GESA
GLOCAL ENVIRONMENT & SOCIAL ASSOCIATION

International Journal of Biological Innovations
http://ijbi.org.in | http://www.gesa.org.in/journals.php
https://doi.org/10.46505/IJBI.2026.8109
IJBI 8(1): 83-93 **(2026)**
E-ISSN: 2582-1032

A BIRD'S EYE VIEW ON PRECISION IMMUNITY UTILIZING GENOME EDITING (CRISPR/Cas) TO ENGINEER INTRINSIC CROP RESISTANCE

Manal Khan and Aparna Pareek*

Department of Botany

Department of Botany, University of Rajasthan, Jaipur (Rajasthan), India

**Corresponding author*: aparna992000@yahoo.com

Article Info:
Review Article
Received
18.12.2025
Reviewed
20.01.2026
Accepted
28.02.2026

Abstract: Plant diseases are still affecting the world food security by lowering the production of large crops. In this review, the precision immunity concept, which involves the specific editing of endogenous plant genes to boost innate immunity, is critically assessed as the use of CRISPR/Cas-mediated genome editing to increase disease resistance. Authors reviewed the current literature on disruption of susceptibility (S) gene, control of immune signaling pathways, and allele-engineering in crops, including rice, wheat, and tomato. The review also covers the developments in multiplex editing, base editing, prime editing, and the unique functions of the system based on Cas12 (DNA targeting) and Cas13 (RNA targeting) in controlling DNA- and RNA-based pathogenesis. With respect to the editing outcomes, a few brief remarks are made on mechanistic aspects of repair pathways such as non-homologous end joining and homology-directed repair. Technical issues, regulatory changes, and biosafety issues are also evaluated. Altogether, as the existing evidence indicates, CRISPR-based approaches possess enormous capacity to aid in the creation of long-lasting and climate-resilient disease resistance in crops.

Keywords: Base and prime editing, Biosafety, CRISPR/Cas genome editing, Plant disease resistance.

Cite this article as: Khan M. and Pareek A. (2026). A bird's eye view on precision immunity utilizing genome editing (CRISPR/Cas) to engineer intrinsic crop resistance. *International Journal of Biological Innovations*. 8(1): 83-93. https://doi.org/10.46505/IJBI.2026.8109

INTRODUCTION

CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9) system is an adaptive immune system found in bacteria. It uses a Cas9 endonuclease driven by a designed RNA sequence to cause targeted breaks (DSBs) in two genomic loci of plants and animals (Khatodia *et al.,* 2016). CRISPR/Cas9 has become a key player in contemporary plant biotechnology because it is accurate, efficient, and cost-effective when compared to previous genome-editing systems, including ZFNs and TALENs (Khatodia *et al.,* 2016; Mushtaq *et al.,* 2019; Chen *et al.,* 2019). It allows fast and target-oriented modification of the genome by causing RNA-guided activity of the Cas9 enzyme which creates targeted and fast double-strand breaks. The CRISPR represents an alternative to traditional breeding in the light of growing global food demand and climate change that is limited by long generation times and restricted genetic diversity. Even though CRISPR/Cas9 technology offers a more targeted and rapid method of creating high-yielding and stress-tolerant crops, multiple technical and practical constraints exist. These are the lack of efficiency of homology-directed repair (HDR), the possibility of off-target and mutations, and difficulties in delivering

genome-editing elements into plant cells (Chen *et al.,* 2019). Moreover, a lack of complete reference genomes of most crop species and fragmented global regulatory frameworks remain the barriers to the mass application of CRISPR-edited crops (Scheben *et al.*, 2017).

One of the best prospective applications of CRISPR technology is to promote the resistance of crops to biotic stresses which could significantly diminish the yield of key crops (Zaidi *et al.,* 2016). Being more precise in gene knockout, more sophisticated methods like base editing and DNA-free ribonucleoprotein (RNP) delivery can now be used to introduce a specific nucleotide substitution with minimal off-target genomic effects (Chen *et al*., 2019). Such strategies allow turning into disease resistance either the pathogen genomes or altering host susceptibility (S) genes and important regulatory elements of plant defense pathways (Zhao *et al.*, 2022; Faizal *et al.*, 2024). Despite its promising results of laboratory research, field translation has proven to be a complicated issue because of the variability of the environment, evolving pathogens, inconsistency in regulations, and the difficulty in gaining public acceptance. These biological, technical, and socio-regulatory limitations must be overcome to enhance CRISPR-based disease resistance systems in experimental systems to a sustainable system in agriculture.

MECHANISM OF CRISPR/Cas IN GENOME EDITING

1. Components of the CRISPR/Cas system: Cas9, Cas12a, gRNA, PAM: An innovative version of the adaptive immune response of *Streptococcus pyogenes*, the CRISPR/Cas9 system has become the most revolutionary instrument in molecular genetics. It enables targeted and precise editing of DNA sequences across a wide range of organisms, from bacteria to plants, through the coordinated action of three core components: the Cas9 nuclease, the guide RNA (gRNA), and the protospacer adjacent motif (PAM). The overall architecture of the CRISPR/Cas9 complex and its mechanism of target recognition and cleavage are illustrated in figure 1.

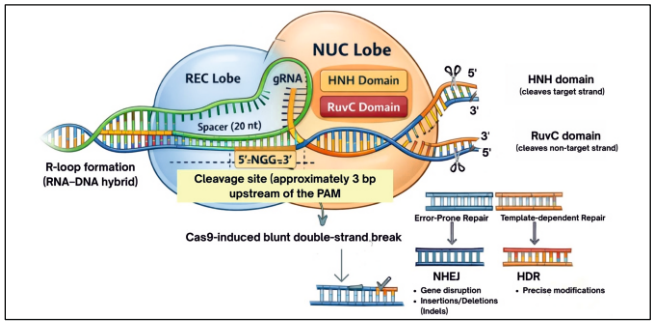

Fig. 1: Schematic representation of the CRISPR/Cas9 mechanism showing gRNA-guided target recognition.

