsCPK8	Agrobacterium	H grom cin	2.56–97.92 0–95.83	C3–C8	–	Wang et al. (2020a)
	hAID-XTEN-nSpRY-UGI	Rice	OsCOI2, OsBSR, OsMPK13, OsGS1, OsGSK4	Agrobacterium	H grom cin	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/H grom cin	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/H grom cin	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	H grom cin	0–21.10 3.50–56.30	C2–C5	–	Zhong et al. (2019)
	nCas9-NG-PmCDA1-UGI	Tomato potato	SlALS	Agrobacterium	H grom cin	32.00	C2–C5	Herbicide resistance	Veillet et al. (2020)
	PmCDA1-nScCas9^{+}$^{+}$-UGI-UGI	Rice	OsWaxy, OsEUI1	Agrobacterium	H grom cin	8.3–86.1	C1–C17	Reduced am lose content	Liu et al. (2021b)
CBE4	FENRY-nCas9-NG-UGI-UGI	Rice	OsCKX2, OsWaxy, OsEUI1, OsSPL4, OsSPL7, OsSPL14,	Agrobacterium	H grom cin	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 efficienc (%)	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/ H grom cin	3.20–59.10	A4–A8	Herbicide resistance	Li et al. (2018)
	TadA-TadA7.10-nCas9	Rice	OsSERK2, OsMPK6, OsWRKY45, OsMPK13	Agrobacterium	H grom cin	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	H grom cin	0–26.00 4.80–61.30	A4–A8	High ield	Hua et al. (2018)
	TadA-TadA7.10-nScCas9	Rice	OsMPK14, OsCPK9, OsMPK15, OsCPK10	Agrobacterium	H grom cin	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	H grom cin	4.50–96.30 0–61.10	A1–A12	High ield; Herbicide resistance	Hua et al. (2020b)
ABE8e	TadA8e(V106W)-nCas9 TadA8e(V106W)-nCas9-NG	Rice	OsEPSPS, OsALS, OsWaxy	Agrobacterium	Herbicide/ H grom cin	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	H grom cin	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	H grom cin	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/ H grom cin	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/ H grom cin	0.40–87.60	C2–C5 A4–A8	–	Xu et al. (2021a)

