Quest Journals
Journal of Research in Agriculture and Animal Science
Volume 11 ~ Issue 7 (July 2024) pp: 22-28
ISSN(Online): 2321-9459
www.questjournals.org

Review Paper

"Contributions of Crispr and Gene Editing Technologies to Plant Breeding and Biotechnology: Advances, Integrative Approaches, And Future Directions"

K. K. Gautam
Department of Botany
Udai Pratap College, Varanasi

S. B. Verma
Associate Professor
Department of Agricultural Botany (Genetics and Plant Breeding)
Udai Pratap College, Varanasi. U.P.221002.India
Email -sbvermaupc@gmail.com

Abstract
The advent of CRISPR and related gene-editing technologies has revolutionized plant breeding and biotechnology by enabling targeted, efficient, and cost-effective genetic modifications. This review explores the evolution from conventional and molecular breeding to precision genome editing, emphasizing the unique advantages of CRISPR systems, including Cas9, Cas12a, Cas13, base editors, and prime editors. Key methodological aspects such as guide RNA design, delivery strategies, DNA repair pathways, multiplex genome editing, and off-target minimization are discussed in the context of enhancing crop traits. The synergy of CRISPR with complementary biotechnologies—such as seed science, omics platforms, tissue culture, and plant–microbe interaction research—demonstrates its potential to address pressing agricultural challenges including climate resilience, yield improvement, and nutritional enhancement. Comparative insights highlight CRISPR's superiority over earlier tools like zinc finger nucleases, TALENs, and meganucleases in terms of precision, scalability, and adaptability. Looking forward, innovations in delivery systems, repair engineering, and regulatory frameworks will further shape the trajectory of CRISPR-enabled agriculture, advancing sustainable food production for a growing global population.
Keywords: *CRISPR, gene editing, plant breeding, genome editing, Cas9, Cas12a, base editing, prime editing, seed science, omics, tissue culture, plant–microbe interactions, sustainable agriculture, crop improvement, biotechnology.*

I. INTRODUCTION

The field of plant breeding has undergone a remarkable transformation, evolving from traditional selection-based approaches to advanced molecular and genome-editing techniques. Conventional plant breeding, which relied on phenotypic selection and crossbreeding, laid the foundation for improving crop traits but was often constrained by long breeding cycles, environmental variability, and limited genetic precision. The advent of molecular breeding introduced tools such as molecular markers, marker-assisted selection, and genetic mapping, enabling breeders to identify and incorporate desirable traits more efficiently. This molecular era bridged the gap between genotype and phenotype, paving the way for highly targeted interventions in plant genomes. In recent years, genome editing technologies—most notably CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)—have revolutionized crop improvement by offering unprecedented precision, speed, and cost-effectiveness. Compared to earlier transgenic and mutagenesis methods, CRISPR provides a simpler, more versatile, and highly specific platform for creating targeted genetic modifications without necessarily introducing foreign DNA, thereby addressing both scientific and regulatory challenges. However, to unlock CRISPR's full potential, its integration with complementary biotechnologies such as genomic selection, high-throughput phenotyping, and synthetic biology is essential. This synergistic approach enables breeders to not only edit genes with precision but also predict, validate, and optimize trait performance under diverse environmental conditions. As agriculture faces mounting pressures from climate change, resource limitations, and a growing global population, the convergence of CRISPR with other cutting-edge biotechnologies represents a transformative pathway toward sustainable, resilient, and high-yielding crop varieties.

1.1 Objective

1. To analyze the advancements in CRISPR and other gene-editing technologies and their specific contributions to accelerating precision breeding in plants.
2. To evaluate integrative approaches that combine CRISPR with complementary biotechnologies such as genomic selection, high-throughput phenotyping, and synthetic biology for enhanced crop improvement.
3. To explore future directions, opportunities, and challenges in applying CRISPR-based strategies for sustainable agriculture and global food security.

