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# Genome Editing: Novel Technique in Plant Disease Resistance

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**Abstract**

The research area of plant pathology has adopted gene editing technology as one of the most important and useful genetic tools. For sustainable agriculture, plant disease resistance must be improved. Plant biology and biotechnology have been revolutionized by gene editing, which enables precise genetic modification. Breeding is a new way to genetically improve plant disease resistance and increase resistance through breeding. It is easier to plan and implement, has a higher success rate, is more adaptable, and is less expensive than other gene editing methods. Diseases have significant effects on crop yield and quality, threatening global food security. Genetic improvement for plant disease resistance is important for sustainable agriculture. Gene editing has revolutionized plant biology and biotechnology by enabling precise and accurate genetic modifications. Transplantation / editing is a new technique for the genetic improvement of plant disease resistance and for accelerating the improvement of resistance.

## ❖ Introduction: Genome editing technology

**Genome editing** (also called gene editing)

It is a set of technologies that allow scientists to change an organism's DNA. These techniques allow genetic material to be added, deleted or changed at specific locations in the genome. Several approaches have been developed for genome editing.

Genetic modification is the process of directly altering the genetic DNA of a cell or organism. The main purpose of gene editing technology is to use sequence-specific nucleases to recognize specific DNA sequences and create DNA double-strand breaks (DSBs) at the target site. DSBs are mainly repaired through two pathways: the non-homologous end-joining (NHEJ) pathway and the homologous recombination (HR) pathway (Voytas and Gao, 2014). In most cases, cells use the non-homologous end-joining pathway to repair DNA double-strand breaks. However, NHEJ is error-prone and often results in insertion or deletion mutations.

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In the presence of a donor DNA template, DSBs are repaired by the HR pathway, resulting in direct base exchange or gene replacement. Current nucleases for gene editing include zinc finger nucleases (ZFNs), effector nucleases (TALENs), and CRISPR-related proteins (CRISPR/Cas) and regularly clustered short palindromic repeats system.

The CRISPR/Cas system is based on the adaptive immune system that destroys foreign plasmids or viral DNA by cleavage in bacteria and archaea (Marraffini and Sontheimer, 2010). The CRISPR/Cas genome editing system consists of a single guide RNA (sgRNA) and a nuclear-active Cas protein. In addition, the gRNA contains a support for Cas protein binding and a user-defined spacer sequence (approximately 20 nt) for gene sequence targeting. Due to its simplicity, high efficiency and ease of use, since its first demonstration in mammalian cells (Mali *et al*., 2013), the application of the CRISPR/Cas9 system has been faster than ZFNs and TALENs in a variety of organisms, including plants (Pennisi, 2013). A limitation of the CRISPR/Cas system is the protospacer adjacent motor (PAM)-dependent cleavage of the target sequence. However, several CRISPR/Cas systems with different types of PAMs (e.g., CRISPR/saCas9, CRISPR/Cpf1 (Cas12a), xCas9) have been identified and engineered beyond CRISPR/Cas9 in Streptococcus pyogenes (Wrighton, 2018). In addition, RNA-targeted CRISPR/Cas systems (e.g., CRISPR/Cas13a (C2c2), CRISPR/Cas13b, CRISPR/Cas13d) have greatly expanded the CRISPR toolbox (Yan *et al*., 2018).

The process of gene editing is known as the sequence of the host gene and the molecular information of the target gene. However, more and more plant species have been sequenced. In addition, genetic and molecular research has revealed molecular information about plant diseases, which is of great interest in the prevention and control of pests and diseases. In particular, negative regulators of plant resistance against disease, called susceptibility (S) genes, are good candidates for gene editing. That's why disease resistance/ immunity is a good way for editing.

❖ **Resistance against fungal pathogens**

Fungal pathogens are a major cause of plant disease and have a significant impact on agriculture. Their diverse lifestyles and high genetic plasticity allow them to rapidly invade new hosts, cross the R gene-mediated barrier, and produce fungicide resistance, a major challenge for disease control (Doehlemann *et al*., 2017). Recently, genome editing has begun to address these issues by altering the host's S gene.

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Powdery mildew is a worldwide fungal disease that affects a wide variety of plants. Fertilizers are the most effective, economical and environmentally friendly way to control powdery mildew. Traditional methods of producing resistant cultivars rely on the introduction of resistance (via R genes) from foreign species into improved cultivars through hybridization. Most of these resistance genes are breed-specific, so as new races of powdery mildew of wheat are developed in the field, their resistance decreases. Therefore, there is a great need to breeding the wheat varieties with a wide and long range.

