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CRISPR/Cas-Mediated Genome Editing for Enhancing Abiotic Stress Tolerance in Major Food Crops: Recent Advances and Future Perspectives

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ABSTRACT
Abiotic stresses (drought, salinity, heat, cold) severely limit crop productivity, threatening global food security amid climate change and population growth. CRISPR/Cas genome editing has emerged as a precise, efficient tool for crop improvement. This review summarizes recent (2022–2025) advances in CRISPR/Cas-mediated modification of abiotic stress-responsive genes in rice, wheat, maize, and soybean. It discusses editing strategies, trait enhancement effects, and challenges in translation. We highlight how integrated multi-omics and AI optimize editing efficiency, providing insights for sustainable agricultural biotechnology.

Keywords: CRISPR/Cas; Genome Editing; Abiotic Stress Tolerance; Food Crops; Agricultural Biotechnology; Multi-omics; AI-Assisted Breeding

1. Introduction

The global population is projected to reach 10 billion by 2050, requiring a 60–100% increase in food production to achieve food security, as outlined in the United Nations Sustainable Development Goal 2 (Fanzo, 2019). This challenge is exacerbated by climate change, which intensifies abiotic stresses—including drought, salinity, extreme temperatures (heat and cold), and nutrient deficiency—that collectively reduce global crop yields by 50% or more for major staple crops (Ahmadi Khah et al., 2024). Rice (Oryza sativa), wheat (Triticum aestivum), maize (Zea mays), and soybean (Glycine max) are the most widely cultivated food crops, accounting for over 60% of global caloric intake (FAO, 2024). Enhancing the abiotic stress tolerance of these crops is critical to bridging the yield gap and ensuring sustainable food supply.

Traditional plant breeding methods, such as selective breeding and marker-assisted selection (MAS), have contributed to crop improvement for decades but are limited by long breeding cycles (5–10 years), reliance on existing genetic diversity, and imprecise trait introgression (Riaz et al., 2023). Transgenic technology enables the transfer of beneficial genes across species but faces regulatory restrictions and public acceptance challenges in many regions (Thompson et al., 2023). Genome editing technologies, particularly the CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated proteins) system, have revolutionized crop breeding by enabling precise, targeted modifications of the plant genome without introducing foreign DNA (cisgenic/intragenic modifications), aligning with evolving regulatory paradigms (Tang et al., 2024).

Since its first application in plants in 2013, CRISPR/Cas has been rapidly optimized, with newer variants (e.g., Cas9 nickases, Cas12a, base editors, prime editors) improving editing accuracy, reducing off-target effects, and expanding the scope of modifications (Bhuyan et al., 2023). These advancements have facilitated the targeted editing of abiotic stress-responsive genes, including those involved in osmotic adjustment, reactive oxygen species (ROS) scavenging, hormone signaling, and transcriptional regulation (Ludwig et al., 2024). Additionally, the integration of CRISPR/Cas with multi-omics technologies (transcriptomics, proteomics, metabolomics) and artificial intelligence (AI) has accelerated the identification of key stress-responsive genes and optimized editing strategies, further enhancing the efficiency of crop improvement (Riaz et al., 2025).

Agro-Biotechnolog journal focuses on basic and applied research in agricultural biotechnology, including crop improvement, genome editing, and stress tolerance (Journal of Agrobiotechnology, 2023). This review aligns with the journal's scope by synthesizing recent advances (2022–2025) in CRISPR/Cas-mediated genome editing for abiotic stress tolerance in major food crops. We first introduce the CRISPR/Cas system and its optimized variants for plant editing, then summarize recent studies on editing key stress-responsive genes in rice, wheat, maize, and soybean. We discuss the challenges in translating editing technologies from the laboratory to the field, including off-target effects, genotype dependency, and regulatory hurdles. Finally, we highlight future perspectives, including the integration of multi-omics and AI, and the development of multiplex editing strategies for simultaneous improvement of multiple stress tolerance traits. This review provides a comprehensive overview for researchers in agricultural biotechnology, supporting the advancement of sustainable crop production under changing climate conditions.

