

Review Article



Challenges and opportunities of the CRISPR/Cas9 system as an efficient genome editing tool in the development of medicinal plants: An updated mini- review

Hossein Soltaninejad^{1*}, Mohadeseh Khoshandam², Elham Karimi³, Reza Sheikhabari⁴, Mojdeh Hosseinpoor Kashani⁵

¹Department of Stem Cells Technology and Tissue Regeneration, Faculty of Interdisciplinary Science and Technologies, Tarbiat Modares University, Tehran, Iran

²Pediatric Cell and Gene Therapy Research Center, Gene, Cell & Tissue Research Institute, Tehran University of Medical Sciences, Tehran, Iran

³Department of Nano Biotechnology, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

⁴Department of Biology, Faculty of Science, University of Qom, Qom, Iran

⁵Department of Reproductive Biology, Academic Center for Education, Culture, and Research (ACECR), Qom Branch, Qom, Iran

*Corresponding Author: Hossein Soltaninejad, Email: hossein.soltani@modares.ac.ir

Summary

CRISPR/Cas9 technology has emerged in recent years as a powerful tool for precise genome editing in a wide range of organisms. The procedure, which offers high productivity, simplicity of design, and affordability as its main advantages, has been extensively utilized in different life science disciplines. In the field of medicinal plants, CRISPR/Cas9 provides a unique opportunity to improve important traits such as increased production of secondary metabolites, enhanced resistance to environmental stresses and diseases, and improved quality of effective compounds. In this article, we first review medicinal plants and their applications, then discuss the mechanism of action of CRISPR/Cas9 and its advantages over other genome editing methods. We also discuss the practical applications of this system in the field of breeding and development of medicinal plants, and the technical, regulatory, and ethical challenges associated with the use of CRISPR/Cas9-edited plants. Finally, we intend to deliberate on the possibilities of how the technology could help in the development of herbal medicines.

Keywords: CRISPR/Cas9, Medicinal plants, Genome editing

Received: November 25, 2025, **Revised:** January 10, 2026, **Accepted:** January 29, 2026, **ePublished:** April 27, 2026

Introduction

Since centuries ago, medicinal plants have been involved in disease treatment and human health to some extent and nowadays, in many cultures, they still are recognized as a part of traditional or complementary medicine.¹ The knowledge about the use of these plants is based on traditional medicine and has been developed over the years through experimenting and watching the healing effects.² The use of medicinal plants in various ethnic groups and cultures shows how plant knowledge has been considered worldwide and each region has cultivated a collection of indigenous species or collected them from nature for the treatment and prevention of diseases, depending on its environmental and climatic conditions.³ Many of the effective compounds in today's chemical drugs were also first identified and extracted from plants, and modern scientific research has attempted to develop more effective drugs for the treatment of diseases by analyzing the structure of these compounds. In addition, direct consumption of some plants without the need for complex chemical processes can have a positive effect on health and reduce the risk of side effects of chemical drugs.⁴

Today, advances in biotechnologies and medicine have enabled researchers to identify effective compounds in medicinal plants, and to achieve higher quality and better therapeutic performance by making genetic modifications or improving cultivation and harvesting methods. These plants contain active compounds that can be effective in a wide range of diseases.⁵ One of the most significant uses is the extraction of medicinal plants and the production of essential oils, which serve as the primary sources for the manufacture of drugs, dietary supplements, and cosmetics.⁶

For the last few years, the combination of healing herbs with standard cancer therapies (chemotherapy, radiotherapy, and surgery) has gained considerable interest from the scientific community.⁷ Many of the effective compounds in anticancer drugs were first discovered from plant sources and then produced and supplied by industrial or semi-industrial methods.⁸ Among the topmost examples in this area are paclitaxel (Taxol) from the yew tree (*Taxus brevifolia*) and vincristine and vinblastine from the *Catharanthus roseus*.⁹ Many medicinal plants are known to contain phenolic compounds, terpenoids, and alkaloids



that can show different types of anticancer activities. Such activities may include cancer cell proliferation inhibition, apoptosis induction, angiogenesis prevention, and inflammation reduction.¹⁰ For instance, curcumin from turmeric, catechin from green tea, and resveratrol from grapes and berries are some of the naturally occurring compounds that scientific research has indicated may possess anticancer effects.^{11,12} Furthermore, some of these compounds are capable of lowering the chemotherapy and radiotherapy side effects by activating protective mechanisms in the healthy cells, thus making the patients more tolerant of the conventional treatments.¹³