1.1. Cas9: The catalytic core of the mechanism is called the protein named Cas9, and it cleaves the DNA in the necessary location. Cas9 consists of two large lobes, the recognition (REC) lobe and the nuclease (NUC) lobe, which interacts with DNA in different ways and cleaves it. The REC lobe is bound to the gRNA-DNA complex, whereas the NUC lobe contains two domains referred to as RuvC and HNH, which cut double-strand breaks (DSBs) on complementary strands of DNA (Jinek *et al*., 2012). When directed to a target by the gRNA, Cas9 unwinds the DNA double helix and introduces a double-strand break (DSB) approximately three base pairs upstream of the PAM sequence. Cas9 introduces a blunt double-strand break approximately three base pairs upstream of the PAM sequence, as depicted in figure 1. This endonuclease activity that is programmable allows the targeted disruption or repair of genes.

After cleavage, a DNA double-strand break (DSB) is formed in plant cells because of a Cas9-mediated cleavage, which can be repaired via two major mechanisms non-homologous end joining (NHEJ) and homology-directed repair (HDR). The most common pathway in plants is known as NHEJ, which proceeds without a homologous template and can lead to small insertions or deletions (indels) that can disable the function of the gene and can be exploited to create knockouts. Instead, HDR employs a homologous DNA template to repair the break accurately, allowing the targeted sequence replacement or insertion; however, HDR is much less frequent in plant cells because of the scarce donor templates and competition with the NHEJ pathway. The knowledge of these repairing processes is significant when explaining the results of CRISPR/Cas9 editing and when establishing methods to increase specific gene editing in plants (Chen *et al.,* 2022). For instance, the OsSWEET14 gene has been effectively knocked out in *Oryza sativa* (rice) with the help of Cas9, which resulted in high resistance to bacterial blight caused by *Xanthomonas oryzae* (Li *et al*., 2013). Additionally, the editing of the PDS3 gene with the adoption of the Cas9 editing method led to albinism in *Arabidopsis thaliana*, which shows that the editing process was effective (Fauser *et al.,* 2014).

There are additional Cas9 orthologs derived from different bacterial species that offer distinct functional advantages. As an example, *Staphylococcus aureus* Cas9 (SaCas9) is smaller than *S. pyogenes* Cas9 (SpCas9) and identifies a longer PAM (NNGRRT) and is simpler to transfer in plant systems with limited vector capacity (Kaya *et al*., 2016). Cas12a (Cpf1), derived from *Francisella novicida*, is another CRISPR variant that creates staggered cuts in DNA and recognizes a T-rich PAM sequence. This feature

expands genome-editing applications in AT-rich plant genomes such as barley and wheat (Tang *et al.,* 2017).

1.2. Cas12a (Cpf1): Cas12a (formerly Cpf1) is a distinct class II CRISPR-associated endonuclease that differs structurally and mechanistically from Cas9. In contrast to Cas9, Cas12a uses only a single CRISPR RNA (crRNA) to identify its target and is not dependent on a trans-activating crRNA (tracrRNA), making its guide RNA design simpler. Cas12a has a RuvC-like nuclease domain but does not have the HNH domain, as found in Cas9. When a protospacer adjacent motif that is rich in thymines is identified, such as 5'-TTVA-3', Cas12a will cleave a staggered double-strand break at the distal end of the PAM site to produce 5' overhangs, as opposed to blunt ends. This pattern of staggered cleavage can be useful in making precise gene insertions using homology-directed repair (Zetsche *et al.,* 2015). Cas12a is effective in genome editing in crops, including rice, maize, barley, and wheat, in plant systems, especially in AT-rich genomic regions, where Cas9 targeting can be constrained by its NGG PAM sequence. Indicatively, Tang *et al*. (2017) have reported successful Cas12a genome editing of rice with *Francisella novicida,* which has been shown to have high mutation efficiency and flexible target sites with high flexibility. These properties render Cas12a a powerful complement of Cas9, particularly in plant genome engineering in vivo research that necessitates alternative PAM recognition and multiplex gene editing plans (Tang *et al*., 2017).

1.3. Guide RNA (gRNA): The guide RNA (gRNA) is an artificial combination of two bacterial RNAs, i.e. the CRISPR RNA (crRNA) defining the DNA target and the trans-activating CRISPR RNA (tracrRNA), which attaches to Cas9. Practically, these two are joined together as one guide RNA (sgRNA) of approximately 100 nucleotides to guide Cas9 to complementary DNA sites through Watson-Crick base pairs.

The gRNA is a GPS to make sure that only the intended location is cut by Cas9. The spacer region is 20 nucleotides long, and it is the one that determines target specificity and the scaffold one that facilitates the binding of Cas9. This flexible design has enabled plants to easily produce many gRNAs that target a group of genes at a time in a process known as multiplex genome editing. As an example, in *Nicotiana tabacum* (tobacco), several gRNAs against ALS (Amyotrophic Lateral Sclerosis), PDS (phytoene desaturase), and NPR1 (natriuretic peptide receptor 1) genes were co-expressed to produce mutants of several genes in one transformation (Gao *et al*., 2014). A successful example of gRNA diversity is tomato (*Solanum lycopersicum*), where multiplex editing of RIN (ripening inhibitor), NOR (non-ripening), and AP2a transcription factors led to an increase in fruit shelf-life and ripening regulation (Wang *et al.,* 2020). These practical uses underscore the way gRNA-mediated specificity has the chance to adjust the physiology and yield of plants.

1.4. PAM: Protospacer adjacent motif (PAM) is a short and conserved DNA fragment that is adjacent to the target site. Not included in the gRNA, PAM recognition is essential in Cas9 binding. The sequence of the PAM of the most common *S. pyogenes* Cas9 is 'NGG', in which N may represent any base (Anders *et al.,* 2014). This PAM serves as a gate keeping mechanism to make sure that Cas9 distinguishes between foreign and self-DNA, which is a critical safety measure in bacteria, to prevent autoimmunity. PAM diversity determines the targetability of genes in plants. Newer Cas variants which include xCas9 and SpCas9-NG have expanded the range of PAM compatibility to include NGN so that previously inaccessible regions of the genome can be edited (Nishimasu *et al*., 2018). As an example, to test the SP Cas9-NG, the gene (Epidermal Patterning Factor Like 9) EPFL9 was efficiently edited in rice, which controls the stomatal density and improves water-use efficiency (Li *et al*., 2024), such as xCas9 and SpCas9-NG, have expanded the range of PAM compatibility to include NGN, enabling editing of previously inaccessible regions of the genome).As a complex, Cas9, gRNA, and PAM establish a very programmable and modular system that can perform surgical-scale rewrites to plant genomes. CRISPR/Cas9 has not only been a tool, but a biological revolution defining plant breeding and genetic studies because of their synergy.

