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ADVANCEMENT OF CROP PRODUCTIVITY VIA CRISPR-NANOPARTICLE INTERFACE

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ABSTRACT

Plant improvement strategies involve diverse techniques, ranging from traditional to marker-assisted methods, as well as chemical and radiation treatments. However, these methods can introduce imprecise changes in plant DNA. Accelerating plant enhancement is crucial to meet global food demand, but current methods are time-consuming. Scientists are revolutionizing plant breeding by employing various techniques to develop crops with specific attributes, such as increased yield and pest resistance, aligning with environmental and societal needs. While these methods offer substantial advantages, they often face challenges and can be less precise than desired. Innovative methods, such as gene editing using CRISPR, offer enhanced precision. CRISPR technology enables precise modifications to a plant's DNA, allowing for targeted improvements without unintended consequences. While CRISPR shows great potential, ensuring its safe and accurate implementation is a priority. Scientists are exploring diverse methods, both viral and non-viral, to effectively deliver CRISPR components into plant cells, with non-viral approaches gaining traction due to their safety and versatility. Nanoparticles play a pivotal role in these advancements by serving as delivery vehicles for CRISPR tools. These particles safeguard and transport the necessary components to specific locations within plants, bolstering growth, yield, and disease resistance. Despite challenges, the synergy of nanotechnology and CRISPR holds promise for revolutionizing plant improvement while safeguarding the environment. This integrated approach offers the potential to enhance crop growth and quality while upholding ecological balance.

Keyword. Precision, Plant Breeding, CRISPR, Gene Editing, Nanotechnology

INTRODUCTION

The agricultural sector confronts a challenging dilemma, the imperative to boost food production to accommodate the rapidly increasing global populace. Nonetheless, a promising avenue for addressing this difficulty lies in the adoption of sensible and environmentally conscientious farming methodologies. This will help us grow more food efficiently while also taking care of the environment. Agroecology is a technique of farming that focuses on growing crops and marketing the produce together in a balanced way. In a balanced way means doing things in a fair and equal manner, considering all the different parts and aspects involved, taking care of the environment, the plants, and the people who depend on them. It's about finding a good middle ground that benefits everyone. Doing things in a balanced way means making sure that none of the important factors, such as the environment, the plants, or the people, are harmed or negatively affected. It's about finding solutions that benefit everyone without causing any harm or negative consequences (Gautam and Kumar 2020; Wezel *et al.*, 2009). Plant breeders are using different techniques to incorporate desired characters in plants. Some methods are conventional, while others use markers to detect favorite traits. They also practice chemicals and radiation to enhance decent traits, but this can sometimes transform the plant's DNA in undesirable ways. These methods have some complications, like not being very precise and occasionally causing too many fluctuations in the plants' DNA. Some methods of modifying plants involve adding new genes to their

DNA, which can sometimes lead to unintended effects if the genes end up in the wrong place. (Mao *et al*., 2019). However, newer methods, like gene editing using CRISPR, are more accurate and effective. CRISPR is like a special tool that can make specific changes to a plant's DNA very precisely. This way, scientists can change plants to have the traits they want without causing problems. CRISPR-Cas9 technology presents a wealth of potential advantages in the realm of agriculture and food production, offering benefits that can revolutionize farming practices and enhance the quality of the food we consume. Firstly, it has the potential to significantly boost crop yields by enabling the crops more resilient to diseases, pests, and adverse environmental circumstances. This not only ensures greater agricultural productivity but also reduces the need for chemical pesticides, thereby lowering costs for growers and diminishing the ecological impact of crop growing. Moreover, CRISPR can be exploited to promote the nutritive content of crops, addressing dietetic scarcities in certain regions. Its capability to accelerate crop development, adapt crops to changing climate conditions, and improve disease resistance further underscores its potential for creating a more sustainable and resilient food system. Nevertheless, the ethical, regulatory, and societal implications of CRISPR in agriculture must be thoughtfully considered to harness its benefits responsibly and effectively.

