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CRISPR/Cas9-Mediated Knockout of Drought-Sensitive Genes Improves Maize Tolerance

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Abstract Maize (*Zea mays* L.) plays a crucial role in ensuring global food security, yet its productivity is severely threatened by recurrent drought stress in many regions. Conventional breeding approaches have achieved limited success in improving drought resilience due to the complex and polygenic nature of drought tolerance. In this study, we explore the application of CRISPR/Cas9 genome editing technology as a precise and efficient strategy for enhancing drought tolerance in maize. By reviewing recent advances, we identified key drought-sensitive genes such as *ZmNAC111*, *ZmPP2C-A10*, and *ZmDREB2A*, which were targeted for knockout using various transformation techniques including Agrobacterium-mediated and biolistic methods. Functional validation and field evaluations of CRISPR-edited maize lines demonstrated significant improvements in physiological and agronomic traits under drought conditions, including enhanced root development, reduced stomatal conductance, better water retention, and higher yield stability compared to wild-type plants. The findings highlight that gene knockouts effectively mitigate drought-induced physiological stress and optimize water use efficiency. Although challenges remain regarding off-target effects, regulatory frameworks, and public acceptance, CRISPR/Cas9 offers a transformative platform for integrating molecular precision with traditional breeding. This study underscores the potential of genome editing in developing drought-resilient maize varieties and anticipates future advancements through multiplex editing and next-generation CRISPR technologies.

Keywords CRISPR/Cas9; Drought tolerance; Maize; Gene knockout; Genome editing

1 Introduction

Corn (*Zea mays* L.) is the star on the dining tables in many countries and an important source of industrial raw materials and feed. Its significance is no longer merely as a staple food; it is related to the nutritional intake and livelihood stability of millions of people around the world. However, the problem is not small either. In recent years, droughts caused by climate change have become increasingly frequent. Sometimes they not only last for a long time but also have a high intensity. Corn has naturally become one of the "victims", with its output declining and the global food system also affected (Sami et al., 2021).

Drought is not a new term, but it is indeed one of the most troublesome abiotic stresses. It not only disrupts the growth rhythm of plants but may also affect the entire physiological process (Kumar et al., 2023). Especially for crops like corn that are more sensitive to water, the blow is even more obvious. In addition to reduced production, drought may also affect grain quality and even plant health. Ultimately, it affects not only farmland, but the entire supply chain and farmers' income (Peer et al., 2024).

However, things are not that simple. Want to solve the problem of drought resistance through breeding? The difficulty is much greater than imagined. On the one hand, behind the drought resistance of corn lies a complex genetic network, with many genes working together (Chen, 2025). On the other hand, although traditional breeding methods are quite experienced in improving some traits, they are inadequate in enhancing drought resistance. The reasons include long time consumption and low efficiency. In addition, alleles with drought resistance characteristics are also rare in natural germplasm (Lorenzo et al., 2022). As for genetically modified organisms? Technically, there is some hope, but in reality, it is often limited by policy restrictions and public concerns (Joshi et al., 2020).

Against this backdrop, CRISPR/Cas9 genome editing technology has gradually been regarded as a breakthrough. It is not like traditional methods that require "slow work to produce fine results", nor is it as controversial as genetically modified organisms. What we want to explore is its application potential in the drought resistance improvement of corn. By precisely knocking out or modifying drought-sensitive genes, this technology may be able to accelerate the breeding process of drought-resistant varieties to a certain extent. This study will discuss the current research progress, key target genes, and the feasibility of incorporating CRISPR/Cas9 into breeding programs, and further analyze the challenges and prospects involved.

2 Mechanisms of Drought Sensitivity in Maize

2.1 Drought perception and signal transduction pathways in maize

When corn is short of water, it first "detects" environmental changes. This reaction is not completed instantaneously but is accompanied by the interweaving of a series of molecular signals. The first to be activated is often the abscisic acid (ABA) signaling pathway-which is mentioned in almost all drought studies, but the response rate and intensity vary in different tissues. When water begins to decrease, ABA accumulation prompts stomata to gradually close to reduce water loss, while simultaneously initiating a series of stress-related genes (Jiang et al., 2025). In this pathway, receptors (PYL/RCAR), protein phosphatases (PP2Cs), and kinases (SnRK2s) act like a relay team, transmitting signals layer by layer and ultimately guiding cells to respond (Cao et al., 2021).