While medicinal plants offer attractive options, it is important to note that the effects of these compounds can vary with dosage, extract type, extraction method, and individual patient characteristics. Thus, it will be very necessary to conduct proper preclinical and clinical research and evaluation on every herbal compound or extract before it can be recommended for use. Also, the arbitrary use of medicinal plants can, in some cases, cause drug interactions or reduce the effectiveness of conventional cancer treatment methods.¹⁴ For this reason, it is recommended that patients with cancer consult their medical team before starting any herbal or complementary treatment to make the best decision by examining their physical condition and the type of treatment being implemented.³ Although the primary source of herbal medicinal compounds is plants found in natural environments or cultivated plants, the preparation of the aforementioned compounds from such sources is associated with limitations, some of which are:

1. The concentration of many of these compounds in plants found in nature or cultivated plants is very low; For example, the concentration of the taxol metabolite is equivalent to one hundredth of a percent of the dry weight of the yew plant, while the concentration of some other plant metabolites is even lower than this amount.¹
2. Sometimes, there are many compounds similar to the desired compound in the plant, whose presence makes the process of separating and purifying the main compounds complicated and costly.¹⁵
3. Production is highly variable, and factors such as environmental conditions have a great impact on the rate of metabolite production, so the production efficiency in this method is associated with large fluctuations.¹⁶
4. The process of producing pharmaceutical Compounds from traditional sources is sometimes very long and may require several years.
5. Some plants producing these compounds are rare and slow-growing, and in such circumstances, collecting them from nature causes damage to the environment.¹⁷

Genome editing in medicinal plants is one of the most recent advances in biotechnology, allowing for the improvement and enhancement of their medicinal properties. This process involves making precise changes

to the DNA of plants to create desirable characteristics, such as increased disease resistance, improved production of active compounds, higher yields, and optimized growth conditions. One of the key tools in this field is the CRISPR-Cas9 technology, which is widely used due to its high precision and high efficiency in gene editing.^{18,19} This review article utilized reliable sources and keywords such as CRISPR/Cas9 technology, medicinal plants, as well as their advantages and disadvantages. The articles reviewed met the necessary inclusion and exclusion criteria, and their results were thoroughly analyzed.

Overview of CRISPR/Cas9 Technology

CRISPR (clustered regular interspersed short palindromic repeats) is a type of adaptive immune system in bacteria and archaea.²⁰ It helps microorganisms defend themselves against viruses and other foreign agents. The mechanism of this system involves storing segments of invading DNA in specific regions of the host genome called CRISPR arrays.¹⁷ These stored sequences are used as a memory to recognize future attacks. During re-invasion, RNAs called crRNAs, transcribed from CRISPR arrays, match the sequence of the invading DNA.²¹ These RNAs, in collaboration with CRISPR-associated proteins, such as Cas9 or Cas12, act as guides and direct the CRISPR enzymes to the target DNA (Figure 1). Once recognized, the Cas protein cuts the invading DNA and inactivates it.

The system allows researchers to edit the genomes of living organisms with high precision. With CRISPR, it is possible to delete, insert, or modify particular gene sequences.²² The innovation has been used in many different areas, such as curing hereditary disorders, improving crops, and manufacturing drugs for humans.²³ CRISPR has several advantages over other technologies, such as TALENs and zinc finger nucleases (ZFNs), including easier design, lower cost, and superior performance. However, it also has drawbacks, such as the risk of unintended mutations in the genome and ethical concerns surrounding its use.²⁴ Overall, CRISPR is recognized as a revolutionary tool in biology and medicine, and much research is underway to further

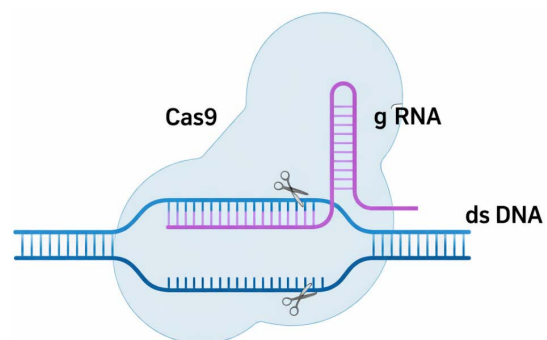


Figure 1. Schematic representation of the CRISPR–Cas system. Guide RNA (gRNA), in association with CRISPR-associated proteins such as Cas9, directs the enzyme to the target double-stranded DNA (dsDNA) sequence, enabling precise recognition and cleavage of the genetic material.