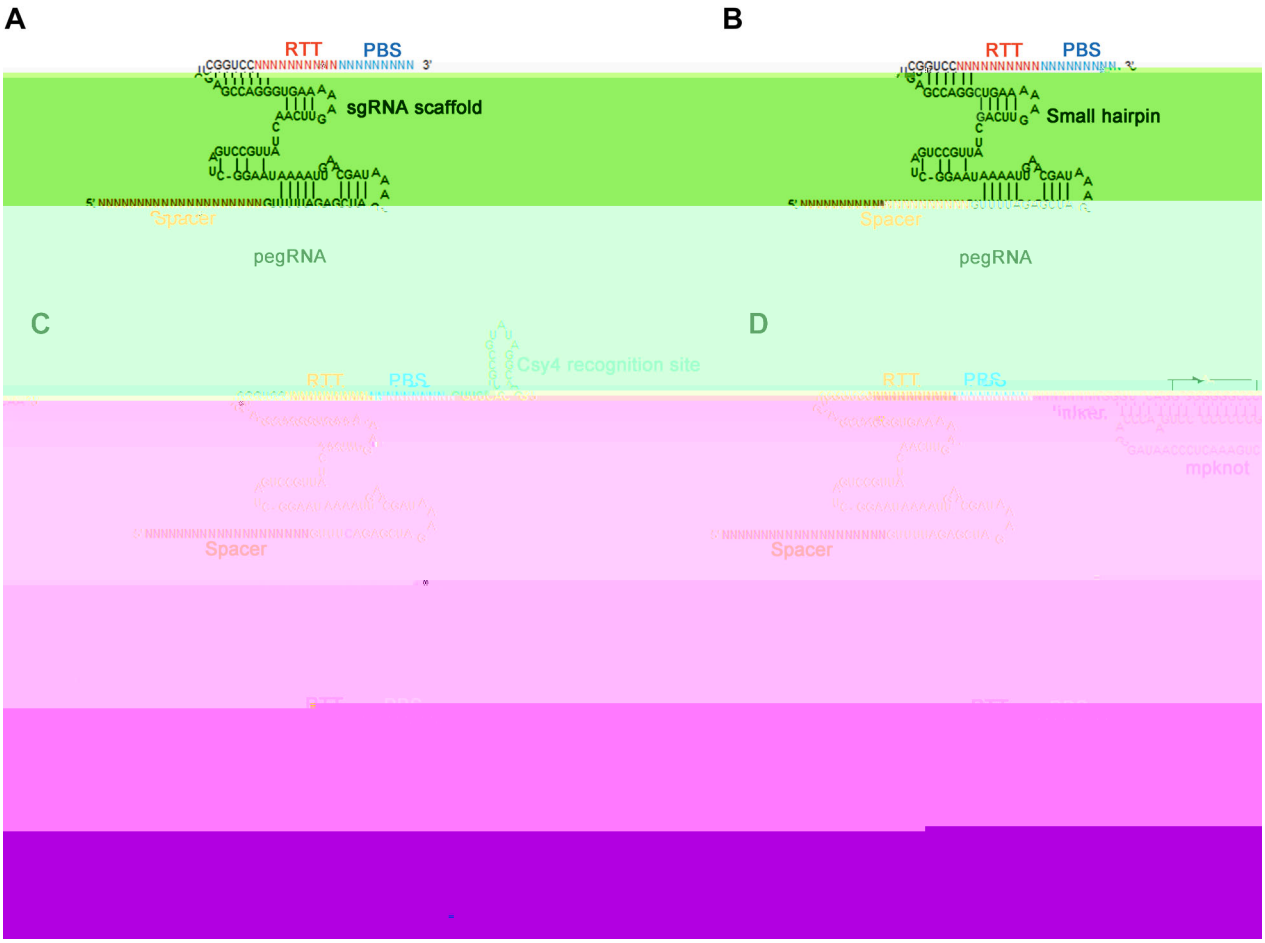
in OsPDS, which encodes a ph toene desaturase, and two targets (S3 and S5) in OsSBEIIb, which encodes a starch branching en me IIb, were successfull edited in rice b using CBE3 with the efficiencies of 19.2%, 10.5%, and 1.0% at the S5, S3, and P2 targets, respectivel (Li et al., 2017) (Table 1). Meanwhile, 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 mai e b using a nCas9-c tidine deaminase fusion (Zong et al., 2017) (Table 1). Although the editing efficiencies of CBEs has been improved to a certain extent, its

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, especiall for the low-efficient ed-

dsDNA, resulting in efficient C:G to T:A conversion in the human mitochondrial genome with high target specificit (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). Recentl , 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 successfull edited without leaving an foreign genes in either the plastid or nuclear genomes in Arabidopsis (Naka ato et al., 2021) (Figure 1O; Table 1). Furthermore, an efficient DdCBE s stem was constructed b fusing a chloroplast transition peptide (CTP) to its N-terminus. This CTP-DdCBE achieved a conserved chloroplast gene chloroph ll A of photos stem I (psaA), for C to T transitions in rice chloroplasts (Li et al., 2021b) (Figure 1O; Table 1). The edited psaA could potentiall improve photos nthetic efficienc and grain ield of crops. The successful implementation of DdCBEs (CRISPR-independent organelle BEs) in plant organelle cells increases the possibilit 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 b ph sical or chemical methods has long been applied to improve traits in plants, but it is labor-intensive and time-consuming. The base editing s stem can enable the artificial evolution of agriculturall important genes in current c



( ) 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. ( ) 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. ( ) The schematic diagram of a Cs 4-processed pegRNA, which protects the 3′ extension from degradation b exonucleases. Cs 4 is a speciali ed ribonuclease that selects clustered regularl interspaced short palindromic repeats (CRISPR) transcripts from the cellular milieu for binding and cleavage. With Cs 4 processing, the hairpin Cs 4 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 Cs 4 recognition site sequence is highlighted in green. ( ) 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. ( ) 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. (,) 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 s 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 (An alone 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 (An alone 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 t pe	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	H grom cin, Bisp ribac sodium	Herbicide resistance, High ield	Butt et al. (2020)
Sp-PE2	13, 13	GFP	2 bp Subs	15.60	NR	Agrobacterium	H grom cin	-	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	H grom cin	-	Xu et al. (2020a)
PE2	13,16; 13, 14–23	OsALS, OsACC	1–3 bp Subs	1.00–7.60	0.00	Agrobacterium	H grom cin	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	H grom cin	Herbicide resistance	Li et al. (2022b)
PPE	8, 17	OsALS	2 bp Subs	2.10	NR	Agrobacterium	H grom cin	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	H grom cin	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	H grom cin	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	H grom cin	Herbicide resistance	Zong et al. (2022)
pZ1WS (drived b 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	H grom cin, Bisp ribac sodium	Herbicide resistance	Hua et al. (2020a)
PE3	13, 28–59	HPTII, OsEPSPS	3–7 bp Subs	2.22–9.38	NR	Bombardment	H grom cin	Herbicide resistance	Li et al. (2020c)

