A New Era in Herbicide-Tolerant Crops Development by Targeted Genome Editing

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ABSTRACT

Weeds are one of the biggest problems that modern agriculture is facing worldwide due to the impact they have on crop productivity. Thus, there is a necessity to develop crop varieties with herbicide resistance or tolerance, which would provide cost-effective tools for helping farmers control weeds in the field. Development of herbicide tolerant crops was initially based on conventional plant breeding and transgenic technology. In recent years, the emerging genome technologies, including ZFNs (zinc-finger nucleases), TALENs (transcription activator-like effector nucleases), and CRISPR (clustered regularly interspaced short palindromic repeat), provide us a new way in crop improvement through precise manipulation of endogenous genes in the plant genomes. Among these, CRISPR technologies, including nuclease systems, base editors, prime editors, are really promising in creating novel crop germplasms with herbicide tolerance since they are simple, easy-to-use, and highly-efficient. In this review, we briefly summarize the latest development and breakthroughs of CRISPR technologies in creating herbicide-tolerant crops. Finally, we discuss the future applications of the CRISPR technologies in developing herbicide-tolerant crops.

KEYWORDS:

herbicide tolerance, genome editing, base editing, prime editing, crop

INTRODUCTION

A foremost threat to world food security are weeds due to the damage they generate in crop production. Within a cultivated field, weeds compete with crops for many factors such as water, sunlight, space, soil nutrients and fertilizer. In addition, weeds can act as reservoirs for pests and diseases.[1, 2] Thus, weeds have a big impact on crops and can cause a decrease in crop production and in crop quality.[3, 4] Indeed, without proper strategies for weed control, crop losses of up to 100% can occur.[5] Among the different strategies available for weed control, the use of herbicide-tolerant crops in combination with effective herbicides is one of the most promising. Since the end of the 20th century, researchers have conducted extensive studies on herbicide-tolerant plants and their tolerance mechanisms. Initially, development of herbicide-tolerant crops was accomplished by conventional mutagenesis breeding, which involves the use of EMS (Ethyl methanesulfonate) as chemical mutagenesis to induce multiple mutations (particularly C to T transitions) in a M1 plant populations (Figure 1A). The main drawback of EMS-mediated mutagenesis is that it is labour-intensive and time-consuming. Thus, in the past few decades, transgenic technology has replaced EMS-mediated mutagenesis as the preferred method of choice for crop improvement.[6-8] The first commercially available herbicide tolerance transgenic crops were developed against the broad-spectrum herbicides: glyphosate and glufosinate, revolutionizing weed management and becoming an essential tool in crop production practices (Figure 1B).[9, 10] Unfortunately, since the beginning of this century, regulatory restrictions on the use of genetically modified herbicide-tolerant crops, due to the presence of transgenes, has limited the use of this technology. Moreover, the approval process of deregulation for commercialized of the currently available transgenic crops are lengthy and require big investments.[11, 12] Consequently, a more feasible and less restrictive crop breeding technology is highly required.

Although traditional mutagenesis and backcrossing have been utilized to develop herbicide-tolerant plants, these methods are not suitable for developing herbicide-tolerant plants based on single-point mutagenesis. Thus, over the past decade, plant genome editing technology using two protein-based DNA targeting systems [zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs)] have been utilized to perform site-directed genome mutagenesis.[13] Currently, the most recently discovered genome editing technology CRISPR/Cas9, which was discovered in many bacteria and most archaea, is the most popular class of plant genome editing technology. CRISPR/Cas9 is based on RNA-programmed DNA cleavage mechanisms, in which Cas9 is directed by a single guide RNA

(sgRNA) to a specific DNA sequence where it generates a double-strand break (DSB). The resulting DSB is repaired by two leading pathways [nonhomologous end-joining (NHEJ) and homology-direct repair (HDR)], resulting in nucleotide insertions/deletions (indels) and/or nucleotide substitutions (Figure 1C).[14] Within the CRISPR systems, the CRISPR/Cas9 is the most widely used system, followed by the more recent CRISPR/Cas12a system.[15, 16] Currently, the CRISPR/Cas9 system is the most advanced due to its wide range of genome editing tools, such as base editors and prime editors which enable more precise and efficient targeted point mutations.[17-19] Perhaps one of the most valuable properties of the CRISPR system is the ability to generate specific base-pair changes and small nucleotide deletions without addition of any foreign DNA into the plant genome (such as plants genetically modified by *Agrobacterium*-mediated transformation). Consequently, in various countries (such as the United States of America, Japan and Canada), CRISPR-edited plants are not considered transgenic and therefore not regulated as such.[20] Thus, the CRISPR system is a promising alternative to traditional and transgenic approaches for the development of novel herbicide tolerant plants through genome editing of endogenous genes. Here, we summarize recent advancements on CRISPR-mediated herbicide tolerance crop trait improvement and provide some insights into the development of novel herbicide tolerance crops.

