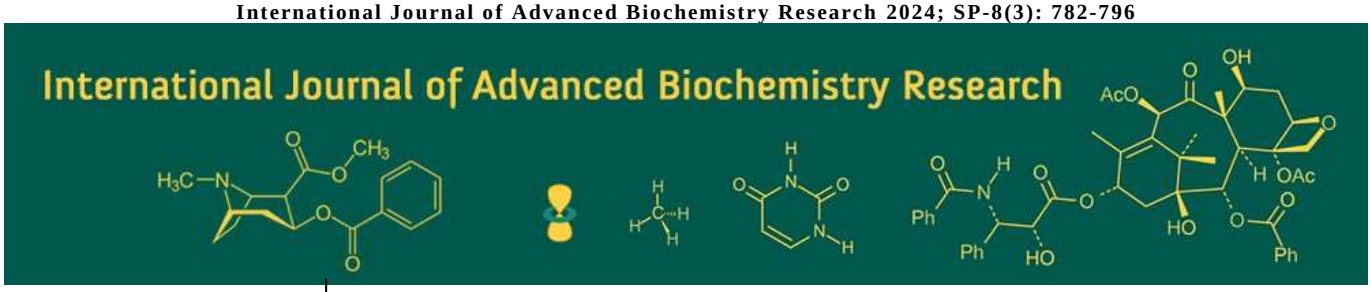

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Viral delivery of CRISPR/Cas9 genome editing for rapid crop improvement: A promising approach to enhance crop resilience against biotic and abiotic stresses

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Abstract
Agricultural production faces challenges from disease, pests, and environmental stresses, necessitating enhanced crop cultivars. Traditional transformation techniques are limited, leading to few cultivars for genetic manipulation. To overcome this, we explore viral delivery of CRISPR/Cas9 for precise plant genome modifications. This method utilizes optimized viruses to deliver CRISPR/Cas9 constructs, enabling targeted changes. By spanning the virus host range, traditional transformation challenges are circumvented. We review virus types used for delivery, recent outcomes, and future potential. Successful cases in various crops show improved disease resistance, insect tolerance, and stress resilience. The technology can target multiple traits simultaneously, streamlining crop improvement. Challenges like off-target effects and regulations need addressing for broader adoption. Viral delivery of CRISPR/Cas9 holds promise for accelerating crop breeding, enhancing resilience, and contributing to global food security and sustainable agriculture.

Keywords: Viral delivery, CRISPR/Cas9, crop improvement, crop resilience, plant stresses

Introduction

Agricultural productivity is increasingly threatened by biotic and abiotic stresses, including disease, pests, and environmental challenges such as drought and heat stress (Fahad *et al*., 2017; Teshome *et al*., 2020) [38, 105]. These stressors can lead to substantial yield losses and pose significant challenges to global food security (Raza & Bebber, 2022; Rivero *et al*., 2022) [91, 92]. To address these issues, there is a pressing need to develop crop cultivars with enhanced resistance or tolerance to these stress factors. Genetic engineering technologies, such as CRISPR/Cas-based genome editing, offer promising avenues for crop improvement by enabling precise modifications to the plant genome (Hsu *et al*., 2014) [51]; Sami *et al*., 2021) [93]. Traditional genetic transformation methods have been valuable for developing genetically modified crops; however, they are not universally optimized for all plant species, limiting their application to specific cultivars (Altpeter *et al*., 2016) [9]; (Eckerstorfer *et al*., 2019) [37]. Moreover, some preferred cultivars may not be amenable to genetic transformation, leading to a slow and laborious introgression process through backcrossing to transfer desired traits (H. Chen *et al*., 2014) [25]. These challenges hinder the rapid development of stress-tolerant crops, necessitating exploring alternative and efficient strategies.

Genetic engineering technologies have significantly advanced over the years, enabling scientists to explore new avenues for crop improvement. One such approach is using CRISPR/Cas9-mediated viral delivery for genome editing in plants, which has shown great promise in enhancing crop resilience against biotic and abiotic stresses (Z. Ali, Abulfaraj, *et al*., 2015a; Wagh & Manoj Baliram Pohare, 2019; Wagh *et al*., 2021) [5, 102]. In recent years, the revolutionary CRISPR/Cas9 genome editing technology has opened new avenues for precise and efficient genetic modifications. To overcome the limitations of traditional methods, researchers have explored viral delivery to introduce CRISPR/Cas9 components into plant cells (Uddin *et al*., 2020) [108].

Utilizing viral vectors allows for targeted and specific genome editing in various crop species. The viral delivery of CRISPR/Cas9 offers a rapid and versatile crop improvement approach that has shown great promise in revolutionizing agriculture. The viral delivery of CRISPR/Cas9 components to model plant species like N. benthamiana has proven to be a valuable tool for system characterization and generation of inoculum for other less amenable plants (Fig. 1A) (Gentzel *et al*., 2022) [43].

The versatility of viral CRISPR/Cas9 delivery to plants allows for targeted studies, facilitating the manipulation of plant and pest genes to increase resistance to biotic and abiotic stresses (Fig. 1B) (Borrelli *et al*., 2018) [20]. Various viruses have been explored as vectors for CRISPR/Cas9 delivery in plants, including potato virus X (PVX) (Ariga *et al*., 2020) [11], tobacco rattle virus (TRV) (C. Zhang *et al*., 2022), foxtail mosaic virus (FoMV) (Brewer *et al*., 2018) [21], and beet necrotic yellow vein virus (BNYVV) ((Jiang *et al*., 2019) [57]. In recent years, viral delivery of CRISPR/Cas-based genome editing technology has emerged as a transformative approach to address these limitations (Montecillo *et al*., 2020) [78]. Viral vectors can efficiently deliver CRISPR/Cas9 constructs to plant cells, enabling targeted and precise modifications to the plant genome (Laforest & Nadakuduti, 2022) [66]. This novel method holds the potential to overcome the restrictions associated with traditional transformation and breeding techniques, as it allows the delivery of genome editing constructs across the entire host range of the virus used (C. Gao, 2021) [41] Consequently, viral delivery of CRISPR/Cas9 offers a broader and more versatile platform for crop improvement. The effectiveness of viral CRISPR/Cas9 delivery has been demonstrated in various crop species, showcasing improved disease resistance, insect tolerance, and abiotic stress tolerance. By harnessing the power of this technology, multiple traits can be simultaneously targeted, streamlining the crop improvement process and expediting the development of stress-resilient crops. This review paper provides an overview of the different types of viruses optimized for CRISPR/Cas9 delivery and examines their application in crop improvement. Furthermore, we highlight the phenotypic outcomes achieved in recent studies and discuss the future potential of viral CRISPR/Cas9 delivery in advancing agriculture. (Table no. 2) We also address the challenges and considerations of this technology, such as off-target effects and regulatory implications, to facilitate its responsible and effective deployment in crop breeding.