understand its capabilities and limitations.¹⁸

Mechanism of Action of Cas9

Mechanism of Action CRISPR naturally functions in bacteria and archaea as an adaptive immune system that can recognize and destroy invading genetic material, such as viral DNA or RNA.²⁵ This system consists of three main steps: adaptation, expression, and intervention. In the adaptation phase, when a virus or another invading agent infects a bacterium, small fragments of the invading DNA, known as protospacer sequences, are recognized and inserted into CRISPR arrays within the bacterial genome.²⁶ These arrays consist of repetitive sequences and distinct spacers, each spacer containing a sequence of the invading genetic material. This process causes the CRISPR arrays to become a type of immune memory. In the expression phase, the CRISPR arrays are transcribed and converted into RNA. These RNAs are known as CRISPR pre-RNAs, or pre-crRNAs. This pre-RNA is then processed into smaller fragments called crRNAs, each containing a guide sequence from the invading genetic material and a portion of the repetitive sequence.²⁷ These crRNAs combine with Cas proteins (such as Cas9, Cas12, or Cas13, depending on the system) to form an active complex. In the intervention phase, the crRNA-Cas complex functions by searching for specific target sequences in the invading genetic material.²⁸ The binding of crRNA to the target sequence specifically depends on the presence of a PAM (protospacer-adjacent motif) sequence near the target. The PAM acts as a marker and ensures that the Cas protein does not attack its own bacterial genetic material. Once the target sequence is recognized, the Cas protein cuts it. In the Cas9 system, this cutting typically occurs on both strands of DNA, resulting in a double-strand break. In systems like Cas12, in addition to cutting DNA, off-target RNA degradation is sometimes observed.^{29,30} This process of destroying invading genetic material prevents infection and ensures the survival of the host cell.

Applications and Challenges of CRISPR/Cas9 Technology in Medicinal Plant Development

Researchers are using CRISPR to discover new ways to improve crop disease resistance and environmental stress tolerance in plants, which could lead to the development of new products to help feed and treat the global population.^{19,31} CRISPR/Cas9 has many advantages over other Genome editing technologies, including multiplexing, high precision, cost-effectiveness, and simplicity. It can be applied directly to the plant and reduces the time required to modify target Genes compared to gene-targeting technologies.²¹ It also has higher specificity and versatility than other mutagenic techniques, such as ZFN and TALEN.³¹ One of the major advantages of genome editing in medicinal plants is the increased production of active pharmaceutical

ingredients. The production of medicinal plants can be boosted and their quality improved by altering the genes that are responsible for metabolic pathways that generate medicinal compounds. To illustrate, in the case of turmeric, which has curcumin as its active ingredient, the editing of the genome can not only boost the production of curcumin but also its anti-inflammatory and anti-cancer properties.³² Moreover, the gene editing technique can not only lead to creating higher amounts of medicinal compounds but also be used to strengthen their resistance to diseases and extreme climates. With the help of pest-resistance, drought-resistance, soil salinity-resistance, or temperature changes, one can alter the genes of medicinal plants for their growth in the desired direction. This will not only unlock the full agricultural potential of these areas but also reduce the use of pesticides and chemical fertilizers, making it a more environmentally friendly practice. However, along with its numerous advantages, genome editing in medicinal plants poses challenges.¹⁷ One such challenge is the ethical and legal problems connected with the use of genetically modified plants. Several nations have very stringent regulations regarding the cultivation and marketing of genetically modified plants, which can further complicate research and development. Additionally, concerns about the environmental and human health impacts of genome editing persist and require thorough and ongoing research. On the other hand, maintaining the therapeutic potential of the plant after genome editing remains a challenge. The genetic changes may lead to unforeseen results on the active plant molecules; thus, there is a need for careful and extensive evaluations to ensure the safety and efficacy of the drugs produced. Also, genome editing processes must be carried out with high precision and quality controls to avoid introducing unwanted changes to the plant genome.⁶ The application of CRISPR/Cas9 in gene editing has several drawbacks, including unintended gene alterations, immune responses, and potential disruptions to ecosystems.³³