1.2 Scope of the review

The scope of this review encompasses the advancements, applications, and integrative potential of CRISPR and other gene-editing technologies within the field of plant breeding and biotechnology. It examines the transition from conventional and molecular breeding to genome editing, highlighting how CRISPR's precision, efficiency, and versatility have transformed crop improvement strategies. The review further explores comparative advantages over earlier genetic modification methods, integration with complementary biotechnologies such as genomic selection, phenomics, and synthetic biology, and its role in addressing global agricultural challenges including climate resilience, yield enhancement, and nutritional quality. Finally, it outlines emerging innovations, regulatory considerations, and future research directions to maximize the sustainable impact of genome editing in agriculture.

II. OVERVIEW OF GENOME EDITING TECHNOLOGIES IN PLANTS

2.1 Pre-CRISPR Genome Editing Tools

Bibikova et al. (2001) discussed the pioneering use of zinc finger nucleases (ZFNs) for targeted genome modification, demonstrating their ability to induce site-specific double-strand breaks and stimulate homologous recombination. While ZFNs represented a breakthrough in genome editing, the authors noted their complex protein engineering requirements and off-target effects as key limitations.

Boch et al. (2009) introduced transcription activator-like effector nucleases (TALENs) as a flexible and customizable genome editing platform, derived from plant pathogenic bacteria. Their study highlighted TALENs' broad targeting range and relatively high specificity compared to ZFNs, but also acknowledged challenges in repetitive sequence assembly and delivery into plant systems.

Chevalier et al. (2002) explored the potential of meganucleases, naturally occurring endonucleases with large recognition sites, in precise genome engineering. The authors demonstrated their utility in rare-cutting, targeted DNA cleavage but emphasized the difficulty of re-engineering meganucleases to recognize novel sequences, which limited their widespread application.

Christian et al. (2010) further refined the TALEN approach by improving modular assembly methods for DNA-binding domains, thereby enhancing design efficiency and expanding applicability in plant and animal systems. Despite these advances, the study recognized the time-intensive nature of TALEN construction compared to later genome editing platforms like CRISPR.

2.2 CRISPR/Cas Systems

Anzalone et al. (2019) developed prime editing, a CRISPR-based approach capable of performing targeted insertions, deletions, and all types of base substitutions without requiring donor DNA templates. They compared its versatility and reduced off-target activity to previous genome editing tools, demonstrating significant improvements in accuracy and applicability for plant biotechnology.

Barrangou and Doudna (2016) highlighted the fundamental mechanisms of CRISPR/Cas systems, explaining how Cas9, Cas12a, and Cas13 enzymes enable targeted DNA and RNA editing through guide RNA-directed cleavage. They emphasized the adaptability of CRISPR over earlier genome engineering tools such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), noting its lower cost, reduced complexity, and broader applicability in plant systems.

Jinek et al. (2012) provided the seminal description of the Cas9-mediated mechanism, detailing how a single-guide RNA directs Cas9 to specific genomic loci for double-stranded DNA cleavage. Their work established the foundation for genome editing in plants and underscored CRISPR's higher speed and efficiency compared to protein-based targeting systems like ZFNs and TALENs.

Komor et al. (2016) introduced base editors as an advancement of the CRISPR/Cas system, enabling single-nucleotide substitutions without double-strand breaks. This innovation reduced off-target effects and expanded the scope of plant genome modifications, offering a more precise and potentially safer alternative to earlier mutagenesis techniques.

