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Application of Base Editing in Maize for Herbicide Resistance Improvement

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Abstract Herbicide resistance is a critical challenge in maize (*Zea mays* L.) production, often leading to yield loss and increased production costs. In this study, we explored the application of base editing technology as a precise genome modification approach to improve herbicide resistance in maize. We first reviewed the principles of cytosine and adenine base editors, highlighting their ability to induce targeted point mutations without double-strand breaks and their advantages over traditional CRISPR-Cas9 systems. Target genes associated with herbicide action pathways, such as acetolactate synthase (ALS) and 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), were identified, and strategies for multiplex editing to broaden resistance profiles were discussed. We proposed application strategies, including the selection of monocot-compatible editor variants, optimization of delivery methods, and validation via molecular and phenotypic assays. A case study on *ALS* gene base editing demonstrated successful point mutation introduction, resulting in enhanced herbicide resistance and stable agronomic performance. Our findings underscore the potential of base editing to deliver precision, shorter breeding cycles, and the ability to stack multiple resistance traits, while addressing challenges such as off-target effects and regulatory considerations. This work lays the foundation for integrating base editing with advanced breeding tools to promote sustainable maize production and reduce chemical herbicide dependence.

Keywords Base editing; Maize; Herbicide resistance; *ALS* gene; Genome modification

1 Introduction

At present, the problem of weeds remains the main threat to global corn production. To solve this problem, farmers usually use herbicides to control weeds. However, the excessive and frequent use of herbicides has led some weeds to evolve resistance (Chen, 2024). As a result, the effectiveness of herbicides deteriorates, which also has an impact on food security. Moreover, the few new ways of herbicide action, coupled with increasingly strict policy supervision, all these have made the management of weeds in corn more difficult (Hussain et al., 2021; Kuang et al., 2024).

Previously, researchers used some traditional genetic methods, such as genetic modification or random mutagenesis, to breed herbicide-resistant corn varieties. Although these methods can bring resistance, they also have many problems, such as low efficiency, the possibility of unexpected changes, as well as some policy restrictions and public non-acceptance. In addition, the commonly used gene editing tools nowadays, such as CRISPR/Cas9, will cause breaks in DNA, and then the genes can be modified by the cells' self-repair or by adding templates (Zhou and Liang, 2024). This approach is not only inefficient but also difficult to precisely control mutation points (Zhu et al., 2000; Li et al., 2020; Kaul et al., 2024).

In contrast, base editing is a new type of gene editing technology. It can directly and permanently modify a certain base without cutting DNA or adding an external template. In corn, scientists have successfully made precise point mutations on key genes such as *ALS* (acetyllactate synthase) and *EPSPS* (5-enolacetone shikimic acid-3-phosphate synthase) using cytosine editors and adenine editors. This enables corn to tolerate sulfonylurea and glyphosate herbicides very well, with almost no impact on yield (Dong et al., 2021; Qiao et al., 2022). This approach also makes it possible to breed new corn varieties that do not carry exogenous genes and are precisely edited.

The objective of this study is to use base editing technology to modify some hereditary point mutations in the genes of corn itself, making corn more resistant to herbicides. This not only enhances the weeding effect but also reduces the usage of herbicides, which is conducive to cultivating more environmentally friendly and better-performing corn varieties. In the future, as long as the base editing technology continues to be optimized and the policy allows it, it is very likely to play a greater role in corn breeding.

2 Principles of Base Editing Technology in Plants

2.1 Mechanism of cytosine and adenine base editors in plants

The key to achieving base substitution lies in a set of specially designed proteins. Scientists combined the functionally inactivated CRISPR-Cas9 (which could be a "cut" version or not cut at all) with deaminases to create a base editing tool. In plants, there are mainly two types of such tools: one is the cytosine base editor (CBE), and the other is the adenine base editor (ABE). CBE converts cytosine (C) into uracil (U), and when cells replicate DNA, they mistake uracil for thymine (T). The result is that C becomes T. ABE, on the other hand, changes adenine (A) to inosine (I), and inosine is read as guanine (G), thus completing the transition from A to G. These alterations do not occur at random locations but are carried to that site of the target gene by a specific sgRNA (single-guide RNA) (Mishra et al., 2019; Hillary and Ceasar, 2024).

