



## CRISPR Beyond Yield: Editing for Climate Resilience in Crops

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### ABSTRACT

Climate change imposes severe threats to global food security by intensifying abiotic and biotic stresses such as drought, heat, salinity, cold, and emerging pest pressures. Traditional breeding approaches have made important contributions to developing climate-resilient crops but are limited by their long timelines, genetic constraints, and unpredictable environmental interactions. The emergence of CRISPR/CAS genome editing systems has revolutionized plant breeding by enabling precise, targeted, and rapid genetic modifications. While much of the early CRISPR research has focused on enhancing yield, there is growing emphasis on editing traits that directly improve resilience to climate stressors. This review synthesizes current knowledge on the role of CRISPR beyond yield improvement, focusing on its application for climate resilience. It covers the fundamental principles of CRISPR/CAS systems, the major climate-related traits targeted for editing, and specific case studies demonstrating successful applications in diverse crops. Additionally, we examine the technical and regulatory challenges associated with CRISPR deployment and explore future opportunities for integrating genome editing with genomic selection, speed breeding, and systems biology approaches. By consolidating recent advances, this review highlights CRISPR's transformative potential in safeguarding agricultural productivity under changing climatic conditions.

**Keywords:** CRISPR/Cas9, Genome editing, Climate resilience, Abiotic stress, Biotic stress, Crop improvement, Drought tolerance, Salinity tolerance, Heat stress, Case studies, and Sustainable agriculture

### INTRODUCTION

Global agriculture faces unprecedented challenges as climate change accelerates shifts in temperature, rainfall patterns, and the frequency of extreme weather events (Kumar et al., 2023; Zaidi, Mahas, Vanderschuren, & Mahfouz, 2020). The Intergovernmental Panel on Climate Change (IPCC) has projected that crop yields in major agricultural regions will decline substantially if adaptive measures are not implemented. In addition to direct temperature effects, climate change amplifies water scarcity, soil salinization, and pest/disease outbreaks, collectively threatening food security and farmer livelihoods.

Conventional plant breeding has achieved remarkable successes in improving yields and adaptability; however, it relies on phenotypic selection and natural genetic variation, which limit the speed and precision of improvement. The breeding cycle for many crops can span 7–12 years, making it difficult to respond quickly to emerging stressors. Furthermore, traits such as drought tolerance and heat resilience are often polygenic and influenced by complex gene-environment interactions, making their improvement through traditional approaches more challenging (Ahmar et al., 2020; Mao, Botella, Liu, & Zhu, 2019).

The development of genome editing technologies, particularly the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system has opened new opportunities for crop improvement. Since its adaptation for eukaryotic genome engineering in 2013 (Gan & Ling, 2022), CRISPR/Cas has gained rapid adoption due to its simplicity, affordability, and high editing efficiency. Unlike transgenic approaches, CRISPR enables precise modifications without necessarily introducing foreign DNA, which may ease regulatory approval in certain jurisdictions. While initial CRISPR applications in agriculture targeted yield-related traits, recent research has expanded to include traits enhancing resilience to abiotic and biotic stresses associated with climate change (Xie et al., 2022).

This review consolidates current advances in applying CRISPR for climate resilience in crops, emphasizing the transition from yield-centered editing to stress tolerance breeding (Alex & Augustine, 2025; Shelake et al., 2022). We first outline the impact of climate change on crop production, followed by a technical overview of CRISPR/Cas systems in plant science. The review then discusses major categories of trait-specific editing for resilience, illustrated with case studies from the past decade. Finally, we address the challenges, limitations, and future prospects of CRISPR-based climate resilience breeding.

### Climate Change and Crop Stress Factors

Climate change intensifies multiple abiotic stresses that directly reduce crop productivity and stability. These stresses often occurring in combination, operate at physiological, cellular, and molecular levels and therefore require different genetic and management solutions. Below are the primary stress categories most relevant to climate resilience breeding, describe their physiological and molecular impacts on plants, and indicate the classes of genes commonly targeted for editing (Alex & Augustine, 2025; Chavhan et al., 2025; Kumar et al., 2023; Shelake et al., 2022; Turnbull, Lillemo, & Hvoslef-Eide, 2021; Zaidi et al., 2020).

#### Drought

Water deficiency restricts cell expansion, reduces leaf area, and limits carbon assimilation by closing stomata to conserve water. Prolonged drought shortens grain-filling periods, reduces biomass and yield, and can trigger premature senescence. Root system architecture (depth, angle, branching) is a major determinant of soil water capture and therefore a key breeding target (Alex & Augustine, 2025; Chavhan et al., 2025; Kumar et al., 2023).

Drought triggers abscisic acid (ABA) synthesis and signalling, which governs stomatal closure, osmotic adjustment and stress-responsive gene expression. Reactive oxygen species (ROS) accumulate under water shortage and activate antioxidant pathways. Transcription factors (e.g., NAC, DREB/CBF families), kinases/phosphatases in signalling cascades, and genes controlling osmolyte biosynthesis (proline, sugars) or root development are central to drought response. Reviews emphasize editing targets that either enhance ABA responsiveness, modulate stomatal kinetics, or alter root architecture strategies that can improve water-use efficiency or drought avoidance/tolerance (Kumar et al., 2023; Sami et al., 2021; Shelake et al., 2022).

#### Heat Stress

High temperatures primarily disrupt reproductive processes (pollen viability, fertilization) and protein stability, leading to reduced grain set and quality. Heat also accelerates phenology, shortening critical developmental windows and lowering yield under chronic warming (Chavhan et al., 2025; Shelake et al., 2022).

Heat triggers heat shock factors (HSFs) and heat shock proteins (HSPs) that act as molecular carrier to maintain protein folding. Membrane fluidity and lipid composition are also altered, affecting cellular integrity. Candidate gene classes for editing include HSFs/HSPs, regulators of reproductive thermotolerance, and genes that stabilize membrane or photosynthetic machinery. Reviews recommend cautious modulation (for example, stress-inducible promoter tuning) because constitutive overexpression of heat-protective genes can incur growth costs under non-stress conditions (Chavhan et al., 2025; Shelake et al., 2022).

#### Salinity

Soil salinization imposes osmotic stress immediately upon exposure and ionic ( $\text{Na}^+/\text{Cl}^-$ ) toxicity over time. High salt concentrations reduce water uptake, disturb nutrient balance (e.g.,  $\text{K}^+/\text{Na}^+$  ratio), impair photosynthesis, and cause leaf necrosis and yield losses, particularly in irrigated and coastal agro-ecosystems (Alex & Augustine, 2025; Kumar et al., 2023; Shelake et al., 2022).