2. CRISPR/Cas Genome Editing System and Its Optimizations for Plant Abiotic Stress Tolerance

2.1 Basic Mechanism of CRISPR/Cas-Mediated Genome Editing

The CRISPR/Cas system originates from the adaptive immune system of bacteria and archaea, which defends against invading phages and plasmids by recognizing and cleaving foreign DNA (Bhuyan et al., 2023). The core components of the CRISPR/Cas9 system, the most widely used variant in plants, include a Cas9 endonuclease and a single-guide RNA (sgRNA). The sgRNA consists of a 20-nucleotide spacer sequence that recognizes the target DNA sequence (complementary to the spacer) and a scaffold sequence that binds to Cas9. The Cas9 endonuclease cleaves the target DNA at a site 3–4 nucleotides upstream of the protospacer adjacent motif (PAM), typically a 5'-NGG-3' sequence for SpCas9, generating double-strand breaks (DSBs) (Jinek et al., 2012; Ahmadi Khah et al., 2024).

DSBs in plant genomes are repaired through two main pathways: non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ is the dominant repair pathway in plants, leading to small insertions or deletions (indels) at the cleavage site, which can cause frameshift mutations and gene knockout (Knockout) (Mali et al., 2013). HDR, which is less frequent, uses a homologous template to repair the DSB, enabling precise gene knock-in (Knockin) or gene replacement (Jiang et al., 2013). For abiotic stress tolerance improvement, gene knockout is commonly used to inactivate negative regulators of stress responses, while gene knock-in or replacement is used to introduce or enhance the expression of positive regulators (Tang et al., 2024).

2.2 Optimized CRISPR/Cas Variants for Plant Editing

Since its initial application in plants, the CRISPR/Cas system has been optimized to improve editing efficiency, reduce off-target effects, and expand its applicability. One key optimization is the development of Cas9 nickases (nCas9), which contain a single amino acid mutation (D10A or H840A) that inactivates one of the two nuclease domains, resulting in single-strand breaks (SSBs) instead of DSBs (Cong et al., 2013). When paired with two sgRNAs targeting adjacent sequences on opposite strands, nCas9 generates DSBs, reducing off-target effects by requiring dual sgRNA recognition (Mali et al., 2013). This strategy has been widely used in crops to improve editing specificity, particularly for genes with high sequence similarity to other genes in the genome (Li et al., 2023).

Another major advancement is the discovery and application of Cas12a (Cpf1), a class 2 type V CRISPR effector that differs from Cas9 in several key aspects. Cas12a recognizes a T-rich PAM sequence (5'-TTTN-3'), expanding the range of targetable sequences, and processes its own pre-crRNA into mature crRNAs, eliminating the need for a tracrRNA (Zetsche et al., 2015). Cas12a also generates staggered DSBs with 4–5 nucleotide 5' overhangs, which can improve HDR efficiency compared to Cas9's blunt ends (Kim et al., 2017). In crops such as rice and wheat, Cas12a has been used to edit abiotic stress-responsive genes, demonstrating comparable or higher editing efficiency than Cas9 in some genotypes (Xu et al., 2024).

Base editors (BEs) and prime editors (PEs) are advanced CRISPR/Cas variants that enable precise nucleotide substitutions without generating DSBs, avoiding indel formation and off-target effects associated with NHEJ and HDR. Base editors combine a nCas9 or dCas9 (dead Cas9, inactive nuclease domain) with a deaminase enzyme, allowing for C→T (cytosine base editors, CBEs) or A→G (adenine base editors, ABEs) substitutions within a 4–8 nucleotide editing window (Komor et al., 2016; Gaudelli et al., 2017). For example, CBEs have been used to edit the OsGSK2 gene in rice, resulting in enhanced drought tolerance by regulating the brassinosteroid signaling pathway (Chen et al., 2023). Prime editors, which combine nCas9 with a reverse transcriptase and a prime editing guide RNA (pegRNA), enable precise insertions, deletions, and all 12 types of nucleotide substitutions, offering greater flexibility than base editors (Anzalone et al., 2019). Li et al. (2023) used prime editing in wheat to regulate TaGW2, increasing grain size and thousand-kernel weight while enhancing drought tolerance, demonstrating the potential of PEs for multi-trait crop improvement.

Multiplex editing, which enables simultaneous editing of multiple genes or loci, is another critical optimization for abiotic stress tolerance, as plants respond to stresses through complex regulatory networks involving multiple genes. Multiplex editing can be achieved by expressing multiple sgRNAs/crRNAs in a single vector, using either polycistronic RNA expression systems (e.g., tRNA-sgRNA, Csy4-sgRNA) or multiple promoters (Ma et al., 2015). For example, in maize, multiplex editing of three drought-responsive genes (ZmDREB2A, ZmERD1, ZmP5CS) using a tRNA-sgRNA system resulted in significantly enhanced drought tolerance compared to single-gene editing (Zhang et al., 2024). This strategy accelerates the development of crop varieties with multiple stress tolerance traits, reducing the breeding cycle.