Scientists are working on how to use CRISPR safely. They're using different tools to carry CRISPR into cells. This is important to make sure the changes happen appropriately and safely inside the cells. Some methods use viruses (Fuentes and Schaffer, 2018), but these can sometimes cause problems. Non-viral methods are safer and can carry larger pieces of DNA, which is good for changing genes (Chen *et al*., 2020; O'Keeffe Ahern *et al*., 2022). Nanoparticles are the tiny heroes in this process. They're like delivery trucks for CRISPR tools (Lee *et al*., 2017; 2018). They protect and transport the tools to where they're needed in plants. Using nanoparticles and CRISPR can make plants better at growing, yielding more, and resisting diseases. This helps farmers and improves the quality of our food. Although there are challenges, this approach of using nanotechnology and CRISPR to improve plants holds a lot of promise. It's a way to help plants grow better and be more useful while also taking care of the environment.

Gene Editing: Innovations & Challenges: Generating plants with wanted traits using certain methods is time consuming and can be complicated. This is problematic since there is a majority of people who need nourishment and things made from plants. Scientists are working to improve plants by changing the way they are bred. They are using various methods to create plants with specific traits that can help meet the needs of people, such as producing more food or being more resistant to pests and diseases. These methods aim to develop plants that are more productive, resilient, and beneficial for both the environment and the people who rely on them. While these methods of changing how plants are bred have their advantages, they also come with some challenges. For example, some of these methods can take a long time to produce the desired results. Additionally, they might not always work as precisely as scientists would like, and this could potentially impact the health and well-being of the plants. It's important for scientists to carefully consider these factors and work towards improving the accuracy and efficiency of these methods to ensure the best outcomes for both the plants and the people who depend on them. Some methods of modifying plants involve adding new genes to their DNA, which can sometimes lead to unintended effects if the genes end up in the wrong place. However, newer techniques have been developed that are more precise and effective (Altpeter *et al*. 2016). One of these advanced methods is gene editing, often referred to as "God's scalpel," can precisely modify DNA or RNA by cutting, replacing, or inserting sequences.

Evolution of Gene Editing: From Random Mutations to Precise Genome Engineering: Since the 1980s, it has advanced significantly and is now used in medicine. First-generation gene-editing techniques primarily involved traditional methods such as irradiation or chemical mutagenesis. These approaches induced random mutations in an organism's DNA to generate genetic diversity, but they lacked precision and control. Researchers had limited ability to target specific genes or genomic regions, resulting in unpredictable and often undesirable changes (MaO *et al*., 2019).

Second-generation gene-editing methods introduced more targeted approaches, notably homologous recombination (HR) and RNA interference (RNAi). HR allowed for the replacement or insertion of specific DNA sequences at desired locations within the genome. RNAi, on the other hand, enabled the selective suppression of gene expression by using small RNA molecules to degrade or inhibit messenger RNA (mRNA). While these second-generation methods were more precise than first-generation techniques, they still had limitations. HR was relatively inefficient and technically challenging, making it unsuitable for many applications. RNAi could suppress gene expression, but it did not provide the capability to make precise changes to the DNA sequence itself (Rajput *et al*., 2021). Technologies like zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), and CRISPR are part of this third generation of gene-editing (Gupta *et al*., 2019).

Gene Editing through CRISPR-Cas9: One of these advanced methods is gene editing, where special tools like CRISPR are used to make specific changes to the plant's DNA. This approach is faster and more accurate, allowing scientists to modify the plants' genes with high precision, ensuring that the changes occur exactly where they are intended to happen. This minimizes the risk of unintended side effects and helps create plants with desired traits more effectively (Altpeter *et al*., 2016). They've propelled life science research forward, where special tools like CRISPR are used to make specific changes to the plant's DNA. This approach is faster and more accurate, allowing scientists to modify the plants' genes with high precision, ensuring that the changes occur exactly where they are intended to happen. This minimizes the risk of unintended side effects and helps create plants with desired traits more effectively.