Nevertheless, the medical use of selected plants becomes more likely than ever, thanks to soon-to-be-considered new methods of genome editing, the development of new CRISPR systems that are exact and can perform more changes with little to no side effects. These methods enable scientists to affect the medicinal characteristics of plants in a very specific and targeted manner, and thus produce plants that are more suitable for medical and pharmaceutical purposes. In the long run, the genetic alteration of the selected plants will lead to new drugs and better health care. The plants with enhanced medicinal qualities will be the result of the collaboration between genetics, biochemistry, and technology, and eventually, more patients will be given access to effective treatments while the cost of production of herbal medicines is made lower. In addition, this technology can also help preserve biodiversity and sustainably use natural resources, which

is especially important in the era of climate change and the need for sustainable solutions.²⁸

Discussion

Plant breeding will undoubtedly benefit from genetic tools, precision breeding methods, and climate-resilient crops to address global food challenges effectively.^{34,35} Technical elements such as genetic markers, reference genomes, databases, transcriptomes, and gene expression profiles are key genomic resources in this context. Advanced methods greatly aid in identifying genes linked to desirable traits, understanding genetic variation in crops, and accelerating the development of new varieties. Selecting such indicators enables faster and more precise work by focusing only on high-quality plants, with the selection of disease-resistant, drought-tolerant, and high-yielding varieties carried out in advance.³⁶ The process of critical traits being obtained in a crop variety will be significantly shortened, and the proportion of breeders' preferred traits becoming common in the population will be raised remarkably.³⁷ Among breeding techniques, genome editing shows the highest precision through CRISPR-Cas9, for instance, in yield improvement. A specific gene modification can be performed on a plant that already possesses the desired trait, or it can be engineered to acquire it, in such a way that no unintended changes occur. CRISPR/Cas-9 and its relatives (such as CRISPR/Cas-12 and CRISPR/Cas-13) are considered revolutionary tools for genome editing due to their precision, efficiency, cost-effectiveness, and simplicity. These gene-editing techniques enable highly accurate modifications within cell nuclei. However, a major limitation remains: the risk of unintended mutations, which may manifest as undesired phenotypes, potentially restricting the application of this technology to only a few genetic loci. To address this issue, newer versions of CRISPR/Cas are being developed and existing systems are being improved to minimize off-target effects. This is accomplished by selecting single guide RNAs (sgRNAs) predicted to have the fewest potential binding sites, based on a comprehensive analysis of the reference genome.

The future of plant breeding promises advances through genetic tools, precision breeding techniques, and climate-resilient crops to address global food security. Key genomic resources include genetic markers, reference genomes, databases, transcriptomes, and gene expression profiles. These tools are critical for identifying genes associated with desirable traits, understanding genetic diversity, and accelerating breeding programs. Molecular markers lead to faster and more accurate crop enhancement and development by enabling the selection of disease-resistant, drought-tolerant, and high-yielding plants. This allows breeders to select and propagate plants with the highest genetic potential, significantly shortening breeding cycles and increasing the efficiency of trait improvement.³⁸ CRISPR/Cas-9 and related systems (such as CRISPR/Cas-12 and CRISPR/Cas-13) are recognized as groundbreaking tools for genome editing due to their

precision, efficiency, cost-effectiveness, and simplicity.³⁹ These technologies facilitate highly precise modifications of nuclear genomes. However, a significant challenge is the potential for off-target mutations, which can lead to deleterious phenotypes and limit the broader application of genome editing. The potential of genome editing is huge when it comes to generating plants with better nutritional value, animals with disease resistance, and even being more resilient to environmental stresses. By using these methods together, it will be possible to have a whole range of plant species that can cope with the problems of a larger world population, a changing climate, and eco-friendly farming.