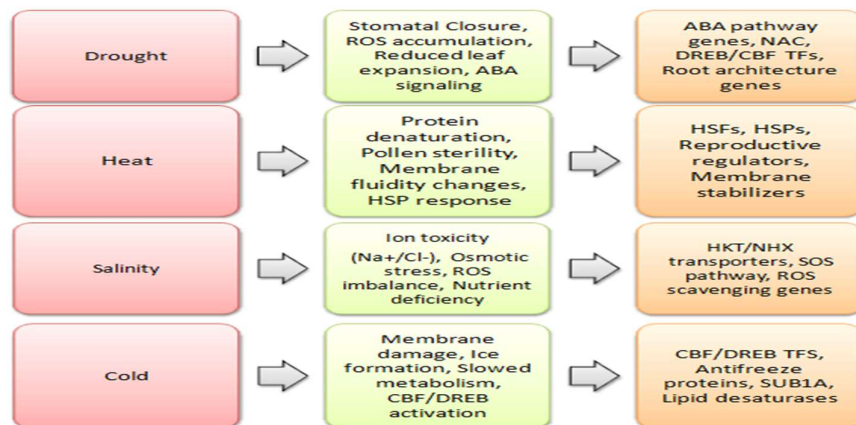
Plants respond via ion transport and compartmentalization (e.g., vacuolar sequestration), osmoprotectant accumulation, and activation of ROS scavenging systems. Key molecular targets for genetic improvement include membrane transporters (HKT, NHX families), SOS pathway components that regulate ion homeostasis, and transcriptional regulators controlling osmotic stress responses. The reviews emphasize deploying editing approaches that either reduce root  $\text{Na}^+$  uptake, enhance sequestration into vacuoles, or increase osmotic adjustment often requiring coordinated (multiplex) edits because salinity tolerance is polygenic (Kumar et al., 2023; Sami et al., 2021; Shelake et al., 2022).

#### Cold and Other Abiotic Stresses (including frost)

Cold stress slows metabolism, disrupts membrane properties, and can cause ice formation in tissues (frost), which leads to cellular rupture. In temperate and high-altitude systems, episodic frosts and late-spring cold snaps are major yield-limiting events for sensitive crops (Kumar et al., 2023; Turnbull et al., 2021).

Cold acclimation involves the CBF/DREB transcriptional cascade that induces protective proteins, osmolyte synthesis and changes in membrane lipid desaturation. Antifreeze proteins, cryoprotectants, and regulators of phenology (to avoid vulnerable stages coinciding with frost) are relevant targets. For stresses like flooding/submergence (also climate-linked), ethylene-responsive factors (e.g., SUB1A in rice) are classical targets. Cold tolerance often has a complex genetic architecture; therefore, cis-regulatory edits or allele refinement (rather

than full knockout) are frequently recommended to balance protection with growth (Kumar et al., 2023; Turnbull et al., 2021).



**Fig 1:** Conceptual framework linking major climate stresses to their physiological effects and representative CRISPR gene targets in crops (Alex & Augustine, 2025; Kumar et al., 2023; Shelake et al., 2022)

## CRISPR/CAS Systems: An Overview

### CRISPR/Cas9 Basics

The CRISPR/Cas9 system, originally discovered as part of the bacterial adaptive immune response, has emerged as a transformative genome editing tool in plants. Its function relies on a programmable single-guide RNA (sgRNA) that directs the Cas9 nuclease to a complementary DNA sequence, inducing a targeted double-strand break (DSB). Repair of the DSB by the plant cell's endogenous mechanisms primarily non-homologous end joining (NHEJ) or, less frequently, homology-directed repair (HDR) enables precise gene knockouts or insertions (Gan & Ling, 2022; Movahedi, Aghaei-Dargiri, Li, Zhuge, & Sun, 2023). Compared with earlier genome editing tools such as zinc finger nucleases (ZFNs) and transcription activator like effector nucleases (TALENs), CRISPR/Cas9 offers higher design flexibility, cost efficiency, and scalability for multiplex targeting (Gan & Ling, 2022; Xie et al., 2022). These features have significantly reduced barriers to its adoption in plant biotechnology.

In plants, CRISPR/Cas9 has been successfully implemented across a range of species, from model crops like rice and maize to complex poly-ploids such as wheat. Its application spans trait improvement for yield, quality, and increasingly, climate resilience, with a growing emphasis on editing tolerance to abiotic stresses such as drought, salinity, heat, and cold (Chavhan et al., 2025; Gan & Ling, 2022; Kumar et al., 2023).

### Beyond Cas9: CAS Variants

While Cas9 remains the most widely applied nuclease, other CRISPR-associated proteins have expanded the range of genome editing possibilities in plants. Cas12a (Cpf1), for example, recognizes T-rich protospacer adjacent motifs (PAMs) and produces staggered DSBs, which can facilitate certain HDR-based strategies (Movahedi et al., 2023; Xie et al., 2022). Cas13 targets RNA rather than DNA, enabling transcriptome engineering without permanent genomic changes; an approach that may prove useful for transient stress adaptation in plants (Y. Li, Wu, Zhang, & Zhang, 2022; Nadakuduti & Enciso-Rodríguez, 2021; Xie et al., 2022).

Additionally, engineered Cas9 variants have been developed to enhance targeting specificity, expand PAM compatibility, or facilitate base and prime editing. Multiplex editing systems, leveraging polycistronic tRNA–sgRNA architectures or ribozyme processing, have allowed simultaneous modification of multiple loci, accelerating the pyramiding of complex traits like combined drought and salinity tolerance (Al-Dossary, 2025; Nadakuduti & Enciso-Rodríguez, 2021; Xie et al., 2022). These advancements broaden the functional reach of CRISPR systems and are essential for addressing multifactorial climate resilience traits.