The Revolutionary CRISPR-Cas9 Gene Editing Technology: Nobel Prize Recognition and its Profound Impact on Science and Medicine: The CRISPR-Cas9 technology for editing genomes won the Nobel Prize in Chemistry in 2020. This award was special because it was given to two women, Emmanuelle Charpentier and Jennifer Doudna. <https://www.nobelprize.org/prizes/chemistry/2020/popular-information/>-They discovered how to use CRISPR-Cas9, often called "genetic scissors," to change DNA. This discovery happened not long ago, but it's incredibly important. With this technology, scientists can change the genes of different living things. It's not only useful for studying organisms and improving crops and animals, but it could also bring big changes in medicine by treating genetic diseases (Zhu, 2012). CRISPR, a powerful gene-editing tool, was initially used in simple organisms. It involves certain genes (Cas genes) that act like tools - some are like builders, some are like mechanics, and others are like cutters. They work together to make precise changes to genes. This technology has been expanding and improving over time (Barrangou, 2015). Using CRISPR, the Cas genes are like detectives that search for specific parts of genes. When they find these parts, they cut them into very small pieces. This cutting process makes it much easier for scientists to make changes to those genes and create new traits in plants or other organisms (Deng *et al*., 2019). The CRISPR/Cas system acts as a defense system in basic organisms, shielding them from harmful intruders like germs. Gradually, scientists have understood how this system functions and found ways to apply it efficiently. One important usage is cutting specific fragments of genes, which is significant in biotechnology. This skill to edit genes helps scientists make positive changes for several purposes (Cong *et al*., 2013).

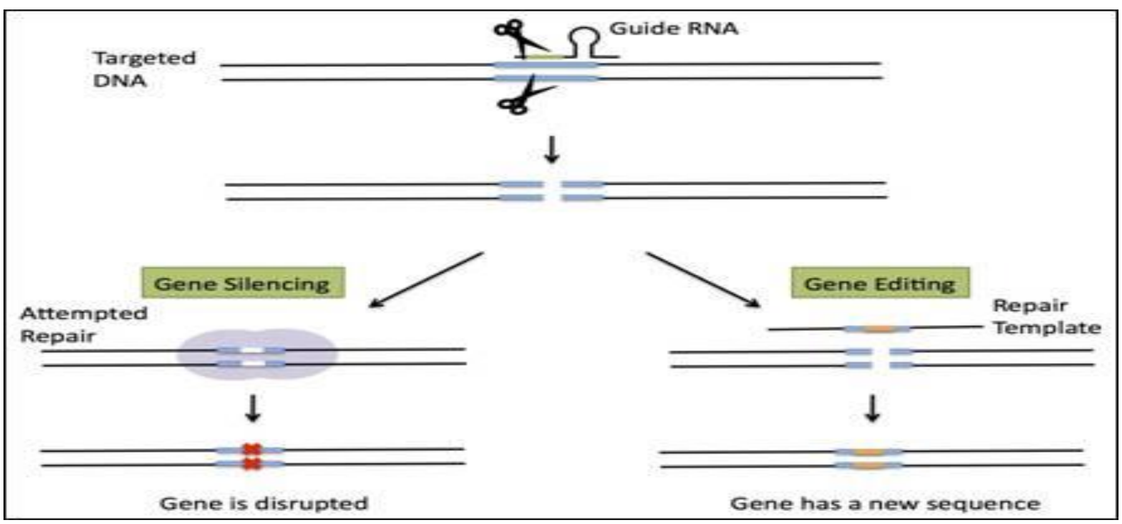

Figure 1. Mechanism of Gene Editing of CRISPR/Cas9.

The Versatility and Promise of CRISPR/ Cas Genome Editing: CRISPR/ Cas genome editing has two parts: Cas9, which is like a cutting tool, and a guide called single guide RNA. These parts team up to locate specific places in our DNA by following specific patterns. This tool is mostly used for medical research because it's flexible, works well, and is simple to use. It helps scientists make precise changes to genes for better understanding and potential treatments (Wu *et al*. 2015). Scientists are still exploring many ways to use CRISPR/Cas. They want to find the best methods for editing genes with rarer errors and improved ways to deliver the editing tools. They're looking into different versions of CRISPR, like CRISPR/Cpf1, CRISPR/Cas13, and CRISPR/saCas9, to see which one works best for specific tasks. This research aims to make gene editing even more accurate and valuable for several drives (Chen *et al*., 2018). CRISPR-Cas9 technology is a groundbreaking advancement in genetic editing with profound implications. To grasp its significance, it's helpful to delve into its components. CRISPR is a natural system found in certain bacteria, serving as a defense mechanism against viruses by storing information about past infections. Cas9, an enzyme, functions as molecular scissors, precisely cutting DNA at specified locations. When these systems are combined, CRISPR-Cas9 becomes a potent genetic editing tool. Its importance is multi-faceted: it provides pinpoint accuracy in editing genes, offering the potential to correct genetic diseases in humans, develop targeted therapies for cancer, and enhance agricultural crops with desired traits. Furthermore, it accelerates genetic research, making it more accessible and cost-effective. This technology is like a genetic Swiss army knife, revolutionizing various fields, but it also necessitates careful ethical and safety considerations in its applications.