Conclusion

Plant breeding is entering a new era, driven by advancements in genetic tools, precision breeding techniques, and the development of climate-resilient crops. Essential genomic resources, including genetic markers, reference genomes, databases, transcriptomes, and gene expression profiles, empower breeders to identify desirable traits, understand genetic diversity, and speed up the creation of improved plant varieties. Molecular markers and genome editing technologies, especially CRISPR/Cas systems, enable precise modifications, helping to select and propagate plants that are disease-resistant, drought-tolerant, high-yielding, and nutritionally enhanced. Although off-target effects pose a challenge, ongoing improvements in CRISPR technology are working to minimize these risks. By integrating these innovative approaches, the future of plant breeding holds the promise of producing crops that can meet the demands of a growing global population, adapt to climate change, and support sustainable, eco-friendly agriculture.

Authors' Contribution

Conceptualization: Hossein Soltaninejad.

Data curation: Mohadeseh Khoshandam, Elham Karimi, Reza Sheikhabari.

Investigation: Mohadeseh Khoshandam, Elham Karimi, Reza Sheikhabari, Mojdeh Hosseinpoor Kashani.

Project administration: Hossein Soltaninejad.

Supervision: Hossein Soltaninejad.

Writing-original draft: Hossein Soltaninejad, Mohadeseh Khoshandam, Elham Karimi, Reza Sheikhabari, Mojdeh Hosseinpoor Kashani.

Writing-review & editing: Hossein Soltaninejad.

Ethical Approval

None.

Funding

None.

Intelligence Use Disclosure

This article uses ChatGPT and Grammarly for language editing according to the journal's policy.

Reference

1. Giannenas I, Sidiropoulou E, Bonos E, Christaki E, Florou-Paneri P. The history of herbs, medicinal and aromatic plants, and their extracts: Past, current situation and future

