Empowering Plant Disease Resistance through Genetic Engineering: A Thorough Analysis of Current Approaches, Challenges, and Future Prospects

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Abstract- **A wide range of symptoms caused by plant diseases can negatively affect the growth, reproduction, and yield of plants. Consequently, genetic engineering presents potential solutions to enhance the resistance of plants against diseases. This paper provides a comprehensive analysis of the current status of genetic engineering methods, including transgenic plants, pathogen resistance genes, RNA interference (RNAi) technology, and genome editing techniques. The challenges associated with genetic engineering for disease resistance are also addressed, such as off-target effects, public perception, and ecological consequences. Furthermore, future prospects and advanced technologies are explored, including next-generation genetic engineering, the integration of multiple defence mechanisms, and disease resistance facilitated by the microbiome. By understanding the current advancements in genetic engineering for disease resistance and overcoming the challenges, we can establish sustainable and eco-friendly agricultural systems.**

Index Terms- **Genetic engineering, Plant Disease Resistance, Transgenic plants, Pathogen resistance genes, RNA interference, Genome editing, Off-target effects, Regulatory frameworks, Ecological impact, Future Prospects.**

I. INTRODUCTION

The potential of a plant to obstruct the proliferation of a pathogen is commonly referred to as plant disease resistance. As the world's population continues to grow, the pressure to ensure food security becomes increasingly significant. However, numerous pathogenic species, such as bacteria, fungi, viruses, and insects, pose continuous threats to agricultural productivity by causing harmful diseases in crops. In response to these challenges, scientists have turned to genetic engineering as a powerful tool to strengthen the plant disease resistance. Genetic engineering techniques involve the precise manipulation of an organism's genome, enabling scientists to introduce specific genes or modify existing ones. Several Genetic Engineering methodologies are employed to establish disease resistance in plants, each with its own unique approach:

Transgenic Plants: One of the most widely used genetic engineering approaches involves the creation of transgenic plants. In this method, a gene of interest, often derived from a naturally resistant organism, is incorporated into the plant's genome. This introduced gene typically acts to suppress the metabolic pathway of the disease, making the plant more resistant to infection.

Pathogen Resistance Genes: Another strategy involves the use of pathogen resistance genes, which are proteins that enable plants to identify and eliminate invading infections more effectively. These genes play a crucial role in empowering plants to resist diseases caused by specific pathogens.

RNAi Technology: RNA interference (RNAi) technology, often referred to as co-suppression, is a technique that inhibits the expression of particular genes by using double-stranded RNA (dsRNA). By targeting key genes in pathogens, RNAi can restrict their ability to cause diseases in plants.

Genome Editing Techniques: Genome editing techniques provide a remarkable level of precision when altering an organism's DNA. Technologies like CRISPR-Cas9, Transcription Activator-Like Effector Nucleases (TALEN), and Zinc-Finger Nucleases (ZFN) enable scientists to precisely remove or replace specific DNA sequences within a plant's genome. This approach offers the opportunity to develop disease-resistant plants with targeted genetic modifications.

While genetic engineering shows great potential, there are some important issues that need attention. One of the main concerns is that genetic modifications may have unintended effects and cause problems in other parts of the organism. We need to be very cautious about this. Moreover, people's opinions and the rules set by authorities regarding genetically modified organisms (GMOs) also greatly influence how we use disease-resistant genetically modified crops. So, we must consider these challenges to make sure we use this technology safely and responsibly.

II. GENETIC ENGINEERING APPROACHES FOR DISEASE RESISTANCE

By 2050, the world's population is expected to increase by at least 9.8 billion people, which will create enormous challenges for modern agriculture in supplying sufficient nutrients to meet the increased demands [1]. Traditional breeding techniques have played a significant role in crop improvement, but their slow and natural process may not be sufficient to address the increasing threats posed by plant diseases. Genetic engineering is a more sophisticated and effective method for enhancing plant disease resistance because it allows targeted alteration of specific genes of interest and facilitates the exchange of genetic material between different species. Plants possess strong defence mechanisms against pathogens and pests, which can be further strengthened through genetic engineering to combat infections effectively. Here are some methods that can be used to alter the genome of plants to make them resistant to harmful diseases:

Transgenic Plants:

Transgenic plants are genetically modified organisms that have one or more foreign genes (transgenes) inserted into their genomes. Transgenes could be resistance genes (R-genes), Quantitative Disease Resistance (QDR) diversity, or modified S-genes sourced from different plant species. By introducing these genes into the crops, they gain enhanced resistance to various pathogens. Over the past three decades, transgenic plants have been cultivated in production agriculture in expanding areas. However, the commercial release of disease-resistant transgenic plant varieties has been relatively limited, despite their significant potential. Some notable successes in disease resistance achieved through transgenic methods include the disruption of a sucrose efflux transporter gene using TALEN-mediated gene editing, conferring resistance to bacterial blight in rice. Additionally, CRISPR-Cas-induced mutations in a rice ethylene-responsive factor gene have been effective in imparting resistance to blast disease. Researchers have successfully developed transgenic plants resistant to a range of Gemini viruses in species like *Nicotiana benthamiana* and *Arabidopsis thaliana*. One of the advantages of this system is its high flexibility, as new sgRNAs can be designed to target mutated viral sequences as they evolve. Transgenic plants have emerged as a powerful tool in plant biotechnology, offering the potential to enhance disease resistance and overall plant performance [2]. While certain achievements have been made in developing disease-resistant transgenic varieties, further research and innovation are essential to overcome challenges and fully unlock the potential of transgenic plants in agriculture.

Pathogen Resistance Genes:

Genetic modification of plants through the incorporation of specific stretches of DNA into their genomes has emerged as a powerful tool for enhancing resistance against plant pathogens. This technology grants plants new or altered characteristics, including improved disease resistance. Pathogens, such as bacteria, fungi, and viruses, can significantly reduce crop yields, with losses ranging from 3% to as high as 30%, depending on the crop and the severity of infection [3]. Traditional methods of disease management often involve the application of chemical agents. However, researchers are increasingly turning to genetic engineering as a sustainable alternative to combat microbial infections. By introducing pathogen resistance genes into plants, scientists can enhance the plant's innate defence mechanisms, making them more effective against various disease-causing agents. The incorporation of pathogen resistance genes not only empowers plants to withstand microbial infections but also reduces the reliance on chemical pesticides, contributing to environmentally friendly agricultural practices. Moreover, genetically engineered crops with enhanced disease resistance have the potential to increase global food security by safeguarding crop yields and minimizing losses caused by diseases.

RNA Interference (RNAi) Technology:

RNA interference (RNAi) is a powerful genetic engineering approach that has opened up new opportunities for enhancing plant disease resistance and dealing with a variety of biotic and abiotic stresses. With the help of this game-changing technology, crops can become more resistant to pathogens, pests, and environmental hazards while also becoming more nutritious and allergy-friendly. The RNAi mechanism involves the introduction of double-stranded RNA (dsRNA) into plant cells. This dsRNA is processed by the enzyme Dicer, or similar enzymes, to produce small interfering RNA (siRNA) molecules consisting of 20–30 nucleotides. These siRNAs are incorporated into the RNA-induced silencing complex (RISC), which includes *Argonaute* family proteins (AGOs), forming the catalytic components for RNAi. The RISC then guides the siRNAs to complementary mRNA sequences, leading to the degradation of the targeted mRNA or inhibition of its translation, effectively silencing the corresponding genes. RNAi technology has demonstrated its efficacy in combating various diseases and pests, making it environmentally beneficial and contributing to overall crop health protection. For instance, the use of RNAi has been successful in eradicating crown gall disease caused by the *Agrobacterium tumefaciens* pathogen in species like *Nicotiana*, *Lycopersicum*, and *Arabidopsis*. Moreover, the versatility of RNAi technology extends beyond disease resistance. It has been applied to address abiotic stresses such as drought, salinity, and extreme temperatures, as well as to improve the nutritional quality of crops and eliminate allergenic components [4]. RNA interference (RNAi) technology has emerged as a revolutionary tool for empowering plant disease resistance. Its ability to silence specific genes and confer resistance against pathogens and stresses opens up exciting possibilities for agriculture, offering a pathway towards food security and environmental sustainability. Through ongoing research and responsible application, RNAi holds the potential to revolutionise crop protection and positively shape the future of agriculture.

Genome Editing Techniques:

Genome editing technologies have revolutionized the field of genetic engineering, offering powerful tools to facilitate pathogen resistance in plants and correct errors in their genomes. CRISPR-Cas9, Zinc Finger Nucleases (ZFNs), and Transcription Activator-Like Effector Nucleases (TALENs) are three well-known genome editing techniques that have gathered a lot of interest for their applications in plant disease resistance.