### Advanced Editing Tools

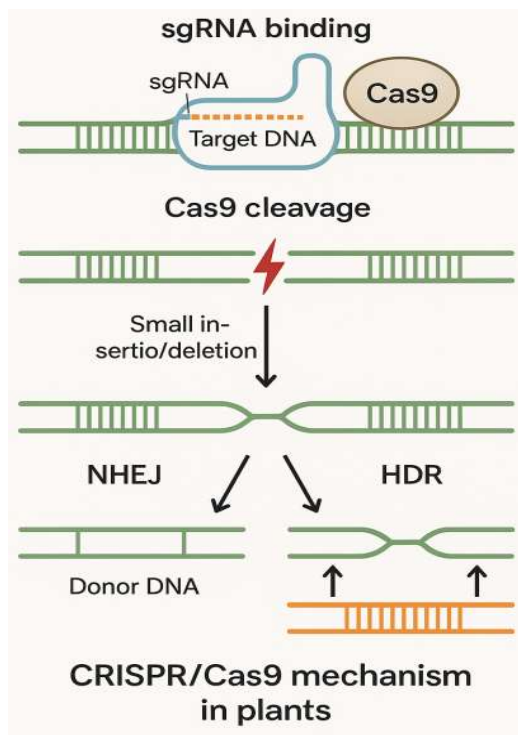
#### Base Editing (CBE, ABE)

Base editors are CRISPR-derived tools that catalyze single-nucleotide substitutions without introducing DSBs. Cytosine base editors (CBEs) convert C-G base pairs into T-A, while adenine base editors (ABEs) convert A-T to G-C (Azameti & Dauda, 2021). In plants, these systems have been used to generate precise point mutations in stress-response genes, modify promoter elements, and adjust protein functions for improved adaptation to environmental stressors (Azameti & Dauda, 2021; Xie et al., 2022). The avoidance of DSBs reduces the risk of large-scale genomic rearrangements, making base editing particularly suited for editing essential genes where knockout would be lethal.

#### Prime Editing

Prime editing, introduced more recently, combines a Cas9 Nickase with a reverse transcriptase to directly write new genetic sequences into target sites, guided by a prime editing guide RNA (pegRNA) (J. Li et al., 2022; Tingting et al., 2023; Vats, Kumar, Sonah, Zhang, & Deshmukh, 2024). This approach allows for targeted insertions, deletions,

and substitutions without requiring donor DNA templates or relying on HDR pathways, which are often inefficient in plants. Optimized plant-specific prime editors, such as the enhanced PE2 systems developed for cereals, have achieved higher editing efficiencies, expanding the potential to correct alleles or install beneficial variants for stress tolerance (J. Li et al., 2022; Tingting et al., 2023). Though still at an early stage compared to Cas9, prime editing holds promise for precision breeding of climate-resilient crops.



**Fig 2:** A Schematic Diagram of CRISPR/Cas Mechanism in Plants (Gan & Ling, 2022; Xie et al., 2022)

### DNA Delivery Methods in CRISPR Editing for Climate-Resilient Crops

Efficient delivery of CRISPR/CAS components into plant cells is a critical step for successful genome editing, particularly when targeting traits linked to climate resilience. The choice of delivery method affects editing efficiency, target specificity, transformation frequency, and ultimately the heritability of edits (Su, Xu, Radani, & Yang, 2023). In crop biotechnology, both DNA-based and DNA-free delivery systems are employed, with each approach offering unique advantages depending on species, tissue type, and desired genetic outcome.

### Agrobacterium-Mediated Transformation

Agrobacterium-mediated transformation is the most widely used approach for stable integration of CRISPR/CAS constructs in plants. This method exploits the natural ability of *Agrobacterium* to transfer T-DNA into the plant genome. For climate resilience traits, such as drought or salinity tolerance, *Agrobacterium*-mediated delivery has been successfully applied in rice, wheat, and tomato. This method is cost-effective, species-adaptable, and well-established, but it can be limited by host genotype specificity and integration of undesired vector backbone sequences. Despite these challenges, optimization of promoters, selection markers, and tissue culture conditions has significantly improved editing efficiency (Rustgi, Naveed, Windham, Zhang, & Demirel, 2022; Zhi et al., 2022).

### Particle Bombardment (Biolistics)

Particle bombardment involves coating microscopic gold or tungsten particles with CRISPR/CAS plasmids or ribo-nucleoproteins (RNPs) and physically propelling them into plant cells using high-pressure helium pulses. This method is particularly useful for monocots and recalcitrant species that are less amenable to *Agrobacterium* transformation. It allows delivery of large DNA constructs and even direct introduction of RNP complexes, reducing the risk of stable transgene integration. However, biolistics can cause extensive tissue damage and result in multiple, random insertions, which require careful screening (Rustgi et al., 2022).

### Protoplast Transfection

Protoplast transfection is a DNA-free method that delivers CRISPR/CAS RNP complexes or plasmid DNA directly into plant protoplasts via polyethylene glycol (PEG) – mediated uptake. Since protoplasts lack a cell wall, the uptake of editing components is efficient, and DNA-free approaches prevent transgene integration, enabling rapid generation of edited lines without regulatory constraints associated with GMOs. This method is advantageous for functional genomics studies in climate resilience genes but requires robust regeneration protocols, which are not yet optimized for many crop species (Sant'Ana, Caprestano, Nodari, & Agapito-Tenfen, 2020).

### Viral Vector–Mediated Delivery

Plant viral vectors, such as geminiviruses and tobacco rattle virus (TRV), can be engineered to deliver CRISPR/CAS components systemically throughout plant tissues. These vectors offer high expression levels, enabling multiplexed gene editing or base editing for complex traits like heat tolerance or multi-stress resilience (Weiss et al., 2025). Their transient nature minimizes the risk of permanent genetic alterations in non-target loci, but viral host range and stability constraints can limit application to specific crops.

### Electroporation and Microinjection

Electroporation involves using electrical pulses to permeabilize cell membranes, allowing CRISPR components to enter plant cells. While more common in microbial and mammalian systems, plant applications have been explored

in specific tissue types. Microinjection, though technically demanding, can directly deliver editing reagents into plant embryos or zygotes, ensuring high precision in certain elite breeding lines (Kost, Galli, Potrykus, & Neuhaus, 1995; Rustgi et al., 2022).

### **Nanoparticle-Mediated Delivery**

Nanotechnology-based systems, including carbon nanotubes, mesoporous silica nanoparticles, and lipid-based carriers, have recently been developed for CRISPR delivery. These approaches can bypass tissue culture by enabling direct delivery into intact plant tissues and have shown potential for editing stress-responsive genes without transgene integration. Although still experimental, nanoparticle-mediated delivery is promising for field-deployable genome editing in climate-resilient crop development (Zhi et al., 2022).

### **Comparative Assessment**

Each delivery method offers trade-offs between efficiency, cost, technical complexity, and regulatory acceptance. Agrobacterium-mediated transformation remains dominant for routine crop improvement, while DNA-free RNP-based methods are gaining traction for avoiding transgene-related regulations. For climate resilience breeding, selecting an optimal delivery method depends on crop species, trait complexity, and breeding pipeline integration (Su et al., 2023).

### **Trait-Specific Genome Editing for Climate Resilience**

CRISPR-based editing enables targeted modification of genes and regulatory elements underlying adaptive responses to abiotic stresses. Depending on the biological role of the target (e.g., signaling component, transcriptional regulator, transporter, or structural protein), different editing strategies such as knockout, promoter editing, base substitution, or precise allele replacement are applied to achieve the desired phenotypic shift. Below, trait classes central to climate resilience are described with representative CRISPR examples that demonstrates the likely physiological basis for improvement, and practical considerations for deploying each approach in breeding programs (Zhou et al., 2023).