In *Arabidopsis thaliana*, EDR1 (Enhanced Disease Resistance 1) negatively regulates resistance to the fungus *Erysiphe cichoracearum* but has little effect on plant growth, suggesting that EDR1 is a good program to enhance powdery mildew resistance. Furthermore, similar to Mlo, EDR1 is highly conserved across plant species (Frye *et al*., 2001). The CRISPR/Cas9 system was used to generate Taedr1 wheat plants by targeting three wheat EDR1 homologues simultaneously. The resulting Taedr1 mutant plants showed resistance to Bgt but no disease from the fungus (Zhang *et al*., 2017). Since *Arabidopsis* edr1 mutant plants are resistant to bacteria and oomycetes, it is reasonable to assume that Taedr1 plants may also be resistant to other wheat pathogens.

The discovery of the barley mlo (mildew resistance locus o) was a major success in plant breeding for broad-spectrum and long-term resistance to powdery mildew (Lyngkjær *et al*., 2000). Mlo resistance has been widely used in spring barley throughout Europe for almost 40 years (Jorgensen, 1992). After the Mlo gene was cloned from barley in 1997, it was discovered that the Mlo orthologues was growing in dicots and monocots. Because bread wheat is allohexaploid, there are three barley Mlo orthologues (TaMlo-A1, B1 and D1). TALENs and CRISPR used to edit these wheat Mlo genes and found that the edited plants showed resistance to the powdery mildew fungus *Blumeria graminis* f. sp. *tritici* (Bgt) occurs only when six copies of TaMlo are mutated simultaneously (Wang *et al*., 2014). These results show that these TaMlo copies are functional and that genome editing/ modification is a useful tool for target modification in polyploid species. In tomato, a Mlo knockout mutant was generated by targeting SlMlo1 using two sgRNAs simultaneously. As expected, the tomato mutants appeared highly resistant to the *Oidium neolycopersici* in tomato (Nekrasov *et al*., 2017).

❖ **Resistance against bacterial pathogens**

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Bacterial infections are very difficult to control. This is because bacterial pathogens are very diverse and can multiply quickly and spread in many ways, especially after an outbreak of an infectious disease. However, due to its great utility in elucidating the molecular mechanisms of pathogen-bacteria interactions, many host plant genes involved in these complex processes have been identified, including some S genes. The S gene in the field has become a popular program for breeding crops which are resistant to bacterial diseases through genome editing.

Bacterial blight of rice is a fungal disease of rice caused by *Xanthomonas oryzae* pv. *oryzae* is a highly contagious disease and is one of the major rice diseases. This results in a yield loss of 10–20% (Ou, 1985), but this loss can exceed 50% under conditions favorable to pathogens (e.g., high humidity) and sometimes results in a complete loss of fertility (Mew *et al*., 1993). *Xanthomonas oryzae* pv. *oryzae* secretes activator-like activating proteins (TALEs) into host cells through a type III secretion system (Makino *et al*., 2006).

Several TALE proteins promote successful infection by targeting the S gene and activating its expression. For example, the TALE protein AvrXa7 from the Philippine strain PXO86 binds to an effector binding element (EBE) in the promoter of OsSWEET14 (also known as Os11N3) and activates its expression. OsSWEET14 encodes a sucrose efflux transporter and is thus captured by PXO86 as a glucose transporter in rice cells to sustain pathogen growth and virulence/ damage (Chen *et al*., 2012). Because OsSWEET14 also plays an important role in plant development, it is not possible to knock down OsSWEET14 to produce *Xanthomonas oryzae* pv. *oryzae* resistance without side effects. Therefore, two TALENs targeting the EBE of the OsSWEET14 promoter were designed to suppress AvrXa7 binding but do not affect the developmental activity of OsSWEET14 (Li *et al*., 2012). PthXo2 is another TALE protein implicated in the virulence of some *Xanthomonas oryzae* pv. *oryzae* strains (Ochiai *et al*., 2005), but the host genes have not been determined. However, OsSWEET13 expression is promoted by PthXo2 binding to the OsSWEET13 promoter in rice. OsSWEET13 knockout mutants were generated by targeting the coding region via the CRISPR/Cas9 system. Unlike OsSWEET14, loss of function of OsSWEET13 did not affect plant development, but increased *Xanthomonas oryzae* pv. *oryzae* resistance in a PthXo2-dependent manner (Zhou *et al*. 2015).