Herbicide working mechanisms

Herbicides predominantly act by disrupting the function of key enzymes or proteins involved in critical metabolic and physiological activities which are crucial for the growth and development of plants.[21] Since the discovery of 2,4-D (2,4-Dichlorophenoxyacetic acid) in 1942, the development of chemical herbicides has lasted nearly 80 years, and hundreds of commercial herbicides have been developed. Up to date, 31 known herbicide sites of action (SOAs) are known (http://www.weedscience.org/Home.aspx). The most common herbicides used in crop production are synthetic auxins, glyphosate, ALS (acetolactate synthase)-inhibitors (sulfonylurea, imidazolinones, triazolopyrimidine, pyrimidinylbenzoates and sulfonylaminocarbonyl triazolinone), glufosinate, dinitroaniline herbicides, acetyl-CoA carboxylase (ACCase)-inhibitors (cyclohexanediones, phenylpyrazolines, and aryloxyphenoxypropionates), PSII (Photosystem II) inhibitors (triazines, phenyl-carbamate,phenyl-pyridazines, amides, triazinones, nitriles, *etc.*), isoxazoles, triketones, and callistemone.[22, 23]

ALS catalyses the primary step of the biosynthesis of branched-chain amino acids (such as valine, leucine, and isoleucine) in plant cells. It is the target of more than 50 commercial and dissimilar herbicides, collectively called the ALS-inhibiting herbicides, which are widely used for weed control in fields.[24] However, the Achilles heel of these herbicides is that weeds easily evolve tolerance to ALS inhibitors due to amino acids substitutions of the ALS protein.[25]

Another enzyme which is targeted by a major group of commercial herbicides (ACCase-inhibitors) is ACCase, which is an enzyme that catalyses the initial step of fatty acid biosynthesis. Within the ACCase, the CT (carboxyltransferase) domain plays an important role in the activity of ACCase-inhibiting herbicides since most tolerant weeds carry mutations in this region.[26] To date, mutations in 10 distinct positions in the CT domain of ACCase have been reported to confer tolerance to ACCase inhibitors.[25]

The broad-spectrum and post-emergence non-selective herbicide glyphosate is one of the most widely used herbicides in the world. Glyphosate disrupts the shikimate pathway for biosynthesis of aromatic amino acids and phenolics by inhibiting EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) in plants.[27] It is believed that glyphosate acts as a competitive inhibitor of PEP (phosphoenolpyruvate), binding to EPSPS and thereby preventing its enzymatic activity. Glufosinate is another broad-spectrum and non-selective herbicide that causes rapid plant death. It inhibits GS (glutamine synthetase), an enzyme present in high abundance in plant leaves, where it plays a vital role in plant nitrogen assimilation.[28] The introduction of glyphosate (glufosinate)-resistant crops has greatly increased the application of glyphosate and glufosinate worldwide. However, fifty-five weed species (27 dicots and 28 monocots) and 5 weed species (1 dicots and 4 monocots) have been identified with evolved resistance to the herbicides glyphosate and glufosinate, respectively, in the fields of HT (herbicide tolerant) crops in the last 20 years. For example, studies show that the double amino acid substitution (T102I + P106S (TIPS)) in EPSPS leads to glyphosate tolerance in goosegrass.[29]

PSII inhibitors (such as phenylcarbamates, pyridazinones, triazines, triazinones, and uracils) are herbicides that inhibit photosynthesis by binding to the Q_B-binding niche on the D1 protein of the photosystem II complex in chloroplast thylakoid membranes. Herbicide binding at this protein location blocks electron transport from Q_A to Q_B and stops CO_2 fixation and production of ATP and NADPH which are all needed for plant growth (http://www.weedscience.org/Pages/SOADescription.aspx).

