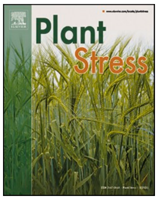
Plant Stress 21 (2026) 101322

Contents lists available at ScienceDirect

Plant Stress

journal homepage: www.sciencedirect.com/journal/plant-stress



Review

# CRISPR/Cas system-mediated gene editing for disease resistance in food crops: current status and future directions

Niranjan Ravindra Thakur [a], Krishnananda Pralhad Ingle [b,*], Sonal Chavan [a], Yashoda Jadhav [a], Swapnil Shamrao Baraskar [a], Ganesh Nikalje [c], Shashi Pandey [d], Jayashri Narayan Papade [e], Sachin Laxman Dhare [f], Stanislaus Antony Ceasar [g,*], Gholamreza Abdi [h,*]

[a] International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telangana, 502324, India
[b] Reliance Industries Limited, Reliance Corporate Park, Ghansoli, Navi Mumbai, Maharashtra, 400701, India
[c] Department of Botany, Seva Sadan's R.K. Talreja College of Arts, Science and Commerce, Ulhasnagar, Maharashtra, 421003, India
[d] Division of Genetics, ICAR-IARI, New Delhi, 110012, India
[e] Swatantrya Vir Ganpatrao Ingle College of Horticulture, Jalgaon, Maharashtra, 443402, India
[f] Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, MS, India
[g] Division of Plant Molecular Biology & Biotechnology, Department of Biosciences, Rajagiri College of Social Sciences, Cochin, Kerala, 683104, India
[h] Department of Biotechnology, Persian Gulf Research Institute, Persian Gulf University, 7516913817, Bushehr, Iran

ARTICLE INFO

*Keywords:*
CRISPR/Cas-9
Food security
Genome editing
Plant disease management
Plant-pathogen interaction
Regulatory challenges
Sustainable agriculture

ABSTRACT

The global population is expected to reach 9.7 billion by 2050, which will put pressure on food production systems that are already vulnerable to climate change. Crop diseases caused by pathogenic bacteria, fungi, and viruses cause significant yield losses in major food crops. Conventional breeding has contributed to developing resistant cultivars, but it is time-consuming and limited by genetic variability. The clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein (Cas) systems have emerged as transformative tools for the precise and rapid improvement of disease resistance in food crops. This review presents a comprehensive overview of the application of CRISPR/Cas-based genome editing platforms such as Cas nucleases, base editors, and prime editor systems for developing disease resistance in food crops. These technologies enable targeted knockout of susceptibility (S) genes, precise nucleotide substitutions, promoter engineering, and direct viral RNA interference for imparting disease resistance. Delivery of DNA-free ribonucleoprotein (RNP) complexes further supports the development of non-transgenic (GM-free) crops for quick regulatory approvals and field cultivation. The review highlights successful applications of CRISPR/Cas tools across major cereals, legumes, and tuber crops for developing disease resistance. Additionally, CRISPR-enabled diagnostics provide new prospects for rapid pathogen detection in field conditions. It also draws in roads for future adoption of CRISPR/Cas tools for developing disease resistance in major crops and extensions to minor cereals, such as millets. Prudent applications of CRISPR/Cas tools will play a pivotal role in promoting sustainable agriculture and achieving the United Nations Sustainable Development Goal of Zero Hunger.

## 1. Introduction

Global food security has become a critical concern in recent years as it is threatened by several factors, such as climate change, an ever-growing world population, and increasing incidences of both biotic and abiotic stress. The global population is projected to reach 9.7 billion by 2050, necessitating an estimated 70 % increase in food production relative to 2005–2007 levels by 2050 (Bruinsma, 2009). The United Nations (UN) has established a framework to address these issues with the Sustainable Development Goals (SDGs) as its cornerstone. SDG 2 specifically targets 'Zero Hunger,' aiming to achieve food security, improve nutritional status, and promote sustainable agriculture (https://sdgs.un.org/goals/goal2). Climate change has also become a major threat to farming around the world. By 2100, the average temperature around the world is expected to rise by 1.4 to 5.8 °C (Arora et al., 2005). The altered patterns in the occurrence of biotic and abiotic stresses have adversely affected plant growth and development by influencing photosynthesis and other physiologically important mechanisms.

---

* Corresponding authors.
*E-mail addresses:* krishnananda.ingle@ril.com (K.P. Ingle), antony_sm2003@yahoo.co.in, saceasar@rajagiri.edu (S.A. Ceasar), abdi@pgu.ac.ir (G. Abdi).

https://doi.org/10.1016/j.stress.2026.101322
Received 30 October 2025; Received in revised form 29 January 2026; Accepted 26 February 2026

Abiotic stresses such as drought, extreme (high and low) temperatures, waterlogging, altered rainfall patterns, and salinity aggravate the biotic stressors (insects, pathogens, and weeds) in crops and weaken the plants' defense responses. Moreover, they may cause minor pests to develop into future threats, causing a serious risk to global food production (Chawla et al., 2024; Pandey et al., 2017; Zahra et al., 2023). The world's primary staple crops, such as rice, wheat, maize, sorghum, millet, soybean, and tuber crops such as potatoes, cassava, yams, and taro, provide the main source of food for most of the global population. Crop diseases and pests are responsible for substantial reductions in agricultural productivity, with estimated global yield losses of 30.0 % (24.6–40.9 %) in rice, 22.5 % (19.5–41.1 %) in maize, 21.5 % (10.1–28.1 %) in wheat, 21.4 % (11.0–32.4 %) in soybean and 17.2 % (8.1–21.0 %) in potato (Savary et al., 2019). Such losses threaten global food security by jeopardizing staple crops that sustain much of the world's population, illustrating the importance of developing biotic stress (disease and pest) resistant cultivars and ensuring sustainable agricultural production. However, resistance breeding is a continuous process, as pathogens evolve and the complexity of host–pathogen interactions changes over time. The conventional approach of breeding for resistant cultivars begins with scouting lines showing resistance to diseases or pests. Sources of resistance may include landraces, obsolete cultivars, or wild relatives. To identify these sources, rigorous characterization is carried out, which can further serve as potential donors for the resistance. Furthermore, the success of breeding for disease resistance depends on understanding the underlying mechanisms at the gene, genotype, and population levels (Nelson et al., 2018).

The nature of the resistance gene, whether it is a major gene or minor gene, plays a crucial role in determining the appropriate breeding strategy. Various approaches, such as backcross breeding, pedigree breeding, recurrent selection and ultimately, mutation breeding, are employed to develop resistant lines. Among these, backcross breeding is the most common method used in breeding for disease resistance. It involves a series of backcrosses to introgress the gene of interest from donor parents along with a gradual decrease in the donor genome content to retain maximum superior agronomic characteristics from the recurrent parent (Hasan et al., 2015). The most popular technique to breed crop plants with specific resistance to pests and diseases is pedigree breeding. Pedigree breeding is particularly effective when resistance traits are controlled by major genes with high heritability (Sahu et al., 2022). In contrast, selection for traits with low heritability is typically deferred to later generations, once the lines have achieved greater homozygosity. Though conventional breeding has played a pivotal role in the development of resistant cultivars, there is a need to deploy the potential biotechnology tools that can supplement the goal to develop disease-resistant cultivars.

Modern plant biotechnology for precision breeding and accelerated crop improvement programs increasingly depends on genome editing tools, such as clustered regularly interspaced short palindromic repeats (CRISPR) and the CRISPR-associated protein (Cas) system (Ingle et al., 2023; Jadhav et al., 2024). The discovery of CRISPR/Cas tools is regarded as one of the most revolutionary breakthroughs in recent years across biology, biotechnology, medicine, and the pharmaceutical and agricultural sectors. CRISPR/Cas tools have opened new avenues for dissecting gene function by enabling precise and targeted modifications, allowing researchers to investigate the biological roles of specific genetic variants. It provides an efficient way to precisely improve crops, leading to rapid development of superior cultivars with desirable traits (Barmukh et al., 2024). CRISPR/Cas9 marked a pivotal turning point in the history of gene editing, owing to its high precision, ease of use, and comparatively low-cost relative to earlier technologies such as zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) (Ayanoğlu et al., 2020). Following the development of CRISPR/Cas9, several additional CRISPR/Cas-based tools emerged, including CRISPR transcriptional activation (CRISPRa), CRISPR transcriptional inhibiton (CRISPRi), CRISPR prime editing, CRISPR base editing, and alternative Cas systems, such as Cas12, Cas13, and Cas14 (Zhu et al., 2020; Ceasar et al., 2022).

The economic viability of present-day agriculture is increasingly compromised by the challenges of biotic stress-related losses and rising expenses associated with chemical control. Plant biotic stresses cost the world over $220 billion a year, which impacts food security and farm profits (FAO, 2022). Farmers have to spend a significant amount of money on synthetic chemicals in disease management. These chemicals also have negative consequences on the environment that could affect long-term ecological resilience (Zhou et al., 2024). In this situation and current era of plant breeding, genome editing using the CRISPR/Cas system has the potential to change the economy by cutting down on the time and money needed to create resistant varieties. Recent analyses suggest that, in some regions where certain genome-edited crops are not regulated as GMOs, they can often be developed and commercialized for about USD 10–15 million within 4–6 years, whereas transgenic traits commonly require investments exceeding USD 130 million and more than a decade to reach the market, largely due to regulatory testing and approval costs (Lassoued et al., 2019; Phillips, 2021). This decrease in the cost of innovation not only makes breeding programs more profitable but also makes advanced genetics more accessible to everyone. This makes it possible to develop disease resistance even for orphan crops and marginal agricultural systems where traditional commercial breeding isn't available.

The demand for disease-resistant plants is growing, and CRISPR/Cas tools have been effectively employed to develop such traits in a wide range of fields and horticultural crops (Ingle et al., 2024). CRISPR/Cas tools have been utilized in plant pathology, especially for enhancing resistance and improving overall agricultural practices (Das et al., 2019; Langner et al., 2018). The CRISPR/Cas system enables the precise introduction of desirable alleles or the targeted editing of endogenous genes based on traits identified in wild relatives, accelerating targeted improvement in plant breeding programs (Thakur et al., 2024). This review aims to elucidate the potential of CRISPR/Cas-based genome editing and its underlying molecular mechanisms for enhancing disease resistance in major food crops of the world, including rice, wheat, maize, sorghum, pearl millet, finger millet, soybean, potato, cassava, and barley.

## 2. CRISPR/Cas systems: components, and engineered applications

Recent advancements in CRISPR/Cas-based gene editing technologies have revolutionized the genetic improvement of food crops, especially for enhancing disease resistance. These tools enable precise manipulation of the genome or transcriptome and have been applied across diverse plant species. The major CRISPR tools like Cas9, Cas12a, Cas13, Cas14, base editors, and prime editors offer a spectrum of editing capabilities, each with unique mechanisms and practical applications.

### *2.1. Components of the CRISPR/Cas system*

The CRISPR/Cas system comprises two principal elements: Cas proteins, which cleave foreign DNA, and the CRISPR array, which serves as a molecular memory against invading viruses. The CRISPR array contains two to several hundred direct repeats (typically 25–36 bp in length) interspersed with spacers (Martynov et al., 2017; Shmakov et al., 2020). These spacers, which are short nucleic acid fragments (28–36 bp) derived from previous encounters with mobile genetic elements (MGEs), form the basis of CRISPR/Cas adaptive immunity. Each spacer is flanked by repeat sequences, and the array is transcribed as a single precursor RNA that is subsequently processed into multiple CRISPR RNAs (crRNAs). The crRNAs are incorporated into effector complexes with Cas proteins, which survey the cell for complementary nucleic acid sequences. Base pairing between the crRNA spacer and a target sequence (protospacer) enables precise recognition, after which the effector complex directs cleavage and degradation of the invading genetic material (Vink et al., 2021).

The effectiveness of CRISPR/Cas9 editing depends on the creation of highly specific single guide RNAs (sgRNAs) (Composed of CRISPR RNA (crRNA) and tracr RNA (tracrRNA)) that guide the Cas9 nuclease to the correct genomic site. In crop plants, sgRNAs are typically designed using bioinformatics platforms such as CHOPCHOP, CRISPR-P, CRISPRdirect, and Benchling (Chen et al., 2021; Jasieniecka and Domingues, 2025). These tools facilitate the selection of target sequences with optimal on-target efficiency while minimizing off-target risks. The protospacer adjacent motif (PAM) is a short DNA sequence required for Cas9-mediated target recognition and cleavage. For *Streptococcus pyogenes* Cas9 *(*SpCas9), the canonical PAM is 5′-NGG-3′ (Adli, 2018).

### 2.2. *Engineered derivatives of CRISPR/Cas systems*

To overcome the limitations of standard DSB-mediated editing, such as off-target effects and the inefficiency of precise homology-directed repair (HDR), researchers have developed engineered derivatives of the Cas9 protein. These innovations range from Cas9 variants designed for enhanced specificity and transcriptional regulation to precise fusion systems like base editing (BE) and prime editing (PE) (Molla et al., 2021). Unlike wild-type Cas9, which generates double-strand breaks (DSBs), BE, and PE enable precise genomic modifications without inducing DSBs or requiring donor DNA. Notably, BE has been reported to boost efficiency over HDR by 200-fold (Yeh et al., 2018).

#### 2.2.1. *Cas9 variants: nickases and transcriptional modulators*

To address concerns regarding off-target effects and to expand the CRISPR toolbox beyond DSBs, engineered variants of the Cas9 protein have been developed. The Cas9 nickase (nCas9) is generated by introducing a point mutation into one of the two catalytic domains (D10A in RuvC or H840A in HNH), retaining the ability to cleave only a single DNA strand. This variant is widely used in a ‘paired nickase’ strategy, where two nCas9-sgRNA complexes generate offset nicks on opposite strands. This approach effectively doubles the specificity of the recognition site, significantly mitigating off-target mutations in crops like rice and *Arabidopsis* (Mikami et al., 2015; Schiml et al., 2014). Furthermore, nCas9 serves as the catalytic chassis for precise editing tools like BE and PE. Another major variant is dead Cas9 (dCas9), created by mutating both catalytic domains (D10A and H840A). The dCas9 lacks endonuclease activity entirely but acts as a robust RNA-guided DNA-binding platform. This property is harnessed for transcriptional modulation: CRISPRi uses dCas9 (often fused with repressors like SRDX) to sterically block transcription of viral genomes or host susceptibility genes (Ji et al., 2015). Conversely, CRISPRa employs dCas9 fused with activators to upregulate plant defense genes. For example, the activation of *SlPR-1* in tomato using a dCas9 system conferred enhanced resistance against bacterial canker (García-Murillo et al., 2023).

#### 2.2.2. *Base editing*

BE is a technique that allows precise modifications at the nucleotide level, where the desired transition mutations (C-T, T-C, A-G, and G-A) can be introduced into the genome or transcriptome with the help of a modified Cas protein (nCas9 or dCas9) and a modifying enzyme. BE is bifurcated into two groups, namely, cytosine base editors (CBEs) and adenine base editors (ABEs). CBE1 catalyzes the deamination of the cytosine base on the target strand, converting cytosine to uracil, which is subsequently read as thymine during replication, resulting in a C-G to T-A transition. To improve efficiency and reduce indel formation, modern CBEs typically utilize nCas9 (D10A) to nick the non-edited strand, stimulating cellular repair mechanisms to favor the edited base. Similarly, ABEs are employed to generate A-T to G-C transitions, where tRNA adenosine deaminase was fused with a dCas9 (or nCas9 in later generations), which gave rise to early generations of ABEs (Gaudelli et al., 2017). ABEs convert adenosine into inosine (I), and during DNA repair or replication, the intermediate I is recognized as G, leading to the transition from A to G. In the context of disease management, a study reported that enhanced resistance against bacterial blight in rice was achieved through base editing, utilizing both ABE and CBE to target the *SWEET14* gene. This approach led to a reduction in *SWEET14* expression by CBE, which subsequently decreased the disease severity (Li et al., 2025).

#### 2.2.3. *Prime editing*

Prime editing is a search-and-replace system that is composed of Cas9 nickase, an engineered reverse transcriptase (RT), and a prime editing gRNA (pegRNA). Three components give rise to pegRNA, namely, a primer binding site (PBS) that initiates reverse transcription, sgRNA that targets the specified locus and a reverse transcript that encodes the desired edit yielding a template (Anzalone et al., 2019). PEs enable precise substitutions, insertions, or deletions without DSBs. PE was utilized to induce resistance against bacterial blight by introducing effector binding element (EBE) in the promoter of gene *xa23* to induce resistance (Gupta et al., 2023a). Also, in another study, the gene *OsSWEET11a* was edited to achieve similar results (Gupta et al., 2023b). PE has also been validated in other staple crops like wheat and maize (Jiang et al., 2020; Lin et al., 2020; Ni et al., 2023; Qiao et al., 2023).

### 2.3. *Delivery of CRISPR/Cas components*

Efficient delivery of CRISPR components to plant cells is a critical step in achieving precise and successful genome editing. The choice of delivery method largely depends on the type of crop, the nature of the target tissue, and the intended application (transient vs. stable expression). One of the most widely used techniques, particularly for dicotyledonous crops such as soybean and potato is *Agrobacterium*-mediated transformation. This method leverages the natural ability of *Agrobacterium tumefaciens* to transfer DNA into plant genomes, making it highly effective for stable integration and regeneration of edited plants (Gelvin, 2017; Hiei et al., 2014). In contrast, for monocotyledonous crops like maize, wheat, and rice, where *Agrobacterium*-mediated transformation is less efficient, particle bombardment (or biolistic transformation) is preferred (Ozyigit and Kurtoglu, 2020). This technique involves the physical delivery of DNA-coated gold or tungsten particles into plant cells using a gene gun, allowing for the transformation of a wider range of tissues and species (Altpeter et al., 2016).

Another promising approach is protoplast transfection, which is especially useful for transient expression studies and high-throughput screening of CRISPR constructs. Protoplasts, which are plant cells with their cell walls enzymatically removed, can readily take up DNA, RNA, or ribonucleoprotein (RNP) complexes via polyethylene glycol (PEG)-mediated methods or electroporation (Lin et al., 2018). Among the most recent and advanced delivery strategies is the use of RNP complexes, where pre-assembled Cas9 protein and guide RNA (sgRNA) are introduced directly into the plant cells. This method offers the significant advantage of minimizing off-target effects and avoiding the integration of foreign DNA into the genome, thus supporting the development of non-GMO edited crops (Ceasar and Ignacimuthu, 2023). Each of these methods plays a crucial role in the broader application of CRISPR/Cas tools in food crops, enabling both fundamental research and crop improvement initiatives.

### 2.4. *Advanced CRISPR/Cas systems for detection of pathogens*

Rapid and accurate pathogen detection is vital for safeguarding global food security, as past outbreaks have demonstrated the devastating impact of crop epidemics. Traditional culture-based detection methods, while reliable, are slow, labor-intensive, and impractical for real-time use. Molecular approaches such as PCR and immunoassays offer improved speed and sensitivity but remain constrained by cost, scalability, and on-site applicability. Rapid pathogen detection is critical for preventing disease outbreaks. CRISPR-based technologies have the potential to change this field by bringing together speed, accuracy, low cost, and portability. Advanced CRISPR-based systems, such as Cas12 and Cas13 systems, enable rapid and precise pathogen detection. Cas12 and Cas13 effectors exhibit collateral (trans) nuclease activity upon recognition of specific DNA or RNA targets, a feature that enables nucleic acid detection through fluorescence or colorimetric readouts. When integrated with isothermal amplification methods and portable biosensors, they offer enhanced field applicability, making them well-suited for decentralized and real-time testing (Liberty et al., 2025). Wang et al. (2023) developed a rapid and sensitive detection tool for on-site and visual detection of rice stripe mosaic virus and sorghum mosaic virus, based on reverse transcription recombinase-aided amplification (RT-RAA) coupled with CRISPR/Cas12a. This assay exhibited higher sensitivity than RT-PCR and demonstrated strong specificity for the target viruses, indicating its potential for field-based detection of major crop viruses. A comparable use of the CRISPR/Cas12a platform was described by Changtor et al. (2023) for rapid detection of the soil-borne phytopathogen *Sclerotium rolfsii*, responsible for dry stem and root rot in cassava. By combining recombinase polymerase amplification (RPA) with CRISPR/Cas12a, the authors developed an efficient assay capable of accurately identifying the pathogen, highlighting its suitability for field-based diagnostics. Overall, CRISPR-based pathogen detection tools hold the potential to transform the technological landscape of nucleic-acid-based detection. The following tools can facilitate the detection of RNA or DNA molecules of pathogenic origin by harnessing the potential of Cas12 and Cas13 variants.

### *2.4.1. SHERLOCK*

The Specific High-sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK) system is a novel diagnostic tool based on CRISPR-Cas technology, first reported by Gootenberg et al. (2017). Based on the discovery of the RNA-guided RNase Cas13a, SHERLOCK was developed to provide a rapid, inexpensive, and highly sensitive method for nucleic acid detection without the need for sophisticated laboratory infrastructure. This system made a paradigm shift in molecular diagnostics by using the properties of CRISPR for signal transduction instead of genome editing, paving the way for next-generation biosensing (Kellner et al., 2019). In the context of pathogen diagnostics, SHERLOCK is particularly valuable because it enables the precise detection of pathogen-specific nucleic acid signatures, including viral RNA, bacterial genes, and fungal or oomycete DNA, thereby allowing early and accurate identification of infectious agents before visible disease symptoms appear.

