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CRISPR-CAS GENOME EDITING TECHNOLOGIES FOR ACCELERATING THE DEVELOPMENT OF CLIMATE-RESILIENT AND HIGH-YIELDING CROP VARIETIES

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Abstract

The world's agriculture system is under an exceptional pressure from population growth and climate change, which requires a 25- to 60-percent increase in food output by 2050 in the face of increasing temperatures, drought, salinity, and new phytopathogens. Traditional breeding and transgenics methodologies have not been adequate to address these challenges due to several factors including genetic bottlenecks, long timelines, and regulatory hurdles. The advent of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein (Cas) system has transformed plant biotechnology through direct, site-specific genome editing in elite germplasm. In this sense, a detailed understanding of the molecular mechanisms of CRISPR-Cas-based platforms, such as SpCas9, Cas12a, base editing, prime editing, epigenome editing, and their potential to be employed to develop crops with tolerance to adverse climatic conditions and elevated productivity is discussed. Significant success stories include: maize ARGOS8 promoter engineering that led to a 5 bushel per acre yield increase under drought, knockouts of susceptibility genes that resulted in resistance to powdery mildew, bacterial blight and citrus canker, and multiplex editing to engineer synthetic apomixis for hybrid vigor preservation. The review provides critical analysis of the international regulation, comparing the product based models (United States, Brazil and Japan) and the process based approaches (European Union), and discusses emerging policies towards exempting transgene-free edits from GMO regulation. Despite technical challenges such as tissue culture recalcitrance, off-target mutations, and the engineering of complex polygenic traits, solutions are emerging in the form of morphogenic regulators, high-fidelity Cas variants, and integration with pangenomics and speed breeding, among others, which are expected to accelerate crop improvement. CRISPR-Cas

technology is a transformative model to develop climate-smart agriculture that provides accurate, effective and transgene-free approaches to improve food security under circumstances of environmental unpredictability.

1. INTRODUCTION

Global farming is dealing with a mix of more people and a changing environment. Experts say the world's population will hit 8.5 billion by 2030 and 9.6 billion by 2050. To feed everyone we'll need to grow 25% to 60% food. At the time climate change caused by humans is making things like droughts, heat waves and pests worse in key farming areas (Khatri et al. 2024). Scientists predict that some places could get up to 8°C hotter by the end of this century. Rain patterns are also shifting, which might raise the drought index from 52.45 to 129. For every 1°C rise in temperature the air can hold more moisture. About 3% more rain variation and 7% more humidity. This creates climate zones that help diseases and insects spread faster in crops (Chen & Frauenfeld 2014).

Rice (Oryza sativa) maize (Zea mays) and wheat (Triticum aestivum) are the sources of calories for people around the world. Rice is the food for nearly half the planet.. About 90% of rice is grown in Asian farming areas that are now at risk from drought, extreme heat, saltwater entering fields and sudden floods (Awika, 2011). For years plant breeders have improved crops by picking the looking plants and using markers to help guide crossings.. This method is slowing down. It's held back by genetic variety, long breeding times and the fact that traits for surviving climate stress are controlled by many genes. Modified crops were a step forward but they take too long to develop many people don't trust them and they face strict rules and high costs (Ahmar et al. 2020).

That's where CRISPR and Cas come in. These are tools based on a system in bacteria that can edit DNA with precision. In plants CRISPR-Cas lets scientists make changes, to genes. Like turning off a gene tweaking its switch or changing how it's controlled without adding foreign DNA. Because it doesn't need genes from outside it avoids many of the problems of older methods. This means we can speed up the creation of crops that handle climate stress better use water and nutrients efficiently and stay productive even under tough conditions (Nazir et al., 2022).