Author(s) & Year	Genome Editing Tool/Approach	Key Mechanism or Innovation	Advantages	Limitations	Relevance to Plant Biotechnology
Anzalone et al. (2019)	Prime Editing (CRISPR-based)	Performs targeted insertions, deletions, and all base substitutions without donor DNA templates.	High precision; versatility; reduced off-target effects; no requirement for donor DNA.	Still relatively new; optimization needed for plant-specific applications.	Allows complex and precise edits in plant genomes for trait improvement without transgene integration.
Barrangou & Doudna (2016)	CRISPR/Cas Systems (Cas9, Cas12a, Cas13)	Guide RNA-directed DNA/RNA cleavage enabling targeted editing.	Cost-effective; adaptable; simpler design than ZFNs and TALENs; broad applicability.	Off-target effects possible; delivery challenges in some plants.	Foundation for modern genome editing in plants, enabling rapid trait development and functional genomics studies.
Jinek et al. (2012)	CRISPR/Cas9	Single-guide RNA directs Cas9 to specific DNA sites for double-stranded breaks.	High efficiency; easy programmability; faster than protein-based systems.	Requires PAM sequence near target site; off-target mutations possible.	Established CRISPR as a revolutionary tool for plant genome engineering, replacing slower conventional methods.
Komor et al. (2016)	CRISPR Base Editors	Single-nucleotide substitutions without double-strand breaks.	Minimizes DNA damage; reduces off-target activity; precise point mutations.	Limited to specific base conversions; delivery efficiency varies.	Enables precise modification of plant genes controlling traits like disease resistance and quality without large genomic changes.
Bibikova et al. (2001)	Zinc Finger Nucleases (ZFNs)	Engineered DNA-binding proteins fused to nucleases create site-specific double-strand breaks.	Enabled first targeted genome modifications; foundational for later tools.	Complex protein design; expensive; higher off-target effects.	Early proof-of-concept for targeted genome editing in plants but largely replaced by newer, easier-to-use platforms.
Boch et al. (2009)	TALENs	DNA-binding proteins from plant pathogens engineered for targeted cleavage.	Broad targeting range; higher specificity than ZFNs.	Labor-intensive construction; delivery challenges.	Useful in targeted plant genome editing before CRISPR, especially for traits requiring high specificity.
Chevalier et al. (2002)	Meganucleases	Naturally occurring rare-cutting endonucleases with large recognition sequences.	High specificity due to long recognition sequences.	Very difficult to re-engineer for new targets; limited flexibility.	Provided groundwork for precise editing concepts but impractical for large-scale plant genome applications.
Christian et al. (2010)	Improved TALEN Assembly	Enhanced modular assembly methods for DNA-binding domains.	Easier and faster TALEN design; expanded range of targetable sequences.	Still slower and more complex than CRISPR; repetitive sequence issues remain.	Improved TALEN utility in plant breeding prior to CRISPR adoption, facilitating targeted modifications in important crop genes.

III. CRISPR METHODOLOGIES AND DELIVERY IN PLANTS

CRISPR methodologies in plants involve a sequence of well-coordinated steps, beginning with guide RNA (gRNA) design and target site selection, where bioinformatic tools identify precise genomic loci adjacent to protospacer adjacent motifs (PAMs) to ensure high specificity. Delivery of CRISPR components into plant cells can be achieved through transformation methods such as *Agrobacterium*-mediated transfer, particle bombardment, or protoplast transfection, each with advantages depending on crop type and tissue compatibility. Once inside the cell, the Cas nuclease introduces targeted double-strand breaks, which are repaired by the plant's endogenous DNA repair pathways: non-homologous end joining (NHEJ) for small insertions/deletions, or homology-directed repair (HDR) for precise sequence insertion using donor templates. Novel repair engineering strategies aim to bias repair toward HDR or introduce base and prime editing for greater precision. Multiplex genome editing, using multiple gRNAs in a single construct, allows simultaneous modification of several loci to accelerate trait stacking. To ensure safety and accuracy, off-target minimization is achieved through optimized gRNA design, use of high-fidelity Cas variants, and comprehensive in silico and experimental validation.