CRISPR-Cas9: CRISPR-Cas9, derived from the bacterial immune system, has emerged as a versatile and efficient genome editing tool in plants. It enables precise modifications within the plant genome and has been successfully applied to various plant species, including *Arabidopsis*, *Nicotiana tabacum*, *Nicotiana benthamiana*, sweet orange, rice, and sorghum. The CRISPR-Cas9 system consists of two main components: Cas9, a DNA endonuclease, and a single-guide RNA (sgRNA). Cas9 is derived from different bacteria, with *Streptococcus pyogenes* being widely used for genome editing in plants. The sgRNA guides Cas9 to the target DNA sequence through Watson-Crick base pairing and identifies the target site using a Protospacer Adjacent Motif (PAM) sequence, typically NGG. Once bound to the target DNA, Cas9 induces a double-stranded break (DSB) at the site, which is subsequently repaired through endogenous mechanisms such as non-homologous end joining (NHEJ) and homology-directed repair (HDR) [5]. The ease of designing sgRNAs and the ability to edit multiple genes simultaneously make CRISPR-Cas9 a cost-effective and accessible technology for commercial applications.

Zinc Finger Nucleases (ZFNs): Zinc Finger Nucleases (ZFNs) were one of the pioneering genome editing tools and have played a crucial role in advancing genetic engineering. They are synthetic restriction enzymes that can cleave long double-stranded DNA sequences. ZFNs consist of a DNA-binding domain derived from *Flavobacterium okeanokoites*, which is fused to the non-specific DNA cleavage domain of the FokI restriction enzyme [6]. ZFNs induce DSBs at specific target sites, leading to repair by either NHEJ or HDR.

Transcription Activator-Like Effector Nucleases (TALENs): Transcription Activator-Like Effector Nucleases (TALENs) are another powerful tool for genome editing in plants. They can target any desired sequence within a genome without PAM site restrictions, making them versatile for site-specific gene modifications. TALENs are based on a fusion of a DNA-binding domain derived from *Xanthomonas* bacteria with the FokI restriction enzyme, allowing for the introduction of DSBs at specific locations. Similar to other genome editing techniques, TALENs activate repair pathways, such as NHEJ or HDR, to fix the DSBs [6], [7]. The precise nature of HDR makes TALENs an attractive tool for achieving targeted modifications in plant genomes against disease.

It is essential to consider potential challenges associated with genetic engineering, such as off-target effects and unintended consequences. Careful evaluation and rigorous testing are crucial to ensuring the safety and efficacy of genetically modified plants before their widespread adoption.

III. Challenges in Genetic Engineering for Disease Resistance

Genetic engineering has paved the way for developing crops with desirable traits, including disease resistance, herbicide tolerance, and insect resistance. Among these traits, disease resistance stands out as a crucial factor in ensuring global food security and sustainable agriculture. However, the deployment of genetically engineered crops with enhanced disease resistance is not without challenges. This section discusses some of the key challenges that need to be addressed to successfully harness the potential of genetic engineering for disease resistance.

Off-Target Effects and Unintended Consequences:

Genetic engineering technologies, particularly CRISPR-Cas9 systems, hold immense promise in conferring disease resistance to plants. However, they are not without challenges, particularly concerning off-target effects and unintended consequences. Off-target effects refer to instances where the CRISPR-Cas9 system may inadvertently act on genomic sites other than the intended target, leading to unintended genetic alterations. Cas9, a commonly used enzyme in CRISPR-Cas9 systems, has been known to tolerate up to three mismatches between the guide RNA and the genomic DNA. Although efforts are made to design guide RNAs with high specificity, the potential for off-target effects remains a concern. To address this issue, researchers have developed tools to predict potential off-target sites in the genome. One approach involves searching the entire genome for sequences similar to the guide RNA and calculating off-target editing probabilities. Another strategy includes using modified CRISPR-Cas9 systems, such as CRISPR-Cas12a (cpf1), which has shown higher editing efficiency and fewer off-target effects in certain cases [8]. In addition to off-target effects, unintended consequences can arise from genetic engineering. For example, a study involving the genetic modification of plants to produce insecticidal substances revealed unintended changes in the leaves of transgenic potatoes. Glycoalkaloids, which are highly toxic to mammals, are typically found in potato leaves and stems, serving as a natural defence against herbivores. Genetic modifications can inadvertently alter the levels of these compounds, raising concerns about potential ecological impacts and unintended effects on non-target organisms. To mitigate off-target effects and unintended consequences, ongoing research is focusing on improving the precision of genome-editing techniques and developing novel tools to accurately predict potential off-target sites. Additionally, extensive risk assessments and rigorous safety evaluations are essential before the commercial release of genetically modified crops to ensure their safety and minimize any potential adverse impacts on the environment and human health [9].