Advancements in CRISPR technologies have further expanded the toolbox for plant genome editing and transcriptional regulation (Lowder *et al*., 2015) [75]; (Wada *et al*., 2022) [113]; (Nambiar *et al*., 2022) [80]. For example, the Cas13a protein has been incorporated, allowing for precise epigenome editing in plants (Dreissig *et al*., 2017) [36]. Additionally, the use of RNA viruses as delivery systems for CRISPR/Cas9 components has demonstrated great potential in achieving heritable gene editing in wheat, maize, and other crops (Uranga *et al*., 2023) [109]; (Beernink *et al*., 2022) [14]; (Begum *et al*., 2019) [15]. This viral delivery system for CRISPR/Cas9 has also been harnessed for targeted gene activation (K. Chen *et al*., 2019; Sasse *et al*., 2019) [94] and targeted DNA demethylation (Ghoshal *et al*., 2020) [44], further expanding its applications in plant research. Moreover, simultaneous gene expression and multi-gene silencing have been achieved in Zea mays using the maize dwarf mosaic virus (Xie *et al*., 2021) [120].

Overall, the combination of CRISPR/Cas9 with viral delivery holds immense potential for rapid and efficient crop improvement, offering a promising approach to enhance crop resilience against both biotic and abiotic stresses (Montecillo *et al*., 2020) [78]; (Ahmad *et al*., 2021) [3]. As more knowledge is gained and techniques are refined, this approach may play a crucial role in ensuring food security and sustainable agriculture in the face of various challenges (Naqvi *et al*., 2022) [81] (Shelake *et al*., 2019) [106].

Gene editing technologies such as CRISPR/Cas9 have emerged as powerful tools for crop improvement. In the context of sugarcane, researchers have explored the use of CRISPR/Cas9 for various applications, including disease resistance, stress tolerance, and yield improvement. Here, I will summarize some of the critical studies related to gene editing in sugarcane. This manuscript aims to comprehensively review the viral delivery of CRISPR/Cas9 genome editing technology in crop improvement. We aim to explore the various types of viruses used for delivery, the mechanisms involved, and the phenotypic outcomes observed in recent studies. Additionally, we will discuss the implications of this technology for crop improvement, food security, and sustainable agriculture. Furthermore, the manuscript will highlight the future potential of viral CRISPR/Cas9 technology (Table no.2) and the challenges that need to be addressed to harness its full benefits in crop enhancement. Through this Review, we hope to emphasize the significance of viral CRISPR/Cas9 delivery as a transformative approach for addressing agricultural challenges and ensuring global food security.

Viral Delivery of CRISPR/Cas9 Genome Editing

By harnessing the natural capabilities of viruses, scientists can package and deliver the CRISPR/Cas9 machinery into target cells with remarkable precision (Xu *et al*., 2019) [132]. This process allows for efficient and targeted gene editing, offering immense potential in treating genetic diseases and developing resilient crops (Zaidi *et al*., 2016) [138]. The viral vectors act as delivery vehicles, transporting the CRISPR/Cas9 components to the desired cells, where the Cas9 enzyme can make precise cuts in the DNA, enabling gene insertion, deletion, or modification. Although viral delivery of CRISPR/Cas9 genome editing is a cutting-edge and promising approach that has revolutionized the field of genetic modification (Abdelnour *et al*., 2021) [1], the technology is still in its early stages. It faces immune response and off-target effects (Bhattacharjee *et al*., 2022) [18]. Ongoing research advances viral delivery systems for safer, more effective genome editing, promising transformative medical and agricultural applications. This Review explores virus types, mechanisms, and recent outcomes, highlighting efforts to improve safety and efficacy (Table no.1.).

Mechanisms of viral delivery of genomic editing constructs

The viral delivery of genomic editing constructs involves several vital mechanisms that enable the efficient transfer of CRISPR/Cas9 components or other gene editing tools into

target cells. These mechanisms are essential for successful gene editing and manipulation. Here are the main steps involved in viral delivery:

Cellular Attachment: The first step in viral delivery is the attachment of the virus to the target cells. Viruses are equipped with specific surface proteins that can recognize and bind to receptors on the surface of the host cells. This binding is particular to certain cell types, allowing targeted delivery.

Cellular Attachment: The initial step in viral delivery entails the attachment of the virus to target cells. Viruses have distinct surface proteins capable of recognizing and binding to receptors on host cell surfaces. This binding exhibits remarkable specificity towards particular cell types, enabling precision-oriented delivery (Maginnis, 2018) [76].

Cell Entry and Endocytosis: Following attachment to the cell surface, the virus gains entry into the target cell. Diverse viruses employ distinct entry mechanisms. Some viruses employ receptor-mediated endocytosis, wherein the cell envelops the virus by forming a vesicle around it (Dimitrov, 2004) [33]. Viral Uncoating: After endocytosis, the viral coat is shed (uncoating), releasing the viral genetic material into the target cell's cytoplasm. In certain instances, the entire virus enters the cell, while in others, solely the viral genome is transported (Yamauchi & Greber, 2016) [134].

Nuclear Entry: For viruses delivering gene-editing tools like CRISPR/Cas9, the viral genome or its components must infiltrate the cell nucleus, where the host DNA is localized. This constitutes a pivotal phase for gene editing, given that the target DNA is housed within the nucleus (Taha *et al*., 2022) [114].

