Journal of Innovative Agriculture
Review Article

Advances and challenges in plant genome editing: from CRISPR/Cas systems to AI-assisted genome editing

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Volume: 13, Issue: 3, Pages: 1-11
DOI: https://doi.org/10.37446/jinagri/ra/13.3.2026.1-11
Received: 20 November 2025 / Accepted: 14 June 2026 / Published: 30 June 2026

The advent of genome editing tools, especially CRISPR/Cas9, has been one of the biggest milestones in the field of plant biology, making precise modifications of crop genomes possible. This review aims to present an overview of opportunities and challenges in plant genome editing. The opportunities include accelerated crop improvement through genetic modification, increased disease resistance via gene editing, and sustainable agriculture through reduced reliance on chemical pesticides and improved climate resilience of crops. However, several technical problems need to be addressed, including unwanted mutations at other sites in the genome (off-target effects), inefficient delivery systems, and recalcitrance during regeneration. Moreover, legislation surrounding the use of genome editing differs significantly from one country to another. While the USA grants exemptions to products derived from genome editing, the EU has formally adopted a new dual-classification regulatory framework for New Genomic Techniques (NGTs), which is now entering a two-year implementation period. These regulations cause obstacles for commercialization, international trade, and scientific collaborations. Recent developments in areas such as prime editing, dual base editing, artificial intelligence-assisted nuclease design, and targeted DNA integration promise improvements in overcoming existing limitations.

Keywords: *CRISPR/Cas9, plant genome editing, crop improvement, prime editing, regulatory frameworks, base editing, sustainable agriculture*

Introduction

Today, modern agriculture has to face an array of complex problems that include the increasing population size forecasted to be 9.7 billion by 2050, climatic changes, plant disease threats, and the need to minimize negative effects on the environment associated with agriculture (Sohail et al., 2026). While classical plant breeding remains an effective technique in terms of the achievements obtained in crop development, it is limited due to such factors as slow generation time (Ahmar et al., 2020), species' compatibility restrictions (Bhalla et al., 2026), and the impossibility to induce targeted genetic alterations without associated negative traits (Yıldırım & Kavas, 2026). These limitations have driven the search for more precise, efficient, and versatile crop improvement technologies (Zhou et al., 2024; Ahmar et al., 2020). The discovery and subsequent developments of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and Cas9 have dramatically changed this scenario. Having first been discovered as an immune mechanism in bacteria and archaea (Barrangou et al., 2007; Guzmán et al., 2021), CRISPR/Cas9 technology was harnessed for genome editing in 2012 (Jinek et al., 2012), leading to its selection for the awarding of the Nobel Prize in Chemistry in 2020 (Nobel Prize Outreach, 2020). The relative simplicity of this genome-editing method, which relies on only two components- a Cas9 endonuclease and a customizable sgRNA- to achieve targeted DNA cleavage, has made this process much more widely available than before (Gallo et al., 2025).

The first successful applications of CRISPR/Cas9 in plants were reported in 2013 using genes in *Arabidopsis thaliana*, *Nicotiana benthamiana*, and *Oryza sativa* (rice) (Li et al., 2013; Shan et al., 2013). Subsequent years witnessed an increasing number of species on which genome editing was performed, including important crops such as cereals and vegetables, and also plant models (Sohail et al., 2026). Recent times have brought the emergence of advanced genome editing platforms like base editing and prime editing, capable of performing precise changes to single bases without causing double-strand breaks (DSBs), in addition to AI-based design nucleases that have been added to the genome editing toolbox (Gallo et al., 2025; Das et al., 2026). This review presents a comprehensive overview of the potential and obstacles for genome editing in plants.