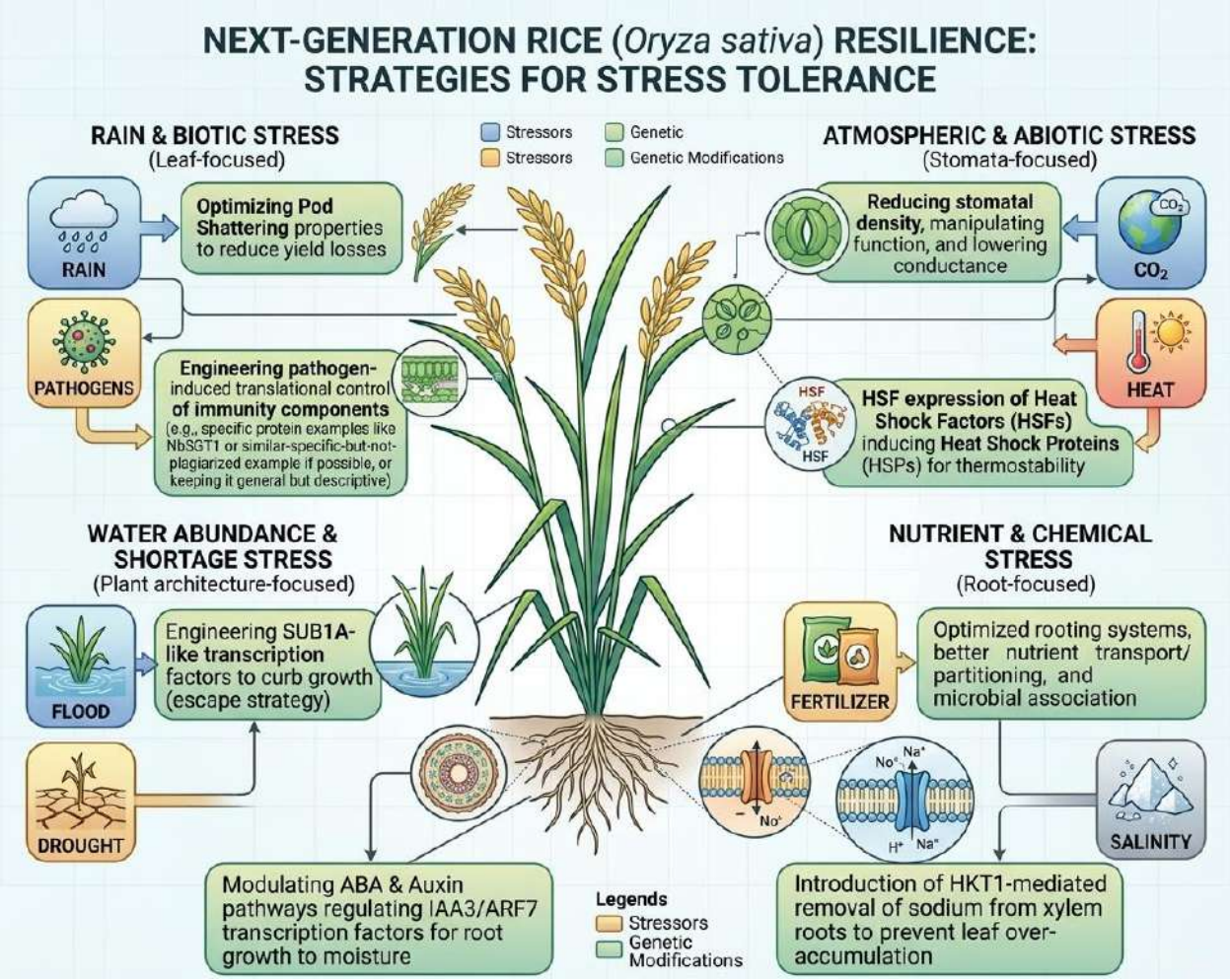


Figure 1: Comparative Paradigms and Timelines of Modern Plant Breeding.