#### **Drought tolerance**

Drought resilience can arise from altered water-use behavior (stomatal regulation), improved root architecture (deeper or more exploratory roots), osmotic adjustment, and enhanced stress signalling (ABA pathway). Key molecular targets therefore include receptors and signalling regulators (e.g., ABA pathway components), transcription factors that rearrange stress responses, and developmental genes controlling root angle, depth and branching (Sami et al., 2021).

Promoter editing and targeted knockouts have both been used to modulate drought responses. A prominent field-relevant example is promoter editing of ARGOS8 in maize, where altering expression reduced ethylene sensitivity, improving grain yield under water shortage without major penalties under non-stress conditions (Shi JinRui et al., 2017). This case highlights promoter engineering as a way to refine gene expression rather than generate complete loss-of-function alleles. In rice, knockout or modification of OsERA1, a negative regulator of ABA signaling has been reported to enhance ABA responsiveness and drought tolerance, demonstrating that disabling negative regulators can strengthen stress signaling networks (Ogata, Ishizaki, Fujita, & Fujita, 2020). Similarly, targeted mutagenesis of the rice DST gene, which impacts stomatal behavior and ROS homeostasis, produced lines with improved tolerance to drought and salinity in experimental settings (Santosh Kumar et al., 2020).

#### **Editing modality choices**

1. Promoter editing (e.g., ARGOS8) is attractive when subtle modulation of expression is needed to avoid pleiotropy (Shi JinRui et al., 2017).
2. Knockouts of negative regulators (e.g., OsERA1, DST) are straightforward with Cas9 and often produce clear phenotypes (Ogata et al., 2020; Santosh Kumar et al., 2020).
3. Base editing can install single-nucleotide variants shown to confer stress-adaptive alleles without introducing DSBs, useful when point changes recapitulate natural tolerant variants (Azameti & Dauda, 2021; Tingting et al., 2023).
4. Prime editing may be used to install complex allele combinations or precise promoter modifications when HDR is inefficient (J. Li et al., 2022).

#### **Phenotypic metrics and trade-offs**

Assessments include water-use efficiency, stay-green, root traits, osmolyte accumulation, ABA sensitivity assays, and grain/yield stability under managed drought trials. Importantly, drought-adaptive edits can have trade-offs (altered growth, reproductive timing), so thorough physiological and field validation is essential (Ogata et al., 2020; Santosh Kumar et al., 2020; Shi JinRui et al., 2017).

#### **Salt tolerance**

Base editing can be used to alter transporter selectivity or regulatory phosphorylation sites. Salt stress tolerance commonly involves control of Na<sup>+</sup>/K<sup>+</sup> homeostasis, sequestration of ions into vacuoles, maintenance of osmotic

balance, and ROS detoxification. Transporters (HKT, NHX families), signalling regulators, and transcriptional controllers are principal targets.

Editing ion transporter genes or their promoters to reduce sodium uptake or increase sequestration has been effective in multiple crops. The rice *DST* mutagenesis example in (Santosh Kumar et al., 2020) illustrates that a single edit can confer cross-tolerance to drought and salinity by altering shared physiological pathways. Other successful strategies include targeted modification of HKT and NHX homologs (reported across reviews and crop-specific studies, (Kumar et al., 2023; Shelake et al., 2022), which reduce cytosolic Na<sup>+</sup> and thereby protect photosynthetic and metabolic processes.

#### Editing modality choices

1. Knockout of susceptibility alleles or allele replacement to favorable variants can reduce ion influx (Kumar et al., 2023; Santosh Kumar et al., 2020; Shelake et al., 2022).
2. Promoter tuning allows tissue-specific or stress-induced expression changes that minimize yield penalties (Al-Dossary, 2025; Xie et al., 2022).

#### Practical considerations

Salinity tolerance often requires coordinated changes in multiple genes; thus, multiplex editing or breeding-compatible allele stacking is frequently necessary. Field trials in saline soils and evaluations across developmental stages are crucial for determining agronomic utility (Kumar et al., 2023; Santosh Kumar et al., 2020; Shelake et al., 2022).

#### Heat tolerance

Heat stress primarily impairs reproductive development (pollen viability, grain set) and protein stability. Targets include heat shock transcription factors (HSFs), chaperone systems (HSP families), membrane stability genes, and regulators of reproductive development.

Compared to drought or salinity, there are fewer well-documented field examples for heat tolerance, but reported approaches include targeting negative regulators of heat shock responses and modifying genes that support reproductive thermo-tolerance (Kumar et al., 2023; Shelake et al., 2022). For example, boosting the expression or activity of HSFs and molecular co-factors through promoter editing or allele replacement can enhance tolerance during critical reproductive stages.

#### Editing modality choices

1. Promoter editing to induce stress-responsive upregulation at high temperature is preferred to avoid constitutive cost (Kumar et al., 2023; Shelake et al., 2022).
2. Multiplex editing may be required because heat tolerance is often polygenic (Xie et al., 2022).
3. Transient RNA targeting (Cas13) could be explored to modulate heat-responsive transcripts during critical periods without permanent genomic change (Y. Li et al., 2022; Nadakuduti & Enciso-Rodríguez, 2021).

#### Phenotyping and deployment challenges

Heat tolerance must be evaluated in realistic diurnal and seasonal temperature regimes, focusing on reproductive standards. Combining heat edits with traits that stabilize phenology (avoidance) or water relations (drought tolerance) often yields better agronomic resilience (Kumar et al., 2023; Shelake et al., 2022).

#### Cold and other abiotic stresses (including frost)

Cold tolerance mechanisms include membrane lipid composition, antifreeze proteins, osmoprotectant accumulation, and induction of the CBF (C-repeat binding factor) regulon. Frost tolerance additionally involves cellular ice-management strategies.

Editing CBF pathway regulators and downstream protective genes has shown promise in model species and horticultural crops; targeted activation or cis-regulatory tuning can induce protective cold-responsive programs without stunting growth (Kumar et al., 2023; Shelake et al., 2022). Where single major effect loci exist (e.g., SUB1A for flooding tolerance), precise allele engineering can be highly effective; for cold tolerance the genetic architecture is often more complex, favoring multiplex or allele-refinement approaches.

#### Editing modality choices

1. CIS-regulatory editing to adjust timing and intensity of cold responses helps mitigate growth penalties (Kumar et al., 2023; Shelake et al., 2022).
2. Base/prime editing can introduce known protective alleles from tolerant germplasm into elite lines (Azameti & Dauda, 2021; Tingting et al., 2023).