HPPD (p-hydroxyphenyl pyruvate dioxygenase) inhibitors (such as callistemones, isoxazoles, and triketones) inhibit HPPD, which converts p-hydroxymethyl pyruvate to

homogentisate and is the latest herbicide SOA identified.[30] The inhibition of this key step in plastoquinone biosynthesis, which is related to carotenoid synthesis, results in bleaching symptoms appearing on new-growth leaves. (http://www.weedscience.org/Pages/SOADescription.aspx).

Dinitroaniline herbicides (such as trifluralin, pendimethalin, and ethalfluralin) have been widely used for selective grass weed control in many crops. Evidence obtained from molecular structural modelling and the relationship between the dinitroanilines tolerance and tubulin mutations in plants, suggest that these herbicides most likely interact with α-tubulin and disrupt meristem development by depolymerising microtubules.[31]

Precision base editing of herbicide target genes with DNA rewriters

Many important agronomic traits are controlled by a single nucleotide polymorphism (SNP) or in some cases by a relatively small group of SNPs. Thus, these traits can be introduced into new varieties by installing the required SNPs. This can be accomplished by conventional mutation breeding and cross breeding, but it is time-consuming, laborious and cost-ineffective, and sometimes even impossible. Fortunately, the rapid advance of the CRISPR-based technologies, especially high-precision base and primer editors, provides a novel genome engineering method for developing next-generation herbicide-tolerant crops in the near future.

1) Gene targeting with artificial endonucleases

So far, all ZFN, TALEN, and CRISPR endonucleases have been reported in installing herbicide-tolerant SNPs into the target genes, mainly based on the HR-mediated DNA repair pathway. In this strategy, a donor DNA fragment containing the desirable nucleotide mutations and homologous arms is delivered together with artificial endonuclease into plant cells after DSB has been induced. Subsequently, the donor DNA fragment is inserted into the target region during the process of DNA repair by homologous recombination, resulting in a precise DNA fragment replacement in genome.[32-35] Thus, with this method, plant endogenous target genes can be precisely modified to confer the herbicide tolerance to crops. For example, P191A, W568L, and S647T mutations in ALS, well known for tolerance to imidazolinones and sulfonamide herbicides, have been individually introduced into tobacco acetohydroxyacid synthese (*ALS SuRA* and *SuRB*) genes using ZFN with different donors.[32] Furthermore, a TALEN-based homologous recombination has been used to create double point mutations (W548L and S627I) in *OsALS* in rice, resulting in bispyribac-sodium-tolerant lines that could be heritable to the T1 generation with normal morphology.[34] Meanwhile, Lanqin Xia's group

has successfully introduced the same two-point mutations into *OsALS* in rice with CRISPR/Cas9 endonuclease.[35] On the other hand, Caixia Gao's group employed CRISPR/Cas9 to target non-coding regions in *OsEPSP*S in rice, fulfilling both DNA fragment replacement and insertion via the NHEJ pathway with donors carrying TIPS, resulting in rice seedlings showing glyphosate tolerance.[36]

2) Gene targeting with cytidine base editors

The newly developed cytidine base editors (CBEs) offer an effective alternative to the CRISPR/Cas9 endonuclease system in modifying herbicide-targeting genes in plants.[37] CBEs use Cas9n-fused cytidine deaminases (rAPOBEC1, PmCDA1, hHID, hAPOBEC3A, *etc.*) to induce C-to-T conversion in target genes without generating double-strand breaks and without the need of a donor DNA fragment (Figure 1D).[17, 37-39] So far, herbicide-tolerant rice, maize, wheat, watermelon, oilseed rape, *Arabidopsis,* and *Solanaceae* plants have been developed successfully using various CBEs. The first report came from Cas9n-PmCDA1, which was employed to edit the A96 site in *OsALS*, resulting in tolerance allele *OsALS(A96V)*.[38] Furthermore, Caixia Gao's group utilized rAPOBEC1-Cas9n and specific sgRNAs targeting the P174 and G631 sites in the endogenous *TaALS* genes, generating various nucleotide substitutions in A, B, D genomes of wheat, which endowed rice seedlings with tolerance to imidazolinone.[40] Meanwhile, the CBE has also been used in wheat to modify the A1992 site in the acetyl-coenzyme A protein, which resulted in tolerances to quizalofop.[40] In the case of the allotetraploid oilseed rape, herbicide tolerance was obtained when the P197 site in *BnALSs* were targeted with the rAPOBEC1 or hAPOBEC3A fused to a Cas9 nickase.[41] CBEs have also been utilized for mutagenizing the endogenous *A*LS gene, generating herbicide-tolerant maize and watermelon plants.[42, 43] In addition, an innovative approach has been used in tomatoes and tetraploid potato to produce transgene-free herbicide-tolerant plants.[44]