Transgene Expression: Post entry into the nucleus, the genetic material delivered by the virus is transcribed and translated by the host cellular machinery. In the context of CRISPR/Cas9, this results in the production of the Cas9 protein and guide RNA, which subsequently combine to form the CRISPR/Cas9 complex accountable for gene editing (Horodecka & Düchler, 2021). Target DNA Binding and Editing: The CRISPR/Cas9 complex identifies the target DNA sequence via the guide RNA and binds to it. Cas9 functions like molecular scissors, generating a double-strand break (DSB) at the target site. This DSB prompts the activation of the cellular repair machinery (Wu *et al*., 2014) [129]. DNA Repair and Editing Outcomes: Two primary DNA repair pathways—Non-Homologous End Joining (NHEJ) and Homology-Directed Repair (HDR)—become operational. NHEJ frequently leads to insertions or deletions (indels) at the DSB site, culminating in gene knockout. On the other hand, HDR, provided with a repair template, can introduce precise nucleotide modifications, thereby enabling meticulous gene editing (Ferreira da Silva *et al*., 2019) [39]. Expression Regulation (Optional): Several viral delivery systems encompass supplementary components, such as promoters or regulatory sequences, to govern the expression of delivered gene-editing tools. This augmentation aids in achieving control over the spatiotemporal aspects of gene editing Expression Regulation (Optional): Several viral delivery systems encompass supplementary components, such as promoters or regulatory sequences, to govern the expression of delivered gene-editing tools. This augmentation aids in achieving control over the spatiotemporal aspects of gene editing (Bulcha *et al*., 2021) [22].

Phenotypic Outcomes of Viral CRISPR/Cas9 Delivery in Recent Studies

Treatment of Genetic Disorders: Viral delivery of CRISPR/Cas9 has shown promising results in correcting genetic mutations associated with various hereditary disorders. For example, in a study targeting Duchenne muscular dystrophy (DMD), researchers successfully restored the expression of the dystrophin gene in mouse models, leading to improved muscle function and reduced disease symptoms (M. Chen *et al*., 2021) [29].

Cancer Therapeutics: CRISPR/Cas9 delivered via viral vectors has been explored as a potential cancer treatment. Studies have demonstrated the successful knockout of oncogenes in cancer cells, inhibiting tumor growth and metastasis (Stefanoudakis *et al*., 2023) [113]. Additionally, CRISPR/Cas9 has been used to enhance the targeting of cancer cells, increasing the specificity and effectiveness of chemotherapy (Balon *et al*., 2022) [13]. Neurological Disorders: Viral CRISPR/Cas9 delivery has shown promise in addressing neurodegenerative diseases. For instance, researchers targeted the mutant huntingtin gene in a study on Huntington's disease. They observed a reduction in mutant protein aggregates, improving motor function in a mouse model (Alkanli *et al*., 2023) [8]. Viral Disease Research: CRISPR/Cas9 delivered by viruses has been instrumental in understanding viral pathogenesis and host-virus interactions. Researchers have used this technology to disrupt viral genes and study their role in viral replication and virulence (Hirano *et al*., 2022) [49]. Drug Discovery: CRISPR/Cas9 delivered via viral vectors has been utilized to investigate the function of specific genes in various biological processes. These studies have identified potential drug targets and provided insights into the mechanisms of certain diseases.

Case studies of successful viral delivery of CRISPR/Cas9 in different crop species

CRISPR/Cas9 has emerged as a revolutionary tool in crop biotechnology, offering precise genome editing capabilities to enhance agricultural productivity and address various challenges in crop cultivation. Several case studies have demonstrated successful viral delivery of CRISPR/Cas9 in different crop species, showcasing its potential to create beneficial genetic modifications. One notable case study (Arazoe, 2021) [10] targeted the susceptibility gene in the rice blast fungus using CRISPR/Cas9 delivered by a viral vector. By doing so, they achieved durable and heritable resistance against rice blast disease in rice plants, one of the most devastating fungal diseases affecting rice crops worldwide. In another research endeavor, (Agarwal *et al*., 2018) [2] utilized a viral delivery system to introduce CRISPR/Cas9 into maize plants to modify the genes responsible for producing seed carotenoids. By enhancing the carotenoid content through precise genome editing, they developed

nutrient-rich maize with potential health benefits for human consumers. Additionally, (Tran *et al*., 2023) [117] demonstrated the successful application of viral delivery of CRISPR/Cas9 in improving drought resistance in tomatoes. By targeting specific genes involved in drought response, they could create transgenic tomato plants with enhanced drought tolerance. These case studies collectively demonstrate the versatility and efficacy of viral delivery for CRISPR/Cas9-mediated genome editing in different crop species. By harnessing the power of this technology, agricultural scientists can work towards developing climate-resilient, nutritionally enriched, and disease-resistant crop varieties to ensure global food security and sustainable agriculture. However, it is essential to note that each crop species and its corresponding genome present unique challenges, and further research is ongoing to optimize the delivery and precision of CRISPR/Cas9 in diverse crops.

Improved disease resistance and insect tolerance

Improved disease resistance and insect tolerance are two crucial traits that can significantly enhance the productivity and sustainability of crops. Where understanding interaction of multiple pathogens with plant and its associated gene to further modified by genome editing (Dandve *et al*., 2019; Jayasinghe *et al*., 2023; Wagh, Daspute, *et al*., 2021) [31, 56, 124]. Gene and through advancements in biotechnology, particularly the utilization of CRISPR/Cas9 genome editing delivered by viral vectors, substantial progress has been made in achieving these desirable traits. With CRISPR/Cas9 technology, researchers can precisely target and modify specific genes associated with crop disease susceptibility. By introducing genetic changes, they have successfully developed plants with enhanced disease resistance. For example, in a case study on potatoes, it was demonstrated that viral delivery of CRISPR/Cas9 targeting essential susceptibility genes conferred resistance against late blight, a devastating disease caused by the pathogen Phytophthora infestans (Kieu *et al*., 2021) [60]. Similarly, viral delivery of CRISPR/Cas9 has also shown promise in imparting insect tolerance to crops. By targeting genes involved in plant-insect interactions, scientists have been able to create plants that are less susceptible to insect pests. One study focused on the diamondback moth, a notorious pest of cruciferous crops. Through CRISPR/Cas9-mediated genome editing, researchers disrupted a gene critical for the moth's survival, increasing resistance in cabbage plants (Shin *et al*., 2022) [107].

The advantage of using viral delivery for CRISPR/Cas9 lies in its ability to introduce the genome-editing machinery into plant cells efficiently. Viruses are highly adept at transferring their genetic material into the host, and by modifying these viral vectors, CRISPR/Cas9 components can be delivered precisely to the target cells without causing harm to the plant. Moreover, viral delivery allows the potential to edit multiple genes simultaneously, creating crops with stacked traits, such as disease resistance and insect tolerance.