2. Comparative Evaluation of CRISPR Nucleases in Plant Systems: Despite several CRISPR-associated nucleases having been implemented in the editing of plant genomes, their efficiency and practical use are widely varying. SpCas9 of *Streptococcus pyogenes* is the most commonly used because it is highly efficient in editing, its behavior has been well characterized, and it has been thoroughly validated in most major crops. It, however, has specifications that limit targetable genomic regions, *namely*, the need of an NGG PAM, which is especially limiting in AT-rich plant genomes (Feng *et al*., 2013). Conversely, SaCas9 derived Staphylococcus aureus is of smaller size, easier to deliver vectors and multiplex, but it has a longer PAM sequence (NNGRRT), which further restricts the availability of targets. Cas12a (Cpf1) of *Francisella novicida* identifies T-containing PAMs and creates staggered double-strand breaks, and thus is especially beneficial in editing AT-rich genomes (e.g. wheat and barley). Besides, Cas12a makes it possible to multiplex edit with just one array of CRISPR.

Although these systems have these benefits, there are certain limitations. Off-target activity may be found in SpCas9, especially with large and repeatable plant genomes. SaCas9's narrower PAM compatibility can reduce editing flexibility, while Cas12a often shows temperature sensitivity and variable efficiency across plant species. (Zetsche *et al.*, 2015) The practical implications of these technical differences are in improving crops: nuclease selection should be specific to genome composition, target locus architecture, delivery mode, and desired breeding strategy. Thus, instead of using one default nuclease, careful matching of CRISPR systems to a particular plant-pathogen situation is necessary to maximize editing specificity and stability of resistance.

ENGINEERED PRECISION IMMUNITY

1. Concept of Precision Immunity: Precision immunity is a precision-based strategy in plant biotechnology that involves CRISPR/Cas system based-engineering of pathogen-specific immunity with minimal effects on plant growth and productivity. This approach, in contrast to the traditional breeding or chemical control, alters the resistance (R) genes or breaks the susceptibility (S) genes used by the pathogens. The most notable one is the editing of the MLO gene family granting wheat and barley resistance to powdery mildew permanently (Wang *et al.,* 2014). Precision immunity processes can be used to promote sustainable crop protection that is in line with the reduced application of pesticides and climate-resilient agriculture through the integration of functional genomics and molecular diagnostics.

2. Strategies for Pathogen-Specific Resistance: The technologies of CRISPR/Cas use several complementary approaches to assemble specific immunity (Pyott *et al.,* 2016). A knockout of susceptibility (S) genes is one method that has been widely used, eliminating factors needed by the host to be infected. Examples are editing MLO to be powdery mildew-resistant and *eIF4E* to be potyvirus-resistant, but they need to be carefully validated to avoid pleiotropic effects (Pyott *et al.*, 2016; Bishnoi *et al.*, 2023). Promoter or cis-regulatory editing is a more sophisticated approach that does not completely silence gene activity but alters effector-binding elements. As an example, editing Xa13 and OsSWEET genes promoters alleviated bacterial blight in rice with no significant growth costs (Shi *et al.*, 2023). Even though OsSWEET14 editing exhibits translational disease resistance, PDS3 editing is a visual reporter mostly used to assess efficacy of genome-editing.

Another level of resistance is direct targeting pathogen genomes, which cleaves the viral DNA or RNA in infected cells (Gosavi *et al.,* 2020). The CRISPR/Cas9-based targeting of tomato yellow leaf curl virus (TYLCV) reduced viral load and viral symptoms severity by a significant margin in tomato (Ali *et al.,* 2016). Multi-locus editing, using multiplex editing, is used to counter the accelerated development of pathogen resistance to produce broad-spectrum and long-lasting resistance. To illustrate, three TaMLO alleles were simultaneously edited in wheat resulting in stable mildew resistance with no penalties (Wang *et al.,* 2014). Moreover, precision introgression is supported by the ability to replace alleles with HDR, base editing, or prime editing, but HDR efficiency is a technical constraint (Xing *et al.,* 2014; Li *et al.,* 2022; Chen *et al.*, 2022). With all these measures, plant protection is transformed as a precise, fast and sustainable process as opposed to the traditional breeding or transgenic methods. Collectively, these strategies redefine plant protection as precise, rapid, and sustainable compared with traditional breeding or transgenic approaches

APPLICATIONS AND CASE STUDIES

1. Virus Resistance of Cassava and Tobacco: Plant viruses are among the greatest threats facing the world in the agricultural sector, especially in the tropics and subtropics. Virus resistance breeding is a traditional breeding method that can take several generations to produce resistance against viruses and may not work well against quickly evolving viruses. CRISPR/Cas 12 systems have changed the paradigm of creating plants resistant to viruses by allowing them to specifically target viral genomes or amend host vulnerability genes. Among the early applications was the production of virus-resistant cassava (*Manihot esculente*) against African cassava mosaic virus (ACMV), a ruthless geminivirus that causes massive losses in the yield of cassava in sub-Saharan Africa. CRISPR/Cas9 was used to mutate cassava plants in the genes of viral replication enhancer protein (AC2) and replication-associated protein (AC3), which lowered viral load and severity of symptoms (Mehta *et al*., 2019).

Equally, in *Nicotiana benthamiana* (a tobacco model), eIF4E (eukaryotic translation initiation factor 4E) and eIF (iso) 4E host susceptibility genes, which Potyvirus needed during replication, were disrupted with CRISPR/Cas9. The engineered plants had high and effective resistance against the Tobacco etch virus (TEV) and Potato virus Y (PVY) (Pyott *et al.*, 2016). These findings show that host-gene editing has the potential to provide long-term resistance and low off-target effects. Moreover, the Cas12a (Cpf1) system has been shown to target single-stranded DNA virus e.g.

Table 1: Overview of CRISPR/Cas applications for pathogen resistance in major crops.