Continued

Table 2. Continued

PEs	PE features (PBS length (nt) and RT template length (nt))	Target gene	Mutation t pe	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	H grom cin	Herbicide resistance	Lin et al. (2020)
pPE3	13, 10	OsWx, OsACC	1 bp Subs	0.00–16.70	NR	Agrobacterium	H grom cin	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	H grom cin	Herbicide resistance	Xu et al. (2020c)
PE3	9–13, 9–28	OsSPL14, OsDHDPS, OsNR2	2–3 bp Subs	0.00–1.00	NR	Bombardment	H grom cin	-	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	H grom cin	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	H grom cin	Herbicide resistance	Zou et al. (2022)
pPE3b	13, 10	OsACC	1 bp Subs	6.25	NR	Agrobacterium	H grom cin	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	H grom cin	Herbicide resistance	Xu et al. (2020c)
pCXPE03 (drived b the RPS5A promoter)	14, 17–18	SlGAI, SlALS, SlPDS	2 bp Subs, 2 bp Ins	0.00–6.70	NR	Agrobacterium	H grom cin	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	H grom cin	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	H grom cin	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	H grom cin	-	Xu et al. (2022)
PE-P3-RT-M (C-terminal M-MLV + multiple s non mous 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	H grom cin	-	Xu et al. (2022)

Table 2. Continued

PEs	PE features (PBS length (nt) and RT template length (nt))	Target gene	Mutation t pe	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	H grom cin	-	Xu et al. (2022)
PE-P2-RT-M (N-terminal M-MLV + multiple s non mous 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	H grom cin	-	Xu et al. (2022)
PE3-HS (h grom - cinY46*-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	H grom cin	-	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	H grom cin, Bisp ribac 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	H grom cin, Bisp ribac 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	H grom cin	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	H grom cin	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	H grom cin	Herbicide resistance	Jiang et al. (2022b)

Continued

Table 2. Continued

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

deletion and repair) method (Jiang et al., 2022a). These prime editing s stems were developed b emplo ing 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 partiall complementar to each other (An alone et al., 2022; Choi et al., 2022; Jiang et al., 2022a; Wang et al., 2022c). Although the above s stems for replacement or knock-in of large DNA fragments through prime editing had been successfull 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 s stem substantiall 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 agriculturall important traits in crops (Li et al., 2020d). Since the first report of prime editing in mammalian cells in 2019 (An alone 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 b further optimi ation 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 summari ed in Table 2.

The feasibilit and efficac of a series of plant codons optimi ed 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 simultaneousl 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 significantl affected the precise editing efficienc of PE2 and PE3 in rice and wheat protoplasts (Lin et al., 2020). The PE2 s stem could also induce programmable editing at different genome sites at a frequenc of 0% to 31.3% in rice stable lines, suggesting that the efficienc of pPE2 varied greatl at different genomic sites and with pegRNAs of diverse structures (Xu et al., 2020a). B using the polII promoter Actin to drive the expression of the tandem repeats of pol cistronic transfer RNAs to simultaneousl produce pegRNA and nicking sgRNA in a PE3, 28 bp and a 59 bp fragments with desired edits were precisel installed into an exogenous inactive h grom cin phosphotransferase (HPT) gene hptII to restore its function, and an endogenous gene OsEPSPS, which encodes a 5-enolp ruv lshikimate-3-phosphate s nthase (EPSPS), to generate a novel allele with TAP-IVS mutations (T173I, A174V, and P177S) which confers rice gl phosate resistance in rice stable lines, respectivel (Li et al., 2020c).