Enhanced abiotic stress tolerance

Enhanced abiotic stress tolerance is another essential area where viral delivery of CRISPR/Cas9 has shown great promise in improving crop resilience. Abiotic stresses, such as drought, salinity, extreme temperatures, and nutrient deficiencies, pose significant challenges to agricultural productivity worldwide (Pawar *et al*., 2020; Siddiki *et al*., 2020) [87, 109]. By leveraging the precision and efficiency of CRISPR/Cas9 technology delivered through viral vectors, researchers have made significant strides in developing crops with increased tolerance to these adverse environmental conditions. One noteworthy study focused on enhancing drought tolerance in rice, a staple food crop for millions of people. Through viral delivery of CRISPR/Cas9, researchers targeted specific genes involved in the plant's response to water deficit. The resulting transgenic rice plants exhibited improved water-use efficiency and sustained growth even under limited water availability (Rai *et al*., 2023) [88]. Similarly, viral delivery of CRISPR/Cas9 has been employed to improve salt tolerance in crops, as salinity is a significant constraint on agricultural land. By editing genes associated with salt sensitivity, scientists were able to produce salt-tolerant tomato plants capable of thriving in saline environments without compromising yield (Tran *et al*., 2021) [116]. Furthermore, researchers have explored viral CRISPR/Cas9 delivery to enhance nutrient uptake and utilization efficiency in crops, addressing nutrient deficiencies in soils. By targeting specific genes responsible for nutrient transport and metabolism, they have created plants with improved nutrient acquisition and utilization, leading to better growth and development even in nutrient-depleted soils (Yadav *et al*., 2021) [133]. The development of crops with enhanced abiotic stress tolerance is of paramount importance in the face of climate change, which is projected to increase the frequency and intensity of extreme environmental conditions. By equipping plants with the ability to withstand droughts, tolerate high salinity, and thrive in suboptimal nutrient conditions, agricultural systems can become more resilient and sustainable, ensuring food security for a growing global population. Despite numerous advancements and successes, challenges remain in scaling up the use of viral delivery of CRISPR/Cas9 in agriculture. Strict regulations, public acceptance, and intellectual property concerns are some of the aspects that need to be addressed to facilitate the deployment of these genetically enhanced crops in real-world farming scenarios. Continued research, collaboration between scientists and policymakers, and public engagement are vital to realizing the full potential of CRISPR/Cas9-mediated abiotic stress tolerance in crop improvement.

Potential to target multiple traits simultaneously

One of the most remarkable features of CRISPR/Cas9 technology delivered through viral vectors is its potential to target multiple traits simultaneously. Traditional breeding methods often face limitations in introducing multiple desirable traits into a crop plant due to the complex genetic inheritance of these traits and the time-consuming nature of conventional breeding programs. However, with the advent of CRISPR/Cas9, it becomes feasible to edit multiple target sites in the plant genome simultaneously, offering an unprecedented opportunity to introduce and stack multiple beneficial traits in a single step. For instance, researchers have successfully utilized viral delivery of CRISPR/Cas9 to create maize plants with stacked traits of disease resistance, insect tolerance, and improved drought tolerance. By editing specific genes associated with each trait, they generated

transgenic maize varieties capable of withstanding insect attacks, resisting common diseases, and maintaining productivity under limited water availability (Hernandes-Lopes *et al*., 2023) [48] Moreover, viral delivery of CRISPR/Cas9 has shown the potential to target multiple genes involved in different metabolic pathways. This capability has been exemplified in the development of biofortified crops with enhanced nutritional value. By simultaneously editing genes responsible for nutrient synthesis, accumulation, and regulation, researchers have produced crops with increased levels of essential vitamins, minerals, and other beneficial compounds.

The ability to target multiple traits in a single CRISPR/Cas9 editing event not only accelerates the breeding process but also enables the creation of tailored crops that address the specific needs of farmers, consumers, and the environment. For example, crops could be designed to have improved nutritional content, reduced susceptibility to pests and diseases, and enhanced adaptability to changing climatic conditions all in one improved variety.

However, it is important to note that simultaneously targeting multiple traits through CRISPR/Cas9 comes with its own set of challenges. Off-target effects, where unintended edits occur in the genome, can be a concern, especially when multiple target sites are involved. Additionally, the regulatory approval process for such multi-trait edited crops can be more complex. Therefore, researchers and regulatory bodies need to work closely together to ensure the safety and efficacy of these multi-trait genetically modified crops before they are introduced into commercial agriculture. In conclusion, the capability of viral delivery of CRISPR/Cas9 to target multiple traits simultaneously holds immense promise for crop improvement. By streamlining the breeding process and allowing the incorporation of multiple beneficial traits, this technology paves the way for the development of more resilient, nutritious, and sustainable crop varieties that can address the challenges of modern agriculture and contribute to global food security.

Disease Resistance

Researchers have targeted genes related to disease resistance in sugarcane using CRISPR/Cas9. For example, they have targeted genes involved in plant-pathogen interactions to enhance resistance against major sugarcane diseases like sugarcane smut, yellow leaf syndrome, and mosaic viruses. By editing specific genes associated with susceptibility, they aim to develop disease-resistant sugarcane varieties.

Stress Tolerance

Sugarcane is often subjected to various abiotic stresses, such as drought, salinity, and low-temperature stress. CRISPR/Cas9 has been used to target genes involved in stress responses and tolerance mechanisms in sugarcane. By modifying genes responsible for stress perception and signaling, researchers aim to develop stress-tolerant sugarcane varieties that can withstand adverse environmental conditions.

Yield Improvement

CRISPR/Cas9 has also been applied to improve sugarcane yield by targeting genes involved in flowering and flowering synchronization. Flowering induction in sugarcane is crucial for seed production and crossbreeding, and precise editing of flowering-related genes can lead to improved seed production and uniform flowering.

Efficient Delivery Methods

To deliver CRISPR/Cas9 components into sugarcane cells, researchers have explored various methods, including the use of viral vectors, protoplast transformation, and particle bombardment. These delivery methods aim to efficiently introduce CRISPR/Cas9 components into sugarcane cells and promote targeted gene editing.

DNA-free Gene Editing

To avoid the integration of foreign DNA into the sugarcane genome, researchers have developed DNA-free gene editing approaches using ribonucleoprotein (RNP) complexes. RNP complexes, consisting of Cas9 protein and synthetic guide RNAs, can be delivered directly into sugarcane cells to achieve gene editing without leaving any residual foreign DNA.