2.2 Advantages over traditional genome editing (e.g., CRISPR-Cas9)

The traditional CRISPR-Cas9 mainly achieves editing by "cutting" the double strands of DNA (Figure 1). The problem is that this kind of breakage needs to be repaired by the cells themselves, and whether it is repaired well or not is beyond the control of researchers. Non-homologous end joins (NHEJ) are prone to errors, and insertions or deletions often occur. However, another repair method - homologous recombination (HDR) - is very inefficient. In contrast, the base editor's approach is much more "gentle". It does not cut DNA and does not require additional templates; it only makes individual base changes at the target position. This method is precise, predictable and highly efficient, and is particularly suitable for traits determined by a single base (Bharat et al., 2020; Molla et al., 2021).

2.3 Potential for targeted point mutations without double-strand breaks

One of the greatest highlights of base editing is that it can directly change one base to another without causing a double-strand break, and this change is irreversible. In this way, the risk is much smaller, but the efficiency is not compromised. For instance, in corn, point mutations resistant to herbicides can be achieved in this way. Because it does not require DNA interruption, this technology has a particular advantage when dealing with some traits where details are very crucial. It has been regarded as a very practical tool for crop breeding, which can help cultivate new varieties with clear genetic improvement goals (Azameti and Dauda, 2021; Min et al., 2022).

3 Target Genes for Herbicide Resistance in Maize

3.1 Key enzymes in herbicide action pathways (e.g., *ALS*, *EPSPS*)

Why can corn tolerate some herbicides? The core lies in just a few enzymes. Enzymes like acetyllactate synthase (*ALS*, also known as AHAS) and *EPSPS* are precisely the "targets" of certain herbicides. If these enzymes are inhibited, for instance, once *ALS* is suppressed by sulfonylurea or imidazolinone herbicides, corn cannot synthesize branched-chain amino acids and will eventually die. However, as long as specific mutations occur in the *ALS* gene, corn can be made resistant (Zhu et al., 2000). Another enzyme, *EPSPS*, is a key point in the action of glyphosate and is very important in the process of synthesizing aromatic amino acids. Studies have shown that if *EPSPS* undergo mutations or are expressed in large quantities, corn can tolerate glyphosate (Liu et al., 2023). In addition, P450 enzymes (such as CYP81A9) are also crucial. They can metabolize some herbicides, and herbicides like metosulfuron can be "detoxified" by them (Zhang et al., 2024). In addition, enzymes such as glutamine synthase or certain aminotransferases (for example, *ZmGHT1*) have also been found to be associated with resistance to glufosinate-ammonium (Bao et al., 2022).

3.2 Known resistance-conferring point mutations in maize genes

Some point mutations can also make corn resistant to herbicides, and research in this area has become relatively clear. Mutations in the *ALS* gene are quite typical. For instance, when a base at a certain site in *ZmALS*1 and

*ZmALS*2 changes from C to T, it shows strong resistance to herbicides like chlorosulfonon. Looking at *EPSPS*, scientists added three amino acid mutations to natural *ZmEPSPS* through base editing. As a result, corn not only tolerated glyphosate but also had its yield unaffected. There are also transgenic routes, such as cp4-epsps or gr79-epsps, which are also common methods for glyphosate resistance breeding at present (Yu et al., 2023). For mesotrione, certain haplotypes of CYP81A9 or their high expression status can make corn more resistant to this herbicide (Zhang et al., 2024). As for *ZmGHT1*, this gene has also begun to attract attention in glufosinate-ammonium resistance, seemingly in connection with a specific one.