The SHERLOCK integrates three components: isothermal nucleic acid amplification, programmable targeting, and collateral cleavage activity of the CRISPR/Cas13 enzyme. The target DNA or RNA is first amplified using RPA or Loop-mediated Isothermal Amplification (LAMP), with a T7 RNA polymerase promoter integrated into the primers to facilitate transcription of DNA into RNA (Kellner et al., 2019). Once the Cas13-crRNA (CRISPR RNA) complex recognizes and binds to its specific target sequence, the enzyme undergoes a conformational change that activates the collateral cleavage, where the enzyme non-specifically degrades nearby single-stranded RNA (ssRNA) reporter molecules. Variants such as SHERLOCKv2 have further refined the system by incorporating Cas12a for DNA detection, utilizing Csm6 to amplify the reporter signal, and enabling the simultaneous detection of up to four distinct targets (Gootenberg et al., 2018; Myhrvold et al., 2018). This programmable targeting capability allows SHERLOCK assays to be customized for the detection of specific plant pathogens by designing crRNAs against conserved or strain-specific genomic regions, enabling discrimination among closely related pathogens, virulent races, or emerging variants in agricultural and biosecurity surveillance.

Despite its significant advantages, several limitations restrict the universal deployment of SHERLOCK in the agricultural context. The main challenge is the heavy reliance on a pre-amplification step, which increases the complexity of the workflow, potentially leading to false-positive results. Additionally, while the specificity is high, the design of efficient crRNAs remains constrained by the preference of certain Cas13 variants for specific protospacer flanking sites (PFS). The cost and stability of reagents, particularly the requirement for a cold chain for enzyme storage and the high price of synthetic RNA reporters, can be expensive for small-scale farmers or resource-limited institutes. These limitations are particularly relevant for field-level pathogen detection in agriculture, where robust, low-cost, and minimal-infrastructure diagnostic platforms are required for routine monitoring of crop diseases, necessitating further optimization of SHERLOCK-based assays for on-site detection of plant pathogens under diverse environmental conditions.

### *2.4.2. DETECTR*

The DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) was introduced as a groundbreaking method for DNA detection (Chen et al., 2018). It utilizes the Cas12a enzyme, which is specifically synthesized for the rapid identification of dsDNA. This technology uses the trans-cleavage biochemical property of Cas12a, which transitioned CRISPR from a gene-editing tool to a diagnostic sensor. The main advantage was its ability to combine the specificity of CRISPR-Cas recognition with isothermal amplification, enabling high-sensitivity detection in less than an hour without specialized laboratory equipment (Harrington et al., 2018). In pathogen diagnostics, DETECTR has been widely adapted for the detection of DNA-based pathogens, including bacterial, fungal, and DNA viral agents, by targeting conserved virulence genes or species-specific genomic regions, thereby enabling rapid identification of infectious organisms in clinical and agricultural samples.

The DETECTR consists of three main components: a Cas12a effector protein, a designed crRNA, and a ssDNA reporter molecule labeled with a fluorophore and a quencher. The programmed Cas12a-crRNA complex scans for a target DNA sequence adjacent to a T-rich PAM, generally a 5′-TTTV-3′ sequence (Paul and Montoya, 2020). Then RuvC nuclease domain of Cas12a initiates the trans-cleavage activity that non-specifically degrades any nearby ssDNA. This cleavage releases the fluorophore from its quencher, generating a measurable signal that confirms the presence of the target. The DETECTR is often paired with RPA or LAMP for higher sensitivity (Kaminski et al., 2021). Variants like HOLMES (One-HOur Low-cost Multipurpose highly Efficient System) also utilize Cas12a or Cas12b but differ in their optimization for specific temperatures or multiplexing capabilities (Li et al., 2019; Paul and Montoya, 2020). This mechanism allows DETECTR assays to be rationally designed for pathogen surveillance by selecting crRNAs against diagnostic loci such as pathogenicity islands, resistance genes, or strain-specific markers, facilitating sensitive detection and differentiation of closely related pathogen species or pathovars.

Despite its efficiency, DETECTR faces several limitations that hinder its use in the agricultural domain. The primary reason is the requirement for a T-rich PAM (TTTV) sequence at the target site, which limits the choice of genomic targets and can complicate the detection of certain SNPs. Additionally, the system requires a pre-amplification step like RPA or LAMP to achieve high sensitivity. There are also concerns regarding the stability of CRISPR reagents, particularly the Cas12a enzyme and synthetic crRNAs, which often require refrigeration that may not be available in every region. These constraints pose particular challenges for field-deployable pathogen diagnostics in agriculture, where target sequence flexibility, reagent robustness, and minimal cold-chain dependence are critical for routine monitoring of crop pathogens and early disease outbreak management.

### *2.4.3. HOLMES*

The One-HOur Low-cost Multipurpose highly Efficient System (HOLMES) is a CRISPR-based diagnostic developed as a rapid-response detector (Li et al., 2019). While CRISPR is traditionally known for its genome-editing capabilities, HOLMES uses the intrinsic trans-cleavage activity of Cas12. The core theme of the HOLMES comprises a Cas12 effector protein, a crRNA for specificity, and a ssDNA reporter. The Cas12-crRNA complex scans for a double-stranded DNA target sequence that possesses PAM sequence. Once the crRNA hybridizes with the complementary target DNA, the Cas12 protein undergoes a conformational change that activates its RuvC nuclease domain. While Cas9 performs only a site-specific cis-cut, the activated Cas12 in the HOLMES system initiates trans-cleavage to degrade nearby ssDNA molecules. This degradation separates the fluorophore from its quencher, resulting in a visible or measurable fluorescent signal. A major advancement in this technology is HOLMESv2, which incorporates the thermophilic Cas12b enzyme from Alicyclobacillus acidoterrestris. As Cas12b remains stable and active at elevated temperatures (60–65 °C), it allows for a one-pot reaction where LAMP and CRISPR detection occur simultaneously. This integration simplifies the workflow, reduces the risk of contamination, and enables quantitative detection of target nucleic acids (Teng et al., 2019). In pathogen diagnostics, HOLMES offers a programmable Cas12-based detection framework that can be adapted to target conserved genomic regions of DNA pathogens, highlighting its potential applicability in agriculture, food safety, and environmental surveillance where rapid decision-making is essential.

HOLMES differs from SHERLOCK and DETECTR primarily through its use of effector proteins and its optimization for one-step detection. While SHERLOCK utilizes Cas13 to target RNA and cleave RNA-based reporters, HOLMES focuses on DNA targets and utilizes DNA reporters. Although DETECTR also uses Cas12a for DNA detection, HOLMESv2 specifically uses the thermophilic properties of Cas12b to enable integration with high-temperature LAMP amplification. This compatibility allows HOLMESv2 to operate more effectively than Cas12a-based DETECTR systems, which often require separate steps or lower-temperature RPA, and reduces the chances of cross-contamination. As a result, the streamlined, single-tube workflow of HOLMESv2 offers practical advantages for rapid DNA detection in time-sensitive diagnostic settings, particularly where simplified assay design and reduced handling steps are critical for minimizing contamination and improving operational robustness.

#### 2.4.4. *iSCAN—OP*

The in vitro Specific CRISPR-based Assay for Nucleic acids detection (iSCAN—OP) is a diagnostic platform to address the need for rapid, on-site detection of plant pathogens, particularly plant RNA viruses like TMV, PVX, and PVY (Aman et al., 2020). Unlike multi-step CRISPR diagnostics, iSCAN—OP represents the consolidation of the entire detection process into a single reaction step. The iSCAN—OP system operates through a one-pot reaction that integrates reverse transcription, isothermal amplification, and CRISPR-mediated detection. The core components include a reverse transcriptase enzyme, the reagents for RPA, Cas12 variants, a target-specific crRNA, and a ssDNA reporter molecule. The viral RNA target is converted into complementary DNA (cDNA) and then amplified into dsDNA amplicons via RPA. As the amplicons are generated, the Cas12-crRNA complex binds to the specific target sequence adjacent to a PAM. This binding event activates the trans-cleavage activity, leading to indiscriminate degradation of the labeled ssDNA reporters in the reaction mixture. The degradation of these reporters releases a fluorescent signal that can be visualized. Variants like iSCAN-V2 have further refined this approach by utilizing thermophilic Cas12b enzymes to enhance reaction stability and sensitivity (Aman et al., 2022). Importantly, the explicit validation of iSCAN—OP for economically important plant RNA viruses establishes it as one of the few CRISPR-based diagnostic platforms directly optimized and experimentally demonstrated for agricultural pathogen detection under near-field conditions. iSCAN—OP distinguishes itself from SHERLOCK and DETECTR primarily by one-pot system and drastically reduces the risk of contamination. While SHERLOCK relies on Cas13 to target RNA and DETECTR often uses a two-step process for DNA detection, iSCAN—OP optimizes the synergy between RPA and Cas12a for a faster workflow. While HOLMES also utilizes Cas12, iSCAN—OP is specifically tailored for rapid agricultural field use, demonstrating superior speed by providing results in a shorter time as compared to other tools. In contrast to more generalized CRISPR diagnostic platforms, the plant-pathogen-specific validation of iSCAN—OP underscores its suitability for on-site crop disease diagnostics, where rapid turnaround time and minimal laboratory infrastructure are critical requirements.

## 3. Mechanism of disease resistance and CRISPR engineering in food crops

Plants have developed a wide range of defense mechanisms to suppress diseases and protect against pathogen-induced damage. The response of plants to pathogen attack depends on their ability to recognize the threat at the cellular level, which then triggers complex signaling pathways (Andolfo and Ercolano, 2015; Jones and Dangl, 2006). When plants perceive pathogen signals such as pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), as well as effectors, they activate stereotypical defense responses (Safaeizadeh and Boller, 2019). The plant innate immune system consists of two distinct but interconnected components: the immunity activation component (IAC) and the immunity modulation component (IMC). The IAC is based on several surveillance receptors, including pattern-recognition receptors (PRRs) and nucleotide-binding leucine-rich repeat receptors (NLRs), which recognize the presence of pathogens and signal invasion. In contrast, IMC relies on phytohormones that play a fundamental role in regulating the plant immune response (Von Essen et al., 2010). Traditionally, resistance traits have been introgressed through classical breeding by exploiting natural genetic variation and undergoing multiple rounds of recombination. However, the efficiency of conventional breeding is frequently constrained by linkage drag, as well as the inherently narrow genetic base of elite germplasm. New alleles may also be introduced via random mutagenesis. However, this approach typically requires time-consuming screening of large populations to identify desirable mutants (Andolfo et al., 2016). Genome editing, particularly CRISPR/Cas systems, enables precise and efficient modification of target genes while minimizing off-target effects (Jhu et al., 2023). These technologies offer considerable potential for enhancing plant disease resistance by precisely manipulating key components of the plant immune system, thereby providing a targeted and versatile approach for crop improvement. The major applications of genome editing for enhancing plant immunity, encompassing four primary strategies and essential delivery methods, are schematically represented in Fig. 1. The mechanisms of resistance engineered via CRISPR/Cas generally fall into three distinct biological categories: the disruption of susceptibility factors (S-genes), the enhancement or engineering of resistance loci (R-genes), and direct molecular interference with viral genomes.

### 3.1. *Disruption of susceptibility (S) genes*

The targeted inactivation of S-genes is a commercially advanced strategy with a broad-spectrum effectiveness for engineered resistance. S genes encode host proteins that are either essential for the pathogen's life cycle, such as nutrient uptake (e.g., SWEET transporters) or cell entry (e.g., Mildew Resistance Locus O (MLO) proteins), or act as negative regulators of plant immunity (e.g., DMR6, which degrades salicylic acid) or function as negative regulators of the plant's immune system (Barka and Lee, 2022). This creates a compatible interaction spot where the plant fails to mount defenses, allowing pathogen proliferation. Pathogens exploit these conditions for compatibility and infection. By removing these conditions, genome editing can confer broad-spectrum, durable resistance, often characterized as recessive resistance. Genome editing technology creates loss-of-function mutations in S genes, conferring recessive resistance that does not rely on recognizing specific pathogen effectors, unlike dominant R genes. This yields broad-spectrum, durable protection, as in MLO mutants providing

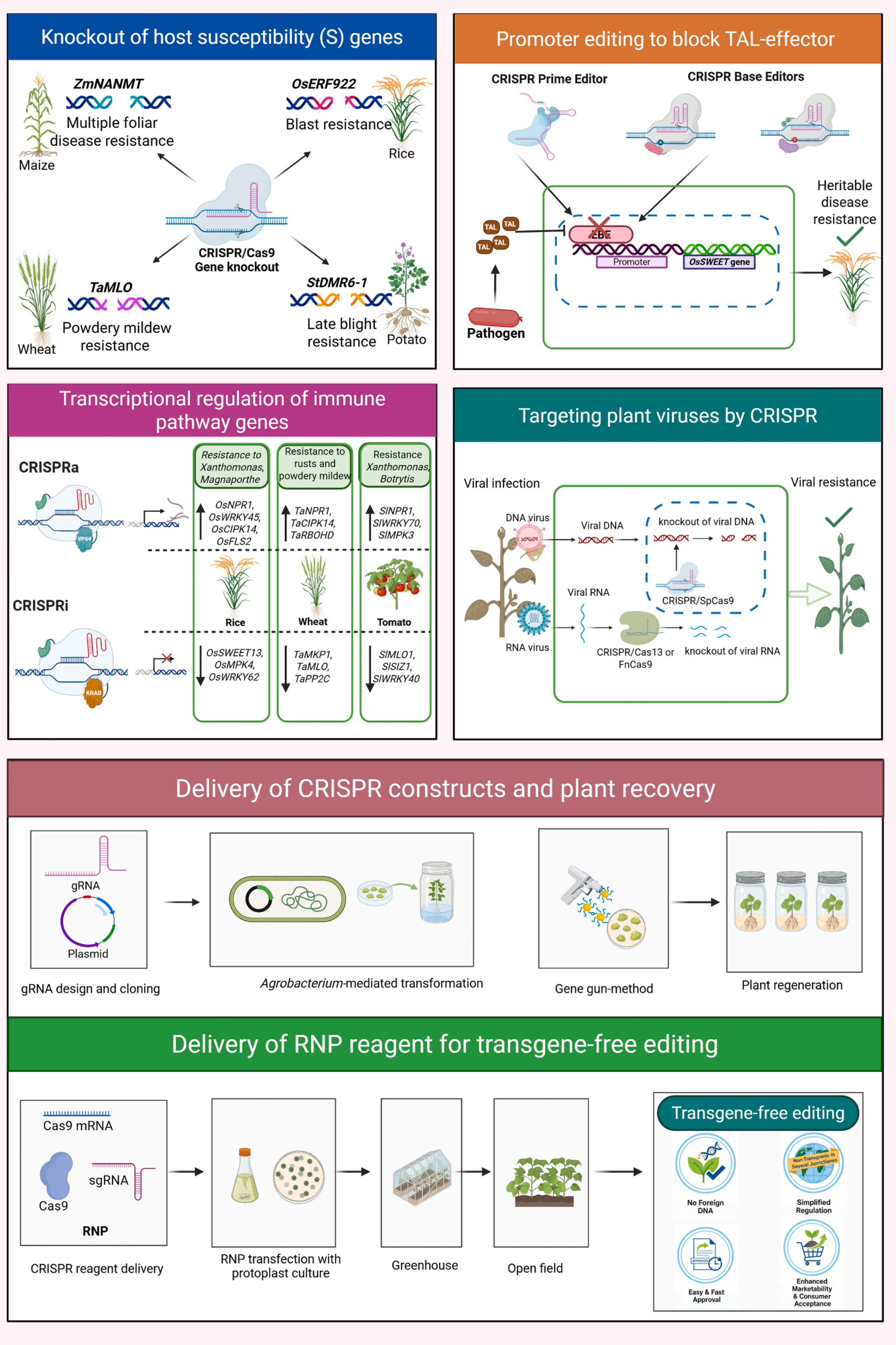


**Fig. 1.** CRISPR-based strategies for engineering plant disease resistance and achieving transgene-free edits. Knockout of susceptibility (S) genes using CRISPR/Cas9 confers broad-spectrum resistance (e.g., *ZmNAMT, OsERF922, TaMLO, StDMR6–1*). Promoter editing disrupts TAL effector binding elements (EBEs) in *SWEET* gene promoters via base or prime editing to block pathogen hijacking. Transcriptional regulation through CRISPRa (activation) or CRISPRi (repression) fine-tunes defense pathways and signaling genes (e.g., *OsWRKY45*, Ta*NPR1, SlNPR1*). CRISPR/Cas9 or Cas13-based systems also target plant DNA and RNA viruses, providing sequence-specific viral resistance. Delivery methods include Agrobacterium-mediated or biolistic transformation and ribonucleoprotein (RNP) transfection for DNA-free, heritable edits. Transgene-free plants offer simplified regulation, consumer acceptance, and faster approval for sustainable crop protection. (Created in www.Biorender.com).

powdery mildew resistance in barley for decades and edited *eIF4E* genes blocking potyvirus replication in melon and cucumber (Thakur et al., 2025). Examples include TALEN-edited *SWEET* promoters in rice for bacterial blight resistance (Li et al., 2025). S gene editing stands out for precision, transgene-free outcomes, and minimal fitness penalties compared to traditional methods, with applications in major food crops including wheat (*TaEDR1* for powdery mildew), tomato (*SlMLO1*), and potato (*CESA3* for late blight). Several studies highlight it as a breakthrough for sustainable breeding, though challenges like pleiotropic effects persist (Garcia-Ruiz et al., 2021; Kusch and Panstruga, 2017; Van Butselaar and Van Den Ackerveken, 2020).

#### 3.1.1. Overcoming trade-offs of the MLO locus

The MLO represents the archetype of S gene-mediated resistance. In wild-type plants, MLO proteins are transmembrane regulators that inhibit vesicle fusion needed for cell wall reinforcement against penetration. Biotrophic powdery mildew fungi (*Blumeria graminis, Erysiphe* spp. in wheat, and *Oidium neolycopersici* in tomato) exploit this suppression for haustorium formation and nutrient extraction, defining S gene dependency (Barka and Lee, 2022; Nekrasov et al., 2017). Early triple knockout (A, B, and D genomes) of wheat *TaMLO* homoeologs conferred robust immunity to *Blumeria graminis* f. sp. *tritici* but induced growth trade-offs, including leaf necrosis and reduced biomass. Recent CRISPR/Cas9 engineering of a 304-kb deletion at the *TaMLO-B1* locus mimics and replicates the natural *Tamlo-R32* allele, decouples resistance from fitness costs. This modification activates the adjacent *TaTMT3B* tonoplast sugar transporter via chromatin remodelling, yielding chlorosis-free plants with sustained yield and broad-spectrum efficacy under field conditions (Li et al., 2022). Similarly, knockout of *SlMLO1* using CRISPR/Cas9 generates stable, transgene-free lines with complete resistance to *O. neolycopersici*. Unlike cereal *mlo* mutants, tomato *Slmlo1* exhibits negligible pleiotropic defects, preserving photosynthesis, fruit quality, and agronomic performance. This facilitates rapid introgression into elite cultivars via marker-assisted or speed-breeding pipelines, positioning *SlMLO1* editing as commercially viable (Nekrasov et al., 2017).

#### 3.1.2. The SWEET transporters and promoter editing

*Xanthomonas oryzae* pv. *oryzae* secretes TALEs that bind specific effector binding elements (EBEs) in the promoters of *SWEET* genes like *OsSWEET11, OsSWEET13*, and *OsSWEET14*, inducing sugar efflux to nourish the bacteria. It promotes bacterial proliferation in the xylem, causing bacterial blight symptoms (Blanvillain-Baufumé et al., 2016; Oliva et al., 2019). Knocking out the coding sequence of the *SWEET* genes is often lethal or may cause severe defects like reduced pollen viability and seed set, making promoter editing preferable to mutate EBEs and block TALE binding while preserving normal gene function (Liu et al., 2024a). Multiplex edits targeting EBEs in *OsSWEET11, OsSWEET13*, and *OsSWEET14* promoters have produced lines with broad-spectrum resistance to diverse *Xoo* strains (Eom et al., 2019). This strategy fundamentally relies on cutting off the pathogen's access to host metabolic pathways. By preventing TALE-induced sugar efflux, the host restricts the carbon supply essential for bacterial nutrition and inoculum reproduction. The fact that multiple TALEs across different *Xanthomonas* species convergently evolve to target *SWEET* family genes underscores the critical necessity of this sugar pathway for successful pathogen colonization (Harris et al., 2023).