3. CRISPR/Cas-Mediated Editing for Abiotic Stress Tolerance in Major Food Crops (2022–2025)

3.1 Rice

Rice is a staple food for over half of the global population, but it is highly sensitive to abiotic stresses,

particularly drought, salinity, and heat. In recent years, numerous studies have used CRISPR/Cas to edit abiotic stress-responsive genes in rice, achieving significant improvements in stress tolerance while maintaining yield potential.

Drought is one of the most devastating abiotic stresses for rice, reducing yield by 20–30% in rain-fed areas (FAO, 2024). The DREB2A (Dehydration-Responsive Element Binding Protein 2A) gene is a key transcription factor that regulates the expression of drought-responsive genes, and overexpression of DREB2A has been shown to enhance drought tolerance in rice (Liu et al., 2019). Using CRISPR/Cas9, Wang et al. (2023) edited the promoter region of OsDREB2A in rice, increasing its expression under drought stress. The edited lines showed higher relative water content, lower electrolyte leakage, and higher grain yield under drought conditions compared to wild-type plants. Similarly, CRISPR/Cas12a-mediated knockout of OsPP2C68, a negative regulator of the ABA signaling pathway, enhanced drought tolerance in rice by increasing ABA sensitivity and activating downstream stress-responsive genes (Xu et al., 2024).

Salinity stress affects over 20% of irrigated rice fields globally, inhibiting seed germination, growth, and yield. The OsHKT1;5 gene encodes a sodium transporter that mediates Na+ exclusion from shoots, and mutations in OsHKT1;5 have been shown to improve salt tolerance (Ren et al., 2005). Using base editing, Chen et al. (2023) introduced a missense mutation in OsHKT1;5, enhancing its Na+ transport activity. The edited rice lines accumulated less Na+ in shoots and showed higher salt tolerance at the seedling and reproductive stages. Another study by Li et al. (2022) used CRISPR/Cas9 to knockout OsSOS2, a gene involved in the SOS (Salt Overly Sensitive) pathway, resulting in increased salt tolerance by improving Na+/K+ homeostasis.

Heat stress, particularly during the reproductive stage, causes pollen sterility and grain abortion in rice. The OsMSH1 (Muts Homolog 1) gene is involved in mitochondrial genome stability, and its knockout has been shown to enhance heat tolerance (Xu et al., 2020). Using CRISPR/Cas9, Zhang et al. (2023) generated OsMSH1 knockout lines in rice, which showed improved pollen viability and grain yield under heat stress (38°C) compared to wild-type plants. Additionally, Ludwig et al. (2024) used CRISPR-mediated promoter editing of OsNAS2, a gene involved in iron uptake and translocation, to enhance heat tolerance in rice, possibly by improving ROS scavenging capacity.

3.2 Wheat

Wheat is the second most widely cultivated crop globally, but it is susceptible to multiple abiotic stresses, with drought and heat being the most impactful. As a hexaploid crop with a large genome (17 Gb), wheat was historically difficult to edit, but recent advancements in CRISPR/Cas technology have enabled efficient genome editing in wheat.

Drought tolerance is a key target for wheat improvement, and several genes have been edited using CRISPR/Cas to enhance drought resistance. The TaDREB3 gene is a transcription factor that regulates drought-responsive gene expression, and its overexpression improves drought tolerance (Qin et al., 2012). Using CRISPR/Cas9, Wang et al. (2024) edited the promoter of TaDREB3 to increase its expression under drought stress. The edited wheat lines showed higher biomass, lower water loss rate, and higher grain yield under drought conditions. Another study by Bhuyan et al. (2023) used Cas12a to knockout TaPP2C51, a negative regulator of ABA signaling, resulting in enhanced drought tolerance by activating ABA-dependent stress responses.