2. CRISPR-Cas Molecular Mechanics and Tool Expansion

The canonical SpCas9 system works with a two-part complex that contains a Cas9 enzyme and a synthetic single-guide RNA called sgRNA. The sgRNA carries a 20-nucleotide spacer that points the SpCas9 enzyme to a matching spot in the genome that sits before a conserved PAM sequence, usually 5′-NGG-3′, for SpCas9. When SpCas9 binds to the target and forms an R-loop, the HNH and RuvC parts of SpCas9 cut the two strands of DNA. This creates a double-strand break that is three bases upstream of the PAM (Chandrashekhar, 2018). Plant cells normally fix these breaks by the Non-Homologous End Joining or NHEJ pathway. NHEJ is a repair system that often adds or removes a few bases, called indels, which can shift the reading frame and knock out the gene. If one brings in a DNA template at the same time SpCas9 can use the Homology-Directed Repair, HDR or Microhomology-Mediated End-Joining, MHEJ, pathways. These pathways can add changes insert new genes or swap promoters into the genome (Cetin et al. 2021).

To overcome target site constraints and enhance editing precision, the molecular CRISPR toolbox has expanded beyond standard SpCas9 nucleases through natural ortholog mining and protein engineering:

- **Cas12a (Cpf1) Orthologs**: Unlike Cas9, Cas12a recognizes thymine-rich PAM sequences (e.g., 5'-

TTTV-3') and uses a single RuvC-like domain to generate staggered DSBs with 4- or 5-nucleotide 5' overhangs. These cohesive ends promote precise HDR-mediated knock-in events over NHEJ indels. Furthermore, Cas12a possesses intrinsic ribonuclease activity capable of processing single crRNA precursor arrays, enabling efficient single-vector multiplex genome editing across extended gene pathways (Parvathy, 2024).

- **Base Editing Systems**: Cytosine Base Editors (CBEs) and Adenine Base Editors (ABEs) combine a catalytically impaired Cas9 nickase (nCas9) or dead Cas9 (dCas9) with single-stranded DNA cytidine or adenosine deaminases. Base editors catalyze targeted C · G → T · A or A · T → G · C transition mutations within a narrow editing window without double-strand break generation or donor DNA templates, avoiding high indel rates (Molla & Yang, 2019).
- **Prime Editing (PE)**: Prime editors integrate an nCas9 fused to an engineered reverse transcriptase, guided by a prime editing guide RNA (pegRNA) that specifies the genomic target locus and encodes the desired edit sequence. Prime editing enables all twelve possible transition and transversion base conversions, targeted micro-insertions, and micro-deletions without donor DNA or DSBs (Zhao et al., 2023).
- **Epigenome Editing**: Fusing dCas9 to transcriptional activators, repressors, or chromatin-modifying enzymes allows locus-specific regulation of gene expression without altering the underlying genomic sequence. For example, coupling dCas9 to the catalytic domain of histone acetyltransferase (HAT) targets the promoter region of *AREB1* (ABRE-binding protein 1), inducing local histone acetylation. This structural chromatin opening increases promoter accessibility for endogenous transcription factors during water stress, upregulating downstream stress-responsive genes like *RD29A* without introducing permanent DNA mutations (Pulecio et al., 2017).

A big problem when using CRISPR technology in different types of crops is getting the pieces needed to make the changes into the cells. This is hard because of the cell walls. Also people still depend on growing plants from tissues in a lab. Even though methods like using Agrobacterium tumefaciens to move DNA into plants and using particles to shoot DNA into cells are still the usual ways there is a better way. Delivering made Ribonucleoprotein (RNP) complexes can avoid adding DNA into the plants own DNA. This makes plants that are not changed in their DNA in the generation. New ways of delivering the CRISPR parts are being used. These include using viruses like Tobacco Rattle Virus. Another new method pairs CRISPR with a line that makes half the number of chromosomes. This allows making changes in the best plant lines. This skips the steps of growing plants, from tissues. (Cardi et al., 2023)

3. Engineering Abiotic Stress Resilience

Abiotic environmental drivers drought, soil salinity, thermal extremes, and nutrient scarcity cause cellular dehydration, reactive oxygen species (ROS) accumulation, photosynthetic disruption, and substantial yield losses. CRISPR-Cas technology provides a platform to systematically re-engineer stress-response pathways, balancing emergency survival mechanisms with agronomic productivity (Chaudhry & Sidhu, 2022).

