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MINI REVIEW

Agricultural applications of CRISPR-Cas9 beyond crop modification

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ABSTRACT

CRISPR-Cas9 technology has transformed genetic research by enabling precise and efficient gene editing, extending its applications beyond crop improvement in agriculture. Recent research explores CRISPR-Cas9's potential in soil microbiome engineering, pest control, disease resistance, and livestock feed improvement, each offering unique contributions to agricultural sustainability and productivity. Additionally, the technology promises to reduce dependency on chemical inputs like fertilizers and pesticides and minimize greenhouse gas emissions from livestock through enhanced feed efficiency. By editing microbial communities in soil, CRISPR-Cas9 can also support healthier crop growth by fostering beneficial nutrient cycles. However, practical, ethical, and regulatory challenges continue to limit widespread application, with concerns about off-target effects, ecological impacts, and public acceptance needing careful attention. This review synthesizes current knowledge on these emerging applications, explores specific benefits and challenges, and discusses regulatory considerations, providing a comprehensive overview of CRISPR-Cas9's expanding role in sustainable agricultural practices. Addressing these challenges through further research and policy innovation is essential to fully harness CRISPR-Cas9's potential in advancing global food security and environmental sustainability.

KEYWORDS

CRISPR-Cas9; Crop improvement; Soil health; Gene editing; Soil pathogens; Sustainable agriculture

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Introduction

CRISPR-Cas9, a powerful gene-editing tool based on bacterial immune systems, has rapidly become a critical