#### Integration across stresses

Crops often face combined stresses (e.g., cold followed by drought), so breeding strategies increasingly aim to combine edits that confer complementary protective mechanisms. This requires careful selection of targets that do not provoke each other physiologically (Alex & Augustine, 2025; Shelake et al., 2022).

### Practical considerations across trait classes

**Multiplexing and stacking:** Many resilience traits are polygenic; multiplex CRISPR designs or sequential editing followed by marker-assisted selection allow stacking of complementary alleles (Al-Dossary, 2025; Xie et al., 2022).

**Editing precision:** Base and prime editors are especially valuable when small sequence changes are sufficient to alter protein function or regulatory logic with minimal collateral effects (Azameti & Dauda, 2021; R. Li et al., 2019; Tingting et al., 2023).

**Off-target and pleiotropy:** Thorough in-silico gRNA design, off-target screening, and phenotyping across developmental stages and environments are essential to identify unintended effects and pleiotropic trade-offs (Le, Kim, Jung, Kang, & Cho, 2022; R. Li et al., 2019).

**Phenotyping and field validation:** Laboratory and greenhouse successes must be validated in multi-location field trials to assess yield stability, agronomic performance, and any other trade-offs (Kumar et al., 2023; Y. Li et al., 2022; Shi JinRui et al., 2017).

**Delivery and breeding fit:** Choice of delivery method (Agrobacterium, RNPs, particle bombardment, nanoparticle) affects downstream breeding strategies and regulatory classification. DNA-free RNP edits can speed deployment where transgene-free status is regulatory advantageous (Laforest & Nadakuduti, 2022; Yan et al., 2022; Zhou et al., 2023).

**Table 1:** Summary of CRISPR Targets and Strategies for Climate Resilience

Stress/Trait	Key Targets	Strategy	Examples	Outcomes
Drought	ABA signaling ( <i>OsERA1</i> ), TFs (NAC, DREB), root genes	Promoter editing, knockout	Maize ( <i>ARGOS8</i> ), Rice ( <i>OsERA1</i> , <i>DST</i> )	Better water use, deeper roots, yield stability
Salinity	Ion transporters ( <i>HKT</i> , <i>NHX</i> ), SOS pathway	Knockout, promoter tuning	Rice ( <i>DST</i> ), cereals	Lower Na <sup>+</sup> uptake, better ion balance
Heat	HSFs, HSPs, membrane genes	Promoter tuning, allele replacement	Wheat, Rice, Tomato	Improved reproductive thermotolerance
Cold/Frost	CBF/DREB, antifreeze proteins, <i>SUB1A</i>	Cis-regulatory edits, base editing	Barley, Rice	Enhanced cold acclimation, frost survival
Multi-stress	Combined targets	Multiplex editing	Cereals, legumes	Tolerance to multiple stresses

Note: This table contains data from (Alex & Augustine, 2025; Kumar et al., 2023; Ogata et al., 2020; Sami et al., 2021; Santosh Kumar et al., 2020; Shelake et al., 2022; Shi JinRui et al., 2017).

### Case Studies in Climate-Resilient Genome Editing

This section highlights representative, crop-level examples where CRISPR-based edits have been used to improve tolerance to climate-linked stresses (Zaidi et al., 2020). Each case is presented with the biological rationale, the editing strategy, reported outcomes (lab/greenhouse and, where available, field), and practical implications or caveats.

#### Drought tolerance in maize ARGOS8 promoter editing

The ARGOS8 example is frequently cited as a proof of concept for how cis-regulatory edits can improve stress resilience without grossly impairing growth under non-stress conditions. In this work, CRISPR was used to modify the regulatory context of the ARGOS8 locus to alter its expression pattern; plants carrying the engineered promoter showed improved performance under water-deficit conditions, with maintenance of grain set and yield stability relative to controls in managed drought trials (Shi JinRui et al., 2017). This case demonstrates an important principle: promoter-level engineering can fine-tune endogenous gene activity to produce agronomically useful phenotypes while avoiding full loss of function or pleiotropy.

#### ZmPL1 (functional validation of stress-relevant regulators)

Targeted editing of ZmPL1 (a maize locus implicated in stress response pathways) produced lines with enhanced tolerance traits in controlled experiments (Tang et al., 2025). Although the exact downstream mechanisms differ by study, ZmPL1 edits typically affect transcriptional networks involved in osmotic regulation and protective metabolite accumulation. These edits illustrate the value of editing transcriptional regulators to produce broad-spectrum physiological adjustments rather than single downstream changes.

#### ZmHDT103 (a cautionary regulatory example)

Functional analyses of ZmHDT103 revealed that it negatively regulates drought stress tolerance in maize: loss-of-function edits increased stress resilience in experimental assays (Wang et al., 2024). This case underscores two points for breeders:

- (1) Targeting negative regulators can yield strong phenotypes.
- (2) Validation of growth and yield under non-stress conditions is essential because removing a negative regulator can have context-dependent costs or developmental consequences.

### **Maize case studies**

Maize examples collectively show promoter engineering and knockouts of negative regulators as effective strategies. Crucially, the ARGOS8 work included managed field evaluations, providing stronger evidence of agronomic benefit than greenhouse-only studies (Shi JinRui et al., 2017; Tang et al., 2025). Maize also highlights genotype dependency, edits that work in one genetic background may require revalidation across elite germplasm due to interaction effects.

### **Rice (drought and ABA response)**

#### **OsERA1 knockout; enhancing ABA responsiveness**

In rice, CRISPR/Cas9 targeting of OsERA1, a negative regulator of abscisic acid (ABA) signaling produces lines with increased ABA sensitivity and improved responses to water shortage (Ogata et al., 2020). Phenotypes included more vigorous stomatal closure and physiological adjustments that conserve water during drought conditions. Because ABA signalling is central to water-use regulation across species, OsERA1 represents a conserved leverage point for drought adaptation.

#### **DST mutagenesis (shared pathways for drought and salinity)**

Targeted mutagenesis of the DST gene in indica rice altered stomatal behaviour and reactive oxygen species (ROS) homeostasis, conferring improved tolerance to both drought and salinity (Santosh Kumar et al., 2020). The DST case exemplifies how single-gene edits can impact multiple stress pathways when the gene occupies an integrative regulatory node. However, because such nodes often influence growth or yield components, detailed phenotyping across environments is required to detect any trade-offs.