3.1 Drought and Osmotic Stress Mitigation

In response to water shortages plants make acid (ABA) and ethylene, which start stomatal closing, root changes and adjustments to the water balance inside the plant. Ethylene helps the plant deal with conditions but too much ethylene during the time the plant is reproducing leads to dried silk failed kernels and lower grain production. The maize ARGOS8 gene acts as a stop signal for the effects of ethylene in the cells. Looking at 400 types of maize scientists found that the natural changes in how ARGOS8 works were not enough to stop the effects of ethylene when the drought was very bad (Aslam et al., 2022).

Using CRISPR-Cas9 scientists replaced the part that controls how the ARGOS8 gene works with a part from the GOS2 gene. This change made the ARGOS8 gene work better in both the leaves and the parts of the plant that are growing. In tests in dry areas during the time the plant is flowering the

new version of ARGOS8 gave about five more bushels of corn per acre than the original version. The new plants also did as well when there was enough water (Shi et al., 2017).

Other parts of the plants system that handle ABA can also be changed. For example changing genes like AREBs/ABFs, SnRK2, DREB2A ZmNAC111 and HaHB4 helps the plant manage water better keep more water and grow longer roots. When some parts that stop ABA from working are removed the plant reacts better to dry conditions. This helps the plant keep its water levels and avoid much damage, from harmful substances (Ayub et al. 2025).

3.2 Thermal, Salinity, and Heavy Metal Tolerance

Heat stress during the phase messes up metabolic balance in cereal crops. This leads to sterility meaning many flowers fail to produce grains. Using multiplex CRISPR editing helps protect cells under conditions like heat and drought. The targets include heat shock transcription factors (HSFs) enzymes that scavenge oxygen species (ROS) and components involved in ethylene signaling. These changes help keep structures intact when plants face multiple stresses (Qian et al. 2025).

In potatoes (Solanum tuberosum) editing two proteins called eIF4E and eIF(iso)4E reduces a problem called induced sweetening. When stored at temperatures potatoes can turn sweeter than desired which affects their quality during processing. This editing fixes that issue. Also changing genes involved in gibberellin production like ZmGA20ox3 leads to sturdier plants. These semidwarf forms resist lodging. Falling over. Better, during winds or heavy rain events (Wakeel, 2013).

4. Biotic Stress Resistance, Yield Architecture, and Domestication

Susceptibility Gene Engineering for Phytopathogen Resistance

Infectious plant diseases driven by fungi, bacteria, and viruses cause significant annual crop loss. Rather than inserting foreign resistance genes, CRISPR-Cas technology enables broad-spectrum resistance by knocking out endogenous Susceptibility (*S*) genes host targets required by pathogens for entry, colonization, or metabolic exploitation:

- **Powdery Mildew Resistance**: Targeted knockout of *EDR1* (Enhanced Disease Resistance 1) in hexaploid wheat (*Triticum aestivum*) confers resistance against *Blumeria graminis* f. sp. *tritici*. Similarly, knocking out *SlMLO1* in tomato (*Solanum lycopersicum*) blocks infection by *Oidium neolycopersici* (Dong & Fan, 2024).
- **Bacterial Blight and Speck Resistance**: In rice (*Oryza sativa*), mutating the promoter region of *OsSWEET13* prevents *Xanthomonas oryzae* pv. *oryzae* from hijacking host sucrose transporters, preventing bacterial blight development. In tomato, knocking out *SlJAZ2* prevents *Pseudomonas syringae* pv. *tomato* from using coronatine toxins to open stomata during entry (Kumari et al., 2021).
- **Citrus Canker Resistance**: Promoter editing of *CsLOB1* in grapefruit (*Citrus paradisi*) and sweet orange (*Citrus sinensis*) abolishes the binding site for the PthA4 effector secreted by *Xanthomonas citri*, establishing bacterial canker resistance (Huang et al., 2022).
- **Rice Blast Control**: Disruption of the transcription factor *OsERF922* in rice significantly increases host resistance against *Magnaporthe oryzae*.