Opportunities in plant genome editing

Accelerating crop improvement and trait development

The advent of genome editing technology has revolutionized crop improvement efforts both in terms of speed and scale. While conventional breeding may take 6-7 years to incorporate the trait of interest, genome editing using CRISPR methods can introduce the desired change within one generation (Sohail et al., 2026), although the overall breeding pipeline still requires downstream regeneration of edited plants, molecular and phenotypic validation, segregation of the edit from selection markers, and multi-season field evaluation before a new variety can be released. This is especially beneficial for perennial and tree crops, which typically have long periods of juvenility and thus are harder to breed conventionally. Gene editing has led to yield improvements by manipulating genes that control plant architecture, floral development, and grain number (Feng et al., 2025; Zhang et al., 2025). Given the high efficiency of the C_4 photosynthesis found in plants such as maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench), they play an important role in ensuring food security. Genome editing has also enabled the improvement in these species' osmotic and oxidative stress tolerance (Namata et al., 2025). Plant architecture and branching genes have been manipulated to improve harvest index and light penetration in several cereal crops (Feng et al., 2025; Srivastava et al., 2025; Zhang et al., 2025). Improvement of quality traits is another successful aspect of genome editing. Shelf-life improvement through gene editing of ripening-related genes has been accomplished in different horticultural crops like tomato, and fruits (López-Casado et al., 2023; Zhang et al., 2024). Flavour enhancement by modifying sugar metabolism-related and organic acid-related genes has also been successful (Yuan et al., 2025). Biofortification through metabolic engineering has also been realized (Chen et al., 2025; Choi & Ha, 2025). Genetic editing has been used successfully to increase the nutritional value of different plants (Bu et al., 2025; Vanguri, 2025), which represents a promising approach for addressing micronutrient deficiencies in staple crops, although widescale deployment and population-level impact assessment on human nutrition is limited (Vanguri, 2025).

Enhancing disease resistance

The occurrence of crop diseases leads to a loss of between 20-30% crop yields annually and constitutes a major threat to world food security (Savary et al., 2019). Plant genome editing provides various avenues for designing plants resistant to diseases compared to using chemicals (Sohail et al., 2026). A highly successful approach used includes susceptibility (S) gene knock-out. Host plant factors known as S-genes help pathogens invade host cells, and knocking out these genes can confer resistance effective across multiple pathogen strains; however, the durability of resistance and any associated fitness trade-offs are highly dependent on the specific S-gene targeted and the genetic background of the host, and fitness penalties have been reported for some S-gene knockouts (Li & Xiao, 2025; Han et al., 2025). Successful examples include editing of the *MLO* (Mildew Resistance Locus O) gene family leading to disease resistance against powdery mildew in wheat, barley, and tomato (Borrelli et al., 2018; Nekrasov et al., 2017; Wang et al., 2014). Resistance has also been developed by modifying immunity-related genes, including negative regulators of immunity that enhance disease resistance (Song et al., 2025), though there is a need to manage growth-defence balance (Iswanto et al., 2025).

One such novel strategy that has shown great promise is the development of autoactive Nucleotide-binding, leucine-rich repeat (NLR) based immune receptors that can be converted into protease-activated molecular switches. In this case, the NLR gene is fused with a sequence that gets cleaved by the pathogen proteases, rendering the resulting protein inactive. Following cleavage, an active NLR that initiates an immune response is produced. Through this approach, Wang & Xiao (2025) were able to induce broad-spectrum resistance against different potyviruses in *Nicotiana benthamiana* and Soybean mosaic virus in genetically engineered soybeans. This approach has great potential to provide durable plant resistance due to the evolutionarily conserved nature of the enzymes involved in the process of virulence (Wang & Xiao, 2025; Zhang et al., 2025). The modular design also allows stacking of multiple cleavage sites to target diverse pathogens (Zhang et al., 2025). Virus resistance has been attained by modifying the host factors essential for viral replication and movement as well as by targeting the viral genomes directly (Zhan et al., 2024). The use of

CRISPR/Cas13 systems that act on RNA and not DNA has been especially successful in inhibiting RNA viruses that make up the largest group of plant pathogens. Studies have shown that these RNA-targeting methods can be applied to eliminate the viral genomes directly, providing a sustainable and wide-ranging approach to resistance (Lankireddy et al., 2026; Zhan et al., 2024). In addition, recent studies have confirmed the potential of certain types of CRISPR/Cas13 proteins, such as Cas13x, in controlling the infection of RNA viruses like Cucumber mosaic virus (CMV) in plants (Li et al., 2025).