Public Perception and Regulatory Frameworks:

Public perception plays a significant role in shaping the acceptance and adoption of genetically modified (GM) crops and foods. Over the years, public attitudes towards genetic engineering in agriculture have been influenced by various factors, including historical events and case studies of specific GM crops. Understanding public opinion is crucial for policymakers and stakeholders to develop effective regulatory frameworks that address public concerns while promoting scientific advancements. In the early years of genetic engineering, the introduction of the first genetically engineered plant, a tobacco plant resistant to viruses, marked the beginning of a revolution in agriculture. As transgenic crops, such as virus-resistant tobacco and "flavour saver tomatoes," entered the market, they faced varying levels of public acceptance. The approval of GE-resistant papaya against the Ring Spot virus demonstrated the potential benefits of GM crops.

However, public perception of GM foods has been mixed. Some individuals perceive GM foods as a serious threat to human health, questioning the safety of consuming products with foreign DNA. Concerns about the unknown health implications and the emergence of unforeseen diseases have led many to avoid GM foods altogether. Studies have sought to identify the factors influencing public opinions regarding GM products in different regions, revealing varied perspectives related to product certification, nutritional value, environmental protection, marketing, cost, and quality.

In various countries, regulatory frameworks have been established to ensure the safe deployment of GM crops. In India, the Ministry of Environment, Forests, and Climate Change (MoEFCC) has enacted the Environment (Protection) Act, providing the foundation for regulations on Genome Engineering Technologies. Several competent authorities, including the Genetic Engineering Appraisal Committee (GEAC), the Review Committee on Genetic Manipulation (RCGM), and the State Biotechnology Coordinator Committee (SBCC), collaborate to enforce the rules for GMO-related activities and products. India's participation in the Cartagena Protocol on Biosafety (CPB) demonstrates the country's commitment to biosafety in GM agricultural research and commercialization processes [10].

The United States has its Coordinated Framework for the Regulation of Biotechnology, which involves three regulatory organisations—the Food and Drug Administration (FDA), the United States Department of Agriculture (USDA), and the Environmental Protection Agency (EPA). The FDA evaluates the safety of foods produced through genetic engineering, the USDA focuses on plant characteristics and environmental effects, and the EPA assesses pesticide-related risks [11].

Similarly, China has a comprehensive regulatory framework involving the State Administration of Market Regulation (SAMR), the General Administration of Customs (GAC), and the Ministry of Agriculture and Rural Affairs (MARA). MARA takes the lead in regulating and ensuring the safety of genetically modified organisms, while SAMR oversees GM labelling for processed foods [12]. Public perception and regulatory frameworks continue to evolve with advancements in genetic engineering technologies. It is essential for regulators to remain vigilant and responsive to public concerns while encouraging innovation in the fields of plant disease resistance and agriculture. Striking a balance between scientific progress and public acceptance is vital for the successful implementation of genetic engineering approaches to combat plant diseases and ensure sustainable food production.

Potential Impact on Ecological Systems:

The adoption of genetically modified (GM) plants has sparked debates concerning their potential ecological, financial, and societal impacts. Among the risks associated with GM crops, one major concern is the loss of genetic diversity, which can result from the widespread cultivation of a limited number of genetically uniform crop varieties. This reduction in genetic variability could make crops more susceptible to evolving pests and diseases, potentially leading to significant crop losses and decreased agricultural resilience. The development of GM crops resistant to specific pests, such as *Bacillus Thuringiensis* (Bt) crops, raises concerns about disrupting ecological balances [13]. Bt crops are engineered to produce toxins that target specific insect pests, but they may inadvertently affect beneficial predator insects that play crucial roles in pest control. The reduced availability of food due to the targeted destruction of insect pests could lead to a decline in the populations of these beneficial predators, impacting the overall ecological equilibrium. Another area of concern is the potential for gene flow between GM crops and related wild plant species. The introduction of viral RNA sequences into plants to confer pathogenic resistance could inadvertently lead to the transfer of these sequences to unrelated viruses that infect the plant. Such recombination events between RNA viruses within a transgenic crop might produce new viral strains, potentially leading to unforeseen disease outbreaks in agricultural ecosystems. To comprehensively assess the overall environmental impact of GM crops compared to conventional crops, it is essential to consider the Environmental Impact Quotient (EIQ). The EIQ is a tool used to measure the potential environmental influence of pesticides, including herbicides used in GM crop management [14]. Evaluating the net amount of herbicide used and its potential impacts on soil, water, and non-target organisms is crucial for understanding the full ecological implications of GM crop cultivation.