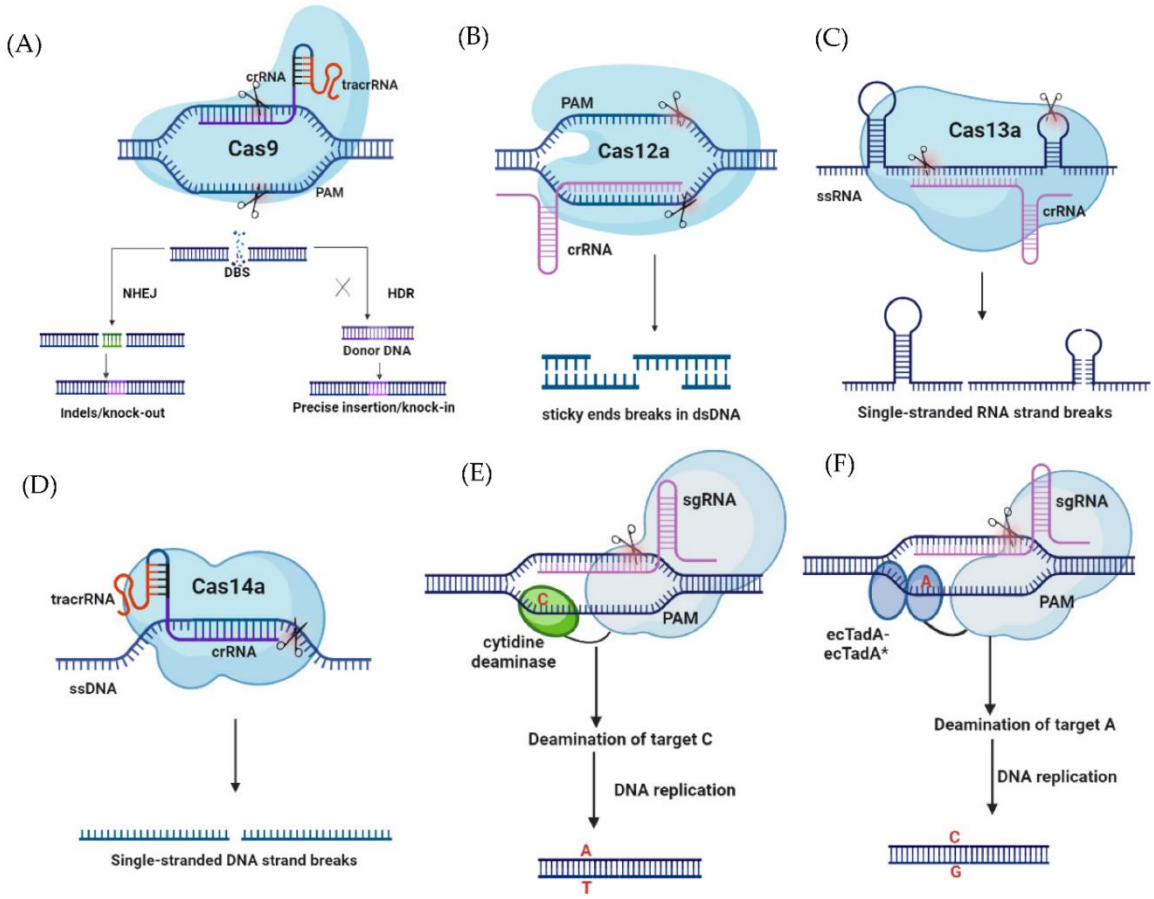

Figure 1 Representative schematic diagrams of CRISPR/Cas editing. (A) Cas9 applies the PAM and sgRNA to cleave the target DNA and produce a DBS, which is repaired by NHEJ or HDR; (B) Cas12a uses a RuvC domain under the guidance of crRNA without the participation of tracrRNA to cleave dsDNA, producing a sticky end; (C) Cas13a targets RNA in the nucleus; (D) CRISPR/Cas14a targets ssDNA cleavage under the direction of sgRNA, and does not require a PAM sequence, producing SSB; (E) CBE system compliments the single base replacement of the target site C-T (G-A), cleaves a single target locus, and produces a staggering cut; (F) ABE system compliments a single base substitution of A-G (T-C), cleaving at the targeted loci, and displaces the DNA fragment, leaving a staggering end in dsDNA (Provided by BioRender.com; accessed on 14 March 2022) (Adopted from Min et al., 2022)