However, ABA is not a "solo". Other hormones such as auxin and cytokinin also get involved. Sometimes they cooperate and sometimes they "go against", thus making the response of corn more flexible. Calcium signals and MAPK cascade reactions act like amplifiers, converting external drought signals into stronger cellular responses (Wang et al., 2022). Overall, this system is more like a constantly fine-tuned network rather than a simple one-way path.

2.2 Role of stress-responsive genes and their regulatory networks

After the drought came, the gene expression in corn was almost "rewritten", and thousands of genes began to change (Zhao et al., 2025). Some genes are responsible for directing-transcription factors such as WRKY, NAC, DREB, bZIP, etc. Some act directly, such as antioxidant enzymes SOD, CAT, and POD, as well as proteins involved in hormone synthesis or signal transduction. Transcription factors check and coordinate with each other, forming a complex regulatory network, which ultimately enables corn to maintain osmotic balance, metabolic adjustment and defense response under stress.

For instance, ZmWRKY30 and ZmDIR11 can enhance antioxidant and hormone metabolism, thereby improving drought resistance (Figure 1). However, there are also opposite examples. For instance, when ZmPL1 or ZmPP2C-A10 is overexpressed, corn becomes more vulnerable instead (Gu et al., 2024). Furthermore, the natural differences in gene regulatory elements and expression dynamics also lead to significant variations in the performance of different varieties under drought conditions (Liu et al., 2020). In other words, drought resistance is not a single "genetic victory", but the result of the entire genetic network.

2.3 Physiological traits influenced by genetic sensitivity to drought

From the appearance, corn with poor drought resistance often has withered, curled leaves, and even yellowing leaves, as well as a decline in photosynthesis and a reduction in biomass-these signs are all familiar to everyone. However, if one looks deeper, it will be found that the accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is the key issue, and they are particularly severe in sensitive corn (Yousaf et al., 2023). If the antioxidant system is weak, the cell structure is prone to damage.

Those genotypes that can hold up usually have stronger antioxidant enzyme activity, more stable osmotic regulation ability (such as accumulation of proline and soluble sugar), and can also maintain a better photosynthetic level under stress. These seemingly physiological differences are actually closely related to the response efficiency of signaling pathways and the regulation of related genes.