3) Gene targeting with adenine base editors

Beside CBE, adenine base editors (ABEs), which consist of Cas9n and adenine deaminases (*E. coli* TadA variants) and capable of inducing A-to-G conversion,[45, 46] have also been developed and used in plants to generate herbicide tolerance. Caixia Gao's group first reported an example of utilizing ABE to inducing C2186R substitution in OsACC in rice, resulting in tolerance to haloxyfop-R-methyl.[47] Furthermore, our team successfully used an ABE system to introduce M268T, a point mutation identified in the alpha-tubulin gene of goosegrass (*Eleusine indica*), into the rice homolog gene *OsTubA2*.[48, 49] This novel rice germplasm exhibits tolerance to both

trifluralin and pendimethalin without fitness penalty.[49] More exciting, with robust ABEs being engineered, four endogenous herbicide-targeting genes, including *OsALS1*, *OsGS1*, *OsTubA2*, and *OsACC*, have been simultaneously edited in rice with high efficiency, implying its great potential in generating broad-spectrum herbicide-tolerant crops.[50]

4) Gene targeting with prime editors

Recently, prime editors (PEs), another type of genome-editing technology, have been developed in mammalian cells and plants. The PE system utilizes a reverse transcriptase (RT) paired with CRISPR/Cas9 nickase (Cas9n) (H840A) and a prime editing guide RNA (pegRNA), which facilitates all 12 possible base-to-base conversions, deletion, and insertion without the need of donor DNA (Figure 1E).[51] Thus, prime editing is a promising system installing multiple nucleotide edits at desired target sites in crops. PEs have been extensively applied in editing herbicide-targeting genes in crops. Xu and co-workers have carried out both C-to-G and C-to-T mutations, corresponding to W548L and P171S, in *OsALS* in rice.[52] Also, G-to-C (G2201A) and A-to-G mutations (D2176G) have been achieved in *OsACC* as well.[53] Similarly, G-to-C (W2125C) transversion in *OsACC* and TIPS change in OsEPSPS in in rice have been fulfilled with PEs.[54] Furthermore, PE has been used to target the *ZmALS1* gene in maize, installing mutations of W542L and/or S621I. [55] To be noted, Plant PE is in the infancy stage due to its significant low editing efficiency reported by many groups, even though 92% efficiency was reported lately on PE-mediated in editing *StALS* in the tetraploid potato.[56]

The discovery of novel herbicide-tolerant alleles for crop breeding

Natural and spontaneous mutation are the natural way by which evolution occurs. However, researchers are now utilizing gene editing to mimic the natural evolution process *in planta* at an accelerated pace. A method called base-editing-mediated gene evolution (BEMGE) has recently been developed by our team to generate entirely new herbicide-tolerance alleles in rice.[57] This method uses both CBEs and ABEs as well as gRNA library that covers the full-length coding region of the herbicide-targeting gene (Figure 1F).[57] This approach can be visualized as a "saturation mutagenesis" procedure, in which the whole coding region is subjected to targeted mutagenesis. In our study, BEMGE was used to target the *OsALS1* gene is rice. As a result, we have identified four amino acid substitutions at two positions (P171 and R190) of the protein that confer herbicide tolerance to the herbicide bispyribac-sodium. These

two unique amino acid positions were previously unknown to influence herbicide tolerance and not considered in rice breeding.[57]

The carboxyltransferase (CT) domain of ACCase is the main site of all known ACCase-inhibiting mutations, which directly interacted with herbicide chemicals. In the study of Li *et al*. (2020), the researchers used dual base editors (STEME-1 and STEME-NG) to induce saturated mutagenesis of the CT domain of ACC2 gene in rice. The saturated mutagenesis was promoted by using a library of 200 sgRNAs.[58] Using this strategy, new *ACC2* gene variants conferring herbicide tolerance to haloxyfop were evolved.[58] Similarly, the CT domain of *OsACC* in rice was also edited with enhanced base editing systems and gRNA libraries. Two new mutations (W2125S and C2186R) in addition to previously-identified I1789V in the OsACC protein were identified in the screen of haloxyfop resistant callus.[59] Unfortunately, the homozygous plants carrying novel SNPs suffered from serious growth retardation and complete sterility.[59]