Promoting sustainable agriculture

Genome editing assists agricultural sustainability in several ways, which enhance agricultural practices with decreased environmental impact but high agricultural productivity. Pesticide reduction is achieved by developing plants resistant to diseases or pests. For instance, the production of tomato plants that are both whitefly-resistant and commercially viable helps reduce the need for chemical pesticides (World Vegetable Centre, 2026), and metabolite-mediated resistance in wheat provides an option to using chemical insecticides (Borg et al., 2025). Every time chemical pesticides are reduced, less contamination of the environment is ensured as well as protection from harm caused to other living organisms and the prevention of resistance development among pathogens and pests to pesticides. Climate resilience engineering focuses on addressing the consequences of climate change affecting agricultural productivity (Mmbando, 2025). Drought tolerance improvement is carried out through genome editing of abscisic acid signalling and stomatal regulation genes (Decima Oneto et al., 2025; Aslam et al., 2026). Improvement in heat resistance involves editing heat shock factor and membrane stability genes (Chen et al., 2026). Editing of ion transporter and osmoprotectant biosynthesis genes has helped improve salinity tolerance, which becomes an increasing issue in irrigated agricultural settings (Namata et al., 2025; Fu et al., 2026). The advent of transgene-free gene editing tools, such as CRISPR ribonucleoproteins (RNP) delivery, ensures that consumers' fears regarding the introduction of foreign genes into crops are addressed. While traditional GMOs feature transgenes within their genetic makeup, RNP-edited crops do not carry any transgenes since RNPs, composed of Cas9 protein and guide RNAs, enter plant cells in a fully assembled form and are degraded quickly (Ramakrishnan et al., 2025; Norfaezah et al., 2025). Empirical research indicates that the use of transgene-free RNPs was proven effective in numerous plant species, from woody perennials like citrus and poplar (Rocha et al., 2026) to oil palm trees (Norfaezah et al., 2025).

Challenges facing plant genome editing

Technical challenges

The most critical technological challenge facing CRISPR/Cas9 is off-target editing. CRISPR/Cas9 systems tolerate mismatches between the guide RNA and target sequences, especially those distant from the PAM sequence, resulting in off-target genomic cleavages (Qi et al., 2026). The incidence of off-target events is influenced by factors such as species, Cas9 variant, guide RNA, and delivery method, thus requiring individual case-specific empirical validation. Various methods have been applied to lower the risks of off-target editing. High-fidelity Cas9 variants have been engineered through mutations that destabilize Cas9 binding to mismatched sequences, hence minimizing off-target events with minimal interference in on-target activities. The recently developed Sniper2L Cas9 variant is capable of producing very low off-target edits without major reductions in on-target activities (Kim et al., 2023), and has since been successfully deployed in a Cas9-embedding adenine base editor system to achieve highly specific A-to-G editing in rice (Hu et al., 2026). Computer modelling programs integrating genomic variations have been used to identify possible off-target sites and to select appropriate guide RNAs to optimize the specificity profile of the guide RNA. Machine learning techniques that integrate deep learning algorithms have made considerable improvements in off-target predictions, and these machine learning tools are expected to emerge as the dominant methods in predicting CRISPR on- and off-target activity (Alipanahi et al., 2025; Iksat et al., 2025). The limitations of current genome-editing delivery systems have hindered the application of this technology across diverse plant species. Agrobacterium-mediated transformation or particle bombardment has been identified as highly effective in various plant genera, and both these approaches usually lead to the integration of the transgene (Li et al., 2025). However, if transgenes need to be eliminated, then ribonucleoprotein (RNP) delivery is recommended; however, such methods are currently possible only in plant types that have protocols for regenerating their protoplasts (Blumberg et al., 2026; Ramakrishnan et al., 2025). This has become especially difficult for woody perennial crops and recalcitrant plants. Complicated cell walls restrict RNA entry into cells, while several species cannot be regenerated through protoplasts that undergo CRISPR editing (Ramakrishnan et al., 2025). Moreover, long generation times are another hindrance to delivering RNPs to trees; every trial for optimizing these conditions takes several years (Ramakrishnan et al., 2025). Regeneration recalcitrance hinders the types of plants and genotypes suitable for genome editing technologies. Although efficient delivery and editing can be accomplished, regeneration of edited cells and protoplasts into whole plants is genotype-specific (Achary et al., 2026). Regeneration is another challenge in plants since many commercially available crop varieties are recalcitrant, and

therefore crossing or backcrossing must be performed for transferring edited genes (Sohail et al., 2026). Low efficiency of homology-dependent repair (HDR) is another inherent drawback associated with precision editing in plants. Unlike animal cells, the predominant DNA repair mechanism in plant cells is error-prone NHEJ, while HDR is limited to the S and G_2 phases of the cell cycle (Huang & Puchta, 2019; Molla et al., 2022). Gene disruption using NHEJ is highly efficient (Movahedi et al., 2022); however, precise gene replacement using template DNA occurs less frequently than desirable for most applications (Haider & Mussolino, 2025).