Yield Architecture Optimization and Weed Management

Agronomic yield is a trait that depends on how plants move resources how their flowers grow how big the seeds are and how nutrients are spread around. Using CRISPR-Cas9 to change the parts of genes that control when they turn on scientists focus on the CLAVATA-WUSCHEL signaling system. This system includes members of the CLE gene family. Changing the parts of these genes that control when they are active can make the growing tip of the plant bigger. In maize and rice this leads to rows of kernels and more total grain in each ear (Tefera & Debele 2026).

Removing the Waxy genes changes the starch in the seeds making it better for processing. Also changing parts of the ALS and EPSPS genes in maize, wheat, potato, soybean and cassava gives plants resistance, to certain weed killers. This helps farmers use tilling and save water when managing weeds (Sun et al. 2017).

De Novo Domestication and Clonal Seed Production

I have seen wild plant species and underutilized orphan crops contain natural stress resilience and genetic diversity. They often have poor agronomic traits, such as fruit shattering, tall architectures, small seed size and low yield. De novo domestication uses CRISPR editing to change domestication gene orthologs in wild germplasm. This method quickly adds yield-related traits while keeping stress tolerance. A clear example of this approach appeared in groundcherry (Physalis peruviana). In that study CRISPR-Cas9 knocked out the ortholog SP5G. The knockout altered stem growth and flowering producing a compact plant architecture and higher fruit yield (Chapman et al. 2022).

We have seen another breakthrough in crop breeding: the fixation of hybrid vigor also known as synthetic apomixis. F1 crop hybrids show yield compared to inbred lines but genetic segregation during meiosis prevents farmers from saving F2 seeds for replanting. By using CRISPR-Cas9 to knock out three genes REC8, PAIR1 and OSD1 researchers turned meiosis into a mitotic division creating the MiMe phenotype. When this MiMe system is combined with a targeted expression of a factor it allows the production of clonal diploid seeds, from F1 hybrids. The result is that hybrid vigor is permanently fixed across generations (Underwood & Mercier 2022).