#### 3.1.3. Emerging targets in non-cereal crops

In a recent study, researchers used CRISPR/Cas9 for knocking out of *TcNPR3* defense genes. As *NPR3* functions as a negative regulator of the immune system, its knockout upregulated reactive oxygen species pathways and pathogenesis-related proteins, achieving a 42 % reduction in lesion size from *Phytophthora* infection (black pod disease). Notably, edited plants were non-transgenic, establishing a regulatory precedent for tree crops like cacao (*Theobroma cacao*) (Guiltinan et al., 2025). Concurrently, the 'Project Oppotunity' consortium reported successful 2025 field trials in Sweden and Denmark for the starch potato variety, *Kuras*, with *polyphenol oxidase* (*PPO*) knockouts, which redirected secondary metabolite flux to boost antimicrobial phenolics and confer field resistance to late blight (*Phytophthora infestans*). This precision editing accelerates breeding by 8–10 years over conventional methods (Pieterse, 2025). However, the function of *PPO* in immunity is multifaceted and context-dependent; while its knockout conferred resistance in *Kuras*, a subsequent study by Jose et al. (2026) revealed that *PPO*-edited lines of *Désirée* and *Balatoni Rózsa* exhibited increased susceptibility to bacterial wilt (*Ralstonia solanacearum*) and no improvement in late blight (*Phytophthora infestans*) resistance, suggesting that *PPO*-mediated quinone production remains essential for defending against specific bacterial pathogens. These contrasting findings highlight the complexity of metabolic editing and reveal critical trade-offs between reduced browning and pathogen defense that must be navigated in future breeding.

### 3.2. Engineering resistance (R) genes and upstream open reading frames (uORFs) fine-tuning

S gene knockout represents a subtractive strategy that removes or inactivates host S genes exploited by pathogens. Whereas, engineering resistance (R) genes takes an additive approach by introducing or modifying these genes to equip plants with new or enhanced immune receptors, predominantly nucleotide-binding NLR receptors (Zhang and Coaker, 2017). These R genes detect specific pathogen effectors to activate effector-triggered immunity (ETI), often triggering hypersensitive response and defense signaling, which supports resistance breeding, especially when integrated with upstream open reading frames (uORF) fine-tuning for precise expression control (Thordal-Christensen, 2020).

#### 3.2.1. CRISPR-activation (CRISPRa)

CRISPRa systems employ a catalytically dCas9 fused to activators like VP64 to boost expression of weakly expressed defense genes, enhancing pathogen resistance without DNA cuts (McLaughlin et al., 2025). García-Murillo et al. (2023) targeted the *SlPR-1* promoter in tomato via CRISPRa upregulated pathogenesis related gene, reducing the infection and symptoms of bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*), pathogen load, and plant damage while preserving agronomic traits. Similarly, Rivera-Toro et al. (2025) used CRISPRa activation of *SlPAL2*, which drove epigenetic changes like H3K4me3 deposition, boosting lignin in cell walls to block bacterial canker ingress more effectively.

#### 3.2.2. Translational control via uORFs

A continual difficulty in resistance breeding pertains to the growth-defense trade-off, wherein improved disease resistance frequently limits plant growth and yield due to the costs associated with resource allocation. Studies indicate that this constitutes a significant barrier in crops, as immune activation incurs fitness costs under normal conditions (Hou and Xu, 2025). In a recent study, researchers targeted uORFs in the 5′ UTR of genes to fine-tune translation without constitutive overexpression. This approach relieves inhibitory effects on downstream main ORFs, enabling controlled gene expression (Sang et al., 2025). Hao et al. (2025) utilized CRISPR/Cas9 editing of uORFs in the *BnVTC2* gene, which is involved in vitamin C production, to enhance ascorbate concentrations in rapeseed (*Brassica napus*). This enhanced tolerance to abiotic stimuli, such as oxidative stress, serves as an indicator of biotic defense mechanisms, while overcoming yield penalties observed in overexpression lines.

This suggests that the combined use of R gene engineering, uORF fine-tuning, and CRISPRa provides accurate, fitness-preserving approaches to address growth-defense trade-offs in resistance breeding. These methods provide flexible immunity, hence fostering the development of resilient crops.

### 3.3. Direct viral interference

CRISPR functions as a bacterial adaptive immune system that prokaryotes utilize to protect against viral attacks by incorporating fragments of historical viral DNA into their genome. Upon the entry of an associated virus, CRISPR transcribes these fragments into guide RNAs (crRNAs) that instruct Cas proteins to identify and cleave the viral genome. Upon viral entry, the Cas enzyme, guided by the crRNA, swiftly attaches to the complementary viral DNA or RNA sequence and destroys it through nuclease activity, thereby inhibiting replication. This direct degradation emulates innate immunity but is sequence-specific and heritable, providing precise antiviral protection regardless of the host's native defenses (Tang et al., 2021).

This adaptable, sequence-specific cleavage mechanism has been effectively repurposed in plants to combat persistent viral infections, utilizing nuclear-localized Cas endonucleases for DNA viruses and cytoplasmic versions for RNA viruses. In crops such as tomato and tobacco, designed CRISPR systems specifically target critical viral components during replication, resulting in significant reductions in viral titer without off-target impacts on the host genome. Geminiviruses, (Tomato Yellow Leaf Curl Virus, TYLCV) encapsulate single-stranded DNA but convert it to double-stranded DNA (dsDNA) intermediates within the host nucleus for rolling-circle replication, rendering them susceptible to nuclear-localized Cas9. Constitutive expression of Cas9 (e.g., through the CaMV 35S promoter) when combined with sgRNAs directed at the Rep gene (a replication initiator protein crucial for cleaving viral DNA) or the intergenic region (IR, which harbors bidirectional promoters and origins) effectively cleaves these double-stranded DNA forms, reducing viral titers up to 90 % in tomato protoplasts and transgenic plants (Faal et al., 2020; Maio et al., 2020).

Shifting to RNA viruses, Cas13 variants offer cytoplasmic targeting. Cas13d/CasRx revolutionized RNA virus resistance in plants, with robust interference against viruses like Turnip mosaic virus in *Nicotiana benthamiana*, outperforming other Cas13 variants. RNAi suppression by viral proteins is common, making Cas13 alternatives appealing (Cao et al., 2021). CasRx exemplifies this advance through its unique properties. CasRx provides HEPN-dependent RNA cleavage without PFS requirements, often stronger than RNAi in transient/stable plant assays. Still, the GVA claim overstates unverified specifics (Mahas et al., 2019).

### 3.4. Understanding plant-pathogen interaction by CRISPR/Cas tools

Pathogens infect plants by using effector molecules that lower the plant's immune system and change the way the plant's metabolism works to get nutrients. Plants fight back with a variety of defense responses, such as antimicrobial compounds, strengthening their cell walls, and stress-signaling pathways. Omics-based technologies have enabled the identification of R-genes, S genes, and effector targets, providing a foundation for CRISPR/Cas-mediated functional validation. These include next-generation sequencing (Arafa et al., 2017), comparative genomics (Nepal et al., 2017), RNA-seq (Ma et al., 2019; Zhang et al., 2019b), proteomics (Prabhukarthikeyan et al., 2017, 2019), and the use of mutant gene resources (O'Malley et al., 2015). These technological developments have helped translate research into practical crop improvements.

The CRISPR/Cas toolbox is an innovative, rapid, precise, and efficient tool for addressing challenges in agriculture and elucidating plant-pathogen interactions in crops. CRISPR/Cas tools facilitate targeted disruption of host or pathogen genes, promoter editing to modify effector-binding elements, and tagging of immune proteins for in vivo interaction studies (Fig. 1). It aids in fundamental studies on plant–pathogen interactions, facilitates the identification of pathogen virulence genes, helps unravel resistance and susceptibility factors in host plants, and enables genome engineering for developing host resistance (Karmakar et al., 2022). By enabling both single and multiplex gene knockouts, as well as large-scale mutant library construction, CRISPR/Cas platforms provide a powerful means to accelerate functional genomics of plant immunity and pathogen virulence. Systematic targeting of key players involved in plant–pathogen interactions, such as resistance gene families, receptor-like kinases, transcription factors, and pathogen-responsive genes, will help in the identification and functional characterization of major defense regulators (Gosavi et al., 2020).

## 4. CRISPR/Cas systems for identification and validation of disease-resistant and susceptible genes

CRISPR/Cas systems serve as powerful tools to enhance broad-spectrum disease resistance in crops by enabling the functional validation and characterization of candidate genes that confer resistance or susceptibility (Fig. 1). *ZmACD6* was found in maize and shown to be the same as *Arabidopsis* ACD6 (ACCELERATED CELL DEATH 6). It works with salicylic acid (SA) in a positive feedback loop to make the plant more resistant. CRISPR/Cas9-mediated mutagenesis confirmed that *ZmACD6* positively regulates resistance to *Ustilago maydis* (Zhang et al., 2019c). Fusarium head blight (FHB) is a devastating disease of cereals, particularly wheat and barley, causing severe yield losses and reduced grain quality. In *Arabidopsis*, the *DMR6* (*Downy Mildew Resistance 6*) gene encodes a putative 2-oxoglutarate Fe(II)-dependent oxygenase (*2OGO*) and functions as a susceptibility factor to downy mildew. Using CRISPR/Cas9-mediated knockout, the barley *2OGO* gene (*Hv2OGO*) was validated as a functional orthologue of *Arabidopsis DMR6* in mediating susceptibility to FHB, thereby providing a molecular basis for proposing *2OGO* as a plant immunity suppressor in *Arabidopsis* and potentially in barley, and establishing a rationale for enhancing FHB resistance through its targeted manipulation (Low et al., 2020). Recent advancements in CRISPR technology have enabled the use of CRISPRa systems to generate gain-of-function mutations through targeted upregulation of endogenous genes. By precisely enhancing the expression of genes associated with plant immunity, CRISPRa offers a powerful strategy for improving disease resilience in crops. Its high specificity and tunable control over gene expression make it particularly valuable for modulating defense pathways without adversely affecting plant growth and development.

### 4.1. CRISPR/Cas for resistance against fungal pathogens

Based on current knowledge of the molecular pathways involved in plant-pathogen interactions, various techniques have been developed to enhance fungal resistance in plant species. These approaches focus on identifying key candidate genes and gene products that contribute to plant defense against fungi, making them prime targets for CRISPR/Cas system-based gene editing (Borrelli et al., 2018). Fungi, the primary root causes of plant diseases, substantially affect agricultural production. Their diverse life cycles and high genetic adaptability make disease management challenging, as they can quickly infect new hosts, overcome R-gene-mediated resistance, and develop resistance to fungicides (Doehlemann et al., 2017).

Recent advances in CRISPR/Cas systems targeting host S- genes have enabled the development of genetically recessive, durable disease resistance in plants (Fig. 1). S- genes are plant genes exploited by pathogens to facilitate host recognition, penetration, nutrient acquisition, proliferation, and spread, or to suppress host immune signaling (Huang et al., 2023). Powdery mildew, a pervasive fungal disease in wheat, is developing wheat varieties with durable and broad-spectrum resistance, which is highly desirable. A breakthrough in breeding for such comprehensive and long-lasting resistance to powdery mildew was the identification of barley MLO mutants (Lyngkjær et al., 2000). Extensive research has focused on negative regulators and S genes involved in defense pathways. For instance, knocking out the wheat homologs of the *MLO* gene (*TaMLOs*) using the CRISPR/Cas9 system rendered wheat resistant to the fungus *Blumeria graminis* f. sp. *tritici*, which causes powdery mildew (Wang et al., 2014). Rice blast, caused by *Magnaporthe oryzae*, is among the most destructive diseases impacting rice production worldwide (Dean et al., 2012). Additionally, the ethylene responsive factors (ERFs) within the APETALA2/ERF (AP2/ERF) superfamily play a crucial role in rice's response to various biotic and abiotic stresses (Mizoi et al., 2012). Several studies on major food crops have been conducted worldwide (Table 1), where CRISPR/-Cas systems (mostly the CRISPR/Cas9 system) were used to functionally knock out fungal disease susceptibility genes, leading to the development of new disease-resistant breeding materials. Most of the studies have been conducted in rice, wheat, potato, and soybean, with a few in maize. However, no reports were found on CRISPR/Cas-mediated knockout of susceptibility genes in sorghum, pearl millet, or finger millet.

### 4.2. CRISPR/Cas for resistance against bacterial pathogens

Traditional management of bacterial diseases in crops relies on genetic resistance, agronomic practices, and biocontrol agents (Kerr, 2016). However, these approaches often fail to provide durable protection due to the rapid evolution and high genetic diversity of bacterial pathogens. CRISPR/Cas-based genome editing offers a precise means to engineer host resistance by targeting S genes or enhancing defense pathways (Fig. 1). One of the most prevalent rice diseases is bacterial blight, caused by the vascular pathogen *Xanthomonas oryzae* pv. *Oryzae*. This disease typically results in yield reductions of 10–20 % (Ou, 1985), but under conditions favorable to the pathogen, such as high humidity, losses can exceed 50 %, sometimes causing complete crop failure (Mew et al., 1993). *OsSWEET14*, a sugar transporter gene, serves as a key S-gene for bacterial blight. Zeng et al. (2020) used CRISPR/Cas9-mediated knockout of *OsSWEET14* in rice cultivar *Zhonghua 11* and demonstrated that disrupting *OsSWEET14* in this genetic background confers strong resistance to both the African *Xanthomonas oryzae* pv. *oryzae* strain AXO1947 and the Asian strain PXO86, while also resulting in increased plant height without any yield trade-off. Enhanced resistance against bacterial blight in rice was also achieved through base editing, utilizing both ABE and CBE to target the *SWEET14* gene, leading to its reduced expression and subsequent reduction in disease severity (Li et al., 2025). In potatoes, zebra chip disease caused by *Candidatus Liberibacter solanacearum* leads to substantial economic losses worldwide. Non-expressors of pathogenesis-related (NPR) proteins function as receptors of the defense hormone salicylic acid (SA), and downregulation of *NPR3* has been associated with enhanced resistance to bacterial and fungal pathogens. Ramasamy et al. (2024) demonstrated that CRISPR/Cas9-mediated genome editing of *StNPR3* conferred resistance to zebra chip disease in potatoes by activating SA-mediated defense responses and promoting jasmonic acid (JA) catabolism. Together, these studies demonstrate how CRISPR/Cas-mediated editing of susceptibility regulators, such as *SWEET* transporters and *NPR* genes, can provide durable, non-transgenic resistance to bacterial pathogens in major food crops.

**Table 1**
List of CRISPR/Cas studies conducted on major food crops on fungal disease resistance.

| SN | Crop | Targeted Gene/ region | Target Disease | Reference |
|---|---|---|---|---|
| 1 | Rice | *Pi21* | Blast | Wang et al., 2016a |
| 2 | Rice | *OsERF922* | Blast | Wang et al., 2016b |
| 3 | Rice | *Bsr-d1, Pi21 and ERF922* | Blast | Zhou et al., 2022 |
| 4 | Rice | *Pi21* | Blast | J Yang et al., 2023 |
| 5 | Rice | *Bsr-d1* | Blast | Zhang et al., 2024 |
| 6 | Rice | *OsS5H* | Broad-spectrum disease resistance | Liu et al., 2023a |
| 7 | Wheat | *TaMLO* | Powdery mildew | Wang et al., 2014 |
| 8 | Wheat | *TaEDR1* | Powdery mildew | Zhang et al., 2017 |
| 9 | Wheat | *TaPsIPK1* | Stripe rust | Wang et al., 2022 |
| 10 | Wheat | *TaCIPK14* | Stripe rust | He et al., 2022 |
| 11 | Wheat | *MLO-B1* | Powdery mildew | Li et al., 2022 |
| 12 | Wheat | *TaMKP1* | Stripe rust and powdery mildew | Liu et al., 2024b |
| 13 | Maize | *ZmNANMT* | Southern leaf blight, Northern leaf blight, Fusarium stalk rot | Li et al., 2023 |
| 14 | Potato | *StNRL1* | Late blight | Nourozi et al., 2024 |
| 15 | Potato | *StDMR6–1* | Late blight | Karlsson et al., 2024 |
| 16 | Potato | *StMC7* | Late blight | Poudel et al., 2025 |
| 17 | Potato | *StSR4* | Late blight | Moon et al., 2022 |
| 18 | Potato | *StDND1, StCHL1, StDMR6–1* | Late blight | Kieu et al., 2021 |
| 19 | Potato | *StERF3* | Late blight | Razzaq et al., 2022 |
| 20 | Soybean | *GmMLO* | Powdery mildew | Bui et al., 2023 |
| 21 | Soybean | *GmARM* | Phytophthora root rot | Luo et al., 2024 |
| 22 | Soybean | *GmTAP1* | Phytophthora root rot | Liu et al., 2023b |

### 4.3. CRISPR/Cas for resistance against plant viruses

Globally, major agricultural crops suffer substantial yield losses due to severe diseases caused by diverse plant viruses that disrupt normal physiological processes, thereby reducing both yield and produce quality. With estimated annual economic losses of approximately US $30 billion, plant viral diseases represent a major concern for farmers, researchers, and policymakers alike, owing to their profound impact on agricultural productivity, food security, and long-term sustainability (Sharma, 2023; Tatineni and Hein, 2023).

Plant pathologists are increasingly exploring CRISPR/Cas systems to combat viral diseases in both host plants and pathogens (Dort et al., 2020). Plant virus pathosystems exemplify host–pathogen interactions that can be dissected through gene-targeting approaches, where CRISPR/Cas systems are employed to disrupt viral genomes or host susceptibility factors (Ali et al., 2016; Zhang et al., 2018). CRISPR/Cas systems have been employed in two major ways to engineer virus resistance: (i) direct targeting and cleavage of viral genomes (DNA viruses by Cas9/Cas12 and RNA viruses by Cas13) (Fig. 1), and (ii) editing host S genes essential for viral replication, movement, or transmission. Cas9 and Cas12 systems have been primarily used to target DNA viruses such as geminiviruses, while Cas13 effectors target RNA viruses like CMV, TMV, and TuMV, offering both preventive and curative potential. Different S gene knockout studies mediated by CRISPR/Cas systems were carried out in maize, Potato, cassava, and barley (Table 2). These non-GMO mutants are highly specific, involve no foreign DNA, and enable precise edits within the native genome, reducing unintended effects (Kanchiswamy, 2016). Additionally, CRISPR/Cas system-mediated engineering of pathogen resistance in plants offers a promising approach to reduce disease outbreaks while also aiding in the development of avirulent strains and improving our understanding of plant-pathogen interactions (Dort et al., 2020).

Prior to the advent of genome editing, pathogen-derived resistance, such as transgenic expression of viral coat proteins, replicase genes, or RNA sequences, was extensively employed to enhance plant virus resistance (Das et al., 2023). Viral RNA genomes cannot be directly

**Table 2**
List of CRISPR/Cas studies conducted on major food crops on bacterial disease resistance.

| SN | Crop | Targeted Gene/region | Target Disease | Reference |
|---|---|---|---|---|
| 1 | Maize | *ZmGDIα* | Maize rough dwarf disease | Liu et al., 2022 |
| 2 | Potato | *coilin* | Potato virus Y | Makhotenko et al., 2019 |
| 3 | Potato | *eIF4E* | Potato virus Y | Noureen et al., 2022b |
| 4 | Cassava | *MeSWEET10a* | Bacterial blight | Elliott et al., 2024 |
| 5 | Cassava | *eIF4E isoforms nCBP-1 and nCBP-2* | Brown streak disease | Gomez et al., 2018 |
| 6 | Barley | *MP, CP, Rep/RepA, LIR* | Wheat dwarf virus disease | Kis et al., 2019 |
| 7 | Barley | EIF4E | Yellow mosaic virus disease | Hoffie et al., 2021 |
| 8 | Barley | *HvPDIL5–1* | Yellow mosaic virus disease | Cheng et al., 2022 |

targeted by traditional CRISPR/Cas9 systems, as these typically cut double-stranded DNA. However, the development of novel CRISPR/Cas9 variants capable of targeting RNA offers new possibilities for engineering plants resistant to RNA viruses. For instance, scientists have used CRISPR/Cas13a to develop potato plants that are resistant to viruses. Transgenic potato lines expressing Cas13a/crRNA constructs exhibited reduced accumulation of potato virus Y (PVY) and attenuated disease symptoms (Zhan et al., 2019). In a similar approach, CRISPR/Cas13 systems have conferred broad-spectrum resistance against multiple PVY strains in potato (Noureen et al., 2022a), while Cas13d-mediated multiplex RNA targeting has been shown to provide broad-spectrum protection against diverse RNA viruses in potato (Zhan et al., 2023). In soybean, CRISPR/RfxCas13d (CRISPR/CasRx)-mediated resistance was reported against the soybean mosaic virus (Gao et al., 2024). These advanced CRISPR/Cas technologies provide promising alternative strategies for breeding crops with enhanced resistance to biotic stresses, facilitating the development of resistant germplasm and accelerating improvements in crop productivity.