3.3 Strategies for multiplex base editing to enhance resistance spectrum

A single gene mutation might not be sufficient; in such cases, multiple base editing needs to be employed. Some studies have attempted to simultaneously edit *ALS* and *EPSPS*, so that corn can develop resistance to several herbicides, no longer relying on a single mechanism of action, and also slow down the rate at which weeds develop resistance (Li et al., 2020). Another strategy is "combined resistance", for instance, by modifying genes such as *ALS*, *EPSPS*, *CYP81A9* and *ZmGHT1* together, or directly introducing multiple genes (such as cp4-epsps, bar, etc.) in a single transgenic event, corn can be resistant to glyphosate, glufosinate-ammonium and sulfonylurea herbicides simultaneously (Li et al., 2023). It is worth noting that nowadays, an increasing number of studies tend to favor methods that do not use exogenous DNA, such as base editing, which is not only precise and hereditary but also easier to regulate and more readily accepted by the public.

4 Application Strategies for Base Editing in Maize

4.1 Selection of suitable base editor variants for monocots

Not all base editing systems are suitable for monocotyledonous plants like corn, and choosing the right tool is crucial. For instance, when Cas9 is fused with cytidine deaminase, the C in the corn gene can be transformed into T, and the editing efficiency is not low. In some experiments, the editing rate of the plants can reach 43.5% (Zong et al., 2017). Sometimes, to expand the target site range, variants with less stringent PAM requirements, such as xCas9 or Cas9-NG, are also selected, although the hit rate may vary depending on the target site (Fierlej et al., 2022). Moreover, for those stubborn corn varieties with very low conversion efficiency, there is not a complete solution. Researchers found that adding some developmental regulatory factors, such as BABY BOOM, WUSCHEL or GRF-GIF fusion protein, to the transformation vector could significantly increase their transformation and regeneration rates (Zhang et al., 2019a).

4.2 Optimization of delivery systems (e.g., Agrobacterium, Biolistics)

To make the editing system truly effective, having editing tools alone is not enough; the key lies in how to incorporate them. The most commonly used methods are still Agrobacterium and gene guns. The transformation efficiency of Agrobacterium was not high before, especially for some difficult-to-transform genotypes. However, with the advent of ternary vectors and genes that regulate morphology, the efficiency in this regard has been enhanced (Vandeputte et al., 2024). There have also been new advancements in gene guns. For instance, by using mRNA or RNP complexes, editing can be accomplished without leaving exogenous DNA (Svitashev et al., 2016; Qiu et al., 2025). There are even faster methods, such as using viral vectors - like barley striped Mosaic virus - to deliver guide RNA. This approach is fast and can target multiple sites at once (Hu et al., 2019). Some studies even bypassed tissue culture and directly edited in superior materials, such as using haploid induction (HI-Edit/IMGE) methods (Kelliher et al., 2019). Of course, nanotechnology is also being experimented with now, such as nanoparticles and cell-penetrating peptides, which might come in handy in the future (Li et al., 2025).

4.3 Validation of edits through molecular and phenotypic screening

The editing is done. Whether it will succeed or not still needs to be verified. On the one hand, it can be viewed at the molecular level. For instance, PCR and next-generation sequencing (NGS) can be combined with bioinformatics processes to determine whether the bases have changed or gone off-target (Char et al., 2016). On the other hand, it is also necessary to observe the actual plants that grow, such as testing their herbicide resistance or other agronomic traits to see if they meet expectations. Before stable transformation, it is actually possible to conduct a quick test using the protoplast system to see how efficient it is (Fierlej et al., 2022). The final step is very important: screen out those individuals containing exogenous genes or selection markers, and through genetic separation, leave clean edited plants. This is quite crucial for compliance with regulations and public acceptance (Yamada et al., 2024).

5 Case Study

5.1 Background of *ALS* gene function and its role in herbicide sensitivity

The *ALS* gene, in the final analysis, its primary function is to enable plants to synthesize several important amino acids, such as valine, leucine and isoleucine. And the first step of this process is accomplished by an enzyme called acetyllactate synthase (also known as acetylhydroxy acid synthase). However, the problem lies precisely here. Some common herbicides on the market-such as sulfonylureas and imidazolinones-are specifically targeted at it. These drugs can interfere with the function of *ALS*. Plants cannot synthesize amino acids and thus cannot grow normally, and may eventually die directly. However, scientists have found that if the *ALS* gene undergoes a minor mutation, especially in the part that affects the herbicide binding ability, it can resist these drugs, while the growth of the plant itself is basically unaffected (Li et al., 2020).