- perspectives. Feed additives: Elsevier; 2020. p. 1-18. doi:10.1016/B978-0-12-814700-9.00001-7.
2. Casagrande A, Ritter M, Kubo R. Traditional knowledge in medicinal plants and intermedality in urban environments: a case study in a popular community in southern Brazil. *Ethnobotany Research and Applications* 2023;25. doi:10.32859/era.25.35.1-34
 3. Sharma A, Khanna S, Kaur G, Singh I. Medicinal plants and their components for wound healing applications. *Future Journal of Pharmaceutical Sciences* 2021;7(1):53. doi:10.1186/s43094-021-00202-w
 4. Abdel-Aziz SM, Aeron A, Kahil TA. Health benefits and possible risks of herbal medicine. *Microbes in food and health*: Springer; 2016. p. 97-116. doi:10.1007/978-3-319-25277-3_6.
 5. Dar RA, Shah Nawaz M, Ahanger MA, Majid Iu. Exploring the diverse bioactive compounds from medicinal plants: a review. *J Phytopharm* 2023;12(3):189-95. doi:10.31254/phyto.2023.12307
 6. Mishra R, Agarwal P, Mohanty A. Applications of genome editing techniques for the improvement of medicinal plants. *Phytochemical genomics: Plant metabolomics and medicinal plant genomics*: Springer; 2023. p. 545-69.
 7. Zafar A, Khatoun S, Khan MJ, Abu J, Naeem A. Advancements and limitations in traditional anti-cancer therapies: a comprehensive review of surgery, chemotherapy, radiation therapy, and hormonal therapy. *Discov Oncol* 2025;16(1):607. doi:10.1007/s12672-025-02198-8
 8. Demain AL, Martens E. Production of valuable compounds by molds and yeasts. *J Antibiot (Tokyo)* 2017;70(4):347-60. doi:10.1038/ja.2016.121
 9. Sagar PK, Meena R, Ahmad MW, Sajwan K. Useful Anti-Cancerous & Anti-tumorous Asian medicinal Plants (*Taxus baccata* L. or *Taxus baccata* Thunb., *Catharanthus roseus* (L.) G. Don, *Annona muricata* L.). *International Journal of Traditional and Complementary Medicine* 2020;5:22.
 10. Khoshandam M, Soheili ZS, Hosseinkhani S, Samiee S, Latifi-Navid H, Ahmadi H, et al. In vivo inhibition of angiogenesis by htsFLT01/MiRGD nano complex. *Transl Oncol* 2025;56:102400. doi:10.1016/j.tranon.2025.102400
 11. Kianamiri S, Shokri E, Maleki P, Soudi S, Soltaninejad H, Sadeghizadeh M. Novel curcumin nanocarrier for targeting drug delivery of mitochondria proves efficacy in in vivo experiments on hepatocellular carcinoma mice models. *Sci Rep* 2025;15(1):35114. doi:10.1038/s41598-025-19147-8
 12. Jabbari K, Khalafizadeh A, Sheikhbahaei M, Soltaninejad H, Babashah S. TET1: The epigenetic architect of clinical disease progression. *Genes Dis* 2025;12(5):101513. doi:10.1016/j.gendis.2025.101513
 13. Sharma M, Koul A, Sharma D, Kaul S, Swamy MK, Dhar MK. Metabolic engineering strategies for enhancing the production of bio-active compounds from medicinal plants. *Natural bio-active compounds: Volume 3: Biotechnology, bioengineering, and molecular approaches*: Springer; 2019. p. 287-316. doi:10.1007/978-981-13-7438-8_12
 14. Pezzani R, Salehi B, Vitalini S, Iriti M, Zuñiga FA, Sharifi-Rad J, et al. Synergistic Effects of Plant Derivatives and Conventional Chemotherapeutic Agents: An Update on the Cancer Perspective. *Medicina (Kaunas)* 2019;55(4):110. doi:10.3390/medicina55040110
 15. Alamgir A. Cultivation of herbal drugs, biotechnology, and in vitro production of secondary metabolites, high-value medicinal plants, herbal wealth, and herbal trade. *Therapeutic use of medicinal plants and their extracts: volume 1: pharmacognosy*: Springer; 2017. p. 379-452. doi:10.1007/978-3-319-63862-1_9.
 16. Ahad B, Shahri W, Rasool H, Reshi ZA, Rasool S, Hussain T. Medicinal Plants and Herbal Drugs: An Overview. In: Aftab T, Hakeem KR, editors. *Medicinal and Aromatic Plants: Healthcare and Industrial Applications*. Cham: Springer International Publishing; 2021. p. 1-40. doi:10.1007/978-3-030-58975-2_1
 17. Zaidi SS, Mahas A, Vanderschuren H, Mahfouz MM. Engineering crops of the future: CRISPR approaches to develop climate-resilient and disease-resistant plants. *Genome Biol* 2020;21(1):289. doi:10.1186/s13059-020-02204-y
 18. Muthukrishnan A, Jayachandran H, Kumaresan K. CRISPR/Cas9: A Novel Genetic Tool to Manipulate Plant Secondary Metabolite Pathways. 2023. p. 45-57. doi:10.1007/978-981-99-4939-7_3
 19. Khoshandam M, Soltaninejad H, Mousazadeh M, Hamidieh AA, Hosseinkhani S. Clinical applications of the CRISPR/Cas9 genome-editing system: Delivery options and challenges in precision medicine. *Genes Dis* 2024;11(1):268-82. doi:10.1016/j.gendis.2023.02.027
 20. Li J, Wu S, Zhang K, Sun X, Lin W, Wang C, et al. Clustered Regularly Interspaced Short Palindromic Repeat/CRISPR-Associated Protein and Its Utility All at Sea: Status, Challenges, and Prospects. *Microorganisms* 2024;12(1):118. doi:10.3390/microorganisms12010118
 21. Khoshandam M, Soltaninejad H, Bhia I, Goudarzi MTH, Hosseinkhani S. CRISPR challenges in clinical developments. *Prog Mol Biol Transl Sci* 2025;210:263-79. doi:10.1016/bs.pmbts.2024.08.001
 22. Zhang F. Development of CRISPR-Cas systems for genome editing and beyond. *Quarterly Reviews of Biophysics* 2019;52:e6. doi:10.1017/S0033583519000052
 23. Schachtsiek J, Stehle F. Nicotine-free, nontransgenic tobacco (*Nicotiana tabacum* L.) edited by CRISPR-Cas9. *Plant Biotechnol J* 2019;17(12):2228-30. doi:10.1111/pbi.13193
 24. Khoshandam M, Soltaninejad H, Hamidieh AA, Hosseinkhani S. CRISPR, CAR-T, and NK: Current applications and future perspectives. *Genes Dis* 2024;11(4):101121. doi:10.1016/j.gendis.2023.101121
 25. Marraffini LA, Sontheimer EJ. CRISPR interference: RNA-directed adaptive immunity in bacteria and archaea. *Nat Rev Genet* 2010;11(3):181-90. doi:10.1038/nrg2749
 26. Newsom S, Parameshwaran HP, Martin L, Rajan R. The CRISPR-Cas Mechanism for Adaptive Immunity and Alternate Bacterial Functions Fuels Diverse Biotechnologies. *Front Cell Infect Microbiol* 2020;10:619763. doi:10.3389/fcimb.2020.619763
 27. Mohanraju P, Saha C, van Baarlen P, Louwen R, Staals RHJ, van der Oost J. Alternative functions of CRISPR-Cas systems in the evolutionary arms race. *Nat Rev Microbiol* 2022;20(6):351-64. doi:10.1038/s41579-021-00663-z
 28. Chen K, Wang Y, Zhang R, Zhang H, Gao C. CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture. *Annu Rev Plant Biol* 2019;70:667-97. doi:10.1146/annurev-arplant-050718-100049
 29. Yang P, Khoshandam M, Bhia I, Raji S, Soltaninejad H, Hosseinkhani S, et al. Integrating CRISPR/Cas technology with clinical trials: Principles, progress and challenges. *Asian J Pharm Sci* 2025;20(6):101068. doi:10.1016/j.ajps.2025.101068
 30. Tuncel A, Pan C, Clem JS, Liu D, Qi Y. CRISPR-Cas applications in agriculture and plant research. *Nat Rev Mol Cell Biol* 2025;26(6):419-41. doi:10.1038/s41580-025-00834-3
 31. Bigini V, Camerlengo F, Botticella E, Sestili F, Savatin DV. Biotechnological Resources to Increase Disease-Resistance by Improving Plant Immunity: A Sustainable Approach to Save Cereal Crop Production. *Plants (Basel)* 2021;10(6):1146. doi:10.3390/plants10061146
 32. Chen K, Gao C. Targeted genome modification technologies and their applications in crop improvements. *Plant Cell Rep* 2014;33(4):575-83. doi:10.1007/s00299-013-1539-6
 33. Zhao F, Ding X, Liu Z, Yan X, Chen Y, Jiang Y, et al. Application of CRISPR/Cas9-based genome editing in ecotoxicology. *Environ*