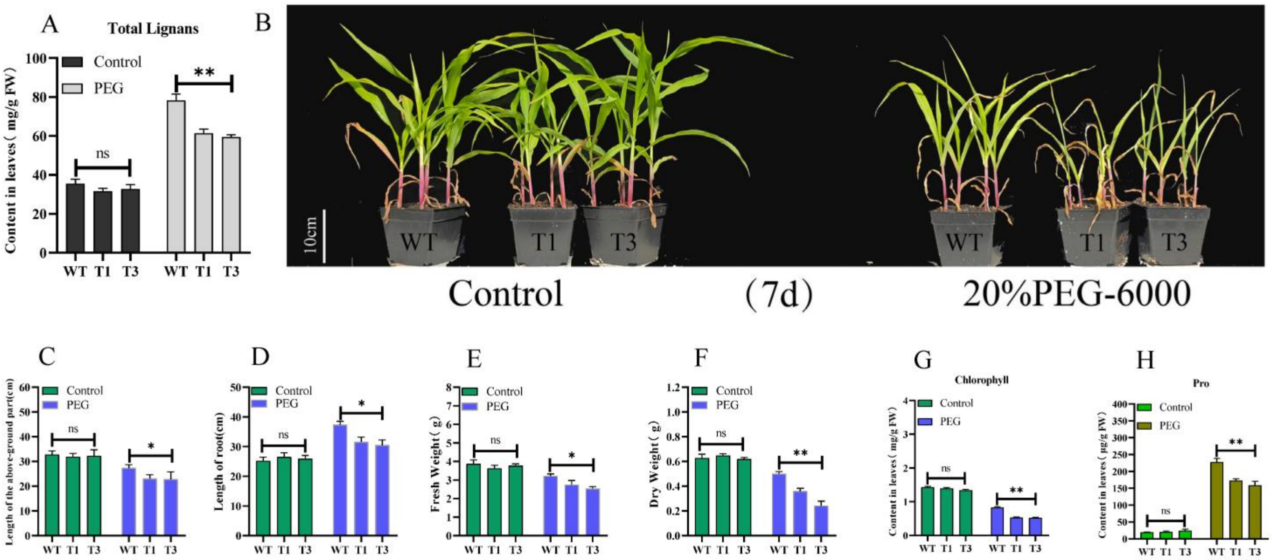

Figure 1 (A). Total lignin content in WT and mutant lines subjected to both normal and drought treatments; (B). Phenotypic photographs of WT and mutant lines after various treatments, T1 and T3, represent two ZmDIR11-EMS mutant lines with different mutation profiles; Scale bar = 10 cm; (C–G). Growth parameters of WT and mutant lines T1 and T3 under normal and drought conditions, which include above-ground length, root length, fresh weight, dry weight, and chlorophyll content in leaves (from C to G, respectively); (H). Proline (Pro) content in the leaves of WT and mutant lines T1 and T3 under normal and drought treatments. Data are presented as the mean of triplicate values, with error represented as standard deviation (SD). Statistical significance is indicated as non-significant (ns), $p < 0.05$ (*), and $p < 0.01$ (**) (Adopted from Zhao et al., 2025)

3 CRISPR/Cas9 as a Genome Editing Tool in Plants

3.1 Mechanism of CRISPR/Cas9: double-strand breaks and repair pathways

In plants, the core of gene editing is actually not "cutting", but "repairing". The reason why the CRISPR/Cas9 system is powerful is that it can create a double-strand break (DSB) at a specific position in DNA, and all of this relies on guide RNA (gRNA) to lead the Cas9 enzyme to the target sequence (Bao et al., 2019). Once a break occurs, cells will naturally attempt to repair it. The commonly used methods mainly include two: non-homologous end joining (NHEJ) and homologous recombination (HR). The former is like random mending, prone to errors. Thus, insertion or deletion (indel) has become a common outcome, which is precisely exploited by scientists to "knock out" a certain gene. The latter is more meticulous and requires providing repair templates that can achieve precise rewriting (Xue and Greene, 2021).

However, most plant somatic cells prefer to use NHEJ, which makes CRISPR/Cas9 particularly adept at creating loss-of-function mutations. Strictly speaking, it is not a "perfect fix", but rather a natural strategy of "winning by mistake".

3.2 Advantages over traditional breeding and RNA interference (RNAi)

To talk about the advantages of CRISPR/Cas9, we first need to look at the limitations of the old methods. Traditional breeding relies on hybridization, screening and backcrossing, which has a long cycle, low efficiency and is also limited by genetic background. CRISPR/Cas9 can precisely "target" and modify, much faster, and can also modify multiple genes at once (Ahmad, 2023).

RNA interference (RNAi) may sound like it can also silence genes, but it usually only reduces expression and does not achieve complete knockout. Sometimes, off-target problems may also occur (Chen et al., 2019). In contrast, CRISPR/Cas9 not only has high specificity but also can yield stable and heritable mutant strains. More importantly, some strategies can even leave no trace of exogenous DNA after editing, thus bypassing the "genetically modified" label and making it much easier in terms of regulation and public acceptance. In other words, this technology makes "precision" and "tracelessness" possible.

3.3 Transformation methods in maize: agrobacterium-mediated, biolistic, and RNP delivery

For CRISPR/Cas9 to truly function in corn, the key lies not in the "scissors", but in "putting the scissors in". There are three common practices, each with its own advantages and disadvantages. Agrobacterium-mediated transformation is the most common, which integrates the CRISPR/Cas9 DNA construct into the plant genome by means of Agrobacterium rhizocarpus. This approach is mature and reliable, but it is not very friendly to certain genotypes of corn, and the insertion position is often random (Li et al., 2021). Another method is the gene gun technique, which uses high-speed particles to directly inject DNA or Cas9-gRNA ribonucleoprotein (RNP) into cells. This method is particularly suitable for monocotyledonous plants like corn, and it can also avoid introducing exogenous DNA when using RNP, achieving true "non-GMO" editing. The third type-RNP delivery-is actually an extension of the gene gun method: Cas9 and gRNA are pre-assembled and then delivered into cells, eliminating the transcription and translation process, improving efficiency, and reducing the risk of off-target. Now it has been proven to be successfully applied in corn, being both clean and efficient.