5.2 Experimental base editing approaches targeting *ALS* in maize

Recently, an experiment attempted to use the CRISPR/Cas9 system, in combination with a cytidine deaminase and UGI protein, to change certain bases in the two *ALS* genes of corn - *ZmALS1* and *ZmALS2* - from C to T. At first, they tested it in corn protoplasts, and later they also used these systems to work on regenerated plants. The result

is quite good, with the highest editing efficiency within the body reaching up to 13.8%. It is worth mentioning that this method can also directly obtain homozygous mutants without exogenous DNA. These plants only carry one or two point mutations on the *ALS* gene.

5.3 Outcomes in resistance phenotype and agronomic performance

Later observations revealed that these plants with the *ZmALS*1 mutation, or those with both *ZmALS*1 and *ZmALS*2 mutations, demonstrated remarkable resistance when confronted with the sulfonylurea herbicide chlorosulfonon. Even when the dosage is increased to 15 times the usual field dosage, they can still withstand it. Moreover, these mutated corns grew normally without showing any growth or development issues, which indicates that the introduced mutations did not affect their agronomic traits. This research shows that base editing technology can indeed be used to precisely breed herbicide-resistant corn varieties, and it is also a non-GMO route, which is quite meaningful for the future direction of breeding.

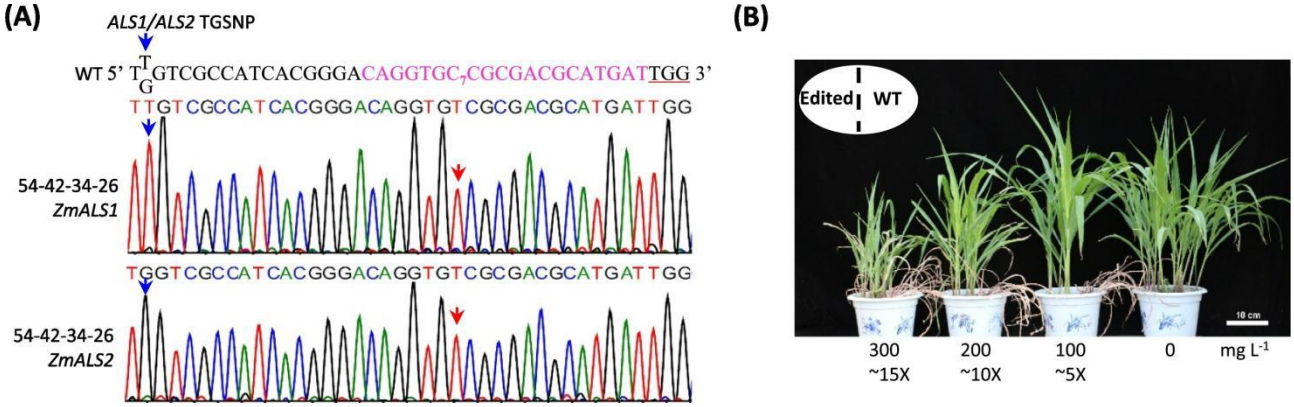

Figure 2 Targeted base editing in two non-allelic *ALS* genes, ZmALS1 and ZmALS2, conferred high-dose chlorsulfuron resistance in maize plants. (A) Sequencing profiles identified a T3 line, 54-42-34-26, with homologous thymine mutations in both ZmALS1 and ZmALS2 at target site 7. Blue arrows indicate the T/G SNP discriminating ZmALS1 and ZmALS2; red arrows indicate the induced target mutation of cytosine to thymine. (B) Three chlorsulfuron concentration level treatments in both ZmALS1 and ZmALS2 targeted CT base edited mutants (on left in pods) and wild-type control (on right in pods) of ZC01. Photographs were made 30 days after herbicide (chlorsulfuron) treatments. Treatments with 500 mL of 0 (empty control), 100, 200, and 300 mg/L chlorsulfuron were applied in each pot of this test. Each pot contained 6 mutant plants on the left and 6 WT plants on the right. 5×, 10×, and 15× indicate the received chlorsulfuron dosage for plants: 5, 10, and 15 times the recommended field application dose, respectively. Scale bar, 10 cm (Adopted from Li et al., 2020)