## 5. Ethical issues and regulations in food crop gene-editing

By enhancing agricultural traits and minimizing waste, plant gene editing via CRISPR offers a range of solutions to food security and climate resilience. However, it also raises complex social and ethical concerns that must be addressed alongside scientific advancements (Venkataraman and Hefferon, 2025). Public perception plays a crucial role in the acceptance of gene-edited crops. Recent studies suggest that consumers tend to view gene-edited foods more favorably than transgenic genetically modified organisms (GMOs), particularly in terms of perceived safety and ethical considerations (Bearth et al., 2022; Yang and Hobbs, 2020). Many regard gene editing as a climate-smart technology, but safety concerns, particularly regarding off-target effects and the potential use of gene drives, remain unresolved (Chavhan et al., 2025). Proponents contend that democratized access to gene editing has diminished corporate supremacy due to lower development costs and regulatory hurdles (Hefferon and Herring, 2017). On the other hand, detractors highlight patent schemes that strengthen corporate dominance over agricultural methods and seeds. Words like ‘precision’ and ‘editing’ have the potential to minimize moral dilemmas and influence public opinion (Helliwell et al., 2019). What risks CRISPR-edited crops offer if the ultimate products of modified plants are transgene-free is a crucial topic. This also applies to some genetically modified products, including canola oil. In the European Union (EU) nations, gene-edited crops were historically regulated under the general GMO framework, which, following the 2018 Justice of the European Union (CJEU) ruling on mutagenesis, placed most gene-edited plants in the same category as GMOs and fueled public resistance to agricultural biotechnology. However, the regulatory landscape shifted with the provisional political agreement on plants produced by certain new genomic techniques in late 2025, introducing a two-tier system that distinguishes New Genomic Techniques (NGT), ‘conventional-like’ NGT-1 plants, treated similarly to conventionally bred plants, from NGT-2 plants, which remain subject to standard GMO rules (Anonymous, 2025a; Zimny and Sowa, 2021). The potential threats to human health and the environment remain central drivers of the primary sources of public concern. Because gene-edited goods might appear just as the non-edited ones; hence, clear labeling is essential to safeguarding consumer rights. To preserve the uniqueness of organic farming, agroecological organizations advocate for more defined boundaries between it and gene editing (Woźniak-Gientka et al., 2022). In support of responsible innovation aligned with broader societal values, recent policy discussions have emphasized the need for non-safety assessments. These assessments would consider the ethical, social, and sustainability implications of gene-edited crops before deregulation (Myskja and Myhr, 2020; Turnbull et al., 2021).

### 5.1. *Sustainability in food crops gene editing*

CRISPR/Cas gene editing has potential for sustainable agriculture by reducing the usage of pesticides, increasing disease resistance, increasing yields, and improving tolerance to drought and other environmental stresses (Purohit et al., 2026). Furthermore, it can improve marketability, shelf life, and nutritional quality, and can also play a significant role in reducing environmental waste and leaving a smaller environmental footprint by enhancing the qualitative features and shelf-life of postharvest crops (Shipman et al., 2021). Consequently, a growing number of gene-edited crops have successfully navigated regulatory pathways to reach the market, as detailed in Table 3.

Since sustainability is a dynamic and evolving process rather than a fixed goal, continuous evaluation of gene-editing outcomes is essential to ensure they align with long-term ecological and societal objectives (Keiper and Atanassova, 2022; Shan, 2024). Large -scale monocultures and farming systems that may be detrimental to the environment, human health, and food accessibility may be sustained and intensified by the commercial dominance that agri-biotech companies have over industrial agriculture systems, farmers, and consumers. Regulations regarding patents could address issues such as environmental degradation and monocultures (Houvener, 2024; Khatoon, 2025).

Achieving sustainability requires a careful balance between ecological preservation and the needs of future generations. Mepham's matrix and other ethical frameworks offer direction but may overlook critical socioeconomic dimensions (Dassler and Myhr, 2021). Environmental protection, social and economic aspects of sustainability, and the connections between them must be considered when managing gene-editing breakthroughs, as research on gene editing and its commercial use is expected to grow rapidly in the near future. To ensure fair, responsible, and inclusive use of gene editing technology, regulatory policies must evolve to incorporate environmental, ethical, and economic considerations (Wray-Cahen et al., 2022).

### 5.2. *Maintaining biodiversity*

Biodiversity is essential for sustainable development because diversity at genetic, species, and ecosystem levels underpins resilient food systems, ecosystem services, and long-term human well-being. Novel molecular breeding approaches, such as CRISPR/Cas systems, offer promising tools to enhance or restore genetic diversity and support conservation and genetic rescue efforts for vulnerable and endangered species. However, modern plant breeding that has prioritized a narrow set of trait-specific crop varieties has often led to the replacement of diverse local landraces and a narrowing of the cultivated gene pool, contributing to genetic erosion and the loss of traditional crop varieties (Khoury et al., 2021; Thakur et al., 2024; Yin et al., 2024). Concerns

**Table 3**
Global regulatory frameworks and approved genome-edited crops by continent and country.

| Continent | Country | Regulatory Agencies | GMO Commercial Cultivation Area (Million Hectares) (2024) | Approved Genome Edited Crops | Approved Year | Regulation Governing the Release of Gene-Edited Crops | SDN-1 | SDN-2 | SDN-3 | References |
|---|---|---|---|---|---|---|---|---|---|---|
| North America | US | United States Department of Agriculture (USDA); Animal and Plant Health Inspection Service (APHIS); Food and Drug Administration (FDA); and Environmental Protection Agency (EPA) | 75.4 | Rapeseed/ Canola<br>Non-browning Mushroom<br>Innate (non-browning) Potato<br>Drought-resistant Maize<br>High-oleic Soybean<br>Less Pungent Mustard Greens<br>Non-browning Lettuce | 2014<br>2016<br>2016<br>2016<br>2019<br>2023<br>2024 | Coordinated Framework for Regulation of Biotechnology (1986, updated 2017, 2020 SECURE Rule); USDA exempts SDN-1 via 7 CFR Part 340 if no plant pest risk | Deregulated | Case-by-case/ Deregulated | Regulated as GMO | Ledesma and Van Eenennaam, 2024; SeedWorld, 2025; Vengadesen et al., 2025; Global Gene Editing Regulation Tracker, Genetic Literacy Project, 2024 |
| | Canada | Canadian Food Inspection Agency (CFIA) | 11.7 | Non-browning Potato<br>Non-browning Apple | 2015<br>2017 | Plants with Novel Traits Regulations (novel trait-based, no method-specific); SDN-1 treated as conventional; Directive94–08 (Dir94–08) | Deregulated/ Novelty-based Regulation | Deregulated/ Novelty-based Regulation | Regulated as GMO/ Novelty-based Regulation | Vengadesen et al., 2025; Global Gene Editing Regulation Tracker, Genetic Literacy Project, 2024 |
| South America | Argentina | National Advisory Commission on Agricultural Biotechnology (Comisión Nacional de Biotecnología Agropecuaria) (CONABIA) | 23.8 | Drought Resistant Wheat<br>High-fiber Wheat | 2020<br>2025 | Resolution No.173/15 (2015) | Deregulated (Case-by-case) | Deregulated (Case-by-case) | Regulated as GMO | Ledesma and Van Eenennaam, 2024; Neocrop, 2025; Nature Biotechnology, 2021; Orroño and Vesprini, 2018; SeedWorld, 2025 |
| | Brazil | Commission for Biosafety (CTNBio) | 67.9 | Drought Tolerant Soybean<br>Sugarcane | 2023<br>2022 | Normative Resolution No.16 (2018) (RESOLUÇÃO NORMATIVA N° 16, 2018) | Deregulated (Case-by-case) | Deregulated (Case-by-case) | Regulated as GMO | Court of Justice of the European Union, 2018; Entine et al., 2021; ISAAA, 2023; Kumar et al., 2024; Ledesma and Van Eenennaam, 2024; SeedWorld, 2025 |
| | Chile | Ministry of Agricultural and Livestock Services (SAG); National Service of Agri-Food Health and Quality (Servicio Nacional de Sanidad y Calidad Agroalimentaria) (SENASA) | <1 | High-fiber Wheat | 2025 | Introduction of methodological procedure (2017) | Deregulated (Case-by-case) | Deregulated (Case-by-case) | Regulated as GMO | AgbioInvestor, 2025; ChileBio, 2025; Sánchez, 2024; Zarate et al., 2023 |
| | Colombia | Colombian Agricutural Institute (ICA) | 0.1 | Drought Tolerant Soybean | 2023 | Resolution No. 00,029,299 (2018) | Deregulated (Case-by-case) | Deregulated (Case-by-case) | Regulated as GMO | Entine et al., 2021; Global Gene Editing Regulation Tracker, Genetic Literacy Project, 2024; Kuiken and Kuzma, 2021; Zarate et al., 2023 |

(*continued on next page*)

**Table 3** (*continued*)

| Continent | Country | Regulatory Agencies | GMO Commercial Cultivation Area (Million Hectares) (2024) | Approved Genome Edited Crops | Approved Year | Regulation Governing the Release of Gene-Edited Crops | SDN-1 | SDN-2 | SDN-3 | References |
|---|---|---|---|---|---|---|---|---|---|---|
| | Honduras | National Committee of Biotechnology and Biosecurity (NCBB); National Service of Agri-Food Health and Quality (Servicio Nacional de Sanidad y Calidad Agroalimentaria) (SENASA); Ministry of Agriculture and the National Biosafety Committee (CTNBio) | <1 | No approved crops | - | Acuerdo No. 1570–98 and Agreement for genome editing SENASA C.D. -008–2019 | Deregulated (Case-by-case) | Deregulated (Case-by-case) | Regulated as GMO | Gatica-Arias, 2020; Zarate et al., 2023 |
| Asia and the Pacific | China | National Biosafety Committee (NBC), Ministry of Agriculture and Rural Affairs (MARA) | 3.5 | Fungal Resistant Wheat<br>High Oleic acid Soybean<br>Wheat<br>Rice | 2024<br>2023<br>2025<br>2025 | Administrative rules for safety of agricultural GMOs, Guidelines issued by MARA (2022) | Simplified Regulation | Simplified Regulation | Regulated as GMO | AgbioInvestor, 2025; Feed Strategy, 2025; Global Gene Editing Regulation Tracker, Genetic Literacy Project, 2024; Reuters, 2023; Sprink et al., 2022; Vengadesen et al., 2025; Zhu, 2022 |
| | India | Genetic Engineering Appraisal Committee (GEAC); and Ministry of Environment, Forest and Climate Change (MoEFCC) | 11.2 | Improved Drought and Salt Tolerance Rice | 2025 | Regulatory Framework and Guidelines for Risk Assessment (2022); 2022 MoEFCC OM (SDN-1/2 exempt from GMO rules) | Deregulated | Deregulated | Regulated as GMO | AgbioInvestor, 2025; Priyadarshini, 2025; Tachikawa and Matsuo, 2024 |
| | Japan | The Ministry of Agriculture, Forestary, and Fishries (MAFF); Ministry of Health, Labour and Welfare (MHLW); Ministry of the Environment (MOE); and Consumer Affairs Agency (CAA) | - | GABA Tomato<br>Waxy high starch Corn | 2021<br>2024 | GMO as defined under the Japanese Cartagena Act (Act No. 97 of 2003) (2014) | Deregulated | Case-by-case (foreign DNA)/ Regulated | Regulated as GMO | Global Gene Editing Regulation Tracker, Genetic Literacy Project, 2024; Sakurai et al., 2022; Tachikawa and Matsuo, 2024 |
| | Pakistan | Ministry of Climate Change and Environmental Coordination (MoCC&EC); National Biosafety Committee (NBC); and Pakistan Environmental Protection Agency (Pak-EPA) | 1.9 | Sugarcane<br>Herbicide Tolerance and Bollworm Resistance Cotton | 2025<br>2025 | Pakistan National Biosafety Rules, 2005 | Under development | Under development | Regulated as GMO | AgbioInvestor, 2025; Babar et al., 2019; Jones et al., 2022; PDI, 2025 |
| European Union | Only Spain and Portugal | National Biosafety Commission (CNB); and Directorate General for Food and Veterinary (Direção-Geral de Alimentação e Veterinária) (DGAV) | <1 | No approved crops | - | Directive18/2001/EC (2001) and court decision in case C-528/16; 2025 NGT Regulation (Category 1 exempt, appl. 2028). | Regulated | Regulated | Regulated as GMO | AgbioInvestor, 2025; European Commission, 2021; European Parliament, 2024; Vengadesen et al., 2025 |
| Oceania | Australia | Office of the Gene Technology Regulator (OGTR) | 1.4 | No approved crops | - | Gene Technology Act 2000 (am. 2019); SDN-1 exempt from GMO rules | Deregulated/ Case-by-case | Regulated | Regulated as GMO | AgbioInvestor, 2025; Ivanov et al., 2025; Pacillo, 2025; Tachikawa and Matsuo, 2024 |
| | New Zealand | Environmental Protection Authority (EPA) | - | No approved crops | - | Hazardous Substances and New Organisms Act (HSNO) (1996); Gene Tech Bill (2025, low-risk exemptions via Schedule 3A) | Regulated/ Case-by-case | Regulated/ Case-by-case | Regulated as GMO | New Zealand Legislation, 2024; Schmidt et al., 2020 |

*Note*: SDN (Site-Directed Nuclease) genome editing creates targeted DNA modifications using programmable nucleases (e.g., CRISPR/Cas9, TALENs, ZFNs), as mentioned previously in this paper, that induce double-strand breaks repaired by endogenous cellular mechanisms. SDN-1: Editing without a donor template; breaks are repaired via Non-Homologous End Joining (NHEJ), leading to random small indels; resulting variants are often indistinguishable from natural mutations. SDN-2: Editing with a small donor template; breaks are repaired via Homology-Directed Repair (HDR) to induce precise nucleotide substitutions or small insertions (mimicking allele conversion); typically uses short DNA templates homologous to the target site. SDN-3: Editing with a large DNA template; involves the site-specific insertion of large sequence stretches (e.g., full genes or promoters) via HDR; often results in the integration of foreign (transgenic) or cisgenic DNA; GMO: Genetically Modified Organisms.

have been raised that the deployment of modern, particularly transgenic, crop varieties in biodiversity hotspots may have direct effects on non-target species and that transgenes can move via gene flow into landraces and wild relatives, potentially altering or eroding their genetic diversity (Tobón-Niedfeldt et al., 2022; Warwick et al., 2009).

The degree of transgene integration in the local gene pool is influenced by several processes and variables, including genetic drift, the degree of selective advantage, the rate of migration, epistatic effects, and the interaction between genotype and environment (Barton, 2016; Simard, 2010). Many domestication-related traits involve recessive alleles that primarily adjust crops to cultivated environments. In contrast, transgenic traits related to insect resistance can modify pests' natural enemy interactions and non-target biodiversity. Organic farming communities, therefore, argue that genome editing technologies may disrupt natural evolutionary dynamics, although the extent of these impacts remains uncertain (Mmbando and Ngongolo, 2024).

### 5.3. *Upholding social justice*

Gene editing raises significant social justice concerns, particularly regarding equitable access to technology and its benefits across different socioeconomic groups. Plant breeders' rights, established under frameworks like the UPOV Convention, aim to compensate breeders for their innovations while still allowing farmers to legally cultivate protected varieties (Anonymous, 2024; Louwaars and Jochemsen, 2021). Although the purpose of plant breeders' rights was to equitably reward innovation, ongoing discussions concerning patents, particularly in relation to gene-edited crops, highlight issues of monopolization and restricted access (Anonymous, 2025b).

Although patent dominance is lessened by European frameworks such as *sui generis* systems and exemptions, ownership of breeding techniques is still permitted (Euroseeds, 2023; Jiang, 2020). Indeed, gene editing has resulted in a significant increase in the number of patents in recent years. This trend has sparked concerns regarding possible social costs stemming from the monopolization of intellectual property, which may affect the profitability aspect of reaching sustainability objectives (Feeney et al., 2021; Geissler et al., 2024). Current intellectual property rules offer only partial solutions, frequently limited by voluntary licensing and bureaucracy. Therefore, as a more open and respectable substitute for ethical licensing, a government-run patenting system was suggested (Scheinerman and Sherkow, 2021). Proposals to remedy the situation include reforming the TRIPS Agreement, establishing a WTO ethics advisory board, and implementing a government-led patent classification system. By taking these actions, the global governance of gene-editing technologies is intended to be equitable, moral, and inclusive (Feeney et al., 2021).

### 5.4. *Obligations of breeders and corporates for food crop genome editing*

Beyond legal compliance, breeders and corporations engaged in plant gene editing bear ethical responsibilities encompassing social justice, environmental stewardship, and corporate accountability (Venkataraman et al., 2025). Although corporate conduct is guided by voluntary norms set by, The Organization for Economic Co-operation and Development, Paris, France, discussions between enforceable obligations and soft-law methods continue. Transparency in breeding practices and labeling becomes especially important when gene editing leaves little to no detectable traces in final products (Woźniak-Gientka et al., 2022). Recent studies emphasize that without clear traceability, consumer trust may be eroded (Spök et al., 2022).

Although breeders encounter challenges when operating in nations with unclear gene-editing rules, organic and biodynamic farming ideologies advocate moral integrity in breeding techniques. Given the technical difficulty of detecting gene-edited traits, supply chain transparency is essential. Tools like private labeling and certification can safeguard consumer rights and encourage educated decision-making

(Spök et al., 2022). Beyond risks, ethical considerations also include moral duty and larger society values. Building public trust in biotechnology requires adherence to ethical frameworks rooted in sustainability, fairness, and precaution, as well as inclusive, continuous communication among stakeholders (Chhikara and Maurya, 2024).

### 5.5. *Regulations pertaining to gene-editing*

Gene editing in food crops can make it difficult to strike a balance between safety and innovation when focusing on process-based versus product-based regulation (Macnaghten and Habets, 2020). CRISPR/Cas technology modifies DNA without introducing foreign genes, prompting questions about the need to waive stringent biosafety regulations. Regulatory approaches vary globally, creating a diverse landscape where countries are categorized by their acceptance of transgene-free edits. This global divergence is illustrated in Fig. 2. Several nations, including the US, Canada (under novelty-based regulation), Brazil, Australia, India, and Argentina, have determined that laws governing GMOs will not apply to specific categories of plants developed from genome editing methods (such as Site-Directed Nuclease (SDN) SDN-1 or SDN-2), provided they do not include foreign DNA (Entine et al., 2021). In contrast, the European Court of Justice (ECJ) ruled in case C-528/16 that plants/organisms/products created by mutagenesis are subject to the GMO directive and regulated as genetically modified organisms (GMOs) because the process of mutagenesis modifies the organism’s genetic material in a way that does not occur naturally. However, the ruling also clarifies that there is a distinction between older mutagenesis techniques (which have a long safety record and may be exempted) and newer mutagenesis methods (such as targeted genome editing), which are not exempt and must comply fully with GMO legislation (Court of Justice of the European Union, 2018).

In the United States, the regulatory framework has faced recent instability. While the 2020 SECURE Rule initially exempted many gene-edited crops, a federal district court vacated the rule in December 2024, forcing the USDA-APHIS to revert to legacy regulations and “Am I Regulated?” inquiries to determine exemption status on a case-by-case basis (Lewis-Burke Associates, LLC, 2025; Rostoum, 2025; USDA, 2024). In contrast, China has accelerated its process-based approval system, granting safety certificates for gene-edited crops including two soybeans, one wheat, one corn, and one rice, alongside 12 GM varieties in 2024 and 2025 to enhance national food security (Rostoum, 2025). Similarly, India’s Ministry of Environment, Forest, and Climate Change solidified its exemption for SDN-1 and SDN-2 crops in 2022, leading to the commercial release of gene-edited rice varieties in May 2025 (Press Information Bureau, Delhi, 2025). In the European Union, a provisional agreement reached in December 2025 aims to establish a dual-track system: 'NGT-1′ plants (equivalent to conventional breeding) will be exempt from GMO rules, while 'NGT-2′ plants will remain strictly regulated. This marks a significant shift from the 2018 ECJ ruling that classified all mutagenesis products as GMOs (Anonymous, 2025a; Stokstad, 2024).

While some countries exempt gene-edited products from strict oversight, others still require case-by-case risk assessments. Intellectual property frameworks like the CRISPR patents owned by MIT and the University of California, Berkeley, also impact ethical regulation through controlled licensing (Guerrini et al., 2017). Trade and technological adoption are hindered by uneven international rules, particularly in view of the EU's rigorous GMO legislation that applies to all gene-edited crops (Anonymous, 2025a). A detailed comparison of these evolving regulatory frameworks, including the specific status of SDN-1, SDN-2, and SDN-3, as well as approved varieties across key countries, is provided in Table 3.