Multi-allelic Gene Targeting

Researchers have also demonstrated the potential of CRISPR/Cas9 for multi-allelic gene targeting in sugarcane. By editing multiple alleles of target genes simultaneously, they aim to achieve desired trait combinations and maximize genetic improvement. Overall, gene editing technologies such as CRISPR/Cas9 hold significant promise for advancing sugarcane breeding and crop improvement efforts. They offer the potential to develop improved sugarcane varieties with enhanced disease resistance, stress tolerance, and yield performance, contributing to the sustainability and productivity of the sugarcane industry. Viral CRISPR/Cas9 technology has already demonstrated its revolutionary potential in advancing genome editing and crop improvement. As researchers continue to refine and expand this technology, its future potential becomes even more promising. Here are some key areas where viral CRISPR/Cas9 technology is expected to make significant contributions:

Precision and Efficiency: Current research is focused on enhancing the precision and efficiency of CRISPR/Cas9 editing. This includes developing more precise Cas9 variants and optimizing delivery methods to ensure that edits are accurately targeted and achieved with high efficiency. Improving precision will minimize off-target effects and maximize the desired changes in the crop's genome.

Expansion to Diverse Crops: While viral CRISPR/Cas9 has been extensively studied in model plant species and major crops, efforts are ongoing to extend its applications to a wider variety of plants. Successfully implementing this technology in diverse crops will unlock new possibilities for agricultural innovation and broaden the range of crops that can be improved for various traits.

Epigenome Editing: Epigenome editing involves making changes to the chemical modifications of DNA or associated proteins without altering the underlying genetic code. This emerging area of research holds great promise for

controlling gene expression without introducing DNA changes. Viral delivery systems can be adapted to deliver epigenome editing tools, allowing for more nuanced control of gene regulation in crops.

RNA Editing: CRISPR/Cas9 technology is primarily known for editing DNA, but there is increasing interest in developing RNA-targeting CRISPR systems for precise RNA editing. This approach can be useful in fine-tuning gene expression levels without permanently altering the genome. Viral delivery methods could facilitate the introduction of RNA-targeting CRISPR systems into plant cells.

Engineering Complex Traits: Viral CRISPR/Cas9 technology will play a crucial role in engineering complex traits, including those controlled by multiple genes or influenced by intricate regulatory networks. By targeting multiple genes simultaneously or sequentially, researchers can design crops with improved agronomic performance and stress tolerance.

Redesigning Photosynthesis: Photosynthesis is a fundamental process that governs a plant's energy conversion and growth. Scientists are exploring the potential to redesign photosynthesis using CRISPR/Cas9 to enhance crop productivity significantly. This could lead to the development of crops that are more efficient in converting sunlight into biomass and yield.

Resistance to Emerging Diseases and Pests: As new diseases and pests emerge due to changing environmental conditions and global trade, viral CRISPR/Cas9 technology can be rapidly deployed to confer resistance to these threats. This will help mitigate the impact of plant diseases and reduce the reliance on chemical pesticides.

Environmental Adaptation: With climate change affecting agricultural regions worldwide, there is a growing need to develop crops that can thrive in new and challenging environments. Viral CRISPR/Cas9 technology can facilitate the introduction of stress-tolerance genes into crops, enabling them to withstand heat, drought, salinity, and other environmental stresses.

Conservation and Biodiversity: CRISPR/Cas9 can also aid in conservation efforts by contributing to the preservation of endangered plant species and enhancing biodiversity. By targeting specific genes associated with key traits in endangered plants, researchers can promote their survival and restoration in their natural habitats.

Accelerating crop breeding for resilience to biotic and abiotic stresses

The revolutionary CRISPR/Cas9 technology has the potential to significantly accelerate crop breeding efforts aimed at enhancing resilience to both biotic and abiotic stresses (Gajardo *et al*., 2023) [40]. Biotic stresses caused by pathogens such as viruses, bacteria, fungi, and pests, as well as abiotic stresses like drought, extreme temperatures, and soil salinity, pose significant challenges to global agriculture and food security (Husaini, 2022) [54]. With CRISPR/Cas9, researchers can precisely target and modify specific genes responsible for stress tolerance in crop plants (Nascimento *et al*., 2023) [82]. By introducing beneficial genetic changes, breeders can develop crop varieties with enhanced resistance to pests and diseases and improved adaptation to challenging environmental conditions (Nerkar *et al*., 2022) [85]. The rapid and precise nature of CRISPR/Cas9-mediated editing enables the creation of stress-tolerant crops in a more time-efficient manner compared to traditional breeding methods. This advancement holds great promise for boosting agricultural productivity and ensuring food security in the face of mounting climate and environmental challenges.

Advantages of rapid crop improvement using viral delivery of CRISPR/Cas9

The viral delivery of CRISPR/Cas9 brings about several advantages in the realm of crop improvement that contribute to greater agricultural productivity. Viral delivery systems offer an efficient means of transporting CRISPR/Cas9 components into plant cells, bypassing the need for time-consuming tissue culture and regeneration steps (Montecillo *et al*., 2020) [78]. This significantly expedites the process of creating genetically edited crops. Additionally, the targeted nature of CRISPR/Cas9 editing minimizes off-target effects and unintended mutations, ensuring that the desired genetic modifications are accurately introduced (Verma *et al*., 2023) [122]. The ability to simultaneously target multiple genes further enables the development of crop varieties with complex traits, such as improved nutritional content and shelf life (Cardi *et al*., 2023) [24]. Moreover, viral delivery allows for the introduction of CRISPR/Cas9 technology into diverse crop species, broadening the scope of agricultural improvements (Hua Liu 2022) [52]. By harnessing the power of viral vectors for CRISPR/Cas9 delivery, researchers and breeders can achieve rapid, precise, and reliable crop improvements that hold immense potential for meeting the growing demands of global food production.

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The revolutionary CRISPR/Cas9 technology can significantly accelerate crop breeding efforts to enhance resilience to both biotic and abiotic stresses (Zaidi *et al*., 2020) [139]. Biotic stresses, caused by pathogens such as viruses, bacteria, fungi, and pests, as well as abiotic stresses like drought, extreme temperatures, and soil salinity, pose significant challenges to global agriculture and food security (Sheikh *et al*., 2020) [105]. With CRISPR/Cas9, researchers can precisely target and modify specific genes responsible for stress tolerance in crop plants. By introducing beneficial genetic changes, breeders can develop crop varieties with enhanced resistance to pests and diseases and improved adaptation to challenging environmental conditions (Nelson *et al*., 2018) [84]. The rapid and precise nature of CRISPR/Cas9-mediated editing enables the creation of stress-tolerant crops in a more time-efficient manner compared to traditional breeding methods (Raj & Nadarajah, 2022) [89]. This advancement holds great promise for boosting agricultural productivity and ensuring food security in the face of mounting climate and environmental challenges.