6 Advantages and Potential of Base Editing in Herbicide Resistance Breeding

6.1 Precision and predictability of trait introduction

Not all herbicide-resistant breeding requires breaking DNA strands. Base editing technology can actually directly modify a single base at a specific position without cutting DNA or requiring a donor template. In this way, herbicide-resistant mutations can be introduced more quickly and accurately. Such changes are very small and look almost no different from natural mutations, and the risk of off-target is also lower. At present, crops such as corn, wheat, soybeans and rapeseed have developed some herbicide-resistant varieties that can be inherited without genetically modified labels through this method (Dong et al., 2021; Shi et al., 2023). In addition, with the addition of specific allele markers, the screening process becomes more convenient (Wu et al., 2020; Wei et al., 2023).

6.2 Shorter breeding cycles compared to conventional methods

In the past, breeding for herbicide resistance relied on mutagenesis or backcrossing generation after generation, and slowness was the norm. Now it's different. With base editing, point mutations can be directly applied to superior varieties, and the speed is very fast. It doesn't take too many generations to obtain homozygous mutants without genetically modified components. This approach significantly saves time and is also easier to promote (Shimatani et al., 2017; Zhang et al., 2019b).

6.3 Opportunities for stacking multiple resistance traits

In the past, it was very complicated to make one crop resistant to multiple herbicides. Now, with base editing, modifications can be made simultaneously or step by step at one or more gene loci. For instance, in wheat, after editing targeting genes such as *ALS* and *ACCase*, the crop has developed resistance to various herbicides (Zhang et al., 2020). This strategy can effectively broaden the range of herbicide options and also reduce the risk of resistance failure, which is of great help to weed management.

7 Challenges and Limitations of Base Editing in Maize

7.1 Off-target effects and unintended base conversions

Base editing sounds quite precise, but in fact, it is not completely without deviation. Sometimes, even if the editors are well designed, they can still cause some unexpected changes nearby. For instance, cytosine and adenine editors are prone to trigger bystander mutations around the target area. Moreover, if certain regions on the genome are only partially similar to the guide RNA, they may also be "mistakenly damaged". Research has found that adenine editors like ZmAYBEv3 are not inefficient in corn, but chimerism and some unwanted edits can still be observed. This indicates that even if sgRNA and the editor itself are optimized, these risks cannot be completely avoided. Another issue is that the "window" of the editor is relatively small. Some areas cannot be edited at all because the sequence is not appropriate. The current editors can mainly achieve C to T and A to G changes. Other types of base changes are still difficult to achieve (Jeong et al., 2020).

7.2 Efficiency variations across maize genotypes

Not all corn varieties are equally "cooperative" with base editing. In other words, some varieties are easy to edit, while others are particularly difficult to handle. The transformation method, the degree of response of tissue culture, and whether chromatin is easily accessible will all affect the editing effect. Especially for some excellent but not easily convertible inbred lines, there are relatively greater limitations in their application. Even if an editor like ZmAYBEv3 performs well in some strains, in others, there may be problems such as incomplete editing or difficulty in passing it on. Some studies have pointed out that in many cases, only a small number of T0 plants exhibit stably inherited target mutations (Fierlej et al., 2022). In addition, whether to use a gene gun or Agrobacterium and other delivery methods, the efficiency will also vary due to the differences between the target genes and the corn itself.

7.3 Regulatory, biosafety, and public acceptance issues

Regarding the regulation of base-edited crops, not all national standards are the same. In some places, these plants are not classified as genetically modified because they do not contain exogenous DNA. But elsewhere, they might still be regarded as genetically modified organisms. This makes it very difficult for developers and breeders to determine how to proceed (Rai et al., 2025). In addition to regulations, biosafety issues cannot be ignored either, such as the ecological impact that off-target mutations may bring, and detailed molecular testing is also needed to confirm that there are no hidden exogenous fragments. Finally, there is another point that is often overlooked: how the public views it. Even if the scientific community has proven that these crops are safe, if consumers do not accept them, it will be very difficult to bring these achievements to the market (Namata et al., 2025).