S. No.	Crop	Target gene / region	Pathogen	Editing strategy	Outcome / impact	Reference
1.	Rice	Os SWEET promoters (EBEs)	*Xanthomonas oryzae* pv. *oryzae* (bacterial blight)	Promoter editing to block TALE binding sites	Broad resistance to bacterial blight across multiple rice lines	Ku and Ha (2020)
2.	Rice	OsSWEET14	*Xanthomonas oryzae*	Knockout of susceptibility gene	Reduced bacterial proliferation and lesion length	Zhang *et al.* (2019)
3.	Wheat	TaMLO (A, B, D homeologs)	*Blumeria graminis* f. sp. *tritici* (powdery mildew)	Multiplex CRISPR/Cas9 knockout	Durable mildew resistance in hexaploid wheat	Wang *et al.* (2014)
4.	Wheat	Viral coat protein (WDV)	Wheat dwarf virus (WDV)	Direct viral genome targeting via Cas9/sgRNA	Strong viral suppression and symptom reduction	Yuan *et al.* (2024)
5.	Tomato	SlMlo1	*Oidium neolycopersici* (powdery mildew)	CRISPR/Cas9 knockout	Generation of transgene-free mildew-resistant tomato	Nekrasov *et al.* (2017)
6.	Tomato	eIF4E1	*Potyviruses* (PepMoV, ToMV)	Susceptibility-gene editing	Broad viral resistance with no growth penalty	Bally *et al.* (2020)
7.	Tomato	XSP10 and SlSAMT	*Fusarium oxysporum* f. sp. *lycopersici*	Multiplex gene knockout	Enhanced resistance to Fusarium wilt	Debbarma *et al.* (2023)
8.	Cassava	AC1 and AC2 (viral genes)	African Cassava Mosaic Virus (ACMV)	CRISPR/Cas9-mediated viral DNA cleavage	Reduced viral load and partial immunity	Khan *et al.* (2022)
9.	Maize	ZmTOM1	*Maize chlorotic* mottle virus (MCMV)	Host factor knockout (loss-of-susceptibility)	Resistance to maize lethal necrosis (MCMV + SCMV)	Zhang *et al.* (2022)
10.	Potato	StDND1	*Phytophthora infestans* (late blight)	S-gene knockout using CRISPR/Cas9	Enhanced late blight resistance; healthy growth maintained	Norouzi *et al.* (2024)
11.	Banana	Musa DMT	*Fungus* (*F. oxysporum* f. sp. *cubense*)	S-gene knockout	Reduced Fusarium wilt susceptibility	Tripathi *et al.* (2021)
12.	Tobacco	TMV Genome	Virus TMV	Multiplex CRISPR targeting	Broad resistance to TMV	Hamza *et al.* (2021)

Tomato yellow leaf curl virus (TYLCV) providing an alternative nuclease with different PAM specifications and higher multiplexing (Shashikala *et al.*, 2025). Together, these researchers affirm that CRISPR-based genome editing is a sustainable approach to the solution of viral epidemics in crops and when used alone with bioinformatics-driven sgRNA design and viral population surveillance.

2. Bacterial Blight Resistance in Rice: Bacterial blight is a disease of rice caused by *Xanthomonas oryzae* pv. *oryzae* and is one of the most devastating diseases of rice in the world particularly in South and Southeast Asia. The pathogen allows transcription activator-like (TAL) effectors to bind to the promoters of plant SWEET sugar transporter genes, which are then activated and help to infect the plant (Hutin *et al.*, 2015). CRISPR/Cas9 system has already been used in the successful effort to edit these promoter regions to prevent TAL effector binding and achieve durable resistance (Li *et al.*, 2022), which was used in genes to induce mutations in promoters of three SWEET gene members (OsSWEET11, OsSWEET13, and OsSWEET14), producing rice lines that were resistant to various strains of Xoo with no yield penalties. This technique is referred to as promoter editing, which is one of the most lauded success stories in crop genome editing. Other susceptibility loci like (Transcription Factor II A) TFIIA 5 have also been altered along with the SWEET genes to enhance defense response (Zhang *et al.*, 2019). Multiplex CRISPR-based strategies, addressing multiple genes at the same time, are currently being implemented to create the next generation of rice varieties with pan-spectrum and durable resistance. Notably, genetically modified rice plants made using CRISPR are genetically equivalent to the traditional bred counterparts and are not considered being genetically modified organisms (GMOs) in several countries. Such regulatory differences can help them gain faster adoption and play a major role in food security in Asia and Africa.

3. Fungal Resistance in Wheat and Banana: Fungal pathogens are a significant limitation to crop production in the world, and such diseases as wheat powdery mildew and banana *Fusarium* wilt jeopardize food staples. CRISPR/Cas9 genome editing provides new possibilities to design specific and hereditary resistance systems in such crops. The knockout of the MLO (mildew resistance locus O) gene in Wheat (*Triticum*

aeostivum), was one of the earliest significant proofs of the potential of CRISPR. Removal of TaMLO homologs in the A, B and D genomes of hexaploid wheat caused a plant to exhibit long-term resistance to *Blumeria graminis* f. sp. *tritici* (powdery mildew), with no effects appearing on plant growth or fertility (Wang *et al.*, 2014). This MLO knockout method has now been transferred to other crops, such as barley, tomato and cucumber, stressing the universality of susceptibility-gene editing in fungal defense.

Another crop that is seriously affected by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc) Tropical Race 4 has caused destruction of plantations in Asia and Africa. Tripathi *et al.* (2021) disabled MusaD14 gene, which is one of the major elements of the strigolactone signaling pathway through CRISPR/Cas9 and examined the effect of gene disabling on pathogen colonization. The banana plants, which were edited, showed slower development of symptoms and fewer fungal colonies under greenhouse conditions. The results are specifically useful in clonally producing crops such as bananas, in which it is almost impossible to use traditional breeding techniques to generate resistance. In addition, recent developments of CRISPR-based editing and Cas13a RNA-targeting technologies have allowed fungal resistance strategies to grow beyond DNA modification. As an example, CRISPR/Cas13a has already been used to silence fungal transcripts in barley and tomato, representing an unprecedented disease resistance on the RNA level (Ali *et al.*, 2018).

DELIVERY METHODS FOR CRISPR/Cas COMPONENTS IN PLANTS

One of the most important steps to successful genome editing is the efficient delivery of the components of CRISPR/Cas to plant cells. Plant cells are not like microbial or mammalian systems since they have a rigid cell wall, tissue-specific variations, and a complicated way of regenerating. A variety of delivery methods have been streamlined to deliver Cas9 and guide RNA (gRNA) into plant cells, each with its own unique benefits and drawbacks based on the species of plant, type of tissue and desired editing result (table 2).