- Pollut 2023;336:122458. doi:[10.1016/j.envpol.2023.122458](https://doi.org/10.1016/j.envpol.2023.122458)
34. Naqvi RZ, Siddiqui HA, Mahmood MA, Najeebullah S, Ehsan A, Azhar M, et al. Smart breeding approaches in post-genomics era for developing climate-resilient food crops. *Front Plant Sci* 2022;13:972164. doi:[10.3389/fpls.2022.972164](https://doi.org/10.3389/fpls.2022.972164)
 35. Hafeez U, Ali M, Hassan S, Akram MA, Zafar A. Advances in Breeding and Engineering Climate-Resilient Crops: A Comprehensive Review. *Journal of Scientific Research and Advances* 2023;2: 85–99.
 36. Sarfraz Z, Zarlashat Y, Ambreen A, Mujahid M, Iqbal MS. Advanced gene editing techniques for enhancing disease resistance and climate resilience in crops. *Funct Plant Biol* 2025;52:FP24357. doi:[10.1071/fp24357](https://doi.org/10.1071/fp24357)
 37. Khan SU, Khan MS, Wang H, Qian M, Javed T, Fahad S, et al. Harnessing nanobiotechnology for drought stress: transforming agriculture's future; what, why and how? *Environmental Science: Nano* 2024;11(7):2861–84. doi:[10.1039/D4EN00112E](https://doi.org/10.1039/D4EN00112E)
 38. Sampath V, Rangarajan N, Sharanappa CH, Deori M, Veeraragavan M, Ghodake BD, et al. Advancing Crop Improvement Through CRISPR Technology in Precision Agriculture Trends-A Review. *International Journal of Environment and Climate Change* 2023;13(11):4683–94. doi:[10.9734/ijecc/2023/v13i113647](https://doi.org/10.9734/ijecc/2023/v13i113647)
 39. Kumar R, Das SP, Choudhury BU, Kumar A, Prakash NR, Verma R, et al. Advances in genomic tools for plant breeding: harnessing DNA molecular markers, genomic selection, and genome editing. *Biol Res* 2024;57(1):80. doi:[10.1186/s40659-024-00562-6](https://doi.org/10.1186/s40659-024-00562-6)