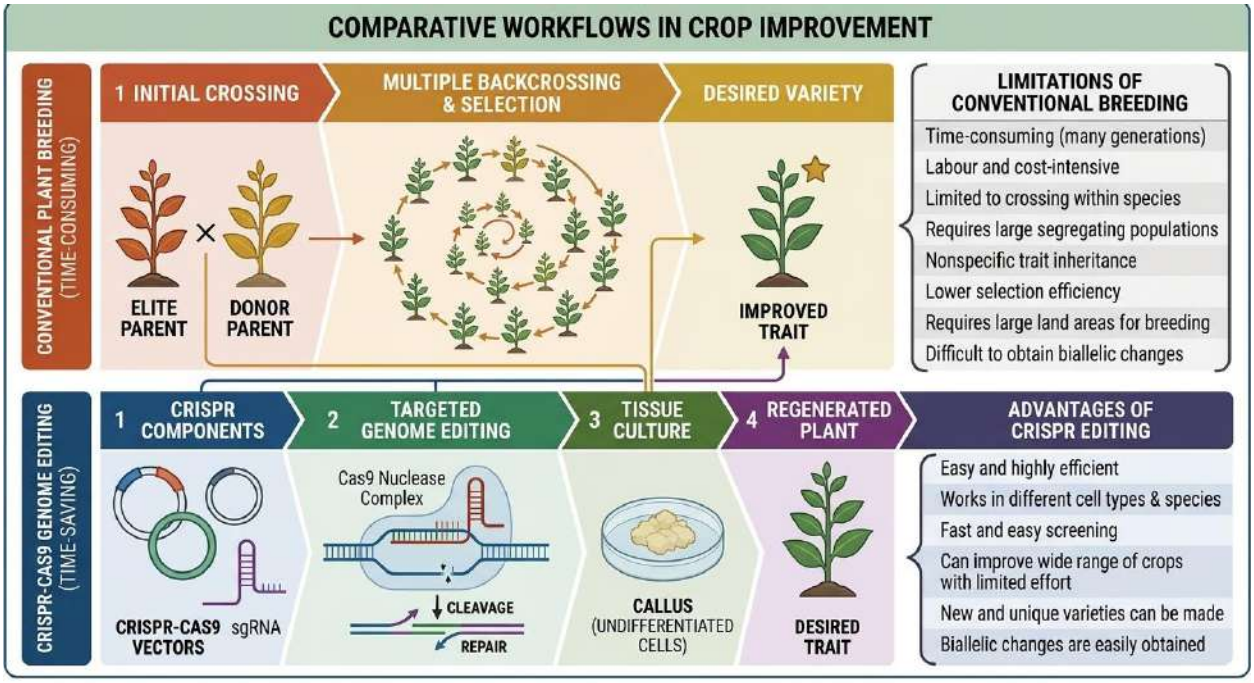


Figure 2: Comparative Workflows and Operational Advantages of Conventional Breeding versus CRISPR-Cas9 Genome Editing.

Table 1. Representative CRISPR-Cas genomic modifications for climate resilience, yield enhancement, and pathogen resistance in crops.

Crop Species	Target Gene(s)	Editing Mechanism / Tool	Targeted Agronomic Phenotype / Trait
Maize (*Zea mays*)	*ARGOS8*	CRISPR-Cas9 (HDR promoter insertion)	Increases grain yield under drought during flowering (~5 bu/acre boost)
Maize (*Zea mays*)	*ZmGA20ox3*	CRISPR-Cas9 (NHEJ knockout)	Yields semidwarf architecture; increases stalk lodging resistance
Maize (*Zea mays*)	*CLE* family	CRISPR-Cas9 (Promoter editing)	Expands shoot meristem; increases kernel row number
Wheat (*Triticum aestivum*)	*EDR1*	CRISPR-Cas9 (NHEJ knockout)	Establishes broad-spectrum resistance to powdery mildew (*B. graminis*)
Rice (*Oryza sativa*)	*OsERF922*	CRISPR-Cas9 (NHEJ knockout)	Increases host defense against rice blast (*M. oryzae*)
Rice (*Oryza sativa*)	*OsSWEET13* promoter	CRISPR-Cas9 (Promoter deletion)	Blocks pathogen exploitation; confers bacterial blight resistance
Rice (*Oryza sativa*)	*REC8*, *PAIR1*, *OSD1*	Multiplex CRISPR-Cas9	Fixes hybrid vigor via clonal, apomictic seed production
Potato (*Solanum tuberosum*)	*eIF4E*, *eIF(iso)4E*	CRISPR-Cas9 (NHEJ knockout)	Reduces viral infection susceptibility and cold-induced sweetening
Tomato (*Solanum lycopersicum*)	*SlMLO1*	CRISPR-Cas9 (NHEJ knockout)	Establishes complete resistance to powdery mildew (*O. neolycopersici*)
Grapefruit (*Citrus paradisi*)	*CsLOB1* promoter	CRISPR-Cas9 (Promoter editing)	Disrupts effector binding; confers citrus canker resistance
Groundcherry (*Physalis peruviana*)	*SP5G*	CRISPR-Cas9 (NHEJ knockout)	Induces compact growth and fruit retention (de novo domestication)

5. Global Regulatory Architectures and Policy Harmonization

The commercial adoption of CRISPR-edited crops depends heavily on global biosafety frameworks and legal classifications. Genome editing techniques using Site-Directed Nucleases (SDNs) are classified internationally into three main categories based on the repair mechanism and whether exogenous DNA remains in the genome:

- **SDN-1**: Induces double-strand breaks repaired by endogenous NHEJ without donor DNA, generating small random insertions or deletions. These edits contain no foreign DNA and are genetically indistinguishable from natural spontaneous mutations or traditional physical/chemical mutagenesis (Movahedi et al., 2023).
- **SDN-2**: Uses a short donor DNA template via HDR or MHEJ to introduce precise nucleotide changes or substitutions without leaving recombinant foreign DNA.
- **SDN-3**: Uses a donor template to integrate large exogenous DNA sequences (transgenes or cisgenes) at a specific target locus. SDN-3 products are universally regulated under traditional Genetically Modified Organism (GMO) frameworks.