1. Agrobacterium-mediated Transformation: The most used plant transformation tool has been into a T-DNA construct and introduced into plants through agrobacterium-mediated transformation, leading to which is a soil bacterium with a natural capacity to transfer DNA to plant genomes. In CRISPR studies, Cas9 and gRNA fragments are cloned in T-DNA construct and inserted into plants with *Agrobacterium* infection, leading to stable insertion of the editing complex. This method has been effective in both dicots (e.g. *Arabidopsis thaliana,* tomato, soybean) and monocots (e.g. rice, maize), but the efficiency of transformation may differ. As an example, agrobacterium-mediated CRISPR/Cas9-mediated delivery has been used to generate high-efficiency gene knockouts of OsPDS and Osmpk2 gene in rice (Shan *et al.,* 2013). On the same note, the SlMlo1 gene was engineered by editing it in tomato to provide resistance against powdery mildew (Nekrasov *et al.,* 2017).

2. Biolistic/Gene Gun: The method involves the physical delivery of CRISPR constructs into plant cells with the aid of microscopic-sized tungsten or gold particles coated with DNA or RNA. The particles are shot at high speed to enter the plant cell wall and inject the editing machinery into the nucleus. This technique is especially useful with monocots like maize, wheat, and barley, which are less efficiently transformed by *Agrobacterium.* As an illustration, CRISPR/Cas9 vectors using the gene gun have managed to produce targeted mutations in the TaMLO gene in wheat, which has made it resistant to powdery mildews (Wang *et al.,* 2014).

3. Polyethylene glycol (PEG)-mediated transfection: A transient expression technique that is DNA-free and involves the introduction of Cas9/gRNA directly into protoplasts in plants- cells that have enzymatically had the walls removed. The method enables quick evaluation of guide RNA performance and short-term gene editing without the need to transform the stable. As an example, PEG-based delivery of CRISPR ribonucleoprotein (RNP) complexes, Cas9 protein pre-charged with sgRNA, has been found to achieve efficient gene editing without foreign DNA integration in *Arabidopsis* and rice (Woo *et al.,* 2015). This approach, therefore, offers a good direction towards the regulation of friendly non-GMO genome editing.

4. Virus-mediated Delivery: Plant viruses have been designed to act as vectors in delivering the components of CRISPR, which can then be distributed systemically within plants without having to use tissue culture. Single-strand RNA (sgRNA) and, in certain instances, Cas9 can be delivered into multiple cells simultaneously using viral vectors like tobacco rattle virus (TRV) and geminivirus-based systems. As an example, CRISPR/Cas9 systems based on TRV have demonstrated efficient gene editing of *Nicotiana benthamiana* and tomato (Ali *et al.,* 2016), and geminivirus replicons have been utilised to achieve a higher efficiency of homology-directed repair (HDR) in tomato and tobacco (Baltes *et al.,* 2014).

5. Nanoparticle-based Delivery: With the advent of nanotechnology, there are now promising prospects of non-integrative, species-independent and precise CRISPR delivery. CRISPR/Cas9 RNPs can be encapsulated and delivered straight into plant tissues by nanoparticles like carbon nanotubes, silica nanoparticles, and lipid-based carriers. As an example, carbon nanotube-based systems have proven to be used to transfer plasmid DNA and RNA into mature plant leaves with the ability to express genes efficiently, but without integrating the transgene into the genome (Demirer *et al*., 2019).

Table 2: Overview of major CRISPR/Cas delivery methods in plants.

S. No.	Delivery method	Integration type	Species range	Key advantages	Limitations	References
1.	Agrobacterium-mediated	Stable	Broad (mainly dicots)	High efficiency, low cost	Transgene integration, time-consuming	Shan *et al.* (2013); Nekrasov *et al*. (2017)
2.	Biolistic (Gene Gun)	Stable/Transient	Broad (esp. monocots)	No bacterial vector needed	Tissue damage, random integration	Lacroix *et al.* (2020)
3.	PEG-mediated Protoplast	Transient/DNA-free	Limited (lab species)	Fast, avoids GMO label	Difficult regeneration Transfection	Hayashimoto *et al*. (1990)
4.	Virus-mediated Delivery	Transient/Systemic	Host-specific	High efficiency, tissue culture-free	Cargo limits, recombination risks	Baltes *et al*. (2014); Ali *et al*. (2016)
5.	Nanoparticle-based Delivery	DNA-free	Potentially universal	Non-GMO, precise, species-independent	Experimental, high cost	Demirer *et al.* (2019)

ETHICAL, BIOSAFETY AND REGULATORY CONSIDERATIONS

The advent of genome editing using CRISPR/Cas has brought about a lot of ethical, biosafety and regulatory debate across the globe. Although gene-edited crops have potential solutions to sustainable agriculture and food supply, there are still fears of off-target mutation, unintended ecological effects, and gene flow to wild family. Even though early CRISPR systems have become a matter of biosafety concern, more recent versions like SpCas9-HF1, eSpCas9, and HypaCas9 have reduced off-target effects by a significant margin (Kleinstiver *et al.,* 2016). Pollen dispersal can have an effect on the biodiversity and ecological balance and require effective biocontainment measures and monitoring of the environment (Hartung and Schiemann, 2014). Meanwhile, CRISPR technology can promote environmental sustainability by decreasing the use of pesticides and the chemical run-off (Kanchiswamy *et al*., 2016). Therefore, sustainable governance should be a balance between innovation and environmental protection (Verma, 2019). Regulatory frameworks around the world are differing on genome-edited crops significantly. The level of public acceptance is critical, and it is influenced by ethical issues like intervention into nature, corporate domination of seed technologies, and transparency and labeling requirements (Shew *et al.,* 2018). It will also be necessary to strengthen the stakeholder engagement, open communication, and international harmonization to provide responsible and socially aligned application of CRISPR in agriculture.