Furthermore, development of a plant PE b 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 efficienc and achieved versatile nucleotide substitutions in rice stable lines (Xu et al., 2020c). Compared with normal PE, this strateg could increase the editing efficienc 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 ield (Butt et al., 2020). An inactive eGFP gene was used as a transgenic reporter. After prime editing, it was restored into a wild-t pe EGFP sequence with two precise base conversions (T-G and G-C) at efficiencies of 15.60% and 17.10% for PE2 and PE3, respectivel (Hua et al., 2020a). Simultaneousl , b using a pegRNA designed to introduce a S627N mutation in OsALS, PE3 was also emplo ed to gen-

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

Directed evolution (DE) is a technolog of making random mutation(s) in a target gene to generate novel germplasms and enrich genetic diversit (Zhang and Qi, 2019). Currentl , base editing can enable artificial evolution of agriculturall 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 t pes of small genetic modifications that can be harnessed for producing all possible substitutions for ke amino acids with improved agronomic performance when combined with a well-designed pegRNA librar . For example, a prime editing librar -mediated saturation mutagenesis (PLSM) method had been developed to identif 16 t pes of herbicide resistance-conferring mutations at six different target residues in OsACC1 using a pegRNA librar with all possible combinations of substitutions (64 t pes), which enabled a more comprehensive screening than that achieved b base editing (Xu et al., 2021b). Among the 16 kinds of mutations, three t pes of mutations were first reported in plants. The PLSM s stem 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 optimi ations 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 (h TadA8e) 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 s stem to restore the defective genes into the functional ones encoding antibiotics or herbicides could be more cost-effective and improve the base editing efficienc (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 s non mous substitutions in RTT could stimulate prime editing efficienc (Chen et al., 2021; Li et al., 2022c; Xu et al., 2022). For example, s non mous 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 stabili e the pegRNAs and thus improve the prime editing efficac (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 efficienc (Jiang et al., 2020). Lastl , using the reporter genes such as antibiotics or herbicides as surrogates to enrich the lines with desired edits improved the prime editing efficienc in a cost-effective and labor-saving wa , especiall 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 ears, the following aspects such as optimi ation of the existing BEs, exploitation of novel BEs, and optimi ation 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 highl desirable in the next few ears.

Optimization of the existing BEs and exploitation of novel BEs

To date, CBE and ABE for base transition have been well optimi ed in terms of improving editing efficienc , 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 efficienc is relativel lower in comparison with other BEs in plants (Koblan et al., 2021; Tian et al., 2022). Thus, it is still necessar to increase the efficienc of CGBE. Most importantl , in order to increase the flexibilit of BEs, exploitation of other t pes of BEs for transversion of A to C (T to G) or A to T (T to A) will certainl be ver beneficial in substitution of an 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 worth of further optimi ation. (i) Narrow the editing window of BE to a single base, reduce the b -products of unintended editing, for example, a more precise adenine base editor ABE9 (Figure 1L), which was developed recentl b introducing two mutations L145T and N108Q in ABE8e, maintained the editing activit and minimi ed the editing window to position 5–6 in mammalian cells (Chen et al., 2022b). Furthermore, b 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 pol merase with different deaminases (c tidine and adenosine deaminase), substantiall 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 strateg to further increase base editing efficac (Yang et al., 2022).

Optimization of PEs

A series of parameters such as stable and properl folded pegRNAs, effective assembl 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 eas to partiall degrade b exoribonucleases inside the cells, resulting in truncated pegRNAs (Feng et al., 2022; Nelson et al., 2022). The truncated pegRNAs can still search and recogni e 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, especiall PE, are not widel used or even impossible in the pol ploid species and agriculturall important food crops such as common wheat due to its complex hexaploid genome, gene redundanc , as well as relativel lower transformation efficienc (Li et al., 2021c). Third, for base editing and prime editing in different plant species, we suggest using the aforementioned optimi ed 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, respectivel (Li et al., 2022b). Finall , it is worth noting that the innate nature of target genes ma affect the editing outcomes of both BEs and PEs in plants; for example, some genes or targets could onl be edited at a ver lower efficienc or even not be accessible (Hua et al., 2022). Understanding the potential mechanism underl ing this phenomenon will certainl benefit the precision genome editing of an targets at will in a user-defined manner in plants. Nevertheless, following the continuous endeavors on optimi ation 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 customi ed precise gene editing tools for both plant fundamental research and crop improvement in the near future.

(2020). Directed evolution of adenine base editors with increased activit and therapeutic application. Nat. Biotechnol. 892–900.

(1999). Unusual transcriptional and translational regulation of the bacteriophage Mu mom operon. Pharmacol. Ther. 367–388.

(2015). Variation in NRT1.1B contributes to nitrate-use divergence betw rice sub-species. Nat. Genet. 834-838.