Lately, a method termed prime-editing-library-mediated saturation mutagenesis (PLSM) has been developed for diversifying amino acid changes at the target sites in rice.[60] In their study, six conserved residues in OsACC1 (I1879, P1927, W2097, I2139, D2176, and G2194), involved in haloxyfop tolerance, were individually targeted with PE and a complete set of pegRNA (64 types of NNN) covering all the possible amino acid changes at each site. Using this novel approach, many functional SNVs (single nucleotide variants) were identified.[60]

Current outlook and future perspective

Although more than 400 herbicide active ingredients have been developed so far, most of them have a narrow spectrum. Moreover, pesticide discovery is a typical process with high investment, high risk and long researching cycle. According to the statistics, about 160,000 compounds need to be synthesized with an investment of US $286 million to create a new herbicide, which takes more than 10 years.[61] The agrochemical industry's investment in herbicide discovery declined in the mid-1990s and no commercial herbicides related to any new molecular target (i.e. SOA) has been introduced in the last 30 years.[30, 62] On the other hand, the number of unique cases of evolved herbicide-resistant weeds has increased by about 500% during this period. [30]

Herbicide tolerant crops offer farmers an excellent choice for weed control and hence higher crop yields. Over the past decades, conventional breeding coupled with mutagenesis and transgenic approaches has made significant contributions in the development of herbicide tolerant plants. Based on USDA survey data, the percent of soybean, cotton and corn acres

planted with HT seeds in the US rose to 94%, 95% and 89% in 2019 to 2020, respectively. Because of the great benefits of genetically modified crops (mainly herbicide tolerant crops) to the environment, human and animal health, as well as to improve the socio-economic conditions of farmers and the public, genetically modified crops are being used all over the world. From 1996 to 2018, GM crops have brought $224.9 billion in economic benefits to the world, benefiting 16-17 million farmers, 95% of them from developing countries (https://www.isaaa.org).

The development of transgene-free herbicide tolerant plants by targeted genome editing is currently the most suitable alternative to traditional genetic engineering approaches. The CRISPR technologies, particularly the base editing and prime editing, have a great potential to generate non-transgenic herbicide tolerant crops. Moreover, in many countries, the genome-edited plants created via CRISPR technologies have been excluded from GMO regulation.

Currently, because of technical limitations, the breeding of herbicide tolerant crops focuses mainly on limited herbicides, such us glyphosate, glufosinate, dicamba, 2,4-D, *etc*. Using conventional breeding and transgenic technology to develop crops tolerant to different herbicides is cumbersome. However, with the breakthroughs of CRISPR-mediated genetic engineering technologies in crops, we are able (to an extent) to conquer these limitations and accelerate improvement of desired traits in crops. Thus, it is conceivable that relevant genes and their regulatory regions involved in the interaction or metabolism of herbicide chemicals, especially environment-friendly and non-selective herbicides, can be artificially evolved using gRNA libraries together with BE and PE systems *in planta*. Currently, researchers around the world are identifying many mutations that are involved in the tolerance to herbicides. With this valuable information, development of new varieties of staple crops (i.e. maize, soybean, rice, *etc*.) with broad-spectrum tolerance to multiple types of herbicides can be achieved through rounds of multiplex gene editing.

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Notes

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FIGURE LEGENDS

Figure 1. Strategies for development of herbicide tolerance plants. A. Crop breeding by traditional mutation breeding. **B**. Transgenic technology to create herbicide tolerance crop. **C**. CRISPR-mediated gene knockout, insertion and replacement used to develop herbicide tolerance plants. **D**. CRISPR/Cas9-mediated base editing which generates non-transgenic (non-GMO) plants. **E**. CRISPR/Cas9-mediated prime editing for development of herbicide tolerance plants. nCas9 (H840A) is fused to a reverse transcriptase (RT) pegRNA that contains an sgRNA and an RNA template will replace sgRNA. **F**. CRISPR/Cas-based genetic editing methods can rapidly direct the artificial evolution of crops to develop herbicides tolerance.

Table. 1. DNA rewriters for herbicide tolerance.