FUTURE DIRECTIONS

CRISPR/Cas9 has revolutionized the field of plant biotechnology because it is possible to target crop enhancement to yield and stress tolerance, as well as to improve resistance to diseases. The sphere is fast developing the next-generation tools that increase precision and multiplex capacity and become more sustainable. Genome editing multiplex genome editing can now be used to edit multiple genes simultaneously, and is especially useful in enhancing complex polygenic traits, including drought tolerance as well as disease resistance. CRISPR/Cas9 has transformed plant biotechnology enhancing accuracy and sustainability in agriculture. The rice and tomato demonstrations demonstrate that CRISPR is capable of engineering regulatory networks and recreating advantageous allelic variation (Rodriguez-Leal *et al*., 2017; Zhang *et al.*, 2019). More efficient methods of sgRNA expression and improved systems of vectors further enhance the efficiency of editing and save time and cost (Xie *et al.,* 2015). A comparative overview of major CRISPR systems and their plant applications is summarized in table 3.

In addition to Cas9, new CRISPR systems add to the editing arsenal. Cas12a (Cpf1) cleaves T-rich PAM repeats, forms staggered DNA cuts, and can be used to multiplex editing because it can self-process (Tang *et al.*, 2017). On the contrary, Cas13 systems act on RNA, not on DNA, enabling temporary control over genes and antiviral resistance without irreversible changes in the genome in response to the Turnip mosaic virus (Aman *et al*., 2018; Mahas *et al*., 2019). Moreover, base

Table 3: Comparison of major CRISPR systems used in plant biotechnology.

S.No.	System	Class / type	Target	Key features	Major applications in plants	Key references
1.	Cas9	Class 2, Type II	DNA	NGG PAM; blunt DSBs; widely validated	Gene knockouts, trait improvement, multiplex editing	Zhang *et al.* (2019)
2.	Cas12a (Cpf1)	Class 2, Type V	DNA	TTTV PAM (T-rich); staggered cuts; self-processing crRNA; efficient multiplexing	Gene stacking, editing AT-rich regions	Tang *et al.* (2017)
3.	Cas13a / Cas13d	Class 2, Type VI	RNA	Targets ssRNA; transient regulation; antiviral defense	Viral resistance (e.g., TuMV), transcript modulation	Aman *et al.* (2018); Mahas *et al.* (2019)
4.	Base Editors (BE)	Modified Cas9	DNA	Single base substitution; no DSB	Precise allele correction	Lin *et al.* (2020)
5.	Prime Editors (PE)	Modified Cas9	DNA	Small insertions, deletions, substitutions; no DSB	Precision breeding	Lin *et al.* (2020)
6.	AI and Nano-technology Integration		Delivery/ Design	gRNA optimization; nano-particle-mediated DNA-free delivery	Improved specificity and transformation efficiency	Chen *et al.* (2018); Demirer *et al.* (2019)

and prime editors allow an accurate change of nucleotides without breaking the strands in two, which is safer and more versatile (Lin *et al.,* 2020). Specificity, efficiency and regulatory acceptance are further increased with the integration of Artificial Intelligence in the optimization of guide RNAs and nanotechnology-based delivery systems such as nanoparticle-mediated DNA-free editing (Chen *et al.,* 2018; Demirer *et al.,* 2019). CRISPR-edited crops tend to produce small, natural-like mutations, compared with conventional GMOs, which require foreign DNA insertion, and are more and more regulated differently in various countries, which alters the future of precision breeding (Waltz *et al*., 2018; Menz *et al.,* 2020).

CONCLUSIONS

During the last decade, the CRISPR/Cas technology revolutionized the field of plant science by moving beyond the field of gene discovery to crop design. It has allowed researchers to abandon random mutagenesis and transgenic methods in favor of an efficient, precise, and flexible method of genome-tailoring of plants. CRISPR has already provided solid returns in enhancing disease resistance to increased levels of abiotic stress tolerance in crops of significance to humanity, including rice, wheat, tomato, banana, and cassava. The introduction of CRISPR 2.0 versions, with multiplex and DNA-free editing, can further expand its ability to transform climate-resilient agriculture. Nevertheless, the potential of CRISPR-based agriculture can be achieved only in a comprehensive system-biosafety insurance, ethical management, and equal availability of technology. With the advent of nanotechnological-enhanced, AI-enhanced, genome editing, agriculture will grow increasingly data-based, precise, and environmentally friendly. The CRISPR innovation and responsible science will have the potential to transform the world of agriculture, to the world of food security, sustainability, and resilience in future generations.

REFERENCES

1. **Ali Z., Ali S., Tashkandi M. *et al.*** (2016). CRISPR/Cas9-mediated immunity to geminiviruses: Differential interference and evasion. *Scientific Reports*. 6(1): 26912. https://doi.org/10.1038/srep26912
2. **Ali Z., Mahas A. and Mahfouz M.** (2018). CRISPR/Cas13 as a tool for RNA interference. *Trends in Plant Science*. 23(5):374-378. https://doi.org/10.1016/j.tplants.2018.03.003
3. **Aman R., Ali Z., Butt H. *et al.*** (2018). RNA virus interference via CRISPR/Cas13a system in plants. *Genome Biology*. 19(1): 1. https://doi.org/10.1186/s13059-017-1381-1
4. **Anders C., Niewoehner O., Duerst A. and Jinek M.** (2014). Structural basis of PAM-dependent target DNA recognition by the Cas9 endonuclease. *Nature*. 513(7519): 569-573. https://doi.org/10.1038/nature13579
5. **Bally J., Fishilevich E., Doran R.L. *et al.*** (2020). Plin-amiR, a pre-microRNA-based technology for controlling herbivorous insect pests. *Plant Biotechnology Journal*. 18(9):1925-1932. https://doi.org/10.1111/pbi.13352
6. **Baltes N.J., Gil-Humanes J., Cermak T. *et al.*** (2014). DNA replicons for plant genome engineering. *The Plant Cell*. 26(1): 151-163. https://doi.org/10.1105/tpc.113.119792
7. **Bishnoi R., Kaur S., Sandhu J. *et al.*** (2023). Genome engineering of disease susceptibility genes for enhancing resistance in plants. *Functional & Integrative Genomics*. 23(3): 207. https://doi.org/10.1007/s10142-023-01133-w

8. **Chen J., Li S., He Y., Li J. and Xia L.** (2022). An update on precision genome editing by homology-directed repair in plants. *Plant Physiology.* 188(4): 1780-1794. https://doi.org/10.1093/plphys/kiac037

9. **Chen K., Wang Y., Zhang R. and Gao C.** (2019). CRISPR/Cas genome editing and precision plant breeding in agriculture. *Annual Review of Plant Biology*. 70(1): 667-697. https://doi.org/10.1146/annurev-arplant-050718-100049