### **Field vs. lab translation**

Rice studies typically progress from molecular validation to greenhouse trials (a subset then advances to confined-field evaluation). The strength of translational evidence depends on whether edits are evaluated under agronomically realistic stress conditions and across genetic backgrounds (Ogata et al., 2020; Santosh Kumar et al., 2020). Together, these rice cases illustrate the value of editing negative regulators of stress signalling and the importance of careful phenotyping to detect pleiotropy.

### **Tomato (stress tolerance modifications)**

#### **SILBD40 knockout (improved drought resilience)**

CRISPR/Cas9-mediated knockout of SILBD40 in tomato resulted in improved drought tolerance in controlled experiments, most likely via changes in root and stomatal physiology that enhance water retention and maintain photosynthetic activity under stress (X. Li et al., 2022; Liu et al., 2020). The tomato example highlights how editing developmental regulators can rapidly generate useful adaptive phenotypes, particularly in horticultural species where trait changes can be evaluated quickly.

#### **SINPR1 (a cautionary counter example)**

In contrast, mutagenesis of SINPR1 reduced drought tolerance in tomato (R. Li et al., 2019), serving as a reminder that gene function can be context-dependent and that not all edits assumed to be beneficial will yield the desired outcome. This cautionary case emphasizes the need for functional validation before deployment and illustrates how interfering with immune or signalling nodes can lead to unintended susceptibility to abiotic stress.

### **Practical takeaways for tomato editing**

Tomato case studies show both the potential for rapid trait gains and the risk of adverse trade-offs. For horticultural crops, rapid phenotyping pipelines enable relatively rapid duplication, but breeders must evaluate fruit quality, shelf life, and other agronomic traits under stress conditions.

### **Other crops: wheat, sorghum, barley (and broader comparisons)**

#### **Review-level evidence and crop diversity**

Comprehensive reviews and crop-specific syntheses (Kumar et al., 2023; Y. Li et al., 2022; Namata et al., 2025) summarize edited loci and candidate genes across wheat, sorghum, barley, and other staples. The bulk of studies in these cereals report promising laboratory and greenhouse phenotypes, including improved root architecture, altered hormone signalling, and modified transporter activity, that are relevant to drought, heat, and salinity resilience. However, the degree of advancement to field trials varies. At the same time, model cases (e.g., maize ARGOS8) include field data, many cereal edits remain at the proof-of-concept stage or are limited to confined evaluation.

### Field versus lab performance: common patterns

Two recurring themes emerge from cross-crop comparisons:

- (1) Edits that perform well in controlled settings sometimes show reduced or variable benefits in the field due to (genotype × environment interactions) and stress complexity.
- (2) Promoter engineering and allelic precision (base/prime edits) often produce more stable agronomic performance than blunt knockouts, because they allow finer modulation of gene function (Kumar et al., 2023; Y. Li et al., 2022; Namata et al., 2025). Multiplex editing and stacking of complementary alleles are increasingly proposed to address multi-factorial field stresses but require advanced delivery and breeding strategies.

**Table 2:** CRISPR Case Studies for Climate Resilience

Crop	Key Targets	Stresses	Outcomes	Evidence
Maize (Shi JinRui et al., 2017; Tang et al., 2025; Wang et al., 2024)	<i>ARGOS8</i> , <i>ZmPLI</i> , <i>ZmHDT103</i>	Drought	Yield stability, osmotic regulation, tolerance with trade-offs	Field/Lab
Rice (Ogata et al., 2020; Santosh Kumar et al., 2020)	<i>OsERA1</i> , <i>DST</i>	Drought, Salinity	ABA responsiveness, stomatal/ROS balance	Lab/Greenhouse
Tomato (R. Li et al., 2019; X. Li et al., 2022; Liu et al., 2020)	<i>SILBD40</i> , <i>SINPR1</i>	Drought	Improved traits or reduced tolerance (trade-off)	Lab
Wheat/Sorghum/Barley (Kumar et al., 2023; Y. Li et al., 2022; Namata et al., 2025)	Multiple	Drought, Salinity, Heat	Stress tolerance; variable field results	Lab/Greenhouse

### Synthesis and Implications

The case studies illustrate a spectrum of CRISPR strategies; promoter engineering (*ARGOS8*) for subtle expression tuning, knockout of negative regulators (*OsERA1*, *ZmHDT103*, *DST*) for stronger stress signalling, and targeted edits of transcriptional regulators (*ZmPL1*, *SILBD40*) for broad physiological shifts. Across crops, four practical lessons stand out:

1. **Choice of edit matters:** Promoter or allele refinement often yields agronomically acceptable trade-offs compared with full knockouts.
2. **Validation in target environments is essential:** Greenhouse results can overestimate field benefits; multi-location trials reveal stability and trade-offs.
3. **Genetic background effects are important:** Edits must be tested across elite germplasm to ensure utility in breeding programs.
4. **Stacking and multiplexing will be needed:** Many resilience traits are polygenic or require combined mechanisms to achieve well-field performance.

### Potential Trade-offs and Limitations

While CRISPR/CAS-based genome editing holds immense promise for enhancing crop climate resilience, its deployment is not without trade-offs and practical constraints. These challenges span biological, technical, ecological, and socio-economic dimensions and need to be addressed before widespread adoption (Mao et al., 2019).

### Off-target Mutations and Genome Stability

A central technical limitation of CRISPR/CAS systems is the risk of off-target cleavage, in which the CAS nuclease binds and edits unintended genomic loci with only partial sequence compatibility with the guide RNA. Such unintended mutations can lead to unpredictable phenotypic changes, potentially compromising crop performance, nutritional quality, or stress responses. Although high-profile CAS variants, truncated guide RNAs, and in silico prediction algorithms have significantly reduced off-target rates, complete elimination remains challenging (Biswas et al., 2022). Moreover, the introduction of double-strand breaks (DSBs) may trigger genomic instability in some species, particularly polyploids, where homoeologous sequences can complicate repair.

### Pleiotropic and Unintended Trait Effects

Target genes involved in stress responses often participate in multiple physiological pathways. Editing these loci to enhance drought, heat, or salinity tolerance can alter undesired traits such as flowering time, biomass allocation, or reproductive fitness. For example, modifications in ABA signaling genes can improve water-use efficiency but may also delay germination or reduce yield under optimal conditions. These pleiotropic effects require comprehensive phenotypic evaluation across multiple environments to ensure that climate resilience does not come at the expense of agronomic performance (R. Li et al., 2019; Ogata et al., 2020).

### Environmental and Ecological Considerations

The release of genome-edited crops into the environment raises ecological questions similar to those associated with conventional transgenics. Gene flow from edited crops to wild relatives may alter local adaptation patterns or disrupt natural gene pools. Furthermore, traits conferring enhanced stress tolerance could unintentionally increase

plant initiation in natural ecosystems, potentially affecting biodiversity. For instance, increased drought tolerance might allow certain species to expand into new habitats, displacing native vegetation (Zhao & Wolt, 2017).