DNA rewriters	Plant	Target Gene	Mutation type	Target Herbicide	Reference
ZFN	Wheat	*TaALS*	S653N	Imidazolinone	[63]
TALEN	Rice	*OsALS1*	W548L/S627I	Bispyribac-sodium	[34]
CRISPR/Cas9	Rice	*OsALS1*	W548L/S627I	Bispyribac-sodium	[35]
CRISPR/Cas9	Rice	*OsALS1*	G628W	Imazethapyr, imazapic	[64]
CRISPR/Cas9	Maize	*ZmALS2*	P165S	Chlorsulfuron	[65]
CRISPR/Cas9	Soybean	*GmALS1*	P178S	Chlorsulfuron	[66]
TALEN, CRISPR/Cas9	Potato	*SlALS1*	W563L/S642T	Imidizolinone	[67]
CRISPR/Cas9	Rice	*OsEPSPS*	T173I/P177S (TIPS)	Glyphosate	[36]
CRISPR/Cas9	Rapeseed	*BnEPSPS*	L169F/ G173A/ A175G/ M176C/ R177L	Glyphosate	[68]
CRISPR/Cas9	Flax	*LuEPSPS*	T178I/P182A	Glyphosate	[69]
CRISPR/Cas9	Cassava	*MeEPSPS*	T102I + P06A, 35S replaces *MeEPSPS* promotor	Glyphosate	[70]
CRISPR/Cas9	Rice	*OsSF3b1*	ΔQ157, ΔD223~G232, ΔK1050, K1049R/ K1050E/G1051H, H1048Q/ΔK1049, H1048Q/A1064S/Δ1049	Herboxidiene	[71]
CRISPR/Cas9	Rice	*OsPPO1, OsHPPD*	Inversion, duplication	FCD, bipyrazone	[72]
CBE (Target-AID)	Rice	*OsALS1*	A96V	Imazamox	[38]
CBE	Rice	*OsALS1*	P171F/G628E/G629S	Nicosulfuron, imazapic, pyroxsulam, Flucarbazone, bispyriba	[73]
CBE	Wheat	*TaALSs*	P174 F/S/C, G631D/G632S, G631D/G632N	Nicosulfuron, Mesosulfuron, Imazapic	[40]
		TaACCase	A1992V	Quizalofop	
CBE	Oilseed rape	*BnALSs*	P197S	Tribenuron-methyl	[74]
CBE	*Arabidopsis*	*AtALS1*	P197L/S/F	Tribenuron	[75]
CBE	Watermelon	*ClALS*	P190S	Tribenuron	[42]
CBE	Maize	*ZmALS1*	P165A/S/L/W	Sulfonylurea	[43]
CBE	Tomato, Potato	*SlALS1, SlALS2, StALS1, StALS2*	P186, P184	Chlorsulfuron	[44]

ABE	Rice	*OsACCase*	C2186R	Haloxyfop-R-methyl	[47]
ABE	Rice	*OsTubA2*	M268T	Dinitroaniline	[49]
ABE	Wheat	*TaTubs*	M268T	Dinitroaniline	[76]
ABE	Rice	*OsALS1, OsACCase, OsGS1, OsTubA2*	S627G, C2186R, H249Y, M268T	Imidazolinone, haloxyfop-R-methyl, glufosinate, *dinitroaniline, etc*	[50]
DuBE	Rice	*OsALS1, OsACCase*	P171F, I1879V	Bispyribac-sodium, Haloxyfop	[77]
ABE and CBE	Rice	*OsACCase*	I1879V, C2186R, W2125SC	Haloxyfop	[78]
Prime editing	Rice	*OsACCase*	W2125C	Haloxyfop-R-methyl	[52]
Prime editing	Rice	*OsALS , OsACCase*	W548L, P171S, D2176G, G2201A	\	[53]
Prime editing	Rice	*OsALS1*	W548L	Bispyribac-sodium	[79]
Prime editing	Rice	*OsEPSPS*	T169I/A170V/P173S (TIAVPS)	Glyphosate	[54]
Prime editing	Maize	*ZmALS*	W542L and S621I	\	[55]
Prime editing	Potato	*StALS1*	P186S	Chlorsulfuron	[56]
BEMGE	Rice	*OsALS1*	P171F/L/S, R190H	Bispyribac-sodium	[57]
STEMEs	Rice	*OsACCase*	P1927F, W2125C, S1866F, A1884P	Haloxyfop	[58]
PLSM	Rice	*OsACCase*	I1879L/T/V/S/M, P1927Y/F, W2097G/S/L, I2139N/V/S, D2176G, G2194S/A	Haloxyfop	[60]