The ecological impact of genetically modified plants is a complex and multifaceted subject. While they offer potential benefits such as improved pest resistance and increased crop yields, their adoption must be accompanied by careful risk assessments to avoid unintended consequences for genetic diversity, non-target organisms, and ecological systems. Striking a balance between harnessing the benefits of GM technology and mitigating its potential ecological risks is critical for sustainable agricultural practices and preserving biodiversity. A comprehensive comparison of traditional agricultural practices, GM crops, and other agricultural strategies is essential to making informed decisions about the most environmentally friendly and sustainable approaches for modern agriculture [15].

IV. FUTURE PROSPECTS AND EMERGING TECHNOLOGIES

The future of genetic engineering holds great promise for revolutionizing agriculture and resolving challenges in food security, nutrition, and sustainable crop production. With the aid of genetic engineering technologies, the development and utilization of genetically enhanced crops that exhibit increased productivity, enhanced nutritional content, and enhanced resistance to pests and diseases can be significantly accelerated. However, it is essential to consider biosafety and public concerns associated with the use of genetically modified (GM) crops.

Next-Generation Genetic Engineering:

The field of genetic engineering has witnessed rapid advancements in recent years, ushering in a new era of precision and efficiency. Next-generation genetic engineering technologies have emerged, building upon the foundation laid by traditional methods. These cutting-edge approaches offer enhanced precision, reduced off-target effects, and greater flexibility in modifying plant genomes. In the context of plant disease resistance, next-generation genetic engineering holds immense promise for creating crops that can withstand a wide range of pathogens with increased effectiveness and sustainability.

Gene Editing with Base Editors: Gene editing technologies, particularly CRISPR-Cas9, have revolutionized genetic engineering. However, traditional CRISPR-Cas9 systems rely on creating double-strand breaks (DSBs) in the DNA, which can lead to unintended mutations at off-target sites. Base editors offer an alternative approach, allowing precise point mutations without introducing DSBs. Base editors can convert specific DNA bases to other bases, enabling targeted changes in the genome with reduced off-target effects. For instance, converting susceptible genes to resistant alleles can confer disease resistance without disrupting essential genes.

Genomic Data and Machine learning: The rapid expansion of genomic data has made it possible to incorporate machine learning techniques into genetic engineering procedures. Machine learning can help identify potential target genes for engineering disease resistance, predict the impact of specific genetic modifications, and optimize gene editing strategies. By leveraging big data and artificial intelligence, next-generation genetic engineering becomes even more efficient and predictive, saving time and resources.

Nanoparticles for Gene Delivery: Nanoparticles, typically ranging from 1 to 100 nanometers in size, can encapsulate and protect genetic materials, such as DNA or RNA, from degradation. They offer an efficient means to deliver these materials into plant cells without the need for invasive techniques like Agrobacterium-mediated transformation or particle bombardment. Various types of nanoparticles, such as liposomes, polymers, and viral vectors, have been explored for their potential in gene delivery.

Integration of Multiple Defence Mechanisms:

Plant pathogens have evolved diverse strategies to overcome plant defences, leading to the emergence of new virulent strains. To counter this constant threat, researchers are exploring innovative approaches that involve the integration of multiple defence mechanisms to enhance plant disease resistance. By combining various genetic engineering strategies and natural defence systems, scientists aim to create crops that exhibit robust and durable resistance against a wide range of pathogens.

Stacking Resistance Genes: One approach to integrating multiple defence mechanisms is stacking resistance genes in a single plant. These genes can be sourced from different species, each conferring resistance against distinct pathogens. The simultaneous expression of multiple resistance genes enhances the plant's ability to withstand infections from multiple pathogens. However, challenges exist in identifying compatible gene combinations and potential gene interactions that might cause unintended consequences.

Phytohormone Crosstalk: Phytohormones play pivotal roles in plant defence responses. Cross-talk between different phytohormone signalling pathways can lead to synergistic effects, preparing the plant for a more potent defence response. Researchers are investigating how to modulate these signalling networks to enhance the activation of defence mechanisms while minimizing any negative impacts on plant growth and development.