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Contributions to global food security and sustainable agriculture

The application of CRISPR/Cas9 in agriculture has far-reaching implications for global food security and sustainable agricultural practices (S. A. Zafar *et al*., 2020) [136]. By enhancing crop resilience to biotic and abiotic stresses, CRISPR/Cas9 can help mitigate losses caused by pests, diseases, and adverse environmental conditions (Nazir *et al*., 2022) [83]. Stress-tolerant crops are more likely to thrive in challenging climates, leading to increased yields and greater stability in food production. Additionally, the development of crop varieties with improved nutritional content can address malnutrition and dietary deficiencies in vulnerable populations (Karavolias *et al*., 2021) [58]. The precise gene editing capabilities of CRISPR/Cas9 also open up possibilities for reducing the use of chemical pesticides and fertilizers, promoting environmentally friendly and sustainable agricultural practices (Van Vu *et al*., 2022) [120]. Moreover, the rapid and efficient crop breeding enabled by viral delivery of CRISPR/Cas9 technology can facilitate the timely development of new cultivars, keeping pace with changing agricultural needs and ensuring a steady food supply for an ever-growing global population (Betz *et al*., 2023) [17]. Overall, the adoption of CRISPR/Cas9 in agriculture holds promise in advancing food security, promoting sustainability, and fostering resilience in the face of evolving agricultural challenges.

Recapitulation of the significance of viral CRISPR/Cas9 delivery in crop improvement

In conclusion, the viral delivery of CRISPR/Cas9 has emerged as a transformative tool in crop improvement, offering unprecedented precision, efficiency, and versatility in genetic editing (Z. Ali, Abulfaraj, *et al*., 2015) [6]. By utilizing viral vectors to deliver the CRISPR/Cas9 components, researchers and breeders can bypass traditional limitations of plant transformation and achieve targeted modifications in a wide range of crop species (Z. Ali, Abul-Faraj, *et al*., 2015) [7] This technology has demonstrated remarkable success in enhancing disease resistance, pest tolerance, abiotic stress tolerance, and multiple trait improvements in various crops. Moreover, its potential to expedite the breeding process and introduce beneficial genetic changes more rapidly and accurately has significant implications for addressing global challenges in agriculture and food security (Ariga *et al*., 2020) [11].

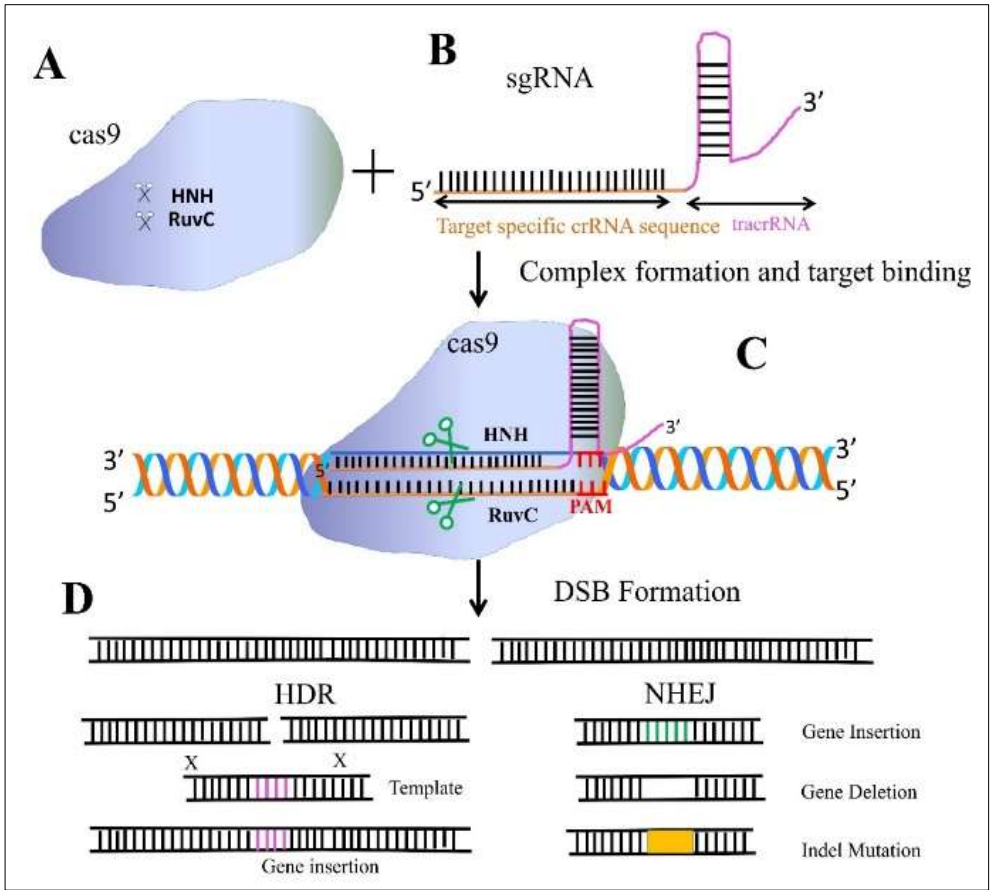
Future prospects and challenges in the application of viral genome editing technology

Looking ahead, the future prospects of viral CRISPR/Cas9 technology in crop improvement are immensely promising (Jaganathan *et al*., 2018) [55]. As research continues, there is potential to expand the host range of viral delivery systems, making it applicable to a broader spectrum of crop plants (Bendix & Lewis, 2018) [16]. Advances in delivery efficiency and off-target reduction techniques will further improve the precision and safety of CRISPR/Cas9 editing (Yip, 2020) [135]. However, challenges remain, such as ensuring proper regulation and addressing ethical considerations related to genetically modified crops (Dizon *et al*., 2016) [34]. Striking a balance between innovation and responsible use will be crucial in harnessing the full potential of this technology.

Overall potential to enhance crop resilience against biotic and abiotic stresses

The ability of viral CRISPR/Cas9 delivery to enhance crop resilience against biotic and abiotic stresses is of paramount importance in securing global food production (Lokhande *et al*., 2019; Wagh, Pohare *et al*., 2021) [74, 112]. By developing stress-tolerant crops, we can mitigate the impact of diseases, pests, and adverse environmental conditions, ultimately leading to increased agricultural productivity and food security (González Guzmán *et al*., 2022) [45]. Moreover, the prospect of simultaneous targeting of multiple traits holds great promise for developing crops with comprehensive resilience and improved nutritional profiles (Zenda *et al*., 2021) [140]. Where Multiomics technological advance will allow to find target for genome editing (Shinde *et al*., 2023; Wagh, Pohare, *et al*., 2021) [108, 112]. The integration of viral CRISPR/Cas9 technology with traditional breeding approaches can further accelerate progress in crop improvement and foster sustainable agricultural practices (Arora & Narula, 2017) [12].