4 Identification and Selection of Drought-Sensitive Genes

4.1 Omics-based screening of candidate genes under drought stress

In corn research, it is not easy to find truly "effective" drought-resistant genes. Scientists often have to dig for clues bit by bit from vast amounts of data. High-throughput technologies such as transcriptome, metabolome and genome-wide association studies (GWAS) are precisely the main tools of this "journey to search for genes". Under drought stress, the transcriptional levels of corn of different genotypes changed astonishingly-thousands of differentially expressed genes (DEGs) were detected, which were involved in signal transduction, antioxidation, hormone synthesis and even metabolic regulation (Zenda et al., 2019; Waititu et al., 2021).

Metabolomics fills in another piece of the puzzle. Some metabolites, such as molecules involved in tryptophan or amino acid metabolism, have been found to be closely related to drought resistance performance (Li et al., 2024). Meanwhile, GWAS and quantitative trait loci (QTL) analyses also identified several key regions, revealing genes related to differences in drought tolerance-including transcription factors and signaling pathway components (Wu et al., 2021). These results collectively indicate that the drought resistance of corn is the product of a multi-level network rather than a story dominated by a single gene.

4.2 Functional studies validating gene roles in drought responses

The candidate genes have been identified. The next step is to confirm that they are "really useful". Researchers usually verify gene functions by means of overexpression, knockout or transgenesis. Taking *ZmPP2C15* and *ZmNAC111* as examples, the CRISPR/Cas9 tool helps researchers directly observe their "role changes" in drought response (Pang et al., 2024).

When positive regulatory factors are overexpressed, corn becomes more resilient-the activity of antioxidant enzymes increases, osmotic protective substances rise, and water use efficiency is also better. Conversely, once those negative regulatory factors or drought-sensitive genes are knocked out, drought resistance can also be improved (Liu et al., 2022). In other words, enhancing drought tolerance is not solely dependent on "strengthening"; sometimes, "weakening" certain genes can be more effective. These experiments have provided empirical evidence for the theory and also offered conclusive evidence for understanding the drought adaptation mechanism of corn.

4.3 Notable drought-sensitive genes targeted in maize: *ZmNAC111*, *ZmPP2C-A10*, *ZmDREB2A*

Among the numerous candidate genes, *ZmNAC111*, *ZmPP2C-A10* and *ZmDREB2A* frequently appear in research reports and have also become key targets for gene editing. *ZmNAC111* is a transcription factor of the NAC family, and its expression is inhibited by the insertion of MITE elements in the promoter. If it is allowed to overexpress, the water use efficiency and drought tolerance of corn will be significantly improved. *ZmPP2C-A10* belongs to Class A PP2C phosphatase, but its function is exactly the opposite-it inhibits drought tolerance response. Research has found that reducing or knocking out this gene can enhance drought resistance by regulating ABA signaling and reducing water loss.

As for *ZmDREB2A*, it is activated almost "instantly" when drought strikes, and then regulates a series of downstream stress genes, enabling plants to maintain homeostasis in adverse conditions (Gulzar et al., 2021). The discovery of these genes not only explains the different performances of corn under drought conditions, but also provides a practical basis for subsequent targeted improvement using CRISPR/Cas9.

5 Case Study: Field Application of CRISPR-Edited Maize Lines

5.1 Description of editing strategy and target gene(s)

Among the numerous attempts at corn gene editing, ARGOS8 is a target that has been repeatedly mentioned. It was originally a negative regulatory factor for the ethylene reaction. That is to say, once its expression level is adjusted, it can indirectly affect the plant's response to stress. Researchers used the CRISPR/Cas9 tool to precisely modify ARGOS8: some inserted the natural GOS2 promoter in its 5 'untranslated region, while others replaced the original promoter with this promoter (Bashir et al., 2021). These treatments enable the genes to be continuously highly expressed in multiple tissues.

After all the modifications were completed, the researchers verified them one by one through PCR and sequencing, confirming that the ARGOS8 site had undergone the expected changes. Although the steps are complex, this idea of "promoter replacement" provides a new entry point for the functional improvement of corn.

5.2 Comparative evaluation: edited vs. wild-type lines under controlled drought conditions

The real test is not in the laboratory but in the field. The research team planted the CRISPR-edited ARGOS8 strain under both drought and normal irrigation conditions, and used the wild type (WT) as a control. It was found that the edited plants showed stable performance under drought stress during the flowering period, and the transcriptional level of ARGOS8 significantly increased (Namata et al., 2025).