Ensuring Safe Passage for Precise Gene Editing: While CRISPR/Cas has many benefits for gene editing, but scientists are also focusing on how to safely put it into cells. They're developing methods using both viruses and non-viral tools to carry the CRISPR/Cas components. This delivery part is crucial to make sure gene editing is done accurately and safely inside cells (Liu *et al*., 2017). Viruses used to carry CRISPR/Cas can trigger immune responses and have size limitations when introduced into living organisms. Because of these challenges, non-viral methods have become more popular. These methods are more effective in delivering CRISPR/Cas, cause fewer immune responses compared to viral methods. Additionally, they have the capacity to carry larger pieces of DNA, making them versatile and useful for precise gene editing (Grimm and Kay, 2003). These non-viral methods, which use polymers and lipids, are particularly well-suited for gene therapies. They offer specific advantages when it comes to delivering significant genetic materials for positive medical treatments.

Unleashing the Power of Nanoparticles and CRISPR for Enhanced Plant Growth and Resilience: The CRISPR/Cas method has brought significant improvements to the field of genomics research, and it lets scientists to add specific and desired traits in plants. This is helpful for making crops more resistant to various challenges, like harsh environments and pests. However, there are still obstacles to overcome. Delivering the CRISPR tools into plants, growing plant tissues in laboratories, and editing genomes in plants are all delicate tasks that scientists are working to improve. Additionally, we still have a lot to learn about the collection of tools that plants have in their genetics and metabolism. This lack of understanding makes it challenging to design and create new desirable traits in plants. The partnership between nanotechnology and biotechnology holds a lot of potential. Nanoparticles (tiny particles) have emerged as the top choice for carrying CRISPR tools in delivery systems (Cao *et al*., 2018; Jiang *et al*., 2021). They're like little delivery vehicles for CRISPR tools. They are positively charged and help protect and transport the CRISPR components. But, sometimes, a few of these nanoparticles can be a bit harmful and not very precise in finding their target. Researchers have made good progress, although they haven't fully achieved their goals yet, in using nanoparticles for CRISPR. They've come a long way, but there's still more work to be done to make this method even better. This technology is being used a lot in agriculture and botany. Using nanoparticles and advanced techniques like CRISPR can make plants grow stronger, high yielding, and be more resistant to diseases. This benefit growers as well as improves the quality of the nutrition we get (Deng *et al*., 2019).

Revealing the Potential of Nanoparticles in Plant Gene Editing with CRISPR: In the exciting world of science, two amazing technologies – CRISPR and nanoparticles – have come together to offer a new way to improve plants. Imagine them working like a powerful team. Nanoparticles are like tiny delivery vehicles that bring CRISPR tools to exactly where they're needed in plants. This teamwork has the potential to make plants better in many ways. Nanoparticles, being super small, are excellent at guiding the important parts of CRISPR to specific places in plants. This helps plants grow better, produce more, and become stronger against diseases. Even though this partnership is full of promise, it also faces challenges that scientists are working to overcome. These challenges are like puzzles that push scientists to become better at using CRISPR and nanoparticles together. Overall, the combination of CRISPR and nanoparticles could bring big changes to how we improve plants. It's like a glimpse into a future where plants are healthier, better at growing, and the environment stays protected. Nanoparticles (NPs) have made it simpler to deliver helpful substances to plants. NPs are skilled at carrying genes and proteins into plant cells, which is especially valuable for creating transgenic plants with fresh characteristics. This information comes from a study by Demirer and colleagues (Demirer *et al*., 2020). NPs can travel around inside plants, even within cells. They work like delivery vehicles, and release them where needed, and shielding them from injury. These nanoparticles are like special couriers designed for specific types of plants. Scientists are trying to use them to make a gene editing technique called CRISPR even better for plants. Unlocking the potential of nanoparticles to enhance plant gene editing with CRISPR is a promising avenue of research in plant science. This could be really helpful for plant research. Despite challenges, researchers are striving to improve how CRISPR edits plant genes using these tiny particles (Khanna *et al*., 2023). Using CRISPR/Cas9 gene editing alongside nanotechnology can solve many issues. This unique form of genetic modification is valuable in agriculture. By managing gene behavior, we can enhance specific traits in crops leading to improved qualities in crops. This approach can make crops more resilient to challenges using CRISPR/Cas9-nanotechnology (Demirer *et al*., 2021, Hussin *et al*., 2022). In addition, these methods can effectively silence unwanted genes, leading to improved crop yields. Without genetic engineering, development of crop varieties is extremely difficult. Traditional methods are slow when it comes to introducing new or resistant traits. This is why genetic modification holds great promise for the future of agriculture. Advanced techniques related to nanotechnology, which are applied in agriculture and biomedicine, prove to be highly effective. These minuscule nanoparticles produce impressive results in enhancing crops (Deng *et al*., 2019).