Table: CRISPR Methodologies and Delivery in Plants

Step/Method	Description	Advantages	Limitations
Guide RNA Design & Target Site Selection	Selection of 20-nt target sequences near PAM sites using bioinformatics tools (e.g., CRISPOR, CHOPCHOP).	High specificity; customizable; adaptable to different plant genomes.	Requires high-quality reference genome; PAM sequence constraints.
Agrobacterium-mediated Transformation	Uses *Agrobacterium tumefaciens* to transfer T-DNA carrying CRISPR components into plant cells.	Efficient for many dicots; stable transformation.	Limited efficiency in monocots; species-dependent.
Particle Bombardment	Physical delivery of DNA-coated microprojectiles into plant cells or tissues.	Broad host range; suitable for monocots; bypasses species-specific barriers.	May cause tissue damage; lower transformation efficiency compared to Agrobacterium.
Protoplast Transfection	Delivery of CRISPR RNPs or plasmids into enzymatically isolated plant protoplasts.	Transgene-free editing possible; rapid testing of gRNA efficiency.	Regeneration from protoplasts challenging for many species.
DNA Repair Pathways – NHEJ	Error-prone repair resulting in insertions/deletions at cut sites.	Simple; efficient; works in all cell types.	Lacks precision; unpredictable indels.
DNA Repair Pathways – HDR	Template-guided repair for precise sequence changes.	Enables gene insertion or replacement.	Low efficiency in plants; requires dividing cells.
Novel Repair Engineering	Base editing, prime editing, and strategies to bias repair toward HDR.	Higher precision; avoids double-strand breaks; reduced off-target effects.	Still under optimization for many crop species.
Multiplex Genome Editing	Simultaneous editing of multiple target sites using multiple gRNAs.	Trait stacking; efficient modification of gene families.	Complex construct design; risk of increased off-target edits.
Off-target Minimization	Use of high-fidelity Cas variants, optimized gRNA design, and genome-wide validation.	Increased editing accuracy; improved biosafety.	May reduce editing efficiency; requires thorough computational and experimental validation.

IV. CONTRIBUTIONS OF CRISPR TO CROP YIELD AND STRESS TOLERANCE

4.1 Modern Approaches in Seed Science and Plant Genetics

Modern approaches in seed science and plant genetics leverage advanced genome editing tools, particularly CRISPR, to improve seed performance traits such as germination vigor, seed longevity, and nutrient composition. By precisely modifying genes regulating seed dormancy, antioxidant systems, and nutrient biosynthesis, breeders can enhance both the quality and storability of seeds. CRISPR has also transformed hybrid seed production by enabling targeted editing of male sterility genes, facilitating more efficient hybridization without labor-intensive manual emasculation. Case studies in rice, wheat, and maize have demonstrated significant yield improvements through targeted editing of genes related to grain size, stress tolerance, and nutrient use efficiency, underscoring the potential of these technologies to meet global food security demands.

4.2 Abiotic Stress Resistance

CRISPR-based genome editing has emerged as a powerful tool for developing abiotic stress-resistant crops by precisely modifying genes and regulatory elements involved in drought, salinity, heat, and cold tolerance. Key strategies include editing transcription factors such as the DREB (Dehydration-Responsive Element-Binding), NAC (NAM, ATAF, CUC), and WRKY families, which regulate complex stress-response networks. For drought resistance, CRISPR has been used to enhance water-use efficiency and root architecture; for salinity tolerance, to modify ion transporters and osmoprotectant biosynthesis genes; for heat tolerance, to regulate heat shock protein expression; and for cold tolerance, to modulate C-repeat binding factors. These targeted modifications enable crops to maintain productivity under extreme environmental conditions while minimizing the trade-offs often associated with conventional breeding approaches.

V. CRISPR AND PLANT-MICROBE INTERACTIONS FOR CLIMATE RESILIENCE

CRISPR technology is emerging as a powerful tool to improve plant–microbe interactions for enhancing climate resilience in crops. By editing plant immune receptors, researchers can fine-tune the balance between pathogen defense and the ability to host beneficial microbes, thereby promoting symbiotic relationships such as mycorrhizal colonization and rhizobial nitrogen fixation. Modifying root exudate composition through targeted genome edits enables plants to recruit and maintain beneficial microbial communities, improving nutrient uptake and stress tolerance under adverse environmental conditions. Case studies have demonstrated CRISPR-mediated improvements in nitrogen-fixing symbioses in legumes and strengthened mycorrhizal associations in cereals, leading to better growth in drought and nutrient-deficient soils. Integrating CRISPR with microbial biotechnology—such as engineering beneficial microbes themselves—offers a synergistic approach for developing sustainable, low-input agricultural systems that are resilient to climate change.