### 5.6. *Transgene-free genome editing with CRISPR/Cas RNP*

Transgene-free genome editing represents a transformative advancement in plant biotechnology that enables precise genome modification without the permanent introduction of foreign DNA (Ceasar and Ignacimuthu, 2023). In this approach, pre-assembled CRISPR/Cas RNP complexes, consisting of purified Cas nuclease and

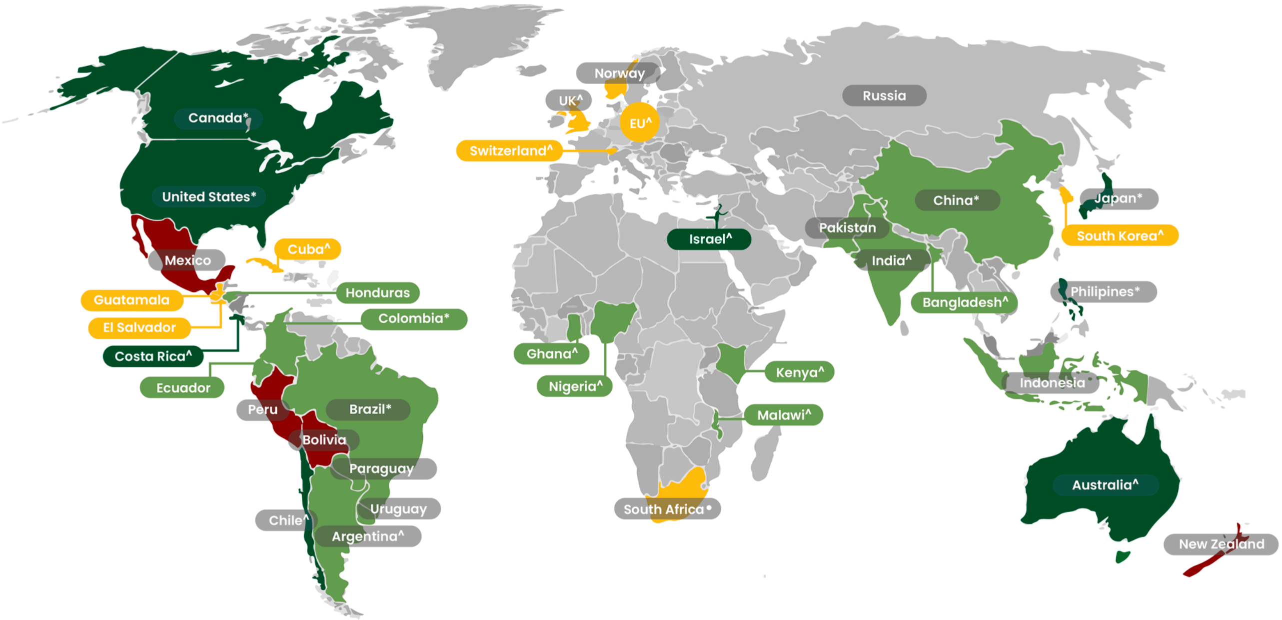


**Fig. 2.** Global regulatory status of gene-edited crops as of May 2024. Countries shaded in dark green (e.g., Japan, Canada, Australia, Philippines, USA, etc.) generally treat transgene-free gene-edited crops as conventional plants with no unique restrictions. Countries in light green (e.g., Argentina, Brazil, China, India, etc.) approve on a case-by-case basis. Regions in yellow (e.g., Cuba, Norway, UK, Switzerland, etc.) have not set any norms yet, and the debate is still ongoing. Whereas countries in grey color indicate that regulations remain unclear (e.g., Russia and several countries in Africa). Countries with red shade have strict norms (e.g., Mexico, New Zealand, etc.). * and ˆ indicate ‘Approved crops’, and research in the ‘Field and/or lab trials’ stage, respectively. (*Note: The authors neither agree nor disagree with the borders or the accuracy of the map, as the figure is directly sourced from the Genetic Literacy Project website.* Source: Global Gene Editing Regulation Tracker, Genetic Literacy Project (2024).

synthetic guide RNA, are directly delivered into protoplasts or embryogenic tissues through PEG-mediated uptake, electroporation, or biolistic methods (Gu et al., 2021). Once inside the cell, the RNP transiently performs site-specific cleavage and is subsequently degraded, leaving behind only the desired genomic change (L. Yang et al., 2023). This strategy minimizes off-target activity, avoids transgene integration, and produces non-GMO edited plants, simplifying regulatory approval and enhancing public acceptance. Compared to conventional *Agrobacterium*-mediated transformation, RNP-based editing offers faster generation of edited lines, reduced somaclonal variation, and compatibility with elite genotypes that are difficult to transform (Ramakrishnan et al., 2025). Moreover, DNA-free editing aligns with evolving global biosafety frameworks that exempt non-transgenic edits from GMO legislation (Zhang et al., 2020). Collectively, this technology bridges precision breeding and regulatory compliance, enabling the rapid development of disease-resistant crops suitable for sustainable agriculture.

## 6. Limitations and prospects of gene editing

Owing to its precision, simplicity, and cost-effectiveness, the CRISPR/Cas system has revolutionized genome engineering across plants, animals, and microorganisms. While its application has advanced rapidly in cereals, oilseeds, and fruit crops, comparable breakthroughs in minor cereals such as millets remain limited (Ceasar, 2022; Jadhav et al., 2024; Papade et al., 2025). We expect the use of this technology in agriculture to continue growing. However, it is crucial to establish social and ethical protection in a manner that considers public opinion. We must promptly take proactive steps to address these issues and ensure fair implementation across agriculture as research on CRISPR/Cas9 applications advances.

Moreover, it is crucial to consider the consequences of CRISPR/Cas9 that go beyond its effects on plants, microorganisms, pests, soil, individuals, etc. This innovative technology has implications for interactions between plants or organisms and the environment, highlighting the need for an assessment of its effects. While ethical and social factors are important, there is also a need to address agricultural issues. Applications of CRISPR/Cas9 go beyond the human realm, prompting concerns about how it may affect ecosystems and biodiversity. Crucial factors to consider in risk assessment are the purposeful harm principle and the application of safety measures to reduce ecological risks (Hirsch et al., 2019; Rodriguez, 2016). For example, there are significant ecological concerns associated with the discharge of genetically modified organisms (GMOs), whether under control or not.

The public's acceptance of GMOs created with CRISPR/Cas9 technology is still a hotly debated topic in agricultural settings. Regardless of the technology employed, historical opposition to GMOs emphasizes how critical it is to address public concerns (Carroll, 2017). Furthermore, safety concerns are raised by the challenge of identifying organisms changed by CRISPR/Cas9 outside of laboratory conditions (Shinwari et al., 2017). Furthermore, the challenge of identifying organisms altered by CRISPR/Cas9 outside of laboratory conditions raises safety concerns. Before items changed with CRISPR/Cas9 are released onto the market, regulatory organizations need to make clear and thorough statements and explanations. This proactive strategy creates strong protection to maintain public safety in addition to preventing misinterpretation. To ethically negotiate the ethical, ecological, and agricultural dimensions of technology, strict precautions and regulatory frameworks must be put in place.

Given the potential influence on the general deployment of this technology, the question of patenting in this field deserves careful thought. Patents have a great deal of power and frequently determine the course and availability of such groundbreaking innovations. Unilateral patenting, however, may potentially make bioethical conundrums worse and give rise to questions about access and justice. Comprehensive legislation and regulations governing these partnerships must be drafted as soon as possible, with a focus on transparency and public involvement. Given the complex issues and ethical nuances surrounding technology, stakeholders from the scientific community, social sciences, law, and government must be involved in creating a strong framework for the responsible use of CRISPR/Cas9 (Cathomen et al., 2019; Otieno, 2015; Shinwari et al., 2017). This collaborative endeavor has the potential to pave the way for a persuasive long-term strategy that advances science while predicting moral dilemmas and developing solutions.

## 7. Conclusion

As the global population grows, the demand for sustainable agriculture increases. CRISPR/Cas gene editing offers a precise way to boost disease resistance in key food crops, reducing reliance on chemicals and supporting environmental health. This technology targets genes linked to pathogen susceptibility, enhancing crop immunity and potentially increasing yields. Despite the potential for global food security and ecosystem stability, we must carefully manage ethical, regulatory, and public perception issues. It holds significant potential to transform plant breeding, making it more resilient and sustainable. By selectively targeting susceptibility genes and enhancing host immunity, CRISPR/Cas makes it possible to create cultivars that need fewer chemicals by selectively targeting S genes and boosting host immunity. This helps keep the environment clean and promotes ecological balance. Beyond improving crop productivity, gene editing holds immense promises for strengthening food and nutritional security, especially in regions reliant on climate-sensitive crops. However, realizing this potential demand is more than technological progress. Ethical considerations, transparent regulatory frameworks, and equitable access must accompany scientific innovation to ensure that its benefits are shared fairly across societies. To build trust and clear up any misunderstandings about genome-edited crops, there needs to be strong governance systems, good communication, and public involvement. In the future, combining CRISPR/Cas tools with multi-omics methods, advanced phenotyping, and data-driven breeding will speed up the creation of new crops that are both high-yielding and environmentally friendly. Collaborative efforts among scientists, policymakers, industry stakeholders, and local communities can transform CRISPR/Cas from a laboratory innovation into a cornerstone of global food security and climate-smart agriculture.

## Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

## Generative AI statement

The authors declare that no Generative AI was used in the creation of this manuscript.

## Ethics approval

This research did not involve human participants, animals, or the use of animal-derived materials. Therefore, no ethics approval is required.

## CRediT authorship contribution statement

**Niranjan Ravindra Thakur:** Conceptualization, Writing – original draft, Writing – review & editing, Visualization. **Krishnananda Pralhad Ingle:** Writing – original draft. **Sonal Chavan:** Writing – original draft, Writing – review & editing. **Yashoda Jadhav:** Conceptualization, Writing – original draft, Writing – review & editing. **Swapnil Shamrao Baraskar:** Writing – original draft, Writing – review & editing. **Ganesh Nikalje:** Writing – original draft. **Shashi Pandey:** Writing – original draft. **Jayashri Narayan Papade:** Writing – original draft. **Sachin**

**Laxman Dhare:** Writing – original draft. **Stanislaus Antony Ceasar:** Writing – original draft, Writing – review & editing, Visualization. **Gholamreza Abdi:** Writing – original draft, Project administration, Writing – review & editing, Supervision, Project administration.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2026.101322.

## Data availability

No data was used for the research described in the article.

## References

Adli, M., 2018. The CRISPR tool kit for genome editing and beyond. Nat. Commun. 9 (1), 1911. https://doi.org/10.1038/s41467-018-04252-2.

AgbioInvestor. 2025. Global GM crop area Review (April 2025). Available at: https://gm.agbioinvestor.com/. Accessed on January 5th, 2026.

Ali, Z., Ali, S., Tashkandi, M., Zaidi, S.S.A., Mahfouz, M.M., 2016. CRISPR/Cas9-mediated immunity to geminiviruses: differential interference and evasion. Sci. Rep. 6, 26912. https://doi.org/10.1038/srep26912.

Altpeter, F., Springer, N.M., Bartley, L.E., Blechl, A.E., Brutnell, T.P., Citovsky, V., Conrad, L.J., Gelvin, S.B., Jackson, D.P., Kausch, A.P., Lemaux, P.G., 2016. Advancing crop transformation in the era of genome editing. Plant Cell 28 (7), 1510–1520. https://doi.org/10.1105/tpc.16.00196.

Aman, R., Mahas, A., Marsic, T., Hassan, N., Mahfouz, M.M., 2020. Efficient, rapid, and sensitive detection of plant RNA viruses with one-pot RT-RPA–CRISPR/CAS12A assay. Front. Microbiol. 11, 610872. https://doi.org/10.3389/fmicb.2020.610872.

Aman, R., Marsic, T., Rao, G.S., Mahas, A., Ali, Z., Alsanea, M., Al-Qahtani, A., Alhamlan, F., Mahfouz, M., 2022. ISCAN-V2: a one-pot RT-RPA–CRISPR/CAS12B assay for point-of-care SARS-COV-2 detection. Front. Bioeng. Biotechnol. 9, 800104. https://doi.org/10.3389/fbioe.2021.800104.

Andolfo, G., Iovieno, P., Frusciante, L., Ercolano, M.R., 2016. Genome-editing technologies for enhancing plant disease resistance. Front. Plant Sci. 7, 1813. https://doi.org/10.3389/fpls.2016.01813.

Andolfo, G., Ercolano, M.R., 2015. Plant innate immunity multicomponent model. Front. Plant Sci. 6, 987. https://doi.org/10.3389/fpls.2015.00987.

Anonymous, 2024. UPOV: Shaping the Protection of Plant Breeders' Rights Globally. General Introductions to IP Rights. TheLaw.Institute. https://thelaw.institute/general-introductions-to-ip-rights/upov-plant-breeders-rights-protection/. Accessed on January 3rd, 2026.

Anonymous, 2025a. New genomic techniques: deal to support the green transition in farming. In: Press Releases. Dated: 04-12-2025. https://www.europarl.europa.eu/news/en/press-room/20251201IPR31710/new-genomic-techniques-deal-to-support-the-green-transition-in-farming. Accessed on December 21st, 2025.

Anonymous, 2025b Ethical implications of gene editing in agriculture. Prism Sustainability Directory. https://prism.sustainability-directory.com/scenario/ethical-implications-of-gene-editing-in-agriculture/#assess. Accessed on January 3rd, 2026.

Anzalone, A.V., Randolph, P.B., Davis, J.R., Sousa, A.A., Koblan, L.W., Levy, J.M., Chen, P.J., Wilson, C., Newby, G.A., Raguram, A., Liu, D.R., 2019. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 576 (7785), 149–157. https://doi.org/10.1038/s41586-019-1711-4.

Arafa, R.A., Rakha, M.T., Soliman, N.E.K., Moussa, O.M., Kamel, S.M., Shirasawa, K., 2017. Rapid identification of candidate genes for resistance to tomato late blight disease using next-generation sequencing technologies. PLoS. One 12 (12), e0189951. https://doi.org/10.1371/journal.pone.0189951.

Arora, M., Goel, N.K., Singh, P., 2005. Evaluation of temperature trends over India/ Evaluation de tendances de température en inde. Hydrol. Sci. J. 50 (1). https://doi.org/10.1623/hysj.50.1.81.56330.

Ayanoğlu, F.B., Elçin, A.E., Elçin, Y.M., 2020 Apr 2. Bioethical issues in genome editing by CRISPR-Cas9 technology. Turk. J. Biol. 44 (2), 110–120. https://doi.org/10.3906/biy-1912-52. PMID: 32256147; PMCID: PMC7129066.

Babar, U., Nawaz, M.A., Arshad, U., Azhar, M.T., Atif, R.M., Golokhvast, K.S., Tsatsakis, A.M., Shcerbakova, K., Chung, G., Rana, I.A., 2019. Transgenic crops for the agricultural improvement in Pakistan: a perspective of environmental stresses and the current status of genetically modified crops. GM. Crops. Food 11 (1), 1–29. https://doi.org/10.1080/21645698.2019.1680078.

Barka, G.D., Lee, J., 2022. Advances in S gene targeted genome-editing and its applicability to disease resistance breeding in selected Solanaceae crop plants. Bioengineered. 13 (6), 14646–14666. https://doi.org/10.1080/21655979.2022.2099599.

Barmukh, R., Thakur, N., Shah, P., 2024. Genomic interventions for improving crop yield and resilience. In: Al-Khayri, J.M., Ingle, K.P., Jain, S.M., Penna, S. (Eds.), Plant Molecular Breeding in Genomics Era. Advances in Plant Breeding Strategies, Vol 3. Springer, Cham. https://doi.org/10.1007/978-3-031-68586-6_3.

Barton, N.H., 2016. How does epistasis influence the response to selection? Hered. (Edinb) 118 (1), 96–109. https://doi.org/10.1038/hdy.2016.109.

Bearth, A., Kaptan, G., Kessler, S.H., 2022. Genome-edited versus genetically-modified tomatoes: an experiment on people's perceptions and acceptance of food biotechnology in the UK and Switzerland. Agric Hum. Values 39 (3), 1117–1131. https://doi.org/10.1007/s10460-022-10311-8.

Blanvillain-Baufumé, S., Reschke, M., Solé, M., Auguy, F., Doucoure, H., Szurek, B., Meynard, D., Portefaix, M., Cunnac, S., Guiderdoni, E., Boch, J., Koebnik, R., 2016. Targeted promoter editing for rice resistance to xanthomonas oryzae pv. oryzae reveals differential activities for SWEET 14 -inducing TAL effectors. Plant Biotechnol. J. 15 (3), 306–317. https://doi.org/10.1111/pbi.12613.

Borrelli, V.M.G., Brambilla, V., Rogowsky, P., Marocco, A., Lanubile, A., 2018. The enhancement of plant disease resistance using CRISPR/Cas9 technology. Front. Plant Sci. 9, 1245. https://doi.org/10.3389/fpls.2018.01245.

Bruinsma, J., 2009. The resource outlook to 2050. Expert Meeting On How to Feed the World in, pp. 1–33. Vol. 2050.

Bui, T.P., Le, H., Ta, D.T., Nguyen, C.X., Le, N.T., Tran, T.T., Van Nguyen, P., Stacey, G., Stacey, M.G., Pham, N.B., Chu, H.H., Tien, P., 2023. Enhancing powdery mildew resistance in soybean by targeted mutation of MLO genes using the CRISPR/Cas9 system. BMC. Plant Biol. 23 (1). https://doi.org/10.1186/s12870-023-04549-5.

Carroll, D., 2017. Focus: genome editing: genome editing: past, present, and future. Yale J. Biol. Med. 90 (4), 653.

Cao, Y., Zhou, H., Zhou, X., Li, F., 2021. Conferring resistance to plant RNA viruses with the CRISPR/CasRx system. Virol. Sin. 36 (4), 814–817. https://doi.org/10.1007/s12250-020-00338-8.

Cathomen, T., Schüle, S., Schüßler-Lenz, M., Abou-El-Enein, M., 2019. The human genome editing race: loosening regulatory standards for commercial advantage? Trends Biotechnol. 37 (2), 120–123. https://doi.org/10.1016/j.tibtech.2018.06.005.

Ceasar, S.A., Maharajan, T., Hillary, V.E., Ajeesh Krishna, T.P., 2022. Insights to improve the plant nutrient transport by CRISPR/Cas system. Biotechnol. Adv. 59, 107963. https://doi.org/10.1016/j.biotechadv.2022.107963.

Ceasar, A., 2022. Genome-editing in millets: current knowledge and future perspectives. Mol. Biol. Rep. 49, 773–781. https://doi.org/10.1007/s11033-021-06975-w.

Ceasar, S.A., Ignacimuthu, S., 2023. CRISPR/Cas genome editing in plants: dawn of agrobacterium transformation for recalcitrant and transgene-free plants for future crop breeding. Plant Physiol. Biochem. 196, 724–730. https://doi.org/10.1016/j.plaphy.2023.02.030.

Changtor, P., Jaroenpol, W., Buddhachat, K., Wattanachaiyingcharoen, W., Yimtragool, N., 2023. Rapid detection of sclerotium rolfsii causing dry stem and root rot disease in cassava by recombinase polymerase amplification technique (RPA) combined with CRISPR/Cas12a. Crop Prot. 172, 106340. https://doi.org/10.1016/j.cropro.2023.106340.

Chavhan, R.L., Jaybhaye, S.G., Hinge, V.R., Deshmukh, A.S., Shaikh, U.S., Jadhav, P.K., Kadam, U.S., Hong, J.C., 2025. Emerging applications of gene editing technologies for the development of climate-resilient crops. Front. Genome Ed. 7, 1524767. https://doi.org/10.3389/fgeed.2025.1524767.

Chawla, R., Karla, U., Chavan, S., Sharma, H., Jattan, M., Phogat, D.S., 2024. Breeding proso millet for biotic stress resistance. Genetic Improvement of Small Millets. Springer Nature Singapore, Singapore, pp. 455–467. https://doi.org/10.1007/978-981-99-7232-6_21.

Chen, J.S., Ma, E., Harrington, L.B., Da Costa, M., Tian, X., Palefsky, J.M., Doudna, J.A., 2018. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science 360 (6387), 436–439. https://doi.org/10.1126/science.aar6245.

Chen, K., Liu, H., Xie, K., ul Qamar, M.T., Chen, L.L., 2021. Advances in guide RNA design for editing plant genomes using CRISPR-Cas systems. Genome Editing For Precision Crop Breeding. Burleigh Dodds Science Publishing, pp. 147–174.

Cheng, C., Kan, J., Li, S., Jiang, C., He, X., Shen, H., Xu, R., Li, B., Feng, Z., Yang, P., 2022. Mutation of barley HvPDIL5-1 improves resistance to yellow mosaic virus disease without growth or yield penalties. Front. Plant Sci. 13, 1018379. https://doi.org/10.3389/fpls.2022.1018379.

Chhikara, M., Maurya, P., 2024. Biotechnology ethics and regulatory frameworks. The Spectrum of Discovery. Multidisciplinary Research in the Modern Era. Volume-1. Redshine Archive. Infinity Publication, UK.

ChileBio. 2025. Chile da luz verde al primer trigo editado genéticamente de las Américas. Available at: https://chilebio.cl/2025/08/04/hito-regulatorio-chile-da-luz-verde-al-primer-trigo-editado-geneticamente-de-las-americas/. Accessed on January 10th, 2026.

Court of Justice of the European Union. 2018. Judgment of the Court (Grand Chamber) of 25 July 2018, Confédération paysanne and Others v Premier ministre and Ministre de l'Agriculture, de l'Agroalimentaire et de la Forêt, case C-528/16. Available at: https://curia.europa.eu/juris/liste.jsf?num=C-528/16&language=EN. Accessed on January 3rd, 2026.

Das, A., Sharma, N., Prasad, M., 2019. CRISPR/Cas9: a novel weapon in the arsenal to combat plant diseases. Front. Plant Sci. 9, 2008. https://doi.org/10.3389/fpls.2018.02008.