10. **Debbarma J., Saikia B., Singha D.L., Das D. *et al.*** (2023). CRISPR/Cas9-mediated mutation in XSP10 and SlSAMT genes impart genetic tolerance to Fusarium wilt disease of tomato (*Solanum lycopersicum* L.). *Genes*. 14(2): 488. https://doi.org/10.3390/genes14020488

11. **Demirer G.S., Zhang H., Matos J.L. *et al.*** (2019). High aspect ratio nanomaterials enable delivery of functional genetic material without DNA integration in mature plants. *Nature Nanotechnology.* 14(5): 456-464. https://doi.org/10.1038/s41565-019-0382-5

12. **Faizal A., Nugroho S., Sembada A.A. *et al.*** (2024). Genome editing in future crop protection: Utilizing CRISPR/Cas9 to improve crop resistance against diseases, pests, and weeds. *Discover Agriculture*. 2:104. doi.org/10.1007/s44279-024-00124-0

13. **Fauser F., Schiml S. and Puchta H.** (2014). Both CRISPR/Cas-based nucleases and nickases can be used efficiently for genome engineering in *Arabidopsis thaliana*. *The Plant Journal.* 79(2): 348-359. https://doi.org/10.1111/tpj.12554

14. **Feng Z., Zhang B., Ding W. *et al.*** (2013). Efficient genome editing in plants using a CRISPR/Cas system. *Cell Research*. 23(10):1229-1232. https://doi.org/10.1038/cr.2013.114

15. **Gao J., Wang G., Ma S. *et al.*** (2015). CRISPR/Cas9-mediated targeted mutagenesis in *Nicotiana tabacum*. *Plant Molecular Biology.* 87(1-2): 99-110. https://doi.org/10.1007/s11103-014-0263-0

16. **Gosavi G., Yan F., Ren B. *et al.*** (2020). Applications of CRISPR technology in studying plant–pathogen interactions: Overview and perspective. *Phytopathology Research.* 2:21. https://doi.org/10.1186/s42483-020-00060-z

17. **Hamza M., Khan M.Z., Mustafa R. *et al.*** (2021). Engineering resistance against cotton leaf curl Kokhran virus-Burewala strain using CRISPR-Cas9 system in *Nicotiana benthamiana* [Preprint]. *Research Square*. https://doi.org/10.21203/rs.3.rs-604666/v1

18. **Hartung F. and Schiemann J.** (2013). Precise plant breeding using new genome editing techniques: Opportunities, safety and regulation in the EU. *The Plant Journal.* 78(5): 742-752. https://doi.org/10.1111/tpj.12413

19. **Hutin, M., Sabot F., Ghesquière A., Koebnik R. and Szurek B.** (2015). A knowledge-based molecular screen uncovers a broad-spectrum *OsSWEET14* resistance allele to bacterial blight from wild rice. *The Plant Journal.* 84(4): 694-703. https://doi.org/10.1111/tpj.13042

20. **Jinek M., Chylinski K., Fonfara I. *et al.*** (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science.* 337: 816-821. https://doi.org/10.1126/science.1225829

21. **Kanchiswamy C.N.** (2016). DNA-free genome editing methods for targeted crop improvement. *Plant Cell Reports. 35*(7): 1469-1474. https://doi.org/10.1007/s00299-016-1982-2

22. **Kaya H., Mikami M., Endo A. *et al.*** (2016). Highly specific targeted mutagenesis in plants using *Staphylococcus aureus* Cas9. *Scientific Reports.* 6(1): 26871. https://doi.org/10.1038/srep26871

23. **Khan Z.A., Kumar R. and Dasgupta I.** (2022). CRISPR/Cas-mediated resistance against viruses in plants. *International Journal of Molecular Sciences.* 23(4): 2303. https://doi.org/10.3390/ijms23042303

24. **Khatodia S., Bhatotia K., Passricha N., Khurana S.M.P. and Tuteja N.** (2016). The CRISPR/Cas genome-editing tool: Application in improvement of crops. *Frontiers in Plant Science.* 7:506. https://doi.org/10.3389/fpls.2016.00506

25. **Kleinstiver B., Pattanayak V., Prew M. *et al.*** (2016). High-fidelity CRISPR–Cas9 nucleases with no detectable genome-wide off-target effects. *Nature.* 529(7587): 490-495. https://doi.org/10.1038/nature16526

26. **Ku H.-K. and Ha S.-H.** (2020). Improving nutritional and functional quality by genome editing of crops: Status and perspectives. *Frontiers in Plant Science.* 11: 577313. https://doi.org/10.3389/fpls.2020.577313

27. **Li J., Chen L., Liang J. *et al.*** (2022). Development of a highly efficient prime editor 2 system in plants. *Genome Biology.* 23(1): 30. https://doi.org/10.1186/s13059-022-02730-x

28. **Li T., Huang S., Jhau J. and Yang B.** (2013). Designer TAL effectors induce disease susceptibility and resistance to *Xanthomonas*

oryzae pv. *oryzae* in rice. *Molecular Plant.* 6(3): 781-789.
https://doi.org/10.1093/mp/sst034

29. **Lin Q., Zong Y., Xue C. *et al.*** (2020). Prime genome editing in rice and wheat. *Nature Biotechnology.* 38(5): 582-585.
https://doi.org/10.1038/s41587-020-0455-x

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

31. **Mehta D., Stürchler A., Anjanappa R.B. *et al.*** (2019). CRISPR-Cas9 interference in cassava to the evolution of editing-resistant geminiviruses. *Genome Biology.* 20(1): 80.
https://doi.org/10.1186/s13059-019-1678-3

32. **Menz J., Modrzejewski D., Hartung F., Wilhelm R. and Sprink T.** (2020). Genome-edited crops touch the market: A view on the global development and regulatory environment. *Frontiers in Plant Science.* 11: 586027.
https://doi.org/10.3389/fpls.2020.586027

33. **Mushtaq M., Mushtaq M., Sakina A., Wani S.H., Shikari A.B., Tripathi P. *et al.*** (2019). Harnessing genome editing techniques to engineer disease resistance in plants. *Frontiers in Plant Science.* 10: 550.
https://doi.org/10.3389/fpls.2019.00550

34. **Nekrasov V., Wang C., Win J. *et al.*** (2017). Rapid generation of a transgene-free powdery mildew–resistant tomato by genome deletion. *Scientific Reports. 7*(1): 10019.
https://doi.org/10.1038/s41598-017-00578-x