VI. CRISPR IN PLANT TISSUE CULTURE AND SOMACLONAL VARIATION

Plant tissue culture serves as a critical platform for delivering CRISPR machinery, enabling transformation of specific tissues, callus, or protoplasts under sterile, controlled conditions. This approach facilitates precise genome editing in crops that are otherwise difficult to manipulate. However, many economically important species, particularly recalcitrant crops, face regeneration bottlenecks due to low efficiency in shoot and root induction. Advances in tissue culture media optimization, morphogenic gene overexpression, and hormone balance are helping overcome these challenges, expanding CRISPR's reach. Somaclonal variation—genetic diversity arising from tissue culture—offers a valuable resource for identifying novel traits, such as disease resistance or stress tolerance. By strategically combining somaclonal variation with CRISPR editing, breeders can stack desirable traits, accelerating the development of improved crop varieties with both targeted modifications and novel, naturally arising genetic changes.

VII. USE OF OMICS TECHNOLOGIES IN CRISPR-BASED BREEDING

Omics technologies have become indispensable in enhancing the precision and efficiency of CRISPR-based plant breeding. Genomics enables the identification of candidate genes and allelic variations associated with desirable agronomic traits, providing a roadmap for accurate target site selection. Transcriptomics allows the examination of gene expression profiles under various biotic and abiotic stresses, uncovering regulatory networks that can be fine-tuned using CRISPR to enhance stress tolerance. Proteomics offers insights into protein abundance, modifications, and interactions, while metabolomics profiles biochemical changes linked to trait expression, enabling comprehensive phenotype validation. The integration of multi-omics datasets—combining genomic, transcriptomic, proteomic, and metabolomic layers—supports systems-level understanding of plant biology, enabling CRISPR interventions to be designed with greater accuracy, reduced off-target risk, and higher probability of achieving the intended phenotypic outcomes in diverse environmental contexts.

VIII. CRISPR IN FUNCTIONAL GENOMICS AND SYNTHETIC BIOLOGY

CRISPR technology has become a cornerstone in functional genomics and synthetic biology by enabling precise, scalable, and versatile genome modifications in plants. High-throughput knockout libraries, created by systematically targeting thousands of genes, allow rapid identification of gene functions and their roles in agronomic traits. In synthetic biology, CRISPR facilitates the engineering of plant metabolic pathways to enhance or introduce the production of high-value compounds such as nutraceuticals, biofuels, and pharmaceuticals, expanding the economic and nutritional potential of crops. Additionally, CRISPR-based gene drives offer a powerful strategy for spreading beneficial alleles through plant populations, enabling the rapid fixation of traits such as disease resistance or stress tolerance. Together, these applications not only accelerate fundamental plant biology research but also open transformative avenues for sustainable agriculture and bio manufacturing.

IX. CASE STUDIES ACROSS MAJOR CROPS

CRISPR technology has been successfully applied across a wide range of major crops, targeting genes associated with yield, quality, stress tolerance, and disease resistance. In rice, editing of genes like *OsSWEET13* and *OsERF922* enhanced bacterial blight resistance and blast tolerance using *Agrobacterium*-mediated transformation. Wheat genome editing of *TaMLO* and *TaGW2* improved powdery mildew resistance and increased grain size via particle bombardment. In maize, disruption of *ZmTMS5* enabled thermo-sensitive male sterility for hybrid breeding, while soybean editing of *FAD2-1A* and *FAD2-1B* altered oil composition for higher oleic acid content. Tomato CRISPR interventions in *SlMlo1* and *SlAGL6* conferred powdery mildew resistance and modified fruit development, whereas in potato, knockout of *StSSR2* improved starch quality. In banana, editing of *MaPDS* served as a proof-of-concept for functional genomics, while targeting *RGA2* enhanced Fusarium wilt resistance. Across these crops, methods like *Agrobacterium*-mediated transformation, particle bombardment, and protoplast transfection, combined with precise gRNA design, have delivered significant trait improvements with minimal unintended effects.