Induced Systemic Resistance (ISR): Induced Systemic Resistance is a plant defence mechanism triggered by beneficial microbes or non-pathogenic microorganisms. These microbes colonise the plant's rhizosphere and activate a systemic immune response, providing resistance against a broad spectrum of pathogens. Integrating ISR into genetically engineered crops holds promise for inducing long-lasting and non-specific resistance against diseases.

Exploration of Microbiome-Mediated Disease Resistance:

Microbiome-mediated disease resistance research has emerged as a promising area in agriculture, leveraging the intricate interactions between plants and their associated microbial communities to enhance plant productivity and combat diseases. Recent advances in genetic information regarding plant-microbiome interactions have provided valuable insights into the molecular aspects of these interactions, enabling better utilization of the plant microbiome in agriculture. Innovative technologies, such as CRISPR, have revolutionized the study of plant-microbiome interactions by facilitating precise genetic changes in both plants and microorganisms, offering potential solutions to strengthen disease resistance in crops.

The precise genetic modification (GM) of plants or microorganisms has been a focus of microbiome-mediated disease resistance research. In this context, genome-editing techniques have become of great interest, as they enable scientists to edit genomic sequences with increased precision without the need for foreign gene incorporation. These techniques, such as transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and CRISPR-Cas9 systems (CRISPR-associated), offer specific advantages, with CRISPR-Cas9 systems standing out due to their simplicity, versatility, cost efficiency, high efficiency, multiplexing capabilities, and specificity [16]. The exploration of microbiome-mediated disease resistance presents exciting opportunities for agriculture. Leveraging the intricate interactions between plants and their microbial partners through technologies like CRISPR-mediated genetic modifications and sustainable microbiome-based approaches can lead to the development of disease-resistant crop cultivars and smarter, environmentally friendly pest control strategies. Continued research and innovation in this field hold the potential to revolutionize agricultural practices and contribute to global food security.

V. CONCLUSION:

We conclude that genetic engineering provides effective solutions for improving plant disease resistance, which is essential for resolving issues with food security and sustainable agriculture. The diverse approaches, including transgenic plants, pathogen resistance genes, RNA interference (RNAi), and genome editing, present potential strategies to combat plant diseases. However, challenges like off-target effects, public perception, and ecological impacts demand careful consideration. We will be able to fully utilize the advantages of genetic engineering by welcoming responsible practices, transparency, and ongoing research. This will pave the way for eco-friendly and sustainable agricultural systems, increase world food production, and ensure a sustainable future.

Abbreviations and Acronyms

1) AGO - Argonaute family proteins
2) Bt - *Bacillus Thuringiensis*
3) CPB - Cartagena Protocol on Biosafety
4) CRISPR - Clustered Regularly Interspaced Short Palindromic Repeats
5) DSB - Double-Stranded Break
6) dsRNA - double stranded RNA
7) EIQ - Environmental Impact Quotient
8) EPA - Environmental Protection Agency
9) FDA - Food and Drug Administration
10) GAC - General Administration of Customs
11) GEAC - Genetic Engineering Appraisal Committee
12) GM - Genetically Modified
13) GMO - Genetically Modified Organisms
14) HDR - Homology-Directed Repair
15) ISR - Induced Systemic Resistance
16) MARA - Ministry of Agriculture and Rural Affairs
17) MoEFCC - Ministry of Environment, Forests, and Climate Change
18) NHEJ - Non-Homologous End Joining
19) PAM - Protospacer Adjacent Motif
20) QDR - Quantitative Disease Resistance
21) RCGM - Review Committee on Genetic Manipulation
22) RISC - RNA-induced silencing complex
23) RNAi - RNA Interference
24) SAMR - State Administration of Market Regulation
25) SBCC - State Biotechnology Coordinator Committee

26) sgRNA - single guide RNA
27) siRNA - small interfering RNA
28) TALEN - Transcription Activator-Like Effector Nucleases
29) USDA - United States Department of Agriculture
30) ZFN - Zinc-Finger Nucleases

REFERENCES:

[1] O. X. Dong, and P. C. Ronald, "Genetic engineering for disease resistance in plants: recent progress and future perspectives," Plant Physiology, vol. 180, no. 1, pp. 26–38, May 2019. doi: 10.1104/pp.18.01224.
[2] Wally, Owen, and Z. K. Punja, "Genetic engineering for increasing fungal and bacterial disease resistance in crop plants," GM crops, vol. 1, no. 4, pp. 199–206, 2010. doi: 10.4161/gmcr.1.4.13225.
[3] H. P. van Esse, T. L. Reuber, and D. van der Does, "Genetic modification to improve disease resistance in crops," New Phytol, vol. 225, no. 1, pp. 70–86, May 2019. doi: 10.1111/nph.15967.
[4] S. Liu, S. Geng, A. Li, Y. Mao, and L. Mao, "RNAi technology for plant protection and its application in wheat," aBIOTECH, vol. 2, pp. 365–374, March 2021. doi: 10.1007/s42994-021-00036-3.
[5] X. Liu, S. Wu, J. Xu, C. Sui, and J. Wei, "Application of CRISPR/Cas9 in plant biology," Acta pharmaceutica Sinica. B, vol. 7, no. 3, pp. 292–302, 2017. doi: 10.1016/j.apsb.2017.01.002.
[6] M. Muntazir, and S. Aafreen et al. "Harnessing genome editing techniques to engineer disease resistance in plants," Frontiers in Plant Science, vol. 10, 2019. doi: 10.3389/fpls.2019.00550.
[7] T. Sprink, J. Metje, and F. Hartung, "Plant genome editing by novel tools: TALEN and other sequence specific nucleases," Current Opinion in Biotechnology, vol. 32, pp. 47–53, April 2015. doi: 10.1016/j.copbio.2014.11.010.
[8] L. Sun, and Nasrullah, et al. "Citrus genetic engineering for disease resistance: past, present and future," International Journal of Molecular Sciences, vol. 20, no. 21, 5256, 2019. doi: 10.3390/ijms20215256.
[9] G. S. Ladics, and A. Bartholomaeus et al. "Genetic basis and detection of unintended effects in genetically modified crop plants," vol. 24, pp. 587–603, August 2015. doi: 10.1007/s11248-015-9867-7.
[10] J. N. Fernandes, P. H. Hemsworth, G. J. Coleman, and A. J. Tilbrook, "Costs and benefits of improving farm animal welfare," Agriculture, vol. 11, no. 2, pp. 104, 2021. doi: 10.3390/agriculture11020104.
[11] M. Shukla, K. T. Al-Busaidi, M. Trivedi, and R. K. Tiwari, "Status of research, regulations and challenges for genetically modified crops in India," GM Crops & Food, vol. 9, no. 4, pp. 173-188, 2018. doi: 10.1080/21645698.2018.1529518.
[12] J. Liang, and X. Yang et al. "The evolution of China's regulation of agricultural biotechnology," aBIOTECH, vol. 3, pp. 237–249, December 2022. doi: 10.1007/s42994-022-00086-1.
[13] P. Scott, and J. Thomson et al. "Genetic modification for disease resistance: a position paper," Food Security, vol. 8, pp. 865–870, June 2016. doi: 10.1007/s12571-016-0591-9.
[14] J. Garcia-Yi, and T. Lapikanonth et al. "What are the socio-economic impacts of genetically modified crops worldwide? A systematic map protocol," Environmental Evidence, vol. 3, no. 24, December 2014. doi: 10.1186/2047-2382-3-24.
[15] O. Sanvido, J. Romeis, and F. Bigler, "Ecological impacts of genetically modified crops: ten years of field research and commercial cultivation," Advances in biochemical engineering/biotechnology, vol. 107, pp. 235–278, 2007. doi: 10.1007/10_2007_048.
[16] R. M. Shelake, D. Pramanik, and J. Y. Kim, "Exploration of Plant-Microbe Interactions for Sustainable Agriculture in CRISPR Era," Microorganisms, vol. 7, no. 8, pp. 1–32, August 2019. doi: 10.3390/microorganisms7080269.
[17] K. Yin, and J. L. Qiu, "Genome editing for plant disease resistance: applications and perspectives," Philosophical transactions of the Royal Society of London, vol. 374, 1767, 2019. doi: 10.1098/rstb.2018.0322.
[18] Chen, and Jiasheng et al. "Off-target effects of RNAi correlate with the mismatch rate between dsRNA and non-target mRNA," RNA biology, vol. 18, no. 11, pp. 1747–1759, 2021. doi: 10.1080/15476286.2020.1868680.
[19] Nora, and L. Czamanski et al. "The art of vector engineering: towards the construction of next-generation genetic tools," Microbial biotechnology, vol. 12, no. 1, pp. 125-147, 2019. doi: 10.1111/1751-7915.13318.
[20] D. B. Collinge, O. S. Lund and H. Thordal-Christensen, "What are the prospects for genetically engineered, disease resistant plants?," Sustainable disease management in a European context, pp. 217–231, September 2007. doi: 10.1007/978-1-4020-8780-6_2.
[21] N. Thakur, and M. Nigam et al. "Host-mediated gene engineering and microbiome-based technology optimization for sustainable agriculture and environment," Functional & Integrative Genomics, vol. 23, no. 57, January 2023. doi: 10.1007/s10142-023-00982-9.
[22] E. J. Andersen, S. Ali, and E. Byamukama, Y. Yen, M. P. Nepal, "Disease Resistance Mechanisms in Plants," Genes, vol. 9, no. 7, 339, July 2018. doi: 10.3390/genes9070339.
[23] S. Yogindran, and M. V. Rajam, "RNAi for Crop Improvement," Plant Biology and Biotechnology, pp. 623–637, 2015. doi: 10.1007/978-81-322-2283-5_31.
[24] A. Younis, M. I. Siddique, C. K. Kim, and K. B. Lim, "RNA Interference (RNAi) Induced Gene Silencing: A Promising Approach of Hi-Tech Plant Breeding," International journal of biological sciences, vol. 10, no. 10, pp. 1150–1158, October 2014. doi: 10.7150/ijbs.10452.
[25] K. Belhaj, A. Chaparro-Garcia, S. Kamoun, N. J. Patron, V. Nekrasov, "Editing plant genomes with CRISPR/Cas9," Current Opinion in Biotechnology, vol. 32, pp. 76–84, 2015. doi: 10.1016/j.copbio.2014.11.007.