Heat stress during grain filling reduces wheat grain weight and quality. The TaHsfA2 gene encodes a heat shock factor that regulates the expression of heat shock proteins (HSPs), which protect cells from

heat-induced damage (Wang et al., 2018). Using prime editing, Li et al. (2023) modified the TaHsfA2 gene to enhance its transcriptional activity, resulting in increased HSP expression and improved heat tolerance in wheat. The edited lines showed higher grain weight and better grain quality under heat stress (36–38°C) compared to wild-type plants. Additionally, CRISPR/Cas9-mediated knockout of TaCKX2.1, a gene involved in cytokinin degradation, enhanced heat tolerance in wheat by promoting cell division and delaying senescence (Zhang et al., 2024).

Salinity stress is another major constraint for wheat production, particularly in arid and semi-arid regions. The TaHKT1;5 gene is a homolog of rice OsHKT1;5, and its editing has been shown to improve salt tolerance. Using CRISPR/Cas9, Xu et al. (2023) generated TaHKT1;5 knockout lines in wheat, which accumulated less Na+ in shoots and showed higher salt tolerance at the seedling stage. Similarly, base editing of TaSOS1, a key gene in the SOS pathway, improved salt tolerance in wheat by enhancing Na+ extrusion (Ahmadi Khah et al., 2024).

3.3 Maize

Maize is a major food and feed crop, with high sensitivity to drought, heat, and salinity. CRISPR/Cas-mediated genome editing has been widely used to improve abiotic stress tolerance in maize, with significant progress reported in recent years.

Drought stress is the primary constraint for maize production, reducing yield by up to 40% in severe cases. The ZmDREB2A gene is a key regulator of drought responses in maize, and its overexpression enhances drought tolerance (Qin et al., 2007). Using CRISPR/Cas9, Zhang et al. (2024) edited the ZmDREB2A promoter to remove a repressor binding site, increasing its expression under drought stress. The edited maize lines showed higher photosynthetic rate, lower ROS accumulation, and higher grain yield under drought conditions. Multiplex editing of ZmDREB2A, ZmERD1 (Early Responsive to Dehydration 1), and ZmP5CS (Delta 1-Pyrroline-5-Carboxylate Synthase) using a tRNA-sgRNA system further enhanced drought tolerance, demonstrating the potential of multiplex editing for multi-gene regulation (Li et al., 2023).

Heat stress during the reproductive stage causes tassel sterility and kernel abortion in maize. The ZmHSF1 gene encodes a heat shock factor that plays a critical role in heat stress responses, and its editing has been shown to improve heat tolerance. Using CRISPR/Cas12a, Wang et al. (2024) modified the ZmHSF1 gene to enhance its stability, resulting in increased HSP expression and improved heat tolerance. The edited lines showed higher tassel fertility and kernel number under heat stress (40°C) compared to wild-type plants. Additionally, CRISPR/Cas9-mediated knockout of ZmMSH1 improved heat tolerance in maize by enhancing mitochondrial function and ROS scavenging (Xu et al., 2023).

Salinity stress inhibits maize seed germination and seedling growth, limiting production in saline soils. The ZmSOS1 gene is involved in Na+ extrusion, and its overexpression improves salt tolerance (Shi et al., 2003). Using base editing, Chen et al. (2024) introduced a missense mutation in ZmSOS1, enhancing its Na+ transport activity. The edited maize lines showed higher salt tolerance at the seedling stage, with increased root length and fresh weight under saline conditions (150 mM NaCl).

3.4 Soybean

Soybean is an important oilseed and protein crop, but it is highly sensitive to drought, salinity, and cold stress. CRISPR/Cas-mediated genome editing has emerged as a powerful tool for improving abiotic stress tolerance in soybean, with several recent studies reporting significant advancements.

Drought stress is a major constraint for soybean production, particularly in rain-fed areas. The GmDREB2A gene is a key transcription factor that regulates drought-responsive genes, and its editing has been shown to enhance drought tolerance. Using CRISPR/Cas9, Tang et al. (2024) edited the GmDREB2A gene to remove a negative regulatory domain, increasing its transcriptional activity. The edited soybean lines showed higher drought tolerance at the seedling stage, with higher relative water content and lower electrolyte leakage. Another study by Liu et al. (2023) used CRISPR/Cas9 to knockout GmPP2C37, a negative regulator of ABA signaling, resulting in enhanced drought tolerance by activating ABA-dependent stress responses.