### **Regulatory and Intellectual Property Barriers**

The global regulatory landscape for CRISPR-edited crops remains fragmented. Some countries, including the US, Japan, and Argentina regulate certain genome-edited plants differently from traditional GMOs, especially if no foreign DNA is present. Others, such as the European Union, classify them under existing GMO legislation, imposing lengthy approval processes. Additionally, patent restrictions on CRISPR tools can limit research and commercial deployment, particularly for small breeding programs and public-sector initiatives (El-Mounadi, Morales-Floriano, & Garcia-Ruiz, 2020; Le et al., 2022; Turnbull et al., 2021).

### **Socio-economic Trade-offs**

While genome editing can accelerate breeding for climate resilience, its benefits may be unevenly distributed. Large agri-businesses with access to CRISPR expertise and infrastructure may adopt the technology faster than smallholder farmers, potentially widening technological and economic gaps. Moreover, public perception and consumer acceptance remain variable; in regions where biotechnology uncertainty is strong, edited crop adoption might face market resistance regardless of scientific evidence (El-Mounadi et al., 2020; Turnbull et al., 2021).

### **Field Performance Gaps**

Many CRISPR-based trait validations occur under controlled growth conditions, which may not fully replicate complex field environments. Traits that perform well in the laboratory or greenhouse can exhibit reduced effectiveness under changing climatic conditions, mixed stress conditions, or pathogen pressures. This “lab-to-field gap” underscores the need for multi-location, multi-year trials before commercial release (Biswas et al., 2022; Kumar et al., 2023).

### **Ethical and Long-term Considerations**

Ethical concerns arise regarding the pace and scope of genetic changes that CRISPR enables. The rapid introduction of novel traits could outpace our understanding of their long-term ecological impacts. Moreover, societal debates persist about the degree to which humans should intervene in crop genomes beyond conventional breeding, especially when targeting traits related to environmental adaptation (El-Mounadi et al., 2020; Turnbull et al., 2021).

### **Regulatory, Biosafety, and Ethical Considerations**

The governance of gene-edited crops sits at the intersection of science, law, and public values. Regulatory frameworks, biosafety assessment practices, and ethical debates differ substantially across jurisdictions, and these differences materially affect research, commercialization, international trade, and public acceptance of CRISPR-derived climate-resilient crop (El-Mounadi et al., 2020; Le et al., 2022; Turnbull et al., 2021). This section synthesizes those global differences, outlines biosafety and traceability challenges, and highlights ethical and policy priorities for responsible deployment.

### **Regulatory approaches: product vs. process-based systems**

Countries adopt two broad regulatory philosophies for novel plant varieties: one is product-based regulation (focus on the traits and final product), and the other is process-based regulation (focus on the method used to develop the plant).

**Product-based regimes** (e.g., Argentina, Brazil, and, in many cases, the United States and Japan) tend to exempt gene-edited plants from burdensome GMO procedures when edits are indistinguishable from naturally occurring mutations or conventional breeding outcomes (Le et al., 2022; Turnbull et al., 2021). These authorities emphasize the characteristics and risks of the developed variety rather than the editing technique itself, enabling faster deployment of transgene-free, edited crops.

**Process-based regimes** (notably the European Union under current jurisprudence) classify organisms produced by certain gene-editing techniques as GMOs regardless of whether foreign DNA persists. This results in more stringent authorization, monitoring, and labelling requirements and often leads to longer, more costly approval pathways (El-Mounadi et al., 2020; Le et al., 2022).

The regulatory choice has practical consequences: product-based systems can accelerate field testing and commercialization of edits such as promoter tuning or small base changes. In contrast, process-based systems may delay or block similar innovations unless additional data or risk management measures are provided.

### **Risk assessment and biosafety evaluation**

Across frameworks, biosafety assessments typically consider molecular characterization of the edit, potential off-target effects, phenotypic stability, agronomic performance, compositional analyses (for food/feed), and ecological impacts (e.g., gene flow, non-target effects). Key biosafety challenges for CRISPR projects include:

**Detecting and characterizing small edits** (single-nucleotide changes or subtle promoter modifications) which can be indistinguishable from natural variation, complicating traceability and regulatory enforcement in process-based systems.

**Evaluating off-target and unintended effects** in polyploid crops or after multiplex editing, where multiple loci have been altered. Thorough genomic, transcriptomic, and phenotypic assays are recommended to reveal unintended consequences (El-Mounadi et al., 2020; Le et al., 2022).

**Ecological risk assessment** for traits that could alter weed traits, invasiveness, or interactions with pests, pathogens, and beneficial organisms; these assessments require field data and ecosystem modelling rather than solely greenhouse assays (Turnbull et al., 2021).

Regulatory agencies increasingly request tiered, hypothesis-driven evidence rather than blanket data packages, emphasizing relevant exposure pathways and possible hazards (El-Mounadi et al., 2020; Le et al., 2022). This approach can streamline evaluation while preserving safety.

### **Detection, traceability, and trade implications**

Divergent regulations create trade friction; a variety considered non-regulated in one country may be classified as a GMO in another, complicating seed movement and commodity exports. Detection is particularly problematic when edits leave no foreign DNA; forensic molecular methods may be unable to distinguish edited alleles from natural variants, making international compliance and monitoring difficult. This uncertainty causes the development of agreed-upon documentation, voluntary registries, and whole supply-chain traceability systems to manage cross-border movement and consumer labelling (Le et al., 2022; Turnbull et al., 2021).

### **Ethical considerations and public engagement**

Ethical issues extend beyond biosafety to questions of equity, stewardship, and public trust. Important dimensions include:

**Access and benefit-sharing:** Ensuring smallholder farmers and public breeding programs can access edited germplasm and that benefits are not monopolized by a few corporations (Turnbull et al., 2021).

**Informed consent and transparency:** Clear communication about the nature of edits, their intended benefits (e.g., climate resilience rather than yield enhancement alone), and the evidence supporting safety and efficiency.

**Socio-economic impacts:** Anticipating and mitigating potential negative effects on farm livelihoods, seed sovereignty, and cultural practices.

**Precaution and intergenerational responsibility:** Considering long-term ecological consequences and exercising precaution where uncertainty is high (El-Mounadi et al., 2020; Turnbull et al., 2021).

Public acceptance is shaped by perceived need (climate mitigation/adaptation framing often increases support), transparency of methods and data, and governance structures that include stakeholder voices. Participatory breeding models and early engagement with farmers, consumer groups, and civil society can improve legitimacy and uptake.