[26] T. Li, B. Liu, C. Y. Chen, and B. Yang, "TALEN-mediated homologous recombination produces site-directed DNA base change and herbicide-resistant rice," Journal of Genetics and Genomics, vol. 43, no. 5, pp. 297–305, 2016. doi: 10.1016/j.jgg.2016.03.005.
[27] Y. Zhang, K. Massel, I. D. Godwin, and C. Gao, "Applications and potential of genome editing in crop improvement," Genome biology, vol. 19, no. 1, 210, 2018. doi: 10.1186/s13059-018-1586-y.
[28] A. S. Bawa, and K. R. Anilakumar. "Genetically modified foods: safety, risks and public concerns-a review," Journal of food science and technology, vol. 50, no. 6, pp. 1035–1046, 2013. doi: 10.1007/s13197-012-0899-1.
[29] E. Woźniak-Gientka, and T. Agata et al. "Public perception of plant gene technologies worldwide in the light of food security," GM Crops & Food, vol. 13, no. 1, pp. 218–241, August 2022. doi: 10.1080/21645698.2022.2111946.
[30] M. A. Altieri, "The Ecological Impacts of Transgenic Crops on Agroecosystem Health," Ecosystem Health, vol. 6, no. 1, pp. 13–23, 2000.
[31] M. Chamberlin, "The Environmental Impact of Genetically Modified Crops," Health & Human Development.
[32] A. Kamthan, A. Chaudhuri, M. Kamthan, and A. Datta, "Genetically modified (GM) crops: milestones and new advances in crop improvement," Theoretical and applied genetics, vol. 129, no. 9, pp. 1639–1655, 2016. doi: 10.1007/s00122-016-2747-6.
[33] N. A. Abdallah, C. S. Prakash, A. G. McHughen, "Genome editing for crop improvement: Challenges and opportunities," GM Crops & Food, vol. 4, no. 4, pp. 183–205, 2015. doi: 10.1080/21645698.2015.1129937.
[34] J. M. Van Emon, "The Omics Revolution in Agricultural Research," Journal of Agriculture and Food Chemistry, vol. 64, no. 1, pp. 36–44, January 2016. doi: 10.1021/acs.jafc.5b04515.
[35] Collinge, D. B. Jørgensen, H. J. L. Lund, O. S. Lyngkjær, F. Michael, "Engineering pathogen resistance in crop plants: current trends and future prospects," Annual review of Phytopathology, vol. 48, no. 1, pp. 269–291, 2023. doi: 10.1146/annurev-phyto-073009-114430
[36] H. Kosack, K. E. Jones, D. G. Jonathan, "Plant disease resistance genes," Annual Review of Plant Physiology and Plant Molecular Biology, vol. 48, no. 1, pp. 575–607, 2023. doi: 10.1146/annurev .arplant .48.1.575.