Das, K., Ayim, B.Y., Borodynko-Filas, N., Das, S.C., Aminuzzaman, F., 2023. Genome editing (CRISPR/Cas9) in plant disease management: challenges and future prospects. J. Plant Prot. Res. 63 (2), 159–172. https://doi.org/10.24425/jppr.2023.145761.

Dassler, T., Myhr, A.I., 2021. The ethical sustainability matrix: a practical tool for assessment of GMOs including genome- edited organisms (Eds.). In: Schübel, H., Wallimann-Helmer, I. (Eds.), Justice and Food Security in A Changing Climate. Wageningen Academic Publishers, the Netherlands, pp. 368–373. https://doi.org/10.3920/978-90-8686-915-2_57.

Dean, R., Van Kan, J.A., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P. D., Rudd, J.J., Dickman, M., Kahmann, R., Ellis, J., Foster, G.D., 2012. The top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 13 (4), 414–430. https://doi.org/10.1111/j.1364-3703.2011.00783.x.

Doehlemann, G., Okmen, B., Zhu, W., Sharon, A., 2017. Plant pathogenic fungi. In: Heitman, J., Howlett, B., Crous, P., Stukenbrock, E., James, T., Gow, N. (Eds.), The Fungal Kingdom. ASM Press, Washington, DC, pp. 703–726. https://doi.org/10.1128/microbiolspec.funk-0023-2016 eds.

Dort, E.N., Tanguay, P., Hamelin, R.C., 2020. CRISPR/Cas9 gene editing: an unexplored frontier for forest pathology. Front. Plant Sci. 11, 1126. https://doi.org/10.3389/fpls.2020.01126.

Elliott, K., Veley, K.M., Jensen, G., Gilbert, K.B., Norton, J., Kambic, L., Yoder, M., Weil, A., Motomura-Wages, S., Bart, R.S., 2024. CRISPR/Cas9-generated mutations in a sugar transporter gene reduce cassava susceptibility to bacterial blight. Plant Physiol. 195 (4), 2566–2578. https://doi.org/10.1093/plphys/kiae243. Jul 31PMID: 38701041; PMCID: PMC11288762.

Entine, J., Felipe, M.S.S., Groenewald, J., Kershen, D.L., Lema, M., McHughen, A., Nepomuceno, A.L., Ohsawa, R., Ordonio, R.L., Parrott, W.A., Quemada, H., Ramage, C., Slamet-Loedin, I., Smyth, S.J., & Wray-Cahen, D. (2021). Regulatory approaches for genome edited agricultural plants in select countries and jurisdictions around the world. pmc.ncbi.nlm.nih.gov. https://doi.org/10.1007/s11248-021-00257-8.

Eom, J., Luo, D., Atienza-Grande, G., Yang, J., Ji, C., Luu, V.T., Huguet-Tapia, J.C., Char, S.N., Liu, B., Nguyen, H., Schmidt, S.M., Szurek, B., Cruz, C.V., White, F.F., Oliva, R., Yang, B., Frommer, W.B., 2019. Diagnostic kit for rice blight resistance. Nat. Biotechnol 37 (11), 1372–1379. https://doi.org/10.1038/s41587-019-0268-y.

European Commission. 2021. Study on the status of new genomic techniques under Union law and in light of the Court of Justice ruling in Case C-528/16. SWD(2021) 92. Available at: https://food.ec.europa.eu/system/files/2021-04/gmo_mod-bio_ngt_exec-sum_en.pdf. Accessed on January 9th, 2026.

European Parliament, 2024. Plants obtained by certain new genomic techniques and their food and feed. EUR-Lex. Off. J. Eur. Union. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52024AP0067. Accessed on January 9th, 2026.

Euroseeds, 2023. Euroseeds statement on IP and NGTs. Euroseeds Embrac. Nat. https://euroseeds.eu/news/euroseeds-statement-on-ip-and-ngts/ Accessed on January 3rd, 2026.

Faal, P.G., Farsi, M., Seifi, A., Kakhki, A.M., 2020. Virus-induced CRISPR-Cas9 system improved resistance against tomato yellow leaf curl virus. Mol. Biol. Rep. 47 (5), 3369–3376. https://doi.org/10.1007/s11033-020-05409-3.

FAO. 2022. FAO's Plant Production and Protection Division. Rome. https://doi.org/10.4060/cc2447en.

Feed Strategy. (2025). China approves 17 more gene-edited crop varieties. Available at: https://www.feedstrategy.com/business-markets/international-trade/news/15711183/china-approves-17-more-geneedited-crop-varieties. Accessed on January 10th, 2026.

Feeney, O., Cockbain, J., Sterckx, S., 2021. Ethics, patents and genome editing: a critical assessment of three options of technology governance. Front. Polit. Sci. 3, 731505. https://doi.org/10.3389/fpos.2021.731505.

Gao, L., Xie, L., Xiao, Y., Cheng, X., Pu, R., Zhang, Z., Liu, Y., Gao, S., Zhang, Z., Qu, H., Zhi, H., Li, K., 2024. CRISPR/CasRx-mediated resistance to soybean mosaic virus in soybean. Crop J 12 (4), 1093–1101. https://doi.org/10.1016/j.cj.2024.07.007.

García-Murillo, L., Valencia-Lozano, E., Priego-Ranero, N.A., Cabrera-Ponce, J.L., Duarte-Aké, F.P., Vizuet-De-Rueda, J.C., Rivera-Toro, D.M., Herrera-Ubaldo, H., De Folter, S., Alvarez-Venegas, R., 2023. CRISPRa-mediated transcriptional activation of the SlPR-1 gene in edited tomato plants. Plant Sci. 329, 111617. https://doi.org/10.1016/j.plantsci.2023.111617.

Garcia-Ruiz, H., Szurek, B., Van Den Ackerveken, G., 2021. Stop helping pathogens: engineering plant susceptibility genes for durable resistance. Curr. Opin. Biotechnol. 70, 187–195. https://doi.org/10.1016/j.copbio.2021.05.005.

Gatica-Arias, A., 2020. The regulatory current status of plant breeding technologies in some Latin American and the Caribbean countries. Plant Cell Tissue Organ Cult. (PCTOC) 141 (2), 229–242. https://doi.org/10.1007/s11240-020-01799-1.

Guiltinan, M.J., Landherr, L., Maximova, S.N., DelVecchio, D., Sebastian, A., Albert, I., 2025. Reduced susceptibility to phytophthora in non-transgenic cacao progeny through CRISPR–CAS9 mediated TCNPR3 mutagenesis. Plant Biotechnol. J. https://doi.org/10.1111/pbi.70365.

Gaudelli, N.M., Komor, A.C., Rees, H.A., Packer, M.S., Badran, A.H., Bryson, D.I., Liu, D. R., 2017. Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. Nature 551 (7681), 464–471. https://doi.org/10.1038/nature24644.

Geissler, A.S., Gorodkin, J., Seemann, S.E., 2024. Patent data-driven analysis of literature associations with changing innovation trends. Front. Res. Metr. Anal. 9, 1432673. https://doi.org/10.3389/frma.2024.1432673.

Gelvin, S.B., 2017. Integration of agrobacterium T-DNA into the plant genome. Annu. Rev. Genet. 51, 195–217. https://doi.org/10.1146/annurev-genet-120215-035320.

Global Gene Editing Regulation Tracker, Genetic Literacy Project. 2024. Available at: https://crispr-gene-editing-regs-tracker.geneticliteracyproject.org/#jet-tabs-control-1403.

Gomez, M.A., Lin, Z.D., Moll, T., Chauhan, R.D., Hayden, L., Renninger, K., Beyene, G., Taylor, N.J., Carrington, J.C., Staskawicz, B.J., Bart, R.S., 2018. Simultaneous CRISPR/Cas9-mediated editing of cassava eIF4E isoforms nCBP-1 and nCBP-2 reduces cassava brown streak disease symptom severity and incidence. Plant Biotechnol. J. 17 (2), 421–434. https://doi.org/10.1111/pbi.12987.

Gootenberg, J.S., Abudayyeh, O.O., Kellner, M.J., Joung, J., Collins, J.J., Zhang, F., 2018. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. Science 360 (6387), 439–444. https://doi.org/10.1126/science.aaq0179.

Gootenberg, J.S., Abudayyeh, O.O., Lee, J.W., Essletzbichler, P., Dy, A.J., Joung, J., Verdine, V., Donghia, N., Daringer, N.M., Freije, C.A., Myhrvold, C., Bhattacharyya, R.P., Livny, J., Regev, A., Koonin, E.V., Hung, D.T., Sabeti, P.C., Collins, J.J., Zhang, F., 2017. Nucleic acid detection with CRISPR-Cas13a/C2c2. Science 356 (6336), 438–442. https://doi.org/10.1126/science.aam9321.

Gosavi, G., Yan, F., Ren, B., et al., 2020. Applications of CRISPR technology in studying plant-pathogen interactions: overview and perspective. Phytopathol. Res. 2, 21. https://doi.org/10.1186/s42483-020-00060-z.

Gu, X., Liu, L., Zhang, H., 2021. Transgene-free genome editing in plants. Front. Genome Ed. 3. https://doi.org/10.3389/fgeed.2021.805317.

Guerrini, C.J., Curnutte, M.A., Sherkow, J.S., et al., 2017. The rise of the ethical license. Nat. Biotechnol. 35 (1), 22–24. https://doi.org/10.1038/nbt.3756.

Gupta, A., Liu, B., Chen, Q., Yang, B., 2023a. High-efficiency prime editing enables new strategies for broad-spectrum resistance to bacterial blight of rice. Plant Biotechnol. J. 21 (7), 1454–1464. https://doi.org/10.1111/pbi.14049.

Gupta, A., Liu, B., Raza, S., Chen, Q., Yang, B., 2023b. Modularly assembled multiplex prime editors for simultaneous editing of agronomically important genes in rice. Plant Commun. 5 (2), 100741. https://doi.org/10.1016/j.xplc.2023.100741.

Hao, M., Li, Y., Sang, S., Song, M., Wen, Y., Wang, H., Wang, W., Mei, D., Liu, J., Li, C., Fu, L., Hu, Q., Cheng, H., 2025. CRISPR/Cas9-mediated editing of uORFs in the BnVTC2 facilitates abiotic stress resilience without yield penalty. Plant Stress 18, 101004. https://doi.org/10.1016/j.stress.2025.101004.

Harrington, L.B., Burstein, D., Chen, J.S., Paez-Espino, D., Ma, E., Witte, I.P., Cofsky, J.C., Kyrpides, N.C., Banfield, J.F., Doudna, J.A., 2018. Programmed DNA destruction by miniature CRISPR-Cas14 enzymes. Science 362 (6416), 839–842. https://doi.org/10.1126/science.aav4294.

Harris, W., Kim, S., Völz, R., Lee, Y., 2023. Nuclear effectors of plant pathogens: distinct strategies to be one step ahead. Mol. Plant Pathol. 24 (6), 637–650. https://doi.org/10.1111/mpp.13315.

Hasan, M.M., Rafii, M.Y., Ismail, M.R., Mahmood, M., Rahim, H.A., Alam, M.A., Ashkani, S., Malek, M.A., Latif, M.A., 2015. Marker-assisted backcrossing: a useful method for rice improvement. Biotechnol. Biotechnol. Equip. 29 (2), 237–254. https://doi.org/10.1080/13102818.2014.995920.

He, F., Wang, C., Sun, H., Tian, S., Zhao, G., Liu, C., Wan, C., Guo, J., Huang, X., Zhan, G., Yu, X., Kang, Z., Guo, J., 2022. Simultaneous editing of three homoeologues of TaCIPK14 confers broad-spectrum resistance to stripe rust in wheat. Plant Biotechnol. J. 21 (2), 354–368. https://doi.org/10.1111/pbi.13956.

Hefferon, K.L., Herring, R.J., 2017. The end of the GMO? Genome editing, gene drives and new frontiers of plant technology. Rev. Agrar. Stud. 7 (1), 1–32. https://doi.org/10.22004/ag.econ.308366.

Helliwell, R., Hartley, S., Pearce, W., 2019. NGO perspectives on the social and ethical dimensions of plant genome- editing. Agric. Hum. Values 36 (4), 779–791. https://doi.org/10.1007/s10460-019-09956-9.

Hiei, Y., Ishida, Y., Komari, T., 2014. Progress of cereal transformation technology mediated by Agrobacterium tumefaciens. Front. Plant Sci. 5, 628. https://doi.org/10.3389/fpls.2014.00628.

Hirsch, F., Iphofen, R., Koporc, Z., 2019. Ethics assessment in research proposals adopting CRISPR technology. Biochem. Med. 29 (2), 206–213. https://doi.org/10.11613/bm.2019.020202.

Hoffie, R.E., Otto, I., Perovic, D., Budhagatapalli, N., Habekuß, A., Ordon, F., Kumlehn, J., 2021. Targeted knockout of eukaryotic translation initiation factor 4E confers bymovirus resistance in Winter Barley. Front. Genome Ed. 3, 784233. https://doi.org/10.3389/fgeed.2021.784233. Nov 29PMID: 34913048; PMCID: PMC8667817.

Houvener, J., 2024. Genetically modified crops and Patent Law: legal considerations. Bold Pat. https://boldip.com/blog/genetically-modified-crops-and-patent-law-legal-considerations/ Accessed on December 21st, 2025.

Huang, Q., Lin, B., Cao, Y., Zhang, Y., Song, H., Huang, C., Sun, T., Long, C., Liao, J., Zhuo, K., 2023. CRISPR/Cas9-mediated mutagenesis of the susceptibility gene *OsHPP04* in rice confers enhanced resistance to rice root-knot nematode. Front., Plant Sci. 14, 1134653. https://doi.org/10.3389/fpls.2023.1134653.

Hou, M., Xu, G., 2025. Growth-defense trade-off in plants: from hypothesis to principle to paradigm. Cell Host. Microbe 33 (8), 1222–1226. https://doi.org/10.1016/j.chom.2025.05.022.

Ingle, K., Thakur, N., Moharil, M.P., Suprasanna, P., Awio, B., Narkhede, B., Kumar, P., Ceasar, S.A., Abdi, G., 2023. Current status and future prospects of molecular marker assisted selection (MAS) in millets. In: Pudake, R.N., Solanke, A.U., Kole, C. (Eds.), Nutriomics of Millet Crops, 1st ed. CRC Press. https://doi.org/10.1201/b22809. eBook ISBN 9781003275657.

Ingle, K.P., Thakur, N.R., Papade, J.N., Kasanaboina, K., Deshmukh, S.S., Abdi, G., Bhalerao, J.B., 2024. Genome editing: revolutionizing horticultural crops improvement (eds.). In: Al-Khayri, Jameel M., Alnaddaf, Lina M., Jain, Shri Mohan, Penna, Suprasanna (Eds.), Innovative Methods in Horticultural Crop Improvement. Advances in Plant Breeding Strategies, Vol 1. Springer, Cham. https://doi.org/10.1007/978-3-031-61081-3_10.

ISAAA, 2023. Brazil and Colombia approve first drought tolerant gene-edited soybeans. Crop Biotech Update. Available at: https://www.isaaa.org/kc/cropbiotechupdate/ged/article/default.asp?ID=19979. Accessed on January 10th, 2026.

Ivanov, M., Buddle, E.A., Ankeny, R.A., 2025. Regulation as key to fulfilling the promises of agricultural genomics: going beyond bottlenecks in plant gene technology development. Plant J. 122 (6), e70277. https://doi.org/10.1111/tpj.70277.

Jadhav, Y., Thakur, N.R., Ingle, K.P., Ceasar, S.A., 2024. The role of phenomics and genomics in delineating the genetic basis of complex traits in millets. Physiol. Plant 176 (3), e14349. https://doi.org/10.1111/ppl.14349. Available from:

Jasieniecka, A., Domingues, I., 2025. CRISPR-CAS9 and its bioinformatics tools: a systematic review. Curr. Issues. Mol. Biol. 47 (5), 307. https://doi.org/10.3390/cimb47050307.

Jhu, M.Y., Ellison, E.E., Sinha, N.R., 2023. CRISPR gene editing to improve crop resistance to parasitic plants. Front. Genome Ed. 5, 1289416. https://doi.org/10.3389/fgeed.2023.1289416. Oct 25.

Ji, X., Zhang, H., Zhang, Y., Wang, Y., Gao, C., 2015. Establishing a CRISPR–Cas-like immune system conferring DNA virus resistance in plants. Nat. Plants. 1 (10), 15144. https://doi.org/10.1038/nplants.2015.144.

Jiang, L., 2020. Commercialization of the gene-edited crop and morality: challenges from the liberal patent law and the strict GMO law in the EU. New Genet. Soc. 39 (2), 191–218. https://doi.org/10.1080/14636778.2019.1686968.

Jiang, Y.Y., Chai, Y.P., Lu, M.H., et al., 2020. Prime editing efficiently generates W542L and S621I double mutations in two ALS genes in maize. Genome Biol. 21, 257. https://doi.org/10.1186/s13059-020-02170-5.

Jones, J.D.G., Dangl, J.L., 2006. The plant immune system. Nature 444, 323–329. https://doi.org/10.1038/nature05286.

Jones, M.G.K., Fosu-Nyarko, J., Iqbal, S., Adeel, M., Romero-Aldemita, R., Arujanan, M., Kasai, M., Wei, X., Prasetya, B., Nugroho, S., Mewett, O., Mansoor, S., Awan, M.J.A., Ordonio, R.L., Rao, S.R., Poddar, A., Hundleby, P., Iamsupasit, N., Khoo, K., 2022. Enabling trade in gene-edited Produce in Asia and Australasia: the developing regulatory landscape and future perspectives. Plants 11 (19), 2538. https://doi.org/10.3390/plants11192538.

Jose, J., Hamow, K.Á., Éva, C., Moncsek, B., Kyrpa, T., Reinoso, L.G., Bozsó, Z., Bakonyi, J., Balázs, E., Sági, L., 2026. CRISPR/Cas-mediated polyphenol oxidase gene knockout in potato reveals divergent roles in resistance to bacterial wilt and late blight. Plant Sci. 364, 112944. https://doi.org/10.1016/j.plantsci.2025.112944.

Kaminski, M.M., Abudayyeh, O.O., Gootenberg, J.S., Zhang, F., Collins, J.J., 2021. CRISPR-based diagnostics. Nat. Biomed. Eng. 5 (7), 643–656. https://doi.org/10.1038/s41551-021-00760-7.

Kanchiswamy, C.N., 2016. DNA-free genome editing methods for targeted crop improvement. Plant Cell Rep. 35, 1469–1474. https://doi.org/10.1007/s00299-016-1982-2.

Karlsson, M., Kieu, N.P., Lenman, M., Marttila, S., Resjö, S., Zahid, M.A., Andreasson, E., 2024. CRISPR/Cas9 genome editing of potato StDMR6-1 results in plants less affected by different stress conditions. Hortic. Res. 11 (7), uhae130. https://doi.org/10.1093/hr/uhae130. May 6PMID: 38974188; PMCID: PMC11224679.

Karmakar, S., Das, P., Panda, D., Xie, K., Baig, M.J., Molla, K.A., 2022. A detailed landscape of CRISPR-Cas-mediated plant disease and pest management. Plant Sci. 323, 111376. https://doi.org/10.1016/j.plantsci.2022.111376.

Keiper, F., Atanassova, A., 2022. Enabling genome editing for enhanced agricultural sustainability. Front. Genome Ed. 4, 898950. https://doi.org/10.3389/fgeed.2022.898950.

Kellner, M.J., Koob, J.G., Gootenberg, J.S., Abudayyeh, O.O., Zhang, F., 2019. SHERLOCK: nucleic acid detection with CRISPR nucleases. Nat. Protoc. 14 (10), 2986–3012. https://doi.org/10.1038/s41596-019-0210-2.

Kerr, A., 2016. Biological control of Crown Gall. Australas, Plant Pathol. 45, 15–18. https://doi.org/10.1007/s13313-015-0389-9.

Khatoon, M., 2025. Intellectual Property Rights in Bioengineered Foods: A Legal Analysis of Patents on GMOs and Lab-Grown Meat in India, Examining Compulsory Licensing, Patent Engrossment Challenges, and the Role of International Frameworks Such As WIPO, USPTO, and EPO in Shaping Sustainable Agricultural Biotechnology and Global Food Security Strategies. CSRIPR. https://tinyurl.com/cjasjvfy. Accessed on December 22nd, 2025.

Khoury, C.K., Brush, S., Costich, D.E., Curry, H.A., De Haan, S., Engels, J.M.M., Guarino, L., Hoban, S., Mercer, K.L., Miller, A.J., Nabhan, G.P., Perales, H.R., Richards, C., Riggins, C., Thormann, I., 2021. Crop genetic erosion: understanding and responding to loss of crop diversity. New Phytol. 233 (1), 84–118. https://doi.org/10.1111/nph.17733.

Kieu, N.P., Lenman, M., Wang, E.S., Petersen, B.L., Andreasson, E., 2021. Mutations introduced in susceptibility genes through CRISPR/Cas9 genome editing confer increased late blight resistance in potatoes. Sci. Rep. 11 (1). https://doi.org/10.1038/s41598-021-83972-w.