Agronomic traits, especially grain yield, are closely monitored-the edited plants not only have enhanced drought resistance but also maintain good yield. Meanwhile, similar strategies have also been applied to the modification of other drought-related genes, such as *ZmPL1* and *ZmGA20ox3*. The researchers respectively evaluated the responses of these plants at the physiological and biochemical levels, and the results also showed positive effects. It can be said that these experiments have truly brought CRISPR from a "laboratory tool" to "farmland application".

5.3 Results and interpretation: improved growth, yield, and stress marker expression

Under drought stress conditions, the ARGOS8 editing strain performed significantly better than the wild type. Field data show that the average yield per unit area increased by approximately 5 bushels, while no negative impact was observed under conditions of adequate moisture (Wang et al., 2024). More detailed observation revealed that the physiological indicators of the edited strain were also more ideal-water loss was slower, reactive oxygen species (ROS) accumulated less, and antioxidant enzyme activity was higher. Similar improvement effects have also emerged in other gene-edited strains. For example, the plants edited with ZmPL1 showed improvements in germination rate and survival rate (Figure 2), while the contents of MDA and ROS decreased and the expression of stress-related genes was stronger (Wang et al., 2025). The ZmGA20ox3 edit strain exhibits an interesting "semi-dwarf" characteristic, which is often accompanied by higher levels of abscisic acid (ABA) and jasmonic acid (JA). They still maintained good yields under drought (Liu et al., 2023). Overall, these results indicate that corn regulated by CRISPR can not only "live better" but also "produce more", providing a practical molecular pathway for drought-resistant breeding.

6 Impacts of Gene Knockouts on Drought Tolerance

6.1Alterations in root system architecture and stomatal regulation

When plants are in drought, the first to "react" are not the leaves but often the roots and stomata. Gene knockout can precisely bring profound changes in these two aspects. Taking corn as an example, researchers knocked out the *DSD1/ZmICEb* gene using CRISPR/Cas9 technology. As a result, stomatal density decreased, water use efficiency improved, and yield loss under stress was significantly reduced (Zhou et al., 2025).

Interestingly, the knockout of the *ZmHDT103* gene did not make plants "weak"; instead, it reduced the pore size of stomata, decreased water loss, and at the same time promoted the accumulation of abscisic acid (ABA) and increased peroxidase activity. These two changes seem independent, but they jointly enhance the regulation of stomata and antioxidant capacity. Overall, these modifications have enabled plants to learn to "conserve" during droughts: reducing transpiration and maintaining internal water content, thus surviving the stage of water deficiency.