In conclusion, viral CRISPR/Cas9 delivery represents a groundbreaking advancement in the field of crop genetics and breeding. Its potential to enhance crop resilience, accelerate breeding efforts, and contribute to global food security highlights the significance of this technology in shaping the future of agriculture (Bohra *et al*., 2022) [19]. As research and development continue, addressing challenges and ethical considerations while capitalizing on the opportunities will be pivotal in maximizing the benefits of viral CRISPR/Cas9 technology for the betterment of agriculture and society as a whole.



A. Complex formation and target binding, B Cas9 nuclease activity introduces a double-strand break (DSB) in the target DNA. C. DSB formation of a double-strand break (DSB) in the target DNA. D. Repair of the DSB by the cell's DNA repair machinery.

Fig 1: Cas9-sgRNA Complex: Mechanisms of DSB Formation and Target Binding

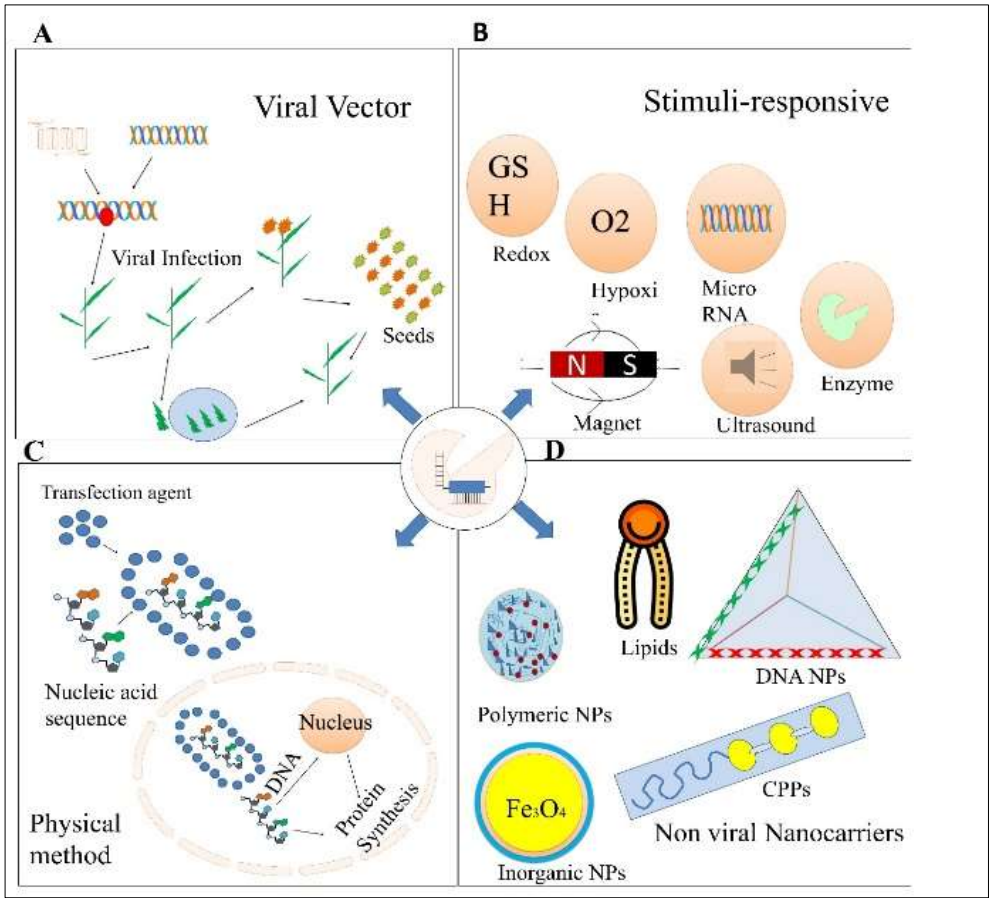


Fig 2: Comparative Analysis of Nucleic Acid Delivery: Viral vs. Non-Viral Approaches

CRISPR-Cas9 systems used for genetic modification in plants, various nano-carriers used in drug delivery systems.

A. Viral Vector Utilizes modified viruses to deliver genetic material into cells, engineered to carry therapeutic genes for targeted gene delivery.
B. Nanocarriers: Engineered for specific triggers like pH or temperature changes, facilitating controlled gene delivery. Stimuli-responsive: Release cargo upon encountering triggers. Redox: Responsive to oxidation state changes for precise delivery. MicroRNA: Tailored for microRNA delivery, regulating cellular processes. Functionalities: Magnet: Guided to sites using external magnetic fields for targeted delivery. Ultrasound: Responsive to ultrasound for controlled, non-invasive delivery.
C. Transfection Agent Chemical compounds facilitating entry of genetic material into cells, overcoming cellular barriers.
D. Lipids Fatty molecules forming nanocarrier structures, encapsulating genetic material. Nucleic Acid Sequence: Delivers specific DNA/RNA sequences, containing genetic instructions for desired outcomes. Polymeric NPs: Nanoparticles composed of polymers, used in nanocarrier fabrication for gene delivery.

Physical Method: Techniques for nanoparticle delivery without chemical agents, including electroporation and sonication. Fe3O4: Magnetite, an iron oxide nanoparticle for magnetically guided delivery. CPPs: Cell-penetrating peptides enhancing nanocarrier efficiency. DNA: Genetic blueprint for protein synthesis and cellular function. Protein Synthesis: Cellular process utilizing DNA instructions for protein production.