Global biosafety policies fall into two main approaches: product-based regulatory frameworks (focusing on the final trait risk profile) and process-based frameworks (regulating crops based on the techniques used to create them) (Alburquerque et al., 2016).

Product-Based Regulatory Paradigms

The United States leads the product-based model through the USDA-APHIS SEC (Sustainable Ecological Consistent) rule under the

Coordinated Framework for Regulation of Biotechnology. Plants with base changes, localized indels (SDN-1) or targeted edits that can be made by conventional breeding (SDN-2) are exempt from biotechnology regulation as long as plants contain no foreign DNA. Similar product-based policies that exempt transgene-free SDN-1 and SDN-2 edits have been adopted in South America for example Brazil under CTNBio Normative Resolution No. 16 And Argentina well as in Japan, India, Australia, the United Kingdom (, through the Genetic Technology Act 2023) the Philippines, Kenya and Nigeria (Sagawa et al, 2023).

Process-Based Models and Policy Shifts

Historically, the European Union was based on a process model, regulated by Directive 2001/18/EC. A similarly restrictive decision was reached in 2018 by the CJEU (Case C-528/16), which also brought all products of directed mutagenesis within the scope of GMO legislation, obliging transgene-free gene-edited crops to undergo comprehensive environmental risk assessments, stringent traceability and compulsory labeling. This regimen imposed compliance-related expenses and trade hurdles and thus discouraged the direction of commercial biotech investments into those areas of the world, i.e., Europe, toward the North American and Asian markets (Guerra, 2022).

In response to climate-driven agricultural challenges, the European Commission proposed a legislative overhaul in July 2023 for New Genomic Techniques (NGTs). The proposed framework introduces a two-tiered classification:

- **Category 1 NGT (NGT1)**: Plants containing targeted edits comparable to natural variation or traditional mutagenesis (defined by specific thresholds, such as a maximum of 20 insertions or deletions) are treated as equivalent to conventional breeding and exempted from GMO regulatory burdens (Koller, 2025).
- **Category 2 NGT (NGT2)**: Plants carrying broader modifications or transgenic insertions (SDN-3) remain subject to standard GMO risk assessment procedures.

China maintains a tiered hybrid regulatory framework under its Safety Evaluation Guidelines, providing a fast-tracked approval pathway for gene-edited plants that contain no foreign transgenic elements (Grantina-Ievina & Rostoks, 2026).

Table 2. International regulatory oversight models and legal classifications for gene-edited crops.

Country / Region	Primary Regulatory Basis	SDN-1 & SDN-2 Legal Status (Foreign DNA-Free)	Key Exemption / Regulatory Thresholds
United States	Product-based (USDA Coordinated Framework / SEC Rule)	Exempt from GMO oversight	Evaluated on trait risk; exempt if achievable via traditional breeding
European Union	Process-based (Directive 2001/18/EC; NGT Proposal 2023)	Historically regulated as GMO; NGT1 proposal aims for exemption	Moving toward exemption for Category 1 NGTs (<20 edits)
United Kingdom	Product-based (Genetic Technology Act 2023)	Exempt from GMO oversight	Exemption granted for Precision Bred Organisms (PBOs)
China	Tiered / Hybrid (Safety Evaluation Guidelines 2022)	Fast-tracked evaluation pathway	Simplified Tier 1 management for non-transgenic edits
India	Product-based (MoEF&CC Revised Guidelines 2022)	Exempt from GMO oversight	Group 1 (SDN-1) and Group 2 (SDN-2) exempt from GEAC assessment