(2022). Improvement of base editors and prime editors advances precision genome engineering in plants. Plant Ph siol. 1795–1810.

(2020a). Precision genome engineering in rice using prime editing s stem. Plant Biotechnol. J. 2167–2169.

(2019). Genome engineering in rice using Cas9 variants that recogni e NG PAM sequences. Mol. Plant 1003–1014.

(2020b). Simplified adenine base editors improve adenine base editing efficienc in rice. Plant Biotechnol. J. 770–778.

(2018). Precise A T to G C base editing in the rice genome. Mol. Plant 627–630.

(2021). CRISPR/Cas s stems: The link between functional genes and genetic improvement. Crop J. 678–687.

(2021). Adapting CRISPR/Cas9 s stem for targeting mitochondrial genome. Front. Genet. 627050.

(2022a). Deletion and replacement of long genomic sequences using prime editing. Nat. Biotechnol. 227–234.

(2022b). Optimi ed prime editing efficientl generates gl phosate-resistant rice plants carr ing homo gous TAP-IVS mutation in EPSPS. Mol. Plant 1646–1649.

(2020). Prime editing efficientl generates W542L and S621I double mutations in two ALS genes in mai e. Genome Biol. 257.

(2010). Regulation of OsSPL14 b OsmiR156 defines ideal plant architecture in rice. Nat. Genet. 541–544.

(2019). C tosine, but not adenine, base editors induce genome-wide off-target mutations in rice. Science 292–295.

(2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunit . Science 816–821.

(2021). Chloroplast and mitochondrial DNA editing in plants. Nat. Plants 899–905.

(2019). Curing c toplasmic male sterilit via TALEN-mediated mitochondrial genome editing. Nat. Plants 722–730.

(2021). Predicting the efficienc of prime editing guide RNAs in human cells. Nat. Biotechnol. 198–206.

(2018). CRISPR-Cas guides the future of genetic engineering. Science 866–869.

(2021). Efficient C*G-to-G*C base editors developed using CRISPRi screens, target-librar anal sis, and machine learning. Nat. Biotechnol. 1414–1425.

(2018). Improving c tidine and adenine base editors b expression optimi ation and ancestral reconstruction. Nat. Biotechnol. 843–846.

(2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature 420–424.

(2017). Improved base excision repair inhibition and bacteriophage Mu Gam protein ields C:G-to-T:A base editors with higher efficienc and product purit . Sci. Adv. eaao4774.

(2020). Base-editing-mediated artificial evolution of OsALS1 in planta to develop novel herbicide-tolerant rice germplasms. Mol. Plant 565–572.

(2021). CRISPR C-to-G base editors for inducing targeted DNA transversions in human cells. Nat. Biotechnol. 41–46.

(2020a). Targeted, random mutagenesis of plant genes with dual c tosine and adenine base editors. Nat. Biotechnol. 875–882.

(2020b). SWISS: multiplexed orthogonal genome editing in plants with a Cas9 nickase and engineered CRISPR RNA scaffolds. Genome Biol. 141.

(2018). Expanded base editing in rice and wheat using a Cas9-adenosine deaminase fusion. Genome Biol. 59.

(2020c). Precise modifications of both exogenous and endogenous genes in rice b prime editing. Mol. Plant 671–674.

(2022a). Multiplex precision gene editing b a surrogate prime editor in rice. Mol. Plant 1077–1080.

(2022b). Development of a highl efficient prime editor 2 s stem in plants. Genome Biol. 161.

(2020d). Toward precision genome editing in crop plants. Mol. Plant 811–813.

(2017). Generation of targeted point mutations in rice b a modified CRISPR/Cas9 s stem. Mol. Plant 526–529.

(2021a). Genome editing mediated b SpCas9 variants with broad non-canonical PAM compatibilit in plants. Mol. Plant 352–360.

(2021b). High-efficienc plastome base editing in rice with TAL c tosine deaminase. Mol. Plant 1412–1414.

(2019). Precise gene replacement in rice b RNA transcript-templated homologous recombination. Nat. Biotechnol. 445–450.

(2020). Precise gene replacement in plants through CRISPR/Cas genome editing technolog : Current status and future perspectives. aBIOTECH 58–73.

(2021c). Present and future prospects for wheat improvement through genome editing and advanced technologies. Plant Commun. 100211.