Synergizing Biotechnology, Nanotechnology, and Genetic Engineering for Enhanced Crop Improvement: Bringing together biotechnology, nanotechnology, and genetic engineering offers more benefits compared to traditional breeding methods. This approach enhances crop varieties resilience to stresses leads to increased yields. This review emphasizes the important role of the CRISPR/Cas system and how it's delivered. Additionally, it focuses on how nanotechnology contributes to the CRISPR/Cas9 system and its use in editing genes both inside and outside of cells. The review primarily discusses the role of genetic engineering in agriculture, the process of editing genes in plants using specific tools, and the development of plants with precise genetic changes. It also addresses the challenges, recent advancements, goals, and limitations of this system for the near future.

CONCLUSION

In the ever-evolving landscape of agricultural innovation, the collaboration between CRISPR technology and nanoparticles shines as a beacon of promise. This partnership is not merely a scientific curiosity; it holds profound significance for agriculture and the environment. By harnessing the precision of CRISPR-Cas9 and the targeted delivery capabilities of nanoparticles, researchers are unlocking a new era in plant enhancement. The ability to precisely edit plant genomes and then deliver these edits to specific cellular locations opens up a world of possibilities. It means enhancing plant growth and resilience in ways previously deemed impossible. Consider the practical benefits: crops engineered to withstand droughts, resist pests without the need for harmful pesticides, and thrive in suboptimal soils. Imagine increased yields to meet the demands of a growing global population and improved nutritional profiles to combat malnutrition. All of this while reducing the environmental footprint of agriculture by minimizing the use of chemicals and conserving water resources. Yet, the journey towards fully realizing this potential is not without its challenges. Researchers struggle with refining delivery mechanisms, ensuring safety, and navigating regulatory landscapes. The complexity of plant biology demands diligent attention to detail and a deep understanding of the ecological consequences. However, it is precisely these challenges that drive scientific exploration and innovation. Scientists are collaborating across disciplines, governments are refining regulatory frameworks, and the agricultural community is increasingly recognizing the transformative power of this technology. In closing, the partnership between CRISPR and nanoparticles is poised to revolutionize agriculture, offering sustainable solutions to some of our most pressing challenges. As we stand at the threshold of this exciting frontier, the future of plant enhancement holds promise for a greener, more resilient, and food-secure world. The synergy between molecular precision and targeted delivery is a beacon of hope, illuminating the path toward a more sustainable and abundant future.

CONFLICT OF INTEREST

The authors showed no conflict of interest.

CONTRIBUTION OF AUTHORS

In the collaborative effort of writing the review article, each author made distinct and valuable contributions. Amir Afzal assumed a central role in conceptualizing and coordinating the entire project. Sairah Syed significantly enriched the article through extensive literature research, skillfully synthesizing information, and actively participating in the writing process, offering invaluable insights along the way. Mishal Khizar played a pivotal role in the research phase by meticulously gathering relevant articles, data, and information pertinent to the review's subject matter. Additionally, she made substantial contributions to the writing and editing of various sections, enhancing the overall quality of the article. Javed Iqbal, brought specialized expertise to the team, contributing valuable insights and knowledge specific to the field or subject under discussion. Sharmin Ashraf and Muhammad Rashid Khan, contributed her organizational skills by structuring the article for a coherent and logical flow of information. Her contributions extended to the meticulous editing of various sections. Aneesa Altaf and Basharat Mahmood, adeptness in writing and editing further elevated the overall quality of the review article. Together, this diverse team of authors harnessed their unique skills and expertise to craft a comprehensive and high-quality review article.

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