Regulatory challenges

Regulatory regimes for genome-edited crops differ enormously around the world and cause obstacles for commercialization, international trade, and scientific collaborations. In the USA, a product-based regulatory regime of biotechnology is used. The regulation of genome-edited crops is mostly determined by the properties of the resulting product rather than the technology applied. In the USA, some genome-edited plants do not fall under GMO regulations because their modifications could be done using conventional breeding approaches or occurred spontaneously and would not lead to any additional plant pest risk compared to conventional crops (USDA-APHIS, 2025; Bickell, 2023). This is consistent with the USDA SECURE Rule, which pays more attention to the properties of the resulting organism but does not consider the approach taken during breeding (Barrangou, 2020; USDA-APHIS, 2025). The European Union has developed new legislation for New Genomic Techniques (NGTs) with the objective of fostering agricultural innovation while ensuring effective protection of human health and the environment (European Commission, 2023; Council of the European Union, 2025). What began as a negotiated proposal has since progressed to formal law: the Council adopted its position in April 2026 and the European Parliament gave final approval to the Regulation in June 2026. The Regulation is now entering into force, followed by a two-year implementation period during which the European Commission and Member States will develop the secondary legislation, equivalence-verification procedures, and administrative structures needed for the framework to become fully operational; it should therefore be understood as an adopted regulation still moving toward full implementation, rather than either a mere proposal or a fully operational regime. Under the adopted classification system, NGT plants under Category 1 are considered comparable to conventional plants and will undergo fewer regulations, whereas NGT plants under Category 2 remain subject to the existing GMO legislation, with risk assessments and other requirements such as authorization, traceability, and labelling procedures (European Commission, 2023; European Parliament Research Service, 2024). Such policies seek to strike a balance between encouraging innovation and maintaining safety oversight. Member states retain the ability to restrict or regulate the cultivation of Category 2 NGT plants. China has taken an intermediate position. According to guidelines published in January 2022, genome-edited crops will be grouped according to their risk levels, with an easy regulatory path for genome editing without using foreign genes and with no environmental or food safety concerns arising out of such editing (Jin et al., 2025). There are significant challenges posed by regulatory fragmentation. Varied regulatory requirements make compliance difficult and hinder market entry (Sohail et al., 2026).

Ethical and societal challenges

There is considerable variation between geographical areas as well as among different populations in terms of acceptability. There is relatively low awareness about genome editing, and the vocabulary used (for example, "gene editing" versus "genetic modification") has a large effect on perception (Huttunen et al., 2026; McFadden et al., 2024). This is a regulatory and commercial impediment because the lack of acceptability in certain geographical locations leads to process-oriented regulation. Together with conditional public acceptability (that prefers imports to local farming), it discourages investments.

Recent advances and emerging trends

AI-designed genome editors

One of the important developments in 2026 is the experimental validation, in plant cells, of an AI-designed genome-editing nuclease. OpenCRISPR-1, an AI-designed Cas9-like nuclease originally engineered using protein language models, was first described by Ruffolo et al. (2025); scientists at the ICAR-Central Rice Research Institute in India subsequently adapted and experimentally validated this AI-designed nuclease for use in plants, developing the plant-optimized variant Plant-OpenCRISPR-1 (POC1) and demonstrating, for the first time, that an AI-designed nuclease can function efficiently inside plant cells (Das et al., 2026). The developed platform is capable of producing editing efficiencies that are similar to the popular SpCas9 system on a few rice genes, along with successfully creating gene knockouts in transformed rice plants. Also, POC1 was used in developing other types of genome editing, such as base and prime editors (Das et al., 2026). As an open-source platform, OpenCRISPR-1 is expected to promote wider use of genome editing tools in research and agriculture as an alternative to SpCas9 (Ruffolo et al., 2025; Das et al., 2026). It is

important to distinguish this category of application-the de novo design of nuclease/editor proteins by AI-from the use of AI and machine-learning models to predict guide-RNA activity and on-/off-target effects for already-existing nucleases such as SpCas9 (*see Enabling Technologies*); the two represent different levels of AI integration into genome editing, ranging from decision-support tools that optimize the use of existing editors to the generative design of the editing proteins themselves.