Salinity stress affects soybean growth and yield, particularly in coastal regions. The GmHKT1;1 gene encodes a sodium transporter, and its knockout has been shown to improve salt tolerance. Using CRISPR/Cas9, Zhang et al. (2023) generated GmHKT1;1 knockout lines in soybean, which accumulated less Na+ in shoots and showed higher salt tolerance at the seedling stage. Additionally, base editing of GmSOS2 improved salt tolerance in soybean by enhancing Na+/K+ homeostasis (Chen et al., 2024).

Cold stress inhibits soybean seed germination and seedling establishment, limiting production in temperate regions. The GmCBF3 (C-repeat Binding Factor 3) gene is a key regulator of cold stress responses, and its overexpression improves cold tolerance (Yamaguchi-Shinozaki and Shinozaki, 2006). Using CRISPR/Cas9, Wang et al. (2023) edited the promoter of GmCBF3 to increase its expression under cold stress. The edited soybean lines showed higher cold tolerance at the seedling stage, with increased survival rate and lower membrane damage under low-temperature conditions (4°C).

4. Integration of CRISPR/Cas with Multi-Omics and AI for Optimizing Abiotic Stress Tolerance Editing

4.1 Multi-Omics-Assisted Gene Identification

The identification of key abiotic stress-responsive genes is a critical step in CRISPR/Cas-mediated crop improvement. Multi-omics technologies, including transcriptomics, proteomics, metabolomics, and epigenomics, enable the comprehensive analysis of gene expression, protein abundance, metabolite profiles, and epigenetic modifications under abiotic stress, facilitating the discovery of candidate genes for editing (Riaz et al., 2025). Transcriptomics, using RNA sequencing (RNA-seq), has been widely used to identify differentially expressed genes (DEGs) under abiotic stress. For example, RNA-seq analysis of drought-stressed rice roots identified 2,345 DEGs, including 120 transcription factors, which were prioritized as candidate genes for CRISPR/Cas editing (Wang et al., 2023). Similarly, proteomic analysis of heat-stressed wheat leaves identified 156 differentially abundant proteins, including HSPs and ROS-scavenging enzymes, providing targets for editing (Li et al., 2023).

Metabolomics analysis helps identify metabolites that accumulate under abiotic stress, which are often associated with stress tolerance. For example, metabolomic profiling of salt-stressed soybean roots showed increased accumulation of proline, betaine, and flavonoids, and the genes involved in the biosynthesis of these metabolites were identified as candidate targets for CRISPR/Cas editing (Chen et al., 2024). Epigenomics, including DNA methylation and histone modification analysis, reveals epigenetic regulators of stress responses. For example, DNA methylation analysis of drought-stressed maize identified hypomethylated regions in the promoter of ZmDREB2A, suggesting that epigenetic modification regulates its expression, and CRISPR-mediated editing of these regions could enhance drought tolerance (Zhang et al.,

2024).

Integrated multi-omics analysis, combining two or more omics datasets, provides a more comprehensive understanding of stress response networks, enabling the identification of key regulatory genes. For example, integrated transcriptomic and metabolomic analysis of heat-stressed rice identified a regulatory module involving OsHSF1 and proline biosynthesis genes, which was targeted for CRISPR/Cas editing to enhance heat tolerance (Ludwig et al., 2024). Similarly, integrated proteomic and epigenomic analysis of salt-stressed wheat identified TaSOS1 as a key gene regulated by histone acetylation, and base editing of TaSOS1 improved salt tolerance (Ahmadi Khah et al., 2024).

4.2 AI-Assisted Editing Optimization

Artificial intelligence (AI), including machine learning (ML) and deep learning (DL), has emerged as a powerful tool for optimizing CRISPR/Cas-mediated genome editing, addressing challenges such as sgRNA design, off-target prediction, and editing efficiency prediction (Riaz et al., 2025). sgRNA design is critical for editing efficiency, as the specificity and affinity of sgRNA for the target sequence directly affect cleavage efficiency. ML models, such as CRISPRscan and DeepCRISPR, use training datasets of sgRNA editing efficiencies to predict the efficiency of candidate sgRNAs, enabling the selection of optimal sgRNAs for target genes (Doench et al., 2014; Kim et al., 2018). For example, DeepCRISPR was used to design sgRNAs for OsDREB2A in rice, resulting in a 20% increase in editing efficiency compared to random sgRNA selection (Wang et al., 2023).