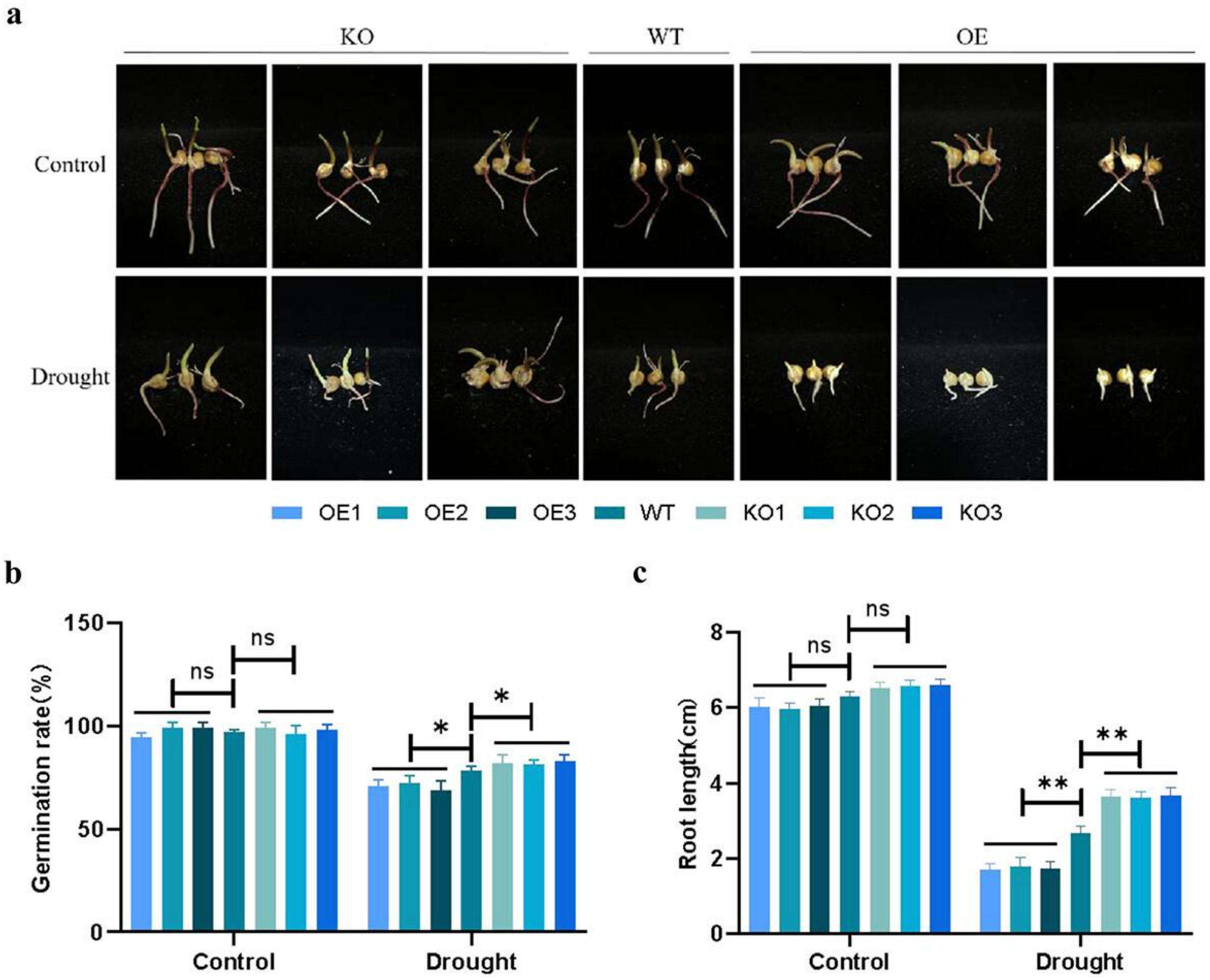

Figure 2 Knockdown of ZmPL alleviate the inhibition of seed germination rate and root length under drought stress.(a) seeds of WT and transgenic lines germinated on filter paper soaked of PEG6000. Scale bar = 0.5 cm. (B, C) statistics of germination rate and root length for different genotypes. Using Student’s t-test, asterisks indicate statistically significant differences (*p <0.05; **p < 0.01). Data are shown as mean ± SD from three independent experiments (Adopted from Wang et al., 2025)

6.2 Enhanced water retention, reduced wilting, and better photosynthetic performance

If the changes in stomata help plants save water, then water retention and photosynthetic performance determine how long they can "survive". In CRISPR-edited strains targeting negative regulatory factors such as ZmPL1 and ZmHDT103, researchers observed higher leaf water content, less wilting, and higher seedling survival rates under drought stress.

The levels of reactive oxygen species (ROS) and malondialdehyde (MDA) in these plants decreased, indicating that cell membrane damage was alleviated and oxidative stress was also reduced. Meanwhile, the activity of antioxidant enzymes and the content of proline increase, making osmotic regulation more effective and the cells more stable. It is precisely for this reason that they can still maintain the efficiency of photosynthesis during droughts.

Similar results have also been repeatedly verified in crops such as rice and wheat-whether overexpressing positive regulatory genes or knocking out negative regulatory factors, it can increase chlorophyll content, reduce stomatal conductance, and ultimately improve survival rate (Usman et al., 2020; Abdallah et al., 2022). In other words, different crops, in different ways, reached similar physiological outcomes.

6.3 Stable yield performance under moderate to severe drought stress

The real test is still the output. No matter how well the laboratory performs, the key lies in whether the field can "hold up". Multiple field and controlled environment trials have shown that gene-edited corn yields are more stable and even slightly increase under drought conditions. For instance, the ARGOS8 variant showed a significant increase in grain yield under drought conditions during the flowering period, while no reduction occurred when there was sufficient water (Rai et al., 2023). Similarly, corn with DSD1/ZmICEb and ZmHDT103 knocked out also reduced yield loss. These results indicate that gene knockout is not merely an experimental phenomenon but a strategy that can be translated into actual agronomic advantages. Overall, these studies reveal a trend-through precise gene editing, corn is expected to achieve "stable production without reduction" in water-scarce environments, which is of great significance to agriculture in areas frequently hit by droughts.