35. **Nishimasu, H., Shi X, Ishiguro S., Gao L., Hirano S., Okazaki S., Noda T. *et al.*** (2018). Engineered CRISPR-Cas9 nuclease with expanded targeting space. *Science.* 361(6408): 1259-1262.
https://doi.org/10.1126/science.aas9129

36. **Norouzi M., Nazarain-Firouzabadi F., Ismaili A., Ahmadvand R. and Poormazaheri H.** (2024). CRISPR/Cas StNRL1 gene knockout increases resistance to late blight and susceptibility to early blight in potato. *Frontiers in Plant Science.* 14: 1278127.
https://doi.org/10.3389/fpls.2023.1278127

37. **Pyott D.E., Sheehan E. and Molnar A.** (2016). Engineering CRISPR/Cas9-mediated potyvirus resistance in transgene-free *Arabidopsis* plants. *Molecular Plant Pathology.* 17(8): 1276-1288.
https://doi.org/10.1111/mpp.12417

38. **Rodriguez-Leal D., Lemmon Z.H., Man J. *et al.*** (2017). Engineering quantitative trait variation for crop improvement by genome editing. *Cell.* 171(2): 470-480.e8.
https://doi.org/10.1016/j.cell.2017.08.030

39. **Scheben A., Wolter F., Batley J., Puchta H. and Edwards D.** (2017). Toward CRISPR/Cas crops: Bringing together genomics and genome editing. *New Phytologist.* 216(3): 682-698.
https://doi.org/10.1111/nph.14702

40. **Shan Q., Wang Y., Li J. and Gao C.** (2014). Genome editing in rice and wheat using the CRISPR/Cas system. *Nature Protocols.* 9(10): 2395-2410.
https://doi.org/10.1038/nprot.2014.157

41. **Shashikala T., Yogi D., Akshay K., Ashok K. *et al.*** (2025). CRISPR/Cas12a-mediated rapid and efficient detection of tomato leaf curl Karnataka virus without amplification. *Biocatalysis and Agricultural Biotechnology.* 64: 103528.
https://doi.org/10.1016/j.bcab.2025.103528

42. **Shew A.M., Nalley L.L., Snell H.A. *et al.*** (2018). CRISPR versus GMOs: Public acceptance and valuation. *Global Food Security. 19*:71-80.
https://doi.org/10.1016/j.gfs.2018.10.005

43. **Shi L., Su J., Myeong-Je Cho, Song H. *et al.*** (2023). Promoter editing for the genetic improvement of crops. *Journal of Experimental Botany.* 74(15): 4349-4366.
https://doi.org/10.1093/jxb/erad175

44. **Tang X., Lowder L., Zhang T. *et al.*** (2017). A CRISPR–Cpf1 system for efficient genome editing and transcriptional repression in plants. *Nature Plants.* 3(3): 17018.
https://doi.org/10.1038/nplants.2017.18

45. **Tripathi, J.N., Ntui V.O., Shah T. and Tripathi L.** (2021). CRISPR/Cas9-mediated editing of *DMR6* orthologue in banana (*Musa* spp.) confers enhanced resistance to bacterial disease. *Plant Biotechnology Journal.* 19(7): 1291-1293.
https://doi.org/10.1111/pbi.13614

46. **Verma A.K.** (2019). Sustainable Development and Environmental Ethics. *International Journal on Environmental Sciences.* 10 (1): 1-5.

47. **Waltz E.** (2018). With a free pass, CRISPR-edited plants reach market in record time. *Nature Biotechnology.* 36(1): 6-7.
https://doi.org/10.1038/nbt0118-6b

48. **Wang Y., Cheng X., Shan Q., Zhang Yi *et al.*** (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature Biotechnology.* 32(9): 947-951.
https://doi.org/10.1038/nbt.2969

49. **Wang, R., Angenent G.C., Seymour G. and Maagd R.A. de** (2020). Revisiting the role of master

regulators in tomato ripening. *Trends in Plant Science*. 25(3): 291-301. https://doi.org/10.1016/j.tplants.2019.11.005

50. **Woo J., Kim J., Kwon S. *et al.*** (2015). DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. *Nature Biotechnology*. 33(11):1162-1164. https://doi.org/10.1038/nbt.3389
51. **Xie K., Minkenberg B. and Yang Y.** (2015). Boosting CRISPR/Cas9 multiplex editing capability with the endogenous tRNA-processing system. *Proceedings of the National Academy of Sciences*. 112(11): 3570-3575. https://doi.org/10.1073/pnas.1420294112
52. **Xing H.L., Dong L., Wang Z.P. *et al.*** (2014). A CRISPR/Cas9 toolkit for multiplex genome editing in plants. *BMC Plant Biology*. 14(1): 327. https://doi.org/10.1186/s12870-014-0327-y
53. **Yuan X., Xu K., Yan F., Liu Z. *et al.*** (2024). CRISPR/Cas9-mediated resistance to wheat dwarf virus in hexaploid wheat (*Triticum aestivum* L.). *Viruses*. 16(9): 1382. https://doi.org/10.3390/v16091382
54. **Zaidi S.S-e-A., Tashkandi M., Mansoor S. and Mahfouz M.M.** (2016). Engineering plant immunity: Using CRISPR/Cas9 to generate virus resistance. *Frontiers in Plant Science*. 7:1673. https://doi.org/10.3389/fpls.2016.01673
55. **Zetsche B., Gootenberg J.S., Abudayyeh O.O. *et al.*** (2015). CPF1 is a single RNA-Guided endonuclease of a Class 2 CRISPR-CAS system. *Cell*. 163(3): 759-771. https://doi.org/10.1016/j.cell.2015.09.038
56. **Zhang P., Du H., Wang J. *et al.*** (2019). Multiplex CRISPR/Cas9-mediated metabolic engineering increases soybean isoflavone content and resistance to soybean mosaic virus. *Plant Biotechnology Journal*. 18(6): 1384-1395. https://doi.org/10.1111/pbi.13302
57. **Zhao Y., Zhu X., Chen X. and Zhou Jian-Min** (2022). From plant immunity to crop disease resistance. *Journal of Genetics and Genomics*. 49(8): 693-703. https://doi.org/10.1016/j.jgg.2022.06.003