Brazil	Product-based (CTNBio Normative Resolution No. 16)	Exempt on a case-by-case basis	Non-GM status assigned if final product lacks recombinant DNA
Japan	Product-based (Genome-Edited Food Guidelines 2019)	Exempt from GMO oversight	Mandatory notification; exempt if foreign genetic material is absent

6. Technical Bottlenecks and Future Perspectives

Despite rapid technological progress, several technical, biological, and economic bottlenecks continue to impact the application of CRISPR-Cas platforms in crop improvement:

1. **Tissue Culture and Transformation Efficiency**: Broad adoption remains constrained by tissue culture limitations. Many commercial elite crop inbreds are recalcitrant to *Agrobacterium*-mediated transformation or plant regeneration. While co-expressing developmental morphogenic regulators (such as *Baby boom*, *Wuschel2*, or *GRF-GIF* chimeras) improves transformation efficiency in monocots, developing tissue-culture-free delivery mechanisms remains a research priority. Direct viral vector delivery, nanoparticle-mediated complexes, and floral-dip transformations offer potential solutions (Mookkan et al., 2017).
2. **Off-Target Mutations**: Unintended cleavage at non-target genomic sites with sequence similarity to the sgRNA remains a technical concern. However, off-target rates in plant systems are lower than in mammalian cells. In addition, unintended mutations introduced during initial editing can typically be segregated out through backcrossing with parental lines. Engineered high-fidelity Cas variants (such as SpCas9-HF1 or HypaCas9) and base editing platforms further minimize off-target risks (Basit & Zheng, 2026).
3. **Complex Polygenic Trait Engineering**: While single-gene knockouts (*S*-genes, yield suppressors) are straightforward, complex traits like drought tolerance and nitrogen-use efficiency are governed by intricate polygenic networks. Advanced multiplex CRISPR platforms using Cas12a crRNA arrays or multiple pol III promoters allow simultaneous targeting of multiple loci across interconnected metabolic pathways (Helliwell & Yang, 2012).
4. **Integration with Pangenomics and Speed Breeding**: Combining pangenomics with high-throughput CRISPR editing enables the identification and targeted introduction of beneficial stress alleles into commercial crop varieties. Integrating these technologies with speed breeding protocols which use controlled growth environments to achieve up to six generations per year can significantly shorten the development cycle for climate-resilient cultivars (Ghorbanzadeh et al., 2025).

CONCLUSION

CRISPR-Cas genome editing is bringing a paradigm shift in plant biotechnology by providing a robust and accurate system for generation of climate-smart, high-yielding crop varieties that are pivotal to ensuring global food security. This review highlights that the CRISPR-Cas toolkits exploiting a variety of nucleases, base editors, prime editors as well as epi-genome editors, allow the execution of precise editing tasks from individual nucleotide modifications to concurrent multi-gene targeting. The proven accomplishments such as drought tolerance in maize via ARGOS8 promoter engineering, powdery mildew resistance in two crops via EDR1 and SlMLO1 knockouts, and synthetic apomixis through multiplex editing, demonstrate CRISPR's potential to improve both abiotic and biotic stress as well as yield architecture. The global regulatory dynamics are evolving and are challenging yet full of opportunities. Product-based regulatory systems in the United States, Brazil, and Japan are progressively exempting from GMO regulations SDN-1 and SDN-2 modifications where no foreign DNA is left in the cells, allowing easier market access whereas the NGT categorization proposed by the European Union is signaling a possible regulatory shift towards science-based/trait-based

regulation. Nevertheless, technical challenges such as low transformation efficiency, off-target effects and engineering complex polygenic traits still remain. To advance the field, CRISPR will need to be combined with novel approaches such as pangenomics, speed breeding and sophisticated delivery mechanisms to shorten breeding cycles and incorporate stress tolerant traits in orphan crops that have been previously overlooked. In the end, unleashing the full power of CRISPR will depend on further advances in molecular tools, global regulatory convergence and farmer engagement to provide equitable delivery of climate-smart crop varieties to farmers globally.

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