(2022c). Highl efficient prime editing b introducing same-sense mutations in pegRNA or stabili ing its structure. Nat. Commun. 1669.

(2021d). The CRISPR/Cas9 revolution continues: From base editing to prime editing in plant science. J. Genet. Genomics 661–670.

(2001). Translational repression and specific RNA binding b the coat protein of the Pseudomonas phage PP7. J. Biol. Chem. 22507–22513.

(1994). Mutations that increase the affinit of a translational repressor for RNA. Nucleic Acids Res. 3748–3752.

(2021). High-efficienc prime editing with optimi ed, paired pegRNAs in plants. Nat. Biotechnol. 923–927.

(2020). Prime genome editing in rice and wheat. Nat. Biotechnol. 582–585.

(2021a). Developing a novel artificial rice germplasm for dinitroaniline herbicide resistance b base editing of OsTubA2. Plant Biotechnol. J. 5–7.

(2021b). The ScCas9(++) variant expands the CRISPR toolbox for genome editing in plants. J. Integr. Plant Biol. 1611–1619.

(2020). A CRISPR-Cas9-mediated domain-specific base-editing screen enables functional assessment of ACCase variants in rice. Plant Biotechnol. J. 1845–1847.

(2021c). Enhancing prime editing b Cs 4-mediated processing of pegRNA. Cell Res. 1134–1136.

(2020). Targeted, efficient sequence insertion and replacement in rice. Nat. Biotechnol. 1402–1407.

(2021). Precise genome modification in tomato using an improved prime editing s stem. Plant Biotechnol. J. 415–417.

(2017). Precise editing of a target base in the rice genome using a modified CRISPR/Cas9 s stem. Mol. Plant 523–525.

(2020). CRISPR/Cas s stems in genome editing: Methodologies and tools for sgRNA design, off-target evaluation, and strategies to mitigate off-target effects. Adv. Sci. 1902312.

(2019). Gene editing in plants: Progress and challenges. Natl. Sci. Rev. 421–437.

(2020). A bacterial c tidine deaminase toxin enables CRISPR-free mitochondrial base editing. Nature 631–637.

(2021). Precise plant genome editing using base editors and prime editors. Nat. Plants 1166–1187.

(2000). RecE/RecT and Redalpha/Redbeta initiate double-stranded break repair b specificall interacting with their respective partners. Genes Dev. 1971–1982.

(2021). Targeted base editing in the plastid genome of Arabidopsis thaliana. Nat. Plants 906–913.

(2022). Engineered pegRNAs improve prime editing efficienc . Nat. Biotechnol. 402–410.

(2022). Evolution of an adenine base editor into a small, efficient c tosine base editor with low off-target activit . Nat. Biotechnol. https://doi.org/10.1038/s41587-022-01533-6

(2016). Targeted nucleotide editing using h brid prokar otic and vertebrate adaptive immune s stems. Science aaf8729.

(1998). DNA strand invasion promoted b Escherichia coli RecT protein. J. Biol. Chem. 12274–12280.

(1993). The RNA binding site of bacteriophage MS2 coat protein. EMBO J. 595–600.

(2022). Prime editing in the model plant Physcomitrium patens and its potential in the tetraploid potato. Plant Sci. 111162.

(2022). Updates on gene editing and its applications. Plant Ph siol. 1725–1730.

(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 s stem. Plant Biotechnol. J. 45–56.

(2019). Cas9-NG greatl expands the targeting scope of the genome-editing toolkit b recogni ing NG and other at pical PAMs in Rice. Mol. Plant 1015–1026.

(2017). A CRISPR/Cas9 toolkit for efficient targeted base editing to induce genetic variations in rice. Sci. China Life Sci. 516–519.

(2018). Improved base editor for efficientl inducing genetic variations in rice with CRISPR/Cas9-guided h peractive hAID mutant. Mol. Plant 623–626.

(2022). A super pan-genomic landscape of rice. Cell Res. 878–896.

(2017). Targeted base editing in rice and tomato using a CRISPR-Cas9 c tidine deam inase fusion. Nat. Biotechnol. 441–443.

(2017). Deep mutational scanning of S. pyogenes Cas9 reveals important functional domains. Sci. Rep. 16836.

(2016). Engineering herbicide-resistant rice plants through CRISPR/Cas9-mediated homologous recombination of acetolactate s nthase. Mol. Plant 628–631.