8 Future Perspectives for Base Editing in Maize Herbicide Resistance Improvement

8.1 Integration of base editing with genomic selection and high-throughput phenotyping

In the future, corn breeding will no longer rely solely on traditional methods. Base editing technology has begun to enter the toolbox of breeders, especially when it is used in combination with genomic selection and high-throughput phenotypic analysis, the effect is more obvious. In fact, breeders have been constantly seeking methods that can precisely introduce target traits. Now, through genomic prediction combined with base editing, it is possible to screen out plants with the ideal combination of traits more quickly. High-throughput phenotypic analysis systems are not just for show. They can quickly test whether the edited plants have truly developed herbicide resistance and also detect other agronomic traits simultaneously (Andorf et al., 2019). Ultimately, this combination of technologies can significantly shorten the breeding time and make it easier to find truly useful resistant varieties.

8.2 Development of next-generation editors with expanded targeting scope

The targets of traditional base editors are still limited, but this problem is being broken. New editors like ZmAYBEv3 have been able to efficiently convert A to T and A to C in corn and other monocotyledonous plants, which was not easy to achieve in the past. These new editors have broadened the area that can be edited, and naturally, more variations related to traits can be introduced (Zhong et al., 2023). Of course, there are still some challenges at present, such as how to reduce chimeras and improve editing efficiency, etc. However, as long as the technology continues to improve, the editor's capabilities will become increasingly powerful, and it may even be possible to introduce multiple resistance sites at once in one generation of plants. This is particularly useful for the improvement of complex traits.

8.3 Potential role in sustainable maize production and reduced herbicide usage

Base editing also has considerable potential in sustainable agriculture. What it can achieve is to precisely endow corn with herbicide resistance or stress tolerance, rather than relying solely on chemical input. The idea of using less pesticides and relying more on one's own genetic resistance has actually been accepted by an increasing number of researchers (Jiang et al., 2024). More importantly, plants obtained through base editing are not necessarily classified as genetically modified, which has obvious advantages in terms of regulatory approval and public acceptance. In the future, this technology will not only be used to resist herbicides, but also has the potential to increase overall production, reduce environmental pressure, and even provide a more powerful guarantee for food security.

9 Conclusion

Base editing technology has indeed opened up a new direction for corn breeding, especially in terms of herbicide resistance. In the past, making corn resistant to drugs might require a long period of repeated trials and selection. However, now, by introducing point mutations, some key genes, such as *ALS*, can be precisely modified. These modifications typically employ a combination of CRISPR/Cas9 and cytidine deaminase, which is not only highly efficient but also capable of generating homozygous plants without exogenous genes.

One obvious benefit is that the tolerance of these corn varieties to herbicides has been greatly enhanced. Even if the dosage far exceeds the original limit, there is no problem, and the growth of the plants and other agronomic traits are basically not affected. In fact, sequencing work on strains like B73 and Palomero has been carried out long ago, which also laid the foundation for the development of base editors. Nowadays, some new editing tools that can achieve A-to-T or A-to-C substitution have further expanded the possible space for improving the traits of corn.

Of course, the changes brought by this technology are not limited to these. It has also saved the breeding process many detours. The previous approach of relying on random mutagenesis and long-term backcrossing might be much slower. Nowadays, breeders can incorporate useful traits more quickly and accurately. Moreover, since the generated varieties are not genetically modified, this is beneficial for passing regulatory reviews and gaining public support.

However, to be fair, base editing is not omnipotent. During the application process, off-target effects, biosafety issues, and even the possible evolution of drug resistance by the weeds themselves are all factors that need to be taken into consideration. Even the most advanced tools may lead to undesirable consequences if they are not combined with ecological management, technical prudence and transparent communication. So, how to use it, when to use it and to what extent to use it all need to be carefully considered.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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