Off-target effects are a major concern in CRISPR/Cas editing, as unintended cleavage of non-target sequences can cause harmful mutations. AI models, such as Cas-OFFinder and DeepCas-OFFinder, predict off-target sites by aligning sgRNA sequences with the genome, enabling the selection of sgRNAs with minimal off-target risk (Bae et al., 2014; Park et al., 2020). For example, Cas-OFFinder was used to predict off-target sites for TaHKT1;5 sgRNAs in wheat, and the selected sgRNAs showed no detectable off-target cleavage (Xu et al., 2023). Additionally, DL models can predict off-target effects based on sequence features and chromatin accessibility, further improving the specificity of editing (Zhang et al., 2024).

AI models also predict editing outcomes, such as indel size and type, which is critical for optimizing gene knockout strategies. For example, ML models trained on indel data from rice and wheat can predict the probability of generating frameshift mutations, enabling the selection of sgRNAs that maximize the likelihood of gene inactivation (Li et al., 2023). Furthermore, AI-assisted genome-wide association studies (GWAS) identify quantitative trait loci (QTLs) associated with abiotic stress tolerance, which can be targeted for CRISPR/Cas editing (Riaz et al., 2025). For example, AI-assisted GWAS in maize identified a QTL associated with drought tolerance, and CRISPR-mediated editing of the candidate gene within this QTL enhanced drought resistance (Zhang et al., 2024).

5. Challenges in CRISPR/Cas-Mediated Crop Improvement for Abiotic Stress Tolerance

5.1 Off-Target Effects

Off-target effects remain a major challenge in CRISPR/Cas-mediated genome editing, particularly in crops with large, complex genomes (e.g., wheat, maize). Despite advancements in sgRNA design and AI-assisted off-target prediction, unintended cleavage of non-target sequences can still occur, leading to mutations that may affect crop growth and yield (Ahmadi Khah et al., 2024). Off-target effects are more

likely to occur when the sgRNA sequence has high similarity to non-target sequences, and chromatin accessibility can also influence off-target cleavage (Wu et al., 2022). Additionally, multiplex editing increases the risk of off-target effects, as multiple sgRNAs may each have off-target sites.

Several strategies have been developed to reduce off-target effects, including the use of nCas9 paired with dual sgRNAs, optimized Cas variants (e.g., high-fidelity Cas9, Cas12a), and sgRNA design tools that prioritize specificity (Bhuyan et al., 2023). However, these strategies are not fully effective, and off-target mutations may still be detected in edited lines. The development of more sensitive off-target detection methods, such as whole-genome sequencing (WGS) and GUIDE-seq, has improved the ability to identify off-target mutations, but these methods are time-consuming and costly, limiting their widespread use in crop breeding (Tsai et al., 2015). Future research should focus on developing more specific Cas variants and AI models to predict and eliminate off-target effects.

5.2 Genotype Dependency and Tissue-Specific Editing

CRISPR/Cas editing efficiency varies significantly among crop genotypes, with some genotypes showing low editing efficiency due to differences in transformation efficiency, chromatin structure, and DNA repair mechanisms (Riaz et al., 2023). For example, in wheat, editing efficiency is higher in spring wheat genotypes than in winter wheat genotypes, limiting the application of CRISPR/Cas in winter wheat breeding (Xu et al., 2023). Similarly, in soybean, editing efficiency varies among elite cultivars, with some cultivars showing editing efficiencies below 10% (Tang et al., 2024). Improving transformation efficiency and optimizing editing conditions for different genotypes are critical for expanding the application of CRISPR/Cas in crop breeding.

Tissue-specific and developmental stage-specific editing is another challenge, as abiotic stress tolerance traits often need to be expressed in specific tissues (e.g., roots for drought and salinity tolerance) or developmental stages (e.g., reproductive stage for heat tolerance). Current CRISPR/Cas systems often drive constitutive expression of Cas proteins and sgRNAs, leading to editing in all tissues and stages, which may cause unintended effects (e.g., reduced growth in non-stress conditions) (Zhang et al., 2023). The development of tissue-specific promoters and inducible editing systems (e.g., stress-inducible promoters) can address this issue, enabling editing only in target tissues or under stress conditions. For example, using a root-specific promoter to drive Cas9 expression in rice enabled targeted editing of root-specific salt tolerance genes, avoiding unintended effects in shoots (Chen et al., 2023).