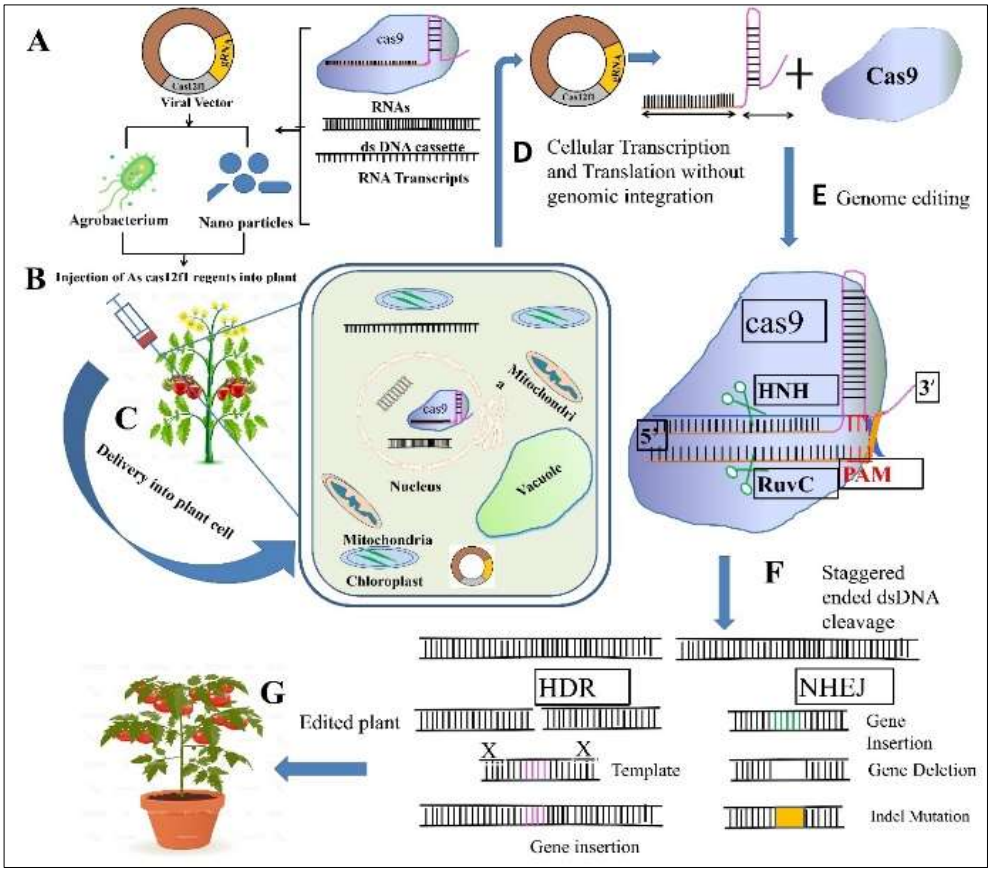


Fig 3: CRISPR-Cas9 delivary through virus genetic modification in plants

(A) Cas9 protein and sgRNA complex Delivery methods, (B) Cas9-sgRNA complex into the plant cell: Agrobacterium and nanoparticles. (F) Target DNA cleavage creating a double-strand break (DSB) at the target DNA sequence. DNA repair (G) Cell repairs the DSB through two main pathways: HDR (homology-directed repair) and NHEJ (non-homologous end joining).

Table 1: Types of viruses optimized for CRISPR/Cas9 delivery

Virus Type	Characteristics	CRISPR Technology	Citation
Adenovirus	Double-stranded DNA virus	CRISPR/Cas9 system	(Didara *et al*., 2023) [32]
Adeno-associated virus (AAV)	Single-stranded DNA virus	CRISPR/Cas9 system	(Y. Wang *et al*., 2022) [128]
Lentivirus	Single-stranded RNA virus	CRISPR/Cas9 system	(Dong & Kantor, 2021) [35]
Retrovirus	Single-stranded RNA virus	CRISPR/Cas9 system	(K. Li *et al*., 2020) [67]
Herpes Simplex Virus (HSV)	Double-stranded DNA virus	CRISPR/Cas9 system	(Karpov *et al*., 2022) [59]
Adeno-associated virus (AAV)	Single-stranded DNA virus	CRISPR/Cas9 with chemically modified sgRNA	(Kim *et al*., 2021) [61]
Lentivirus	Single-stranded RNA virus	CRISPR-Cpf1 system	(Huang *et al*., 2022) [53]
Sendai Virus	Single-stranded RNA virus	CRISPR/Cas9 system	(Park *et al*., 2016) [86]

Table 2: Future Potential of Viral CRISPR/Cas9 Technology

Future Potential of Viral CRISPR/Cas9 Technology in Agriculture	Description	Citations
Precision and Efficiency	Enhancing the precision and efficiency of CRISPR/Cas9 editing for accurate and targeted modifications	(Hua *et al*., 2022) [52]
Expansion to Diverse Crops	Extending CRISPR/Cas9 applications to a wide range of plant species for diverse agricultural improvements	(J. Gao *et al*., 2018) [42]
Epigenome Editing	Modifying chemical modifications of DNA or associated proteins for precise control of gene expression	(X. Liu & Liu, 2016) [73]
RNA Editing	Developing CRISPR systems for precise RNA editing to fine-tune gene expression	(Cox *et al*., 2017) [30]
Engineering Complex Traits	Targeting multiple genes to engineer complex traits in crops for improved agronomic performance	(K. Zafar *et al*., 2020) [136]
Redesigning Photosynthesis	Using CRISPR/Cas9 to redesign photosynthesis for enhanced crop productivity	(Caddell *et al*., 2023) [23]
Resistance to Emerging Diseases and Pests	Rapid deployment of CRISPR/Cas9 to confer resistance to new and emerging plant diseases and pests	(L. Chen *et al*., 2023) [28]
Environmental Adaptation	Introducing stress-tolerance genes to help crops adapt to changing environmental conditions	(A. Zhang *et al*., 2019) [141]
Conservation and Biodiversity	Contributing to the preservation and restoration of endangered plant species and promoting biodiversity conservation	(Mendes *et al*., 2013) [77]
Expanding the Host Range for Viral Delivery	Developing novel viral delivery systems to target a broader range of plant species	(C. Zhang *et al*., 2022) [142]
Overcoming Off-Target Effects and Unintended Mutations	Implementing strategies to minimize off-target effects and unintended mutations during CRISPR/Cas9 editing	(He *et al*., 2020) [143]
Regulatory and Ethical Considerations in Crop Genome Editing	Addressing the regulatory and ethical aspects of using CRISPR/Cas9 in agricultural biotechnology	(M. Kumar *et al*., 2023) [144]
Integrating Viral CRISPR/Cas9 Technology with Traditional Breeding Approaches	Integrating CRISPR/Cas9 with conventional breeding methods for accelerated crop improvement	(Rasheed *et al*., 2021) [90]

Author Contributions

AMP and SGW are the originators of this Review. GBJ was responsible for the Figure designs, AMP and SGW wrote the draft of the manuscript, with critical feedback from BDP, and AAD contributed equally.

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