Although it is one of the most important economic crops worldwide, tomato production and quality is still limited by many important pathogens, including *Pseudomonas syringae*,

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*Phytophthora* spp. and *Xanthomonas* spp. Recent studies have shown that mutations in a single gene in *Arabidopsis thaliana*, downy mildew resistance 6 (DMR6), increase salicylic acid levels and increase resistance to many plant pathogens, including bacteria and oomycetes (Zeilmaker *et al*., 2015). Interestingly, the tomato orthologue SlDMR6-1 also modulates the response to infection by *P. syringae* pv. *tomato* and *Phytophthora capsici*. SlDMR6-1 null mutants xerated in the CRISPR/Cas9 system showed resistance to *P. syringae*, *P. capsici* and *Xanthomonas* spp. It has no negative effect on tomato growth and development (de Toledo *et al*., 2016). Taken together, these results suggest that knockdown of DMR6 is a useful strategy to control plant-wide diseases.

❖ **Resistance against viruses**

Plant virus infections are difficult to control because the viruses multiply quickly and often involve insect pests. Over the past 30 years, transgenic expression of viral proteins or RNA has been widely used to improve virus resistance in plants, and the result is called pathogen-derived resistance. Recently, double-stranded RNA-guided RNAi has been suggested as an effective method to confer virus resistance in plants (Ding and Voinnet, 2007). Genome editing technology is a new weapon against plant viruses.

Geminivirus, have a single-stranded circular DNA genome that replicates in the host nucleus through a double-stranded DNA (dsDNA) medium (Hanley-Bowdoin *et al*., 2013). This dsDNA intermediate is targeted by sequence-specific endonucleases. Previously, engineered an artificial zinc finger protein (AZP) lacking a nuclear domain to target the origin of replication of the *Beet severe curly top virus* (BSCTV) by inhibiting binding of the viral replication protein (Rep). AZP *Arabidopsis* plants showed increased resistance to BSCTV and more than 80% of these transgenic plants showed no signs of virus infection (Sera, 2005). Similarly, ZFNs were designed to target the conserved regions of the Rep genes of *Tomato yellow leaf curl China virus* (TYLCCNV) and *Tobacco curly shoot virus* (TbCSV). Experiments in tobacco have shown that these ZFNs disrupt the target sequence and inhibit viral replication (Chen *et al*., 2014).

Recently, several studies have reported the successful use of CRISPR/Cas9 to generate geminivirus resistance in plants. Delivery of gRNA targeting the intergenic region (IR), coat protein (CP), or Rep in Cas9-expressing tobacco reduced viral accumulation of several large DNA viruses, including *Tomato yellow leaf curl virus* (TYLCV). Furthermore, these studies

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showed that a gRNA targeting a conserved sequence (TAATATTAC) in the IR region can be used to target geminivirus simultaneously (Ali *et al*., 2015). Consistently, gRNA targeting Rep, CP or IR significantly reduced or eliminated disease markers against *Bean yellow dwarf virus* (BeYDV) and *Beet severe curly top virus* (BSCTV) (Ji *et al*., 2015).

**Table 1. Genome editing technologies developed for disease resistance in plants**

| Sr. No. | Crops | Disease | Causal organism | Target gene | References |
|---|---|---|---|---|---|
| 1. | Wheat | Powdery mildew | *Blumeria graminis* f. sp. *tritici* | TaMLO/exon | Wang *et al*. (2014) |
| 2. | Tomato | Powdery mildew | *Oidium neolycopersici* | SlMlo1/exon | Nekrasov *et al*. (2017) |
| 3. | Rice | Blast | *Magnaporthe oryzae* | OsERF922/exon | Wang *et al*. (2016) |
| 4. | Rice | Bacterial blight | *Xanthomonas oryzae* pv. *oryzae* | OsSWEET14/promoter | Li *et al*. (2012) |
| 5. | Citrus | Canker | *Xanthomonas citri* subsp. *citri* | CsLOB1/exon | Jia *et al*. (2017) |
| 6. | Arabidopsis | DNA viral disease | *Beet severe curly top virus* (BSCTV) | Replication origin | Sera (2005) |
| 7. | Tobacco | DNA viral disease | *Tobacco curly shoot virus* (TbCSV) | Replication associated protein (Rep) | Chen *et al*. (2014) |

**Conclusion**

In the area of crop improvement and genetics (functional genomics), gene/ genome editing is rapidly becoming a widely used and adaptable technology. Continued development of CRISPR/Cas9 technology in plant diseases will help improve its critical level of effectiveness, as the technology is poised to work in a variety of species. So far, most

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CRISPR/Cas9 research in plant diseases has focused on agricultural diseases or patho-systems. Overall, genome editing has become a powerful tool for plant-microbe molecular interactions and disease resistance breeding. Undoubtedly, eco-friendly sustainable agriculture will benefit from these advances in genetic modification through genome editing.

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