7 Challenges and Considerations in CRISPR-Based Drought Tolerance

7.1 Off-target effects and their detection and mitigation

In gene editing, what often worries researchers the most is not "unable to cut", but "cutting in the wrong place". The off-target problem of CRISPR/Cas9 is just like this-when the guide RNA is too similar to other genomic sequences, unexpected mutations may occur. However, the good news is that in a complex genome like that of corn, as long as the guide RNA is properly designed and has at least three base misaligns with non-target regions, especially in the seed region near the PAM, the risk of off-target is usually extremely low (Young et al., 2019).

There are also more and more methods for detecting such unexpected edits. Computational prediction tools, whole-genome testing (such as CLEAVE-Seq), and direct validation in plants can all help scientists "identify" these minor biases (Erdogan et al., 2023). In addition, high-fidelity Cas9 variants, shorter grnas, and the delivery method in the form of RNP have also been proven to effectively enhance specificity. Overall, these improvements often result in less off-target variation than natural mutations in traditional breeding. In other words, this "precision revolution" is more controllable than people imagine.

7.2 Regulatory landscape and public acceptance of gene-edited crops

A scientific breakthrough does not mean that one can immediately go to the fields. The regulation and public acceptance of CRISPR-edited crops remain the key factors determining their fate. The attitude in the United States is relatively open-if exogenous DNA is not introduced into plants, they are often not classified as strictly genetically modified (Ahmar et al., 2023), which has accelerated the pace of commercialization.

But not all countries are so optimistic. In regions such as the European Union and New Zealand, gene-edited crops are still treated as genetically modified, with slow approval and numerous restrictions (Ansari et al., 2020). The public's attitude is also complex: what they care about is not the technical principle, but safety, transparency, and "whether it is genetically modified or not". Therefore, using RNP delivery to obtain edited corn without exogenous fragments might alleviate some concerns and bring science closer to society.

7.3 Integration with breeding programs and scaling for field use

The maturity of technology does not mean that it can be immediately transformed into breeding achievements. Integrating CRISPR/Cas9 into the traditional breeding system requires more collaboration and patience. Take multi-genome editing strategies such as BREEDIT as an example. They can simultaneously modify multiple genes, accelerate trait improvement, and also help screen out the optimal drought-resistant gene combination (Shelake et al., 2022).

However, problems also arise: corn of different genotypes show significant differences in transformation and regeneration, and the editing efficiency varies. Some laboratory achievements fail to adapt to the local environment when they reach the fields. Therefore, for these technologies to truly take root, molecular biologists, breeders and regulatory authorities must act together to bridge the last mile from the laboratory bench to the farmland (Ying et al., 2025). Otherwise, the breakthroughs in the laboratory might still remain at the level of papers.

8 Concluding Remarks and Future Directions

CRISPR/Cas9 is no longer a new term in the research of corn drought resistance, but the changes it has brought are still impressive. By targeting and regulating negative regulatory factors such as ZmPL1, ZmHDT103 and ARGOS8, researchers have successfully obtained corn materials that can still achieve stable yields in arid environments. Unlike traditional breeding, these edited plants are not only drought-tolerant but also maintain a good physiological state-less oxidative stress, higher water content and survival rate, while with almost no yield loss under normal conditions. It can be said that this efficient and precise tool has brought the climate-adaptive breeding of corn into an "accelerated stage".

It is worth noting that the research has not been confined to a single gene. Multiple CRISPR strategies are emerging as a new direction, such as the BREEDIT process, which can edit multiple genes or gene families at once, helping researchers deal with complex polygenic traits like drought resistance. This approach has produced a richer combination of mutations, providing the possibility of screening out the optimal gene pairings. Meanwhile, the new generation of editing systems-base editing, guide editing and non-GMO RNP delivery-have further enhanced accuracy, reduced the risk of off-target, and also gained greater flexibility at the regulatory level. Together, they have broadened the boundaries of crop improvement, making it possible to deal with multiple stresses.

However, to truly bring these achievements into the fields, cross-disciplinary cooperation is still needed. Molecular methods alone are far from enough; the combination of genomics, physiology and agronomy is the key. Multi-omics analysis is helpful in identifying new candidate targets, while the accumulation of field phenotypic and physiological data can verify the performance of these editing strains in real-world environments. In the future, the collaboration among molecular biologists, breeders and regulatory agencies will determine whether CRISPR-edited corn can truly be applied on a large scale. Only when science, policy and public awareness keep pace simultaneously can this technology have a sustained impact on global agriculture.

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Conflict of Interest Disclosure

The author affirms 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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