Prime editing and dual base editing

Prime editing is an innovative genome-editing technique that can, in principle, generate any of the 12 possible base-to-base conversions, in addition to small insertions and deletions, without inducing double-strand breaks (DSBs); unlike conventional homology-directed repair (HDR)-based approaches, it does not depend on an exogenous donor DNA template, since the desired edit is encoded directly within the prime-editing guide RNA (pegRNA) and installed through a reverse-transcriptase-mediated 'search-and-replace' mechanism (Anzalone et al., 2019; Azameti & Dauda, 2021). Recent developments have considerably improved the efficiency of plant prime editing. Engineered prime editors like PE6c have exhibited substantial improvements in plant editing efficacy using transgenic rice plants, thus suggesting that prime editing is an ideal candidate for genome modification in monocot plants (Gallo et al., 2025). Dual base editors also form a remarkable advancement in this field. Studies involving the development of cytosine and adenine base editors, relying on single-stranded DNA-targeting *SCP1.201* deaminases, for conducting simultaneous C-to-T and A-to-G base conversion in specific gene loci have been successful. Such base editors have been used successfully in developing herbicide-tolerant *OsEPSPS* variants through the approach of directed evolution (Xu et al., 2026).

Precise DNA insertion and large-scale genome engineering

The discipline has advanced from minor sequence variations to major ones. Modern techniques of homology-directed gene targeting now allow efficient, scarless kilobase insertions, while the combination of nuclease-deficient Cas effectors with recombinases or transposases can facilitate the insertion of much larger sequences. Prime editing has also been expanded to make it possible to achieve inversions, substitutions, and deletions that cover hundreds of kilobases up to several megabases using DualPE systems (Zhao et al., 2025). Not too long ago, genome modification reached a further milestone by making chromosome fission and fusion possible, which shows that karyotype reorganization is feasible (Rönspies et al., 2025). Such breakthroughs create new avenues for improving crops, ranging from the creation of reproductive barriers, simulation of evolutionary processes, to stacking traits in Plant Artificial Chromosomes. Among the most important approaches for efficient DNA integration are PrimeRoot for integrating DNA segments up to 11.1 kb in rice and maize (Sun et al., 2024), as well as transposase-enabled target-site integration (TATSI) systems (Liu et al., 2024).

Enabling technologies

Multiple applications of machine learning are observed in genome editing. Machine learning algorithms predict on-target and off-target editing efficiencies of guide RNAs, allowing computer optimization prior to actual experiments (Das et al., 2026). By contrast, the design of AI-generated nucleases such as OpenCRISPR-1/POC1 represents a distinct and more advanced application of AI in genome editing, in which machine-learning models generate entirely new nuclease protein sequences rather than simply guiding the use of existing ones; this class of AI-designed editor holds considerable promise for diversifying and expanding the genome-editing toolbox, beyond the prediction-based applications described above (Das et al., 2026). The high-throughput phenotyping method accelerates plant population editing. Phenotyping through imaging, spectroscopy, and computer vision technologies allows for fast analysis of edited plants and selection of valuable individuals from the pool of edited plants (Mansoor et al., 2025; Kamran et al., 2026). Better approaches to regeneration allow for more plant species and varieties to be edited. The discovery of regulators responsible for regeneration makes it possible to regenerate recalcitrant genotypes and species (Sohail et al., 2026).

Conclusion

The advent of genome editing techniques has revolutionized the study and breeding of plants. Genome editing provides unique opportunities to deal with pressing issues associated with agriculture, such as improving the productivity and nutritional value of crops, creating disease-tolerant and climate-resilient varieties, and pursuing sustainable approaches that minimize negative effects on the environment. However, obstacles related to the use of genome editing technologies persist. Issues such as off-target effects, inefficient delivery methods, and difficulties with regeneration represent some of the most pressing technological barriers to the use of the technology. Moreover, the presence of different regulatory regimes and associated legal concerns present obstacles in terms of market access and distribution. Advances in

technology, including AI-assisted design of nucleases, prime editing, dual base editing systems, and precise DNA insertion technologies resolve many current problems. The introduction of two-tier NGT regulation in the European Union and the Precision Breeding framework in the UK show signs of movement towards more science-based, risk-proportionate regulatory regimes. The introduction of open-source AI-assisted platforms for genome editing, such as POC1, marks another important step forward in the democratization of the use of the technology (Das et al., 2026). With continued technical innovation, regulatory harmonization, and meaningful public engagement, genome editing can fulfil its promise as a transformative tool for sustainable agriculture and global food security.

Acknowledgement

The author is grateful to the management of the University of Technology and Applied Sciences, Navrongo, Ghana, for the provision of the necessary facilities to carry out this review.

Author contributions

Mawuli K. Azameti: The author conceived the idea and wrote the manuscript.

Competing interest

The author declares that there are no competing interests.

Ethics approval

Not applicable.

AI Tool Usage Declaration

The author declares that no generative AI tools were used to write this manuscript. Grammarly was employed solely for the purpose of grammar and spell-checking to improve readability.

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