(2022). PhieABEs: A PAM-less/free high-efficienc adenine base editor toolbox with wide target scope in plants. Plant Biotechnol. J. 934–943.

(2019). Off-target effects and the solution. Nat. Plants 341–342.

(2020). Plant prime editors enable precise gene editing in rice cells. Mol. Plant. 667–670.

(2022). Efficient C-to-G editing in rice using an optimi ed base editor. Plant Biotechnol. J. 1238–1240.

(2020). Expanding the CRISPR toolbox in P. patens using SpCas9-NG variant and application for gene and base editing in Solanaceae crops. Int. J. Mol. Sci. 1024.

(2020). Unconstrained genome targeting with near-PAMless engineered CRISPR-Cas9 variants. Science 290–296.

(2021a). Microbial single-strand annealing proteins enable CRISPR gene-editing tools with improved knock-in efficiencies and reduced off-target effects. Nucleic Acids Res. e36.

(2022a). dCas9-based gene editing for cleavage-free genomic knock-in of long sequences. Nat. Cell Biol. 268–278.

(2022b). Base editing-mediated targeted evolution of ACCase for herbicide-resistant rice mutants. J. Integr. Plant Biol. 2029–2032.

(2022c). Efficient targeted insertion of large DNA fragments without DNA donors. Nat. Methods 331–340.

(2018a). A single transcription factor promotes both ield and immunit in rice. Science 1026–1028.

(2021b). Spelling changes and fluorescent tagging with prime editing vectors for plants. Front. Genome Ed. 617553.

(2020a). Targeted base editing in rice with CRISPR/ScCas9 s stem. Plant Biotechnol. J. 1645–1647.

(2020b). Cas12a base editors induce efficient and specific Editing with low DNA damage response. Cell Rep. 107723.

(2019). CRISPR technolog is revolutioni ing the improvement of tomato and other fruit crops. Hortic. Res. 77.

(2018b). Genomic variation in 3,010 diverse accessions of Asian cultivated rice. Nature 43–49.

(2021). Efficient generation of homo gous substitutions in rice in one generation utili ing an rABE8e base editor. J. Integr. Plant Biol. 1595–1599.

(2021). The power and versatilit of genome editing tools in crop improvement. J. Integr. Plant Biol. 1591–1594.

(2017). CRISPR-GE: A convenient software toolkit for CRISPR-based genome editing. Mol. Plant 1246–1249.

(2019). Genome editing for horticultural crop improvement. Hortic. Res. 113.

(2021a). Development of an efficient plant dual c tosine and adenine editor. J. Integr. Plant Biol. 1600–1605.

(2020a). Development of plant prime-editing s stems for precise genome editing. Plant Commun. 100043.

(2021b). Identification of herbicide resistance OsACC1 mutations via in planta prime-editing-librar screening in rice. Nat. Plants 888–892.

(2020b). Discriminated sgRNAs-based surrogate s stem greatl enhances the screening efficienc of plant base-edited cells. Mol. Plant 169–180.

(2022). A design optimi ed prime editor with expanded scope and capabilit in plants. Nat. Plants 45–52.

(2020c). Versatile nucleotides substitution in plant using an improved prime editing s stem. Mol. Plant 675–678.

(2021c). SpRY greatl expands the genome editing scope in rice with highl flexible PAM recognition. Genome Biol. 6.

(2021). High-efficienc and multiplex adenine base editing in plants using new TadA variants. Mol. Plant 722–731.

(2018). Highl Efficient A.T to G.C base editing b Cas9n-guided tRNA adenosine deaminase in rice. Mol. Plant 631–634.

(2022). Pioneer factor improves CRISPR-based C-To-G and C-To-T base editing. Adv. Sci. e2202957.

(2020). PhieCBEs: Plant high-efficienc c tidine base editors with expanded target range. Mol. Plant 1666–1669.

(2021). Genome editing for plant research and crop improvement. J. Integr. Plant Biol. 3–33.

(2021). Expanding base editing scope to near-PAMless with engineered CRISPR/Cas9 variants in plants. Mol. Plant 191–194.

(2019). Generation of herbicide tolerance traits and a new selectable marker in wheat using base editing. Nat. Plants 480–485.

(2019). CRISPR enables directed evolution in plants. Genome Biol. 83.

(2021). Gl cos lase base editors enable C-to-A and C-to-G base changes. Nat. Biotechnol. 35–40.

(2019). Improving plant genome editing with high-fidelit xCas9 and non-