### **Capacity, governance, and harmonization needs**

To responsibly scale CRISPR for climate resilience, several policy priorities emerge:

**Regulatory harmonization:** International dialogue (e.g., through FAO, OECD) to align definitions, risk assessment principles, and data requirements would reduce trade barriers and uncertainty (Le et al., 2022; Turnbull et al., 2021).

**Proportionate oversight:** Risk-proportionate, science-based evaluation that differentiates low-risk edits (small, cisgenic changes) from those with larger ecological footprints.

**Investment in detection and monitoring tools:** Improved molecular assays, traceability systems, and transparent registries to enable compliance and consumer choice.

**Capacity building:** Supporting low- and middle-income countries with regulatory expertise, biosafety infrastructure, and breeding capabilities so that climate-resilient gene editing benefits are equitably distributed (El-Mounadi et al., 2020).

**Ethical frameworks and stakeholder engagement:** Embedding ethics review, participatory decision-making, and benefit-sharing mechanisms in research and deployment plans.

### **Concluding remarks on governance**

Regulatory diversity reflects difference in societal values and risk tolerances, but it also creates practical obstacles to deploying gene-edited climate solutions at scale. A practical path forward balances rigorous biosafety assessment with flexible, evidence-based regulation that recognizes the nature of the edit and its possible risks. Coupled with

transparent dialogue, capacity building, and harmonized standards, such governance can enable responsible use of CRISPR tools to support resilient agriculture while addressing legal biosafety and ethical concerns (El-Mounadi et al., 2020; Le et al., 2022; Turnbull et al., 2021).

**Future Prospects and Integration into Breeding Pipelines**

The integration of CRISPR-based genome editing into mainstream crop improvement programs holds significant promise for accelerating the development of climate-resilient varieties. With increasing precision, reduced off-target effects, and expanding editing toolkits such as base editors, prime editors, and CRISPR-associated nucleases beyond Cas9, plant scientists can target traits that are traditionally challenging to modify through conventional breeding. The future pathway of CRISPR research in climate resilience is likely to be shaped by three interconnected trends; technical refinement, trait stacking, and data-driven breeding integration. From a technical perspective, continued improvements in delivery methods, particularly non-transgenic approaches like ribonucleo-protein (RNP) delivery will help streamline regulatory approval and public acceptance (Ahmar et al., 2020). These advances, combined with the possibility of transient expression systems, could allow for precise genome modifications without stable integration of foreign DNA, thereby addressing biosafety concerns while maintaining efficiency.

Another emerging focus is multi-trait engineering, where several loci associated with complex stress responses are edited in parallel. This “trait stacking” approach is particularly relevant for climate resilience, as drought, heat, salinity, and pathogen pressures often occur simultaneously (Gao, 2021). Combining CRISPR edits with traditional quantitative trait loci (QTL) mapping and genomic selection could enhance the cumulative resilience of elite cultivars. For example, breeding pipelines could integrate CRISPR edits that confer improved root architecture, enhanced antioxidant systems, and optimized flowering time into a single genotype.

Data-driven strategies, particularly the use of multi-omics platforms will likely become integral to identifying novel targets. Transcriptomic, proteomic, and metabolomic datasets from stress-response studies can guide CRISPR target selection, while machine learning models could predict the phenotypic impact of edits before field trials. This integration of computational biology with genome editing may substantially reduce breeding cycles (Ahmar et al., 2020).

Despite these opportunities, the pathway to widespread deployment will depend on difference between lab and field performance gaps. Many CRISPR-edited plants perform optimally under controlled conditions but display variable performance in heterogeneous field environments. Future research must prioritize large-scale, multi-location field trials to ensure that edits confer consistent advantages under real-world climate stress scenarios (Gao, 2021).

Long-term, CRISPR-based climate-resilience breeding could become a routine step in cultivar development, working in tandem with speed breeding and doubled-haploid production to deliver improved varieties in a fraction of the traditional timeline. However, success will require collaborative frameworks that unite molecular biologists, breeders, computational scientists, and policy makers to ensure that innovation reaches farmers in a timely and socially acceptable manner.

Opportunities	Challenges
<ul style="list-style-type: none"> <li>• Crop Adaptation (drought tolerance)</li> <li>• Reducing emissions (low-methane rice)</li> <li>• Enhanced Carbon Capture</li> <li>• Disease Resistance</li> </ul>	<ul style="list-style-type: none"> <li>• Regulatory Hurdles</li> <li>• Public Acceptance</li> <li>• Ethical Concerns</li> <li>• Unintended Effects</li> </ul>

**Fig 3:** Dual Outlook on CRISPR Applications (Gao, 2021; Zhao & Wolt, 2017).

**Conclusion**

Climate change poses a complex challenge to global agriculture, threatening yield stability and food security through increased frequency of droughts, heat waves, salinity invasion, and irregular rainfall patterns (Kumar et al., 2023). Conventional breeding, though historically successful, is often too slow to keep pace with these evolving threats. In this context, CRISPR-based genome editing represents a transformative opportunity, enabling precise, targeted, and relatively rapid genetic improvements tailored to climate resilience.

Over the past decade, CRISPR/Cas systems have evolved from a single-tool concept into a versatile platform encompassing base editing, prime editing, and a range of Cas nucleases with unique capabilities. These innovations have already demonstrated success in modulating key traits, including stomatal regulation, osmoprotectant synthesis, reactive oxygen species scavenging, and root system architecture, across diverse crops. Importantly, many of these edits can be designed to avoid transgene integration, which has significant implications for regulatory acceptance and public trust.

However, realizing CRISPR’s full potential in breeding pipelines will require overcoming persistent barriers. Technical challenges such as efficient delivery in resistant crops, precise multiplex editing without unintended trade-offs, and maintaining edit stability under field conditions remain priorities (Gao, 2021). Equally critical are socio-economic considerations, including equitable access to the technology, farmer-centric trait prioritization, and transparent communication about the safety and benefits of genome-edited crops.

Looking forward, CRISPR is expected to become an integral part of a diverse climate adaptation strategy, working in collaboration with speed breeding, genomic selection, and data-driven decision-making. The technology’s

adaptability enables continuous refinement as new stress-response genes are discovered and climate challenges shift geographically and temporally. Ultimately, if coupled with inclusive policies and global collaboration, CRISPR can enable the development of resilient crop varieties that not only sustain yields but also safeguard livelihoods in a warming world.

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Wadia Fatima conceived the idea and the review scheme and drafted the manuscript.

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The authors confirm that no generative artificial intelligence-based tools were employed for content generation or interpretation. All intellectual content, interpretations, and conclusions are the sole responsibility of the authors.

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