Kis, A., Hamar, É., Tholt, G., Bán, R., Havelda, Z., 2019. Creating highly efficient resistance against wheat dwarf virus in barley by employing CRISPR/Cas9 system. Plant Biotechnol. J. 17 (6), 1004–1006. https://doi.org/10.1111/pbi.13077. JunEpub 2019 Feb 5. PMID: 30633425; PMCID: PMC6523583.

Kuiken, T., Kuzma, J., 2021. Genome editing in Latin America: regional regulatory overview. Assessment of the Regulatory and Institutional Frameworks For Agricultural Gene Editing via CRISPR-Based Technologies in Latin America and The Caribbean (March 2023). Genetic Engineering and Society Center. NC State University. Available at: https://ges.research.ncsu.edu/research/idb-crispr/#top.

Kumar, T., Wang, J., Xu, C., Lu, X., Mao, J., Lin, X., Kong, C., Li, C., Li, X., Tian, C., Ebid, M.H.M., Liu, X., Liu, H., 2024. Genetic engineering for enhancing sugarcane tolerance to biotic and abiotic stresses. Plants 13 (13), 1739. https://doi.org/10.3390/plants13131739.

Kusch, S., Panstruga, R., 2017. mlo -based resistance: an apparently universal "weapon" to defeat powdery mildew disease. Mol. Plant-Microbe Interact. 30 (3), 179–189. https://doi.org/10.1094/mpmi-12-16-0255-cr.

Langner, T., Kamoun, S., Belhaj, K., 2018. CRISPR crops: plant genome editing toward disease resistance. Annu Rev. Phytopathol. 56, 479–512. https://doi.org/10.1038/nbt.4192.

Lassoued, R., Phillips, P.W., Smyth, S.J., Hesseln, H., 2019. Estimating the cost of regulating genome edited crops: expert judgment and overconfidence. GM. Crops. Food 10 (1), 44–62. https://doi.org/10.1080/21645698.2019.1612689.

Ledesma, A.V., Van Eenennaam, A.L., 2024. Global status of gene edited animals for agricultural applications. Vet. J. 305, 106142. https://doi.org/10.1016/j.tvjl.2024.106142.

Lewis-Burke Associates, LLC, 2025. Policy update: federal judge vacates rule on biotech crops. ASBP Plant Sci. Today. Available at: https://blog.aspb.org/policy-update-federal-judge-vacates-usda-rule-regulating-biotech-crops/. Accessed on January 6th, 2026.

Li, Y.J., Gu, J.M., Ma, S., Xu, Y., Liu, M., Zhang, C., Liu, X., Wang, G.F., 2023. Genome editing of the susceptibility gene ZmNANMT confers multiple disease resistance without agronomic penalty in maize. Plant Biotechnol. J. 21 (8), 1525–1527. https://doi.org/10.1111/pbi.14078. AugPMID: 37254611; PMCID: PMC10363755.

Li, C., Liu, B., Dong, H., Yang, B., 2025. Enhancing resistance to bacterial blight in rice using CRISPR-based base editing technology. Crop J 13 (1), 115–124. https://doi.org/10.1016/j.cj.2024.09.003.

Li, L., Li, S., Wu, N., Wu, J., Wang, G., Zhao, G., Wang, J., 2019. HOLMESV2: a CRISPR-CAS12B-assisted platform for nucleic acid detection and DNA methylation quantitation. ACS. Synth. Biol. 8 (10), 2228–2237. https://doi.org/10.1021/acssynbio.9b00209.

Li, S., Lin, D., Zhang, Y., Deng, M., Chen, Y., Lv, B., Li, B., Lei, Y., Wang, Y., Zhao, L., Liang, Y., Liu, J., Chen, K., Liu, Z., Xiao, J., Qiu, J., Gao, C., 2022. Genome-edited powdery mildew resistance in wheat without growth penalties. Nature 602 (7897), 455–460. https://doi.org/10.1038/s41586-022-04395-9.

Liberty, J.T., Bromage, S., Peter, E., Ihedioha, O.C., Alsalman, F.B., Odogwu, T.S., 2025. CRISPR revolution: unleashing precision pathogen detection to safeguard public health and food safety. Methods 240, 180–194. https://doi.org/10.1016/j.ymeth.2025.04.018.

Lin, C.S., Hsu, C.T., Yang, L.H., Lee, L.Y., Fu, J.Y., Cheng, Q.W., Wu, F.H., Hsiao, H.C.W., Zhang, Y., Zhang, R., Chang, W.J., 2018. Application of protoplast technology to CRISPR/Cas9 mutagenesis: from single-cell mutation detection to mutant plant regeneration. Plant Biotechnol, J 16 (7), 1295–1310. https://doi.org/10.1111/pbi.12867.

Lin, Q., Zong, Y., Xue, C., et al., 2020. Prime genome editing in rice and wheat. Nat. Biotechnol. 38, 582–585. https://doi.org/10.1038/s41587-020-0455-x.

Liu, L., Li, Y., Wang, Q., Xu, X., Yan, J., Wang, Y., Wang, Y., Shah, S.M.A., Peng, Y., Zhu, Z., Xu, Z., Chen, G., 2024a. Constructed rice tracers identify the major virulent transcription activator-like effectors of the bacterial leaf blight pathogen. Rice 17 (1), 30. https://doi.org/10.1186/s12284-024-00704-0.

Liu, S., Zhang, F., Su, J., Fang, A., Tian, B., Yu, Y., Bi, C., Ma, D., Xiao, S., Yang, Y., 2024b. CRISPR-targeted mutagenesis of mitogen-activated protein kinase phosphatase 1 improves both immunity and yield in wheat. Plant Biotechnol, J. https://doi.org/10.1111/pbi.14312.

Liu, T., Ji, J., Cheng, Y., Zhang, S., Wang, Z., Duan, K., Wang, Y., 2023b. CRISPR/Cas9-mediated editing of GmTAP1 confers enhanced resistance to phytophthora sojae in soybean. J. Integr. Plant Biol 65 (7), 1609–1612. https://doi.org/10.1111/jipb.13476.

Liu, X., Yu, Y., Yao, W., Yin, Z., Wang, Y., Huang, Z., Zhou, J., Liu, J., Lu, X., Wang, F., Zhang, G., Chen, G., Xiao, Y., Deng, H., Tang, W., 2023a. CRISPR/Cas9-mediated simultaneous mutation of three salicylic acid 5-hydroxylase (OsS5H) genes confers broad-spectrum disease resistance in rice. Plant Biotechnol, J 21 (9), 1873–1886. https://doi.org/10.1111/pbi.14099.

Louwaars, N., Jochemsen, H., 2021. An ethical and societal analysis for biotechnological methods in plant breeding. Agronomy 11 (6), 1183. https://doi.org/10.3390/agronomy11061183.

Low, Y.C., Lawton, M.A., Di, R., 2020. Validation of barley 2OGO gene as a functional orthologue of Arabidopsis DMR6 gene in Fusarium head blight susceptibility. Sci. Rep. 10, 9935. https://doi.org/10.1038/s41598-020-67006-5.

Luo, T., Ma, C., Fan, Y., Qiu, Z., Li, M., Tian, Y., Shang, Y., Liu, C., Cao, Q., Peng, Y., Zhang, S., Liu, S., Song, B., 2024. CRISPR-Cas9-mediated editing of GmARM improves resistance to multiple stresses in soybean. Plant Sci. 346, 112147. https://doi.org/10.1016/j.plantsci.2024.112147.

Lyngkjær, M.F., Newton, A.C., Atzema, J.L., Baker, S.J., 2000. The barley mlo-gene: an important powdery mildew resistance source. Agronomie 20, 745–756. https://doi.org/10.1051/agro:2000173.

Ma, Y., Liu, M., Stiller, J., Liu, C., 2019. A pan-transcriptome analysis shows that disease resistance genes have undergone more selection pressure during barley domestication. BMC Genom. 20 (1), 12. https://doi.org/10.1186/s12864-018-5357-7. PMID: 30616511.

Macnaghten, P., Habets, M.G.J.L., 2020. Breaking the impasse: towards a forward-looking governance framework for gene editing with plants. Plants. People Planet. 2 (4), 353–365. https://doi.org/10.1002/ppp3.10107.

Mahas, A., Aman, R., Mahfouz, M., 2019. CRISPR-Cas13d mediates robust RNA virus interference in plants. Genome Biol. 20 (1), 263. https://doi.org/10.1186/s13059-019-1881-2.

Maio, F., Helderman, T.A., Arroyo-Mateos, M., Van Der Wolf, M., Boeren, S., Prins, M., Van Den Burg, H.A, 2020. Identification of tomato proteins that interact with replication initiator protein (REP) of the geminivirus TYLCV. Front. Plant Sci. 11, 1069. https://doi.org/10.3389/fpls.2020.01069.

McLaughlin, J.E., Foka, I.C.K., Lawton, M.A., Di, R., 2025. CRISPR activation: identifying and using novel genes for plant disease resistance breeding. Front. Genome Ed. 7, 1596600. https://doi.org/10.3389/fgeed.2025.1596600.

Makhotenko, A.V., Khromov, A.V., Snigir, E.A., Makarova, S.S., Makarov, V.V., Suprunova, T.P., Kalinina, N.O., Taliansky, M.E., 2019. Functional analysis of coilin in virus resistance and stress tolerance of potato solanum tuberosum using CRISPR-

Cas9 editing. Dokl, Biochem, Biophys 484 (1), 88–91. https://doi.org/10.1134/S1607672919010241.

Martynov, A., Severinov, K., Ispolatov, I., 2017 Dec 18. Optimal number of spacers in CRISPR arrays. PLoS. Comput. Biol. 13 (12), e1005891. https://doi.org/10.1371/journal.pcbi.1005891. PMID: 29253874; PMCID: PMC5749868.

Mew, T.W., Alvarez, A.M., Leach, J.E., Swings, J., 1993. Focus on bacterial blight of rice. Plant Dis. 77, 5–12. https://doi.org/10.1094/PD-77-0005.

Mikami, M., Toki, S., Endo, M., 2015. Comparison of CRISPR/Cas9 expression constructs for efficient targeted mutagenesis in rice. Plant Mol, Biol 88 (6), 561–572. https://doi.org/10.1007/s11103-015-0342-x.

Mizoi, J., Shinozaki, K., Yamaguchi-Shinozaki, K., 2012. AP2/ERF family transcription factors in plant abiotic stress responses. Biochim. Biophys. Acta 1819, 86–96. https://doi.org/10.1016/j.bbagrm.2011.08.004.

Mmbando, G.S., Ngongolo, K., 2024. The current status of the use of genetic modification and editing to improve biodiversity and ecological sustainability. All. Life 17 (1). https://doi.org/10.1080/26895293.2024.2417191.

Molla, K.A., Sretenovic, S., Bansal, K.C., Qi, Y., 2021. Precise plant genome editing using base editors and prime editors. Nat. Plants. 7 (9), 1166–1187. https://doi.org/10.1038/s41477-021-00991-1.

Moon, K.-B., Park, S.-J., Park, J.-S., Lee, H.-J., Shin, S.Y., Lee, S.M., Choi, G.J., Kim, S.-G., Cho, H.S., Jeon, J.-H., Kim, Y.-S., Park, Y-I, Kim, H.-S., 2022. Editing of StSR4 by Cas9-RNPs confers resistance to phytophthora infestans in potato. Front, Plant Sci 13, 997888. https://doi.org/10.3389/fpls.2022.997888.

Myhrvold, C., Freije, C.A., Gootenberg, J.S., Abudayyeh, O.O., Metsky, H.C., Durbin, A. F., Kellner, M.J., Tan, A.L., Paul, L.M., Parham, L.A., Garcia, K.F., Barnes, K.G., Chak, B., Mondini, A., Nogueira, M.L., Isern, S., Michael, S.F., Lorenzana, I., Yozwiak, N.L., Sabeti, P.C., 2018. Field-deployable viral diagnostics using CRISPR-Cas13. Science 360 (6387), 444–448. https://doi.org/10.1126/science.aas8836.

Myskja, B.K., Myhr, A.I., 2020. Non-safety assessments of genome-edited organisms: should they be included in regulation? Sci. Eng. Ethics 26 (5), 2601–2627. https://doi.org/10.1007/s11948-020-00222-4.

Biotechnology, Nature, 2021. Argentina first to market with drought-resistant GM wheat. Nat, Biotechnol 39 (6), 652. https://doi.org/10.1038/s41587-021-00963-y.

Nekrasov, V., Wang, C., Win, J., Lanz, C., Weigel, D., Kamoun, S., 2017. Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. Sci. Rep. 7 (1), 482. https://doi.org/10.1038/s41598-017-00578-x.

Nelson, R., Wiesner-Hanks, T., Wisser, R., Balint-Kurti, P., 2018. Navigating complexity to breed disease-resistant crops. Nat. Rev. Genet. 19 (1), 21–33. https://doi.org/10.1038/nrg.2017.82.

Neocrop. 2025. Neocrop obtains “green light” for the first CRISPR-edited wheat in Argentina. Available at: https://neocroptech.com/en/neocrop-obtains-green-light-for-the-first-crispr-edited-wheat-in-argentina/. Accessed on January 10th, 2026.

Nepal, M.P., Andersen, E.J., Neupane, S., Benson, B.V., 2017. Comparative genomics of non-TNL disease resistance genes from six plant species. Genes. (Basel) 8 (10), 249. https://doi.org/10.3390/genes8100249.

New Zealand Legislation, 2024. Gene Technology Bill. Government Bill – New Zealand Legislation. Available at: https://www.legislation.govt.nz/bill/government/2024/0110/latest/whole.html. Accessed on January 9th, 2026.

Ni, P., Zhao, Y., Zhou, X., et al., 2023. Efficient and versatile multiplex prime editing in hexaploid wheat. Genome Biol. 24, 156. https://doi.org/10.1186/s13059-023-02990-1.

Noureen, A., Khan, M.Z., Amin, I., Zainab, T., Mansoor, S., 2022b. CRISPR/CAS9-mediated targeting of susceptibility factor EIF4E-enhanced resistance against potato virus Y. Front. Genet. 13, 922019. https://doi.org/10.3389/fgene.2022.922019.

Noureen, A., Zuhaib Khan, M., Amin, I., Zainab, T., Ahmad, N., Haider, S., Mansoor, S., 2022a. Broad-spectrum resistance against multiple PVY-strains by CRSIPR/Cas13 system in solanum tuberosum crop. GM. Crops. Food 13 (1), 97–111. https://doi.org/10.1080/21645698.2022.2080481. Dec 31PMID: 35652435; PMCID: PMC9176253.

Nourozi, M., Nazarain-Firouzabadi, F., Ismaili, A., Ahmadvand, R., Poormazaheri, H., 2024 Jan 18. CRISPR/Cas StNRL1 gene knockout increases resistance to late blight and susceptibility to early blight in potato. Front. Plant Sci. 14, 1278127. https://doi.org/10.3389/fpls.2023.1278127.

O'Malley, R.C., Barragan, C.C., Ecker, J.R., 2015. A user's guide to the Arabidopsis T-DNA insertion mutant collections. Plant Functional Genomics. Humana Press, New York, NY, pp. 323–342. https://doi.org/10.1007/978-1-4939-2444-8_16.

Oliva, R., Ji, C., Atienza-Grande, G., Huguet-Tapia, J.C., Perez-Quintero, A., Li, T., Eom, J., Li, C., Nguyen, H., Liu, B., Auguy, F., Sciallano, C., Luu, V.T., Dossa, G.S., Cunnac, S., Schmidt, S.M., Slamet-Loedin, I.H., Cruz, C.V., Szurek, B., Yang, B., 2019. Broad-spectrum resistance to bacterial blight in rice using genome editing. Nat, Biotechnol 37 (11), 1344–1350. https://doi.org/10.1038/s41587-019-0267-z.

Orroño, D.I., Vesprini, F., 2018. Directives and requirements for genetically modified (GM) crop regulation in Argentina. Crop Breed. Appl. Biotechnol. 18 (3), 301–308. https://doi.org/10.1590/1984-70332018v18n3r44.

Otieno, M.O., 2015. CRISPR-Cas9 human genome editing: challenges, ethical concerns and implications. J. Clin. Res. Bioeth. 6 (6), 253–255. https://doi.org/10.4172/2155-9627.1000253.

Ou, S.H., 1985. Rice Diseases. Commonwealth Mycological Institute, Kew, UK, pp. 330–380.

Ozyigit, I.I., Kurtoglu, K.Y., 2020. Particle bombardment technology and its applications in plants. Mol, Biol, Rep 47 (12), 9831–9847. https://doi.org/10.1007/s11033-020-06001-5.

Pacillo, L., 2025. Why Regulations in Plant Gene Editing Are a Must. The University of Adelaide. Available at: https://www.adelaide.edu.au/newsroom/news/list/2025/06/23/why-regulations-in-plant-gene-editing-are-a-must. Accessed on January 9th, 2026.

Pandey, P., Irulappan, V., Bagavathiannan, M.V., Senthil-Kumar, M., 2017. Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. Front. Plant Sci. 8, 537. https://doi.org/10.3389/fpls.2017.00537.

Papade, J.N., Ingle, K.P., Thakur, N.R., Gomashe, S.S., Padmavathy, M., Ceasar, S.A., 2025. Tapping into the potential of underutilized Niger (*Guizotia abyssinica* (L. f.) Cass.) Through breeding and biotechnological tools. Agriculture 15, 350. https://doi.org/10.3390/agriculture15030350.

PDI, 2025. Pakistan achieves milestone in agricultural biotechnology: NBC approves GM sugarcane and cotton varieties for commercialization. PR No. 223. In: Press Information Department. Ministry of Information and Broadcasting, Government of Pakistan. Available at: https://pid.gov.pk/site/press_detail/30733. Accessed on January 10th, 2026.

Paul, B., Montoya, G., 2020. CRISPR-Cas12a: functional overview and applications. Biomed. J. 43 (1), 8–17. https://doi.org/10.1016/j.bj.2019.10.005.

Phillips, P.W.B., 2021. Economic consequences of regulations of GM crops. Genet. Lit. Proj. https://geneticliteracyproject.org/2021/11/19/economic-consequences-of-regulations-of-gm-crops/.

Pieterse, L. 2025. Project Oppotunity completes first CRISPR-Cas field trials of late blight-resistant starch potatoes in Sweden and Denmark. Potato News Today. Available at: https://www.potatonewstoday.com/2025/11/14/project-oppotunity-completes-first-crispr-cas-field-trials-of-late-blight-resistant-starch-potatoes-in-sweden-and-denmark/. Accessed on January 13th, 2026.

Poudel, B., Sathe, A., Bede, J.C., Kushalappa, A.C., 2025. Editing metacaspase (StMC7) gene enhances late blight resistance in Russet Burbank potato. PLoS. One 20 (6), e0325702. https://doi.org/10.1371/journal.pone.0325702. Jun 18PMID: 40531937; PMCID: PMC12176156.

Prabhukarthikeyan, S.R., Manikandan, R., Durgadevi, D., Keerthana, U., Harish, S., Karthikeyan, G., Raguchander, T., 2017. Biosuppression of turmeric rhizome rot disease and understanding the molecular basis of tripartite interaction among Curcuma longa, Pythium aphanidermatum and Pseudomonas fluorescens. Biol. Control 111, 23–31. https://doi.org/10.1016/j.biocontrol.2017.05.003.

Prabhukarthikeyan, S.R., Yadav, M.K., Anandan, A., Aravindan, S., Keerthana, U., Raghu, S., Baite, M.S., Parameswaran, C., Panneerselvam, P., Rath, P.C., 2019. Bio-protection of brown spot disease of rice and insight into the molecular basis of interaction between oryza sativa, Bipolaris oryzae and Bacillus amyloliquefaciens. Biol. Control 137, 104018. https://doi.org/10.1016/j.biocontrol.2019.104018.

Press Information Bureau, Delhi, 2025. Union Agriculture Minister Shri Shivraj Singh Chouhan Announces Two Genome-Edited Rice Varieties Developed in India. Ministry of Agriculture & Farmers Welfare. Available at: https://www.pib.gov.in/PressReleaseIframePage.aspx?PRID=2126802&reg=3&lang=2. Accessed on January 6th, 2026.

Priyadarshini, S., 2025. India approves first genome-edited rice varieties. Nat. India. https://doi.org/10.1038/d44151-025-00078-2.

Purohit, A., Kounain, S., Madala, C., Chavan, S., Guguloth, N., Kethavathu, S., Behera, P. P., Penna, S., Thakur, N.R., 2026. Sustainable crops for future food security: challenges, innovations, and solutions. In: Penna, S., Varshney, R.K. (Eds.), Improving Crops for Future Sustainability and Climate Resilience. Sustainability Sciences in Asia and Africa (SSAA). Springer, Singapore. https://doi.org/10.1007/978-981-95-2754-0_1.

Qiao, D., Wang, J., Lu, M.H., Xin, C., Chai, Y., Jiang, Y., Sun, W., Cao, Z., Guo, S., Wang, X.C., Chen, Q.J., 2023. Optimized prime editing efficiently generates heritable mutations in maize. J. Integr. Plant Biol. 65 (4), 900–906. https://doi.org/10.1111/jipb.13428.