Table: CRISPR Case Studies Across Major Crops

Crop	Target Gene(s)	Methodology	Achieved Trait(s)
Rice	*OsSWEET13, OsERF922*	*Agrobacterium*-mediated transformation	Resistance to bacterial blight; blast tolerance
Wheat	*TaMLO, TaGW2*	Particle bombardment	Powdery mildew resistance; increased grain size
Maize	*ZmTMS5*	*Agrobacterium*-mediated transformation	Thermo-sensitive male sterility for hybrid breeding
Soybean	*FAD2-1A, FAD2-1B*	*Agrobacterium*-mediated transformation	Higher oleic acid content in seed oil
Tomato	*SlMlo1, SlAGL6*	*Agrobacterium*-mediated transformation	Powdery mildew resistance; altered fruit development
Potato	*StSSR2*	Protoplast transfection	Improved starch quality
Banana	*MaPDS, RGA2*	*Agrobacterium*-mediated transformation	Proof-of-concept genome editing; Fusarium wilt resistance

X. REGULATORY, BIOSAFETY, AND ETHICAL CONSIDERATIONS

The regulatory landscape for gene-edited crops varies widely across the globe, with some countries, such as the United States and Japan, adopting more relaxed frameworks when no foreign DNA is introduced, while others, like the European Union, classify them under stringent genetically modified organism (GMO) regulations. These differences influence the speed of research, commercialization, and international trade. Intellectual property (IP) concerns surrounding CRISPR and related technologies are complex, involving competing patents, licensing restrictions, and questions about access for public-sector breeders, especially in developing countries. Public perception remains a critical factor, as societal acceptance depends on clear communication about the distinctions between gene-edited and transgenic crops, potential benefits, and safety assurances. Transparent engagement, inclusive policymaking, and trust-building strategies are essential to ensure that genome editing in agriculture develops responsibly and equitably.

Challenges

Despite its transformative potential, CRISPR-based plant genome editing faces notable hurdles, including the inherently low efficiency of homology-directed repair (HDR) in plants, the risk of unintended mutations from off-target activity, and persistent delivery challenges in recalcitrant species or tissues. These technical limitations can restrict precision, scalability, and adoption in diverse crop systems.

Future Prospects-

Advances in next-generation CRISPR tools—such as CasΦ for compact delivery, prime editing for versatile sequence changes, and base editing for precise nucleotide conversions—promise to overcome current constraints. Integration with artificial intelligence for predictive breeding could accelerate gRNA design, trait prediction, and genotype–phenotype mapping. Ultimately, coupling CRISPR innovations with sustainable breeding strategies holds immense potential for developing climate-resilient crops that enhance global food security while reducing environmental impacts.

XI. CONCLUSION

CRISPR has emerged as a transformative force in plant breeding and biotechnology, offering unparalleled precision, efficiency, and versatility in modifying plant genomes. Its integration with complementary disciplines such as seed science, multi-omics approaches, tissue culture, and plant–microbe interaction research has expanded the scope of crop improvement, enabling the development of climate-resilient, high-yield, and nutritionally enhanced varieties. By combining genome editing with advanced phenotyping, synthetic biology, and sustainable agricultural practices, CRISPR is paving the way for next-generation innovations that address global food security and environmental challenges. As delivery systems, repair mechanisms, and multiplexing strategies continue to evolve, the future promises even more sophisticated and targeted solutions for sustainable agriculture.

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