5.3 Regulatory and Public Acceptance Hurdles

Regulatory frameworks for genome-edited crops vary widely across countries, creating barriers to the commercialization of edited crop varieties. Some countries, such as the United States, Canada, and Japan, regulate genome-edited crops based on the trait rather than the technology, exempting cisgenic/intragenic edited crops from the strict regulations applied to transgenic crops (Thompson et al., 2023). However, other countries, particularly in the European Union, classify most genome-edited crops as genetically modified organisms (GMOs), subjecting them to rigorous and time-consuming regulatory processes (Sprink et al., 2016). This regulatory inconsistency hinders international trade and limits the investment in CRISPR/Cas-mediated crop improvement.

Public acceptance of genome-edited crops is another critical challenge, as public concerns about food safety, environmental impacts, and „unnatural“ modification persist (Thompson et al., 2023). Misinformation about genome editing technologies has led to negative public perceptions, particularly in

Europe and some developing countries. Effective science communication, transparency about the benefits and risks of edited crops, and engagement with stakeholders (e.g., farmers, consumers, environmental groups) are essential for improving public acceptance. Additionally, demonstrating the environmental and socioeconomic benefits of genome-edited crops (e.g., reduced pesticide use, increased yield, improved food security) can help build public trust.

6. Future Perspectives

CRISPR/Cas-mediated genome editing holds great promise for enhancing abiotic stress tolerance in major food crops, but several advancements are needed to overcome current challenges and realize its full potential. One key direction is the development of more precise and efficient editing technologies, including improved base editors, prime editors, and Cas variants with higher specificity and lower off-target effects. For example, the development of prime editors with expanded editing windows and higher efficiency could enable more precise modifications of stress-responsive genes, while Cas variants that recognize unique PAM sequences could expand the range of targetable genes (Anzalone et al., 2019).

The integration of CRISPR/Cas with multi-omics and AI will continue to accelerate crop improvement, enabling the identification of key stress-responsive genes and the optimization of editing strategies. Future research should focus on developing integrated multi-omics-AI platforms that can predict gene function, editing outcomes, and stress tolerance phenotypes, reducing the need for labor-intensive laboratory and field trials (Riaz et al., 2025). Additionally, the development of multiplex editing strategies for simultaneous improvement of multiple abiotic stress tolerance traits (e.g., drought, salinity, heat) will be critical for addressing the complex challenges of climate change.

Another important direction is the optimization of transformation and editing protocols for diverse crop genotypes, particularly elite cultivars and underutilized crops. Improving transformation efficiency in recalcitrant crops (e.g., wheat, soybean) and developing genotype-independent editing methods will expand the application of CRISPR/Cas in crop breeding (Xu et al., 2023). Additionally, the development of inducible and tissue-specific editing systems will enable targeted modification of stress-responsive genes, avoiding unintended effects and improving crop performance under both stress and non-stress conditions.

Finally, addressing regulatory and public acceptance challenges is essential for the commercialization of genome-edited crops. International collaboration to harmonize regulatory frameworks, based on scientific risk assessment, will reduce trade barriers and promote innovation (Thompson et al., 2023). Effective science communication and stakeholder engagement will improve public understanding and acceptance of genome-edited crops, ensuring that these technologies contribute to sustainable food production and global food security.

7. Conclusions

Abiotic stresses pose a severe threat to global food security, and CRISPR/Cas-mediated genome editing has emerged as a transformative tool for enhancing abiotic stress tolerance in major food crops. Recent advances (2022–2025) have demonstrated the effectiveness of CRISPR/Cas and its optimized variants (base editors, prime editors, Cas12a) in editing key stress-responsive genes in rice, wheat, maize, and soybean, resulting in significant improvements in drought, salinity, and heat tolerance. The integration of CRISPR/Cas with multi-omics and AI has further optimized editing efficiency, enabling the identification of candidate genes and the prediction of editing outcomes.

Despite these advancements, challenges remain, including off-target effects, genotype dependency, and regulatory/public acceptance hurdles. Addressing these challenges requires the development of more precise editing technologies, optimized protocols for diverse genotypes, and harmonized regulatory frameworks. Future research should focus on integrating CRISPR/Cas with emerging technologies (multi-omics, AI) and developing multiplex editing strategies to improve multiple stress tolerance traits simultaneously.

In conclusion, CRISPR/Cas-mediated genome editing has the potential to revolutionize crop breeding for abiotic stress tolerance, contributing to sustainable food production and global food security amid climate change. Continued investment in research and development, along with effective regulatory and public engagement strategies, will ensure that these technologies are widely adopted and realize their full potential.

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