Ramakrishnan, M., Kaul, R., Sharma, A., Ahmad, Z., Vijayakanth, V., Keerthana, K., Gao, Z., Zhou, M., Wei, Q., 2025. CRISPR RNP-mediated transgene-free genome editing in plants: advances, challenges and future directions for tree species. Plant Cell Environ. https://doi.org/10.1111/pce.70176.

Ramasamy, M., Rajkumar, M.S., Bedre, R., Irigoyen, S., Berg-Falloure, K., Kolomiets, M. V., Mandadi, K.K., 2024. Genome editing of NPR3 confers potato resistance to Candidatus Liberibacter spp. Plant Biotechnol. J. (9), 2635–2637. https://doi.org/10.1111/pbi.14378.

Razzaq, H.A., Ijaz, S., Haq, I.U., Khan, I.A., 2022. Functional inhibition of the StERF3 gene by dual targeting through CRISPR/Cas9 enhances resistance to the late blight disease in Solanum tuberosum L. Mol, Biol, Rep 49 (12), 11675–11684. https://doi.org/10.1007/s11033-022-07958-1.

Reuters. 2023. China approves safety of first gene-edited crop. Available at: https://www.reuters.com/science/china-approves-safety-first-gene-edited-crop-2023-05-04/. Accessed on January 10th, 2026.

Rivera-Toro, D.M., De Folter, S., Alvarez-Venegas, R., 2025. CRISPR/dCas12a-mediated activation of SlPAL2 enhances tomato resistance against bacterial canker disease. PLoS. One 20 (3), e0320436. https://doi.org/10.1371/journal.pone.0320436.

Rodriguez, E., 2016. Ethical issues in genome editing using Crispr/Cas9 system. J. Clin. Res. Bioeth. 7 (2), 266. https://doi.org/10.4172/2155-9627.1000266.

Rostoum, E., 2025. The New China challenge: Gene Editing On the Farm. CEPA. Available at: https://cepa.org/article/the-new-china-challenge-gene-editing-on-the-farm/. Accessed on January 6th, 2026.

Safaeizadeh, M., Boller, T., 2019. Differential and tissue-specific activation pattern of the AtPROPEP and AtPEPR genes in response to biotic and abiotic stress in Arabidopsis thaliana. Plant Signal. Behav. 14 (5), e1590094. https://doi.org/10.1080/15592324.2019.1590094.

Sang, S., Ma, T., Zhang, Y., Zhang, S., Wang, Y., Zhang, J., Yao, G., Feng, L., Ji, S., Cheng, H., Li, J., Yan, P., 2025. Variations in the OSGGP UORF fine-tune vitamin C content and confer resistance to osmotic stress in rice. Rice 18 (1), 98. https://doi.org/10.1186/s12284-025-00848-7.

Sakurai, A., Kanzaki, S., Honda, F., 2022. Japanese pharmaceutical regulations of engineered viral vectors for medical use compared with those in the United States and the European Union. Clin. Pharmacol. Ther. 113 (5), 960–962. https://doi.org/10.1002/cpt.2788.

Savary, S., Willocquet, L., Pethybridge, S.J., et al., 2019. The global burden of pathogens and pests on major food crops. Nat. Ecol. Evol. 3, 430–439. https://doi.org/10.1038/s41559-018-0793-y.

Sánchez, M.A., 2024. The global advance of genome-edited plants to the market: the key role of Chile in its development. Plants 13 (24), 3597. https://doi.org/10.3390/plants13243597.

Scheinerman, N., Sherkow, J.S., 2021. Governance choices of genome editing patents. Front. Polit. Sci. 3. https://doi.org/10.3389/fpos.2021.745898.

Schiml, S., Fauser, F., Puchta, H., 2014. The CRISPR/Cas system can be used as nuclease for in planta gene targeting and as paired nickases for directed mutagenesis in Arabidopsis resulting in heritable progeny. Plant J. 80 (6), 1139–1150. https://doi.org/10.1111/tpj.12704.

Schmidt, S.M., Belisle, M., Frommer, W.B., 2020. The evolving landscape around genome editing in agriculture. EMBo Rep. 21 (6), e50680. https://doi.org/10.15252/embr.202050680.

SeedWorld. 2025. Adoption record: transgenic crops reached 210 million hectares in 2024. Available at: https://www.seedworld.com/latam/2025/07/21/adoption-record-transgenic-crops-reached-210-million-hectares-in-2024/. Accessed on January 5th, 2026.

Shan, P., 2024. Impact of gene editing technology on ecological balance: case studies and assessments. GMO Biosaf. Res. https://ecoevopublisher.com/index.php/gmo/article/html/3933/.

Sharma, P., 2023. Epidemiology of potyviruses infecting crops of Cucurbitaceae. Plant RNA Viruses. Academic Press, pp. 213–227. https://doi.org/10.1016/B978-0-323-95339-9.00016-8.

Shinwari, Z.K., Tanveer, F., Khalil, A.T., 2017. Ethical issues regarding CRISPR-mediated genome editing. Curr. Issues. Mol. Biol. 26, 103–110. https://doi.org/10.21775/9781910190630.09.

Shipman, E.N., Yu, J.W., Zhou, J.Q., et al., 2021. Can gene editing reduce postharvest waste and loss of fruit, vegetables, and ornamentals? Hortic. Res. 8, 1. https://doi.org/10.1038/s41438-020-00428-4.

Shmakov, S.A., Utkina, I., Wolf, Y.I., Makarova, K.S., Severinov, K.V., Koonin, E.V., 2020 Dec. CRISPR arrays away from cas genes. CRISPR. J. 3 (6), 535–549. https://doi.org/10.1089/crispr.2020.0062. PMID: 33346707; PMCID: PMC7757702.

Simard, M., 2010. Gene flow between crops and their wild relatives. Evol. Appl. 3 (4), 402–403. https://doi.org/10.1111/j.1752-4571.2010.00138.x.

Spök, A., Sprink, T., Allan, A.C., Yamaguchi, T., Dayé, C., 2022. Towards social acceptability of genome-edited plants in industrialised countries? Emerging evidence from Europe, United States, Canada, Australia, New Zealand, and Japan. Front. Genome Ed. 4, 899331. https://doi.org/10.3389/fgeed.2022.899331.

Sprink, T., Wilhelm, R., Hartung, F., 2022. Genome editing around the globe: an update on policies and perceptions. Plant Physiol. 190 (3), 1579–1587. https://doi.org/10.1093/plphys/kiac359.

Stokstad, E., 2024. European Parliament votes to ease regulation of gene-edited crops. Science. Available at: https://www.science.org/content/article/european-parliament-votes-ease-regulation-gene-edited-crops. Accessed on January 6th, 2026.

Tachikawa, M., Matsuo, M., 2024. Global regulatory trends of genome editing technology in agriculture and food. Breed, Sci 74 (1), 3–10. https://doi.org/10.1270/jsbbs.23046.

Tang, N., Zhang, Y., Shen, Z., Yao, Y., Nair, V., 2021. Application of CRISPR-CAS9 editing for virus engineering and the development of recombinant viral vaccines. CRISPR J. 4 (4), 477–490. https://doi.org/10.1089/crispr.2021.0017.

Tatineni, S., Hein, G.L., 2023. Plant viruses of agricultural importance: current and future perspectives of virus disease management strategies. Phytopathology® 113 (2), 117–141. https://doi.org/10.1094/PHYTO-05-22-0167-RVW.

Teng, F., Guo, L., Cui, T., Wang, X., Xu, K., Gao, Q., Zhou, Q., Li, W., 2019. CDetection: cRISPR-Cas12b-based DNA detection with sub-attomolar sensitivity and single-base specificity. Genome Biol. 20 (1), 132. https://doi.org/10.1186/s13059-019-1742-z.

Thakur, N.R., Ingle, K.P., Sargar, P.R., Baraskar, S.S., Kasanaboina, K., Awio, B., Pranati, J., Abdi, G., 2024. Sustainable utilization of wild germplasm resources. In: Al-Khayri, J.M., Jain, S.M., Penna, S. (Eds.), Sustainable Utilization and Conservation of Plant Genetic Diversity. Sustainable Development and Biodiversity, Vol 35. Springer, Singapore. https://doi.org/10.1007/978-981-99-5245-8_16. ISBN: 978-981-99-5245-8.

Thakur, S., Kaur, S., Adhikari, S., Sabharwal, P., Fu, Y., Meru, G., 2025. Turning susceptibility into strength: a new era of durable resistance in plants through genome editing. Plants 14 (19), 3080. https://doi.org/10.3390/plants14193080.

Thordal-Christensen, H., 2020. A holistic view on plant effector-triggered immunity presented as an iceberg model. Cell. Mol. Life Sci. 77 (20), 3963–3976. https://doi.org/10.1007/s00018-020-03515-w.

Tobón-Niedfeldt, W., Mastretta-Yanes, A., Urquiza-Haas, T., Goettsch, B., Cuervo-Robayo, A.P., Urquiza-Haas, E., Orjuela-R, M.A., Gasman, F.A., Oliveros-Galindo, O., Burgeff, C., Rivera-Rodríguez, D.M., De Jesús Sánchez González, J., Alarcón-Guerrero, J., Aguilar-Meléndez, A., Cuevas, F.A., Alavez, V., Alejandre-Iturbide, G., Avendaño-Arrazate, C., Pérez, C.A., Koleff, P., 2022. Incorporating evolutionary and threat processes into crop wild relatives conservation. Nat. Commun. 13 (1), 6254. https://doi.org/10.1038/s41467-022-33703-0.

Turnbull, C., Lillemo, M., Hvoslef-Eide, T.A.K., 2021. Global regulation of genetically modified crops amid the gene edited crop boom – A review. Front. Plant Sci. 12, 630396. https://doi.org/10.3389/fpls.2021.630396.

USDA, 2024. APHIS acknowledges new court ruling vacating updates to biotechnology regulations. Animal and Plant Health Inspection Service. US Dep. Agric. Available at: https://www.aphis.usda.gov/news/program-update/aphis-acknowledges-new-court-ruling-vacating-updates-biotechnology-regulations Accessed on January 6th, 2026.

Van Butselaar, T., Van Den Ackerveken, G., 2020. Salicylic acid steers the growth–Immunity tradeoff. Trends. Plant Sci. 25 (6), 566–576. https://doi.org/10.1016/j.tplants.2020.02.002.

Vengadesen, S., Bin Abdul Aziz, M.F., Jones, M., 2025. Rethinking gene-edited crop regulation: advancing a principle-based framework for modern biotechnology governance. GM. Crops. Food 16 (1), 852. https://doi.org/10.1080/21645698.2025.2576734.

Venkataraman, S., Hefferon, K., 2025. Editorial: social aspects of crop genome editing. Front. Genome Ed. 7. https://doi.org/10.3389/fgeed.2025.1740380.

Venkataraman, S., Zaruk, D., Hefferon, K., 2025. Recent developments, challenges and opportunities in genome editing for crop science from a societal perspective. Front. Genome Ed. 7, 1568072. https://doi.org/10.3389/fgeed.2025.1568072.

Vink, J.N.A., Baijens, J.H.L., Brouns, S.J.J., 2021. PAM-repeat associations and spacer selection preferences in single and co-occurring CRISPR-Cas systems. Genome Biol. 22, 281. https://doi.org/10.1186/s13059-021-02495-9.

Von Essen, M.R., Kongsbak, M., Schjerling, P., Olgaard, K., Ødum, N., Geisler, C., 2010. Vitamin D controls T cell antigen receptor signaling and activation of human T cells. Nat, Immunol 11 (4), 344–349. https://doi.org/10.1038/ni.1851.

Wang, J., Huang, X., Chen, S., Chen, J., Liang, Z., Chen, B., Yang, X., Zhou, G., Zhang, T., 2023. On-site and visual detection of sorghum mosaic virus and rice stripe mosaic virus based on reverse transcription-recombinase-aided amplification and CRISPR/Cas12a. Front. Genome Ed 5, 1124794. https://doi.org/10.3389/fgeed.2023.1124794.

Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., Qiu, J.L., 2014. Simultaneous editing of three homoeoalleles in hexaploidy bread wheat confers heritable resistance to powdery mildew. Nat, Biotechnol 32, 94–951. https://doi.org/10.1038/nbt.2969.

Wang, F., Wang, C., Liu, P., Lei, C., Hao, W., Gao, Y., Liu, Y.-G., Zhao, K., 2016b. Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. PLoS. One 11 (4), e0154027. https://doi.org/10.1371/journal.pone.0154027.

Wang, F.Q., Fan, F.J., Li, W.Q., Zhu, J.Y., Wan, J., Zhong, W.G., Yang, J., 2016a. Knock-out efficiency analysis of Pi21 gene using CRISPR/Cas9 in rice. Chin, J. Rice Sci 30, 469–478.

Wang, N., Tang, C., Fan, X., He, M., Gan, P., Zhang, S., Hu, Z., Wang, X., Yan, T., Shu, W., Yu, L., Zhao, J., He, J., Li, L., Wang, J., Huang, X., Huang, L., Zhou, J., Kang, Z., Wang, X., 2022. Inactivation of a wheat protein kinase gene confers broad-spectrum resistance to rust fungi. Cell 185 (16), 2961–2974. https://doi.org/10.1016/j.cell.2022.06.027. .e19.

Warwick, S.I., Beckie, H.J., Hall, L.M., 2009. Gene flow, invasiveness, and ecological impact of genetically modified crops. Ann, N. Y. Acad, Sci 1168 (1), 72–99. https://doi.org/10.1111/j.1749-6632.2009.04576.x.

Woźniak-Gientka, E., Tyczewska, A., Twardowski, T., 2022. Public opinion on biotechnology and genetic engineering in the European Union: polish consumer study. BioTechnologia 103 (2), 185–201. https://doi.org/10.5114/bta.2022.116212.

Wray-Cahen, D., Bodnar, A., Rexroad, I.II.C., et al., 2022. Advancing genome editing to improve the sustainability and resiliency of animal agriculture. CABI. Agric. Biosci. 3, 21. https://doi.org/10.1186/s43170-022-00091-w.

Yang, J., Fang, Y., Wu, H., Zhao, N., Guo, X., Mackon, E., Peng, H., Huang, S., He, Y., Qin, B., Liu, Y., Liu, F., Chen, S., Li, R., 2023a. Improvement of resistance to rice blast and bacterial leaf streak by CRISPR/Cas9-mediated mutagenesis of *Pi21* and *OsSULTR3;6* in rice (*Oryza sativa* L.). Front. Plant Sci. 14, 1209384. https://doi.org/10.3389/fpls.2023.1209384. Jul 17PMID: 37528980; PMCID: PMC10389665.

Yang, Y., Hobbs, J.E., 2020. Supporters or opponents: will cultural values shape consumer acceptance of gene editing? J Food Prod Mark 26 (1), 17–37. https://doi.org/10.1080/10454446.2020.1715316.

Yang, L., Machin, F., Wang, S., Saplaoura, E., Kragler, F., 2023b. Heritable transgene-free genome editing in plants by grafting of wild-type shoots to transgenic donor rootstocks. Nat, Biotechnol 41 (7), 958–967. https://doi.org/10.1038/s41587-022-01585-8.

Yeh, W., Chiang, H., Rees, H.A., Edge, A.S.B., Liu, D.R., 2018. In vivo base editing of post-mitotic sensory cells. Nat. Commun. 9 (1). https://doi.org/10.1038/s41467-018-04580-3.

Yin, K., Chung, M.Y., Lan, B., Du, F.K., Chung, M.G., 2024. Plant conservation in the age of genome editing: opportunities and challenges. Genome Biol. 25 (1), 279. https://doi.org/10.1186/s13059-024-03399-0.

Zahra, N., Hafeez, M.B., Al Shukaily, M., Al-Sadi, A.M., Siddique, K.H., Farooq, M., 2023. Influence of abiotic stresses on disease infestation in plants. Physiol, Mol, Plant Pathol 127, 102125. https://doi.org/10.1016/j.pmpp.2023.102125.

Zarate, S., Cimadori, I., Jones, M.S., Roca, M.M., Barnhill-Dilling, S.K., 2023. Assessing agricultural gene editing regulation in Latin America: an analysis of how policy windows and policy entrepreneurs shape agricultural gene editing regulatory regimes. Front. Bioeng. Biotechnol. 11, 1209308. https://doi.org/10.3389/fbioe.2023.1209308.

Zeng, X., Luo, Y., Vu, N.T.Q., Shen, S., Xia, K., Zhang, M., 2020. CRISPR/Cas9-mediated mutation of OsSWEET14 in rice cv. Zhonghua11 confers resistance to xanthomonas oryzae pv. oryzae without yield penalty. BMC. Plant Biol. 20, 313. https://doi.org/10.1186/s12870-020-02524-y.

Zhan, X., Liu, W., Nie, B., Zhang, F., Zhang, J., 2023. Cas13d-mediated multiplex RNA targeting confers a broad-spectrum resistance against RNA viruses in potato. Commun. Biol. 6 (1), 855. https://doi.org/10.1038/s42003-023-05205-2. PMID: 37591976; PMCID: PMC10435558.

Zhan, X., Zhang, F., Zhong, Z., Chen, R., Wang, Y., Chang, L., Bock, R., Nie, B., Zhang, J., 2019. Generation of virus-resistant potato plants by RNA genome targeting. Plant Biotechnol. J. 17 (9), 1814–1822. https://doi.org/10.1111/pbi.13102. SepEpub 2019 Mar 8. PMID: 30803101; PMCID: PMC6686122.

Zhang, Z., Ge, X., Luo, X., Wang, P., Fan, Q., Hu, G., Xiao, J., Li, F., Wu, J., 2018. Simultaneous editing of two copies of GH 14-3-3D confers enhanced transgene-clean plant defense against verticillium dahliae in allotetraploid upland cotton. Front. Plant Sci. 9, 842. https://doi.org/10.3389/fpls.2018.00842.

Zhang, D., Hussain, A., Manghwar, H., Xie, K., Xie, S., Zhao, S., Larkin, R.M., Qing, P., Jin, S., Ding, F., 2020. Genome editing with the CRISPR-Cas system: an art, ethics and global regulatory perspective. Plant Biotechnol, J 18 (8), 1651–1669. https://doi.org/10.1111/pbi.13383.

Zhang, M., Coaker, G., 2017. Harnessing effector-triggered immunity for durable disease resistance. Phytopathology. 107 (8), 912–919. https://doi.org/10.1094/phyto-03-17-0086-rvw.

Zhang, Y., Bai, Y., Wu, G., Zou, S., Chen, Y., Gao, C., Tang, D., 2017. Simultaneous modification of three homoeologs of TaEDR1 by genome editing enhances powdery mildew resistance in wheat. Plant J. 91 (4), 714–724. https://doi.org/10.1111/tpj.13599.

Zhang, Y., Lin, X., Li, L., Piao, R., Wu, S., Song, A., Gao, M., Jin, Y., 2024. CRISPR/Cas9-mediated knockout of bsr-d1 enhances the blast resistance of rice in Northeast China. Plant Cell Rep. 43 (4). https://doi.org/10.1007/s00299-024-03192-0.

Zhang, Y., Yao, J.L., Feng, H., Jiang, J., Fan, X., Jia, Y.F., Wang, R., Liu, C., 2019b. Identification of the defense-related gene VdWRKY53 from the wild grapevine Vitis davidii using RNA sequencing and ectopic expression analysis in Arabidopsis. Hereditas 156 (1), 14. https://doi.org/10.1186/s41065-019-0089-5.

Zhang, Z., Guo, J., Zhao, Y., Chen, J., 2019c. Identification and characterization of maize ACD6-like gene reveal ZmACD6 as the maize orthologue conferring resistance to Ustilago maydis. Plant Signal. Behav. 14 (10). https://doi.org/10.1080/15592324.2019.1651604.

Zhou, Y., Xu, S., Jiang, N., Zhao, X., Bai, Z., Liu, J., Yao, W., Tang, Q., Xiao, G., Lv, C., Wang, K., Hu, X., Tan, J., Yang, Y., 2022. Engineering of rice varieties with enhanced resistances to both blast and bacterial blight diseases via CRISPR/Cas9. Plant Biotechnol, J 20 (5), 876–885. https://doi.org/10.1111/pbi.13766.

Zhou, W., Li, M., Achal, V., 2024. A comprehensive review on environmental and human health impacts of chemical pesticide usage. Emerg. Contam. 11 (1), 100410. https://doi.org/10.1016/j.emcon.2024.100410.

Zhu, H., Li, C., Gao, C., 2020. Applications of CRISPR–Cas in agriculture and plant biotechnology. Nat. Rev. Mol. Cell Biol. 21, 661–677. https://doi.org/10.1038/s41580-020-00288-9.

Zhu, J., 2022. The future of gene-edited crops in China. Natl. Sci. Rev. 9 (4), nwac063. https://doi.org/10.1093/nsr/nwac063.

Zimny, T., Sowa, S., 2021. Potential effects of asymmetric legal classification of gene edited plant products in international trade, from the perspective of the EU. EFB Bioeconomy J. 1, 100016. https://doi.org/10.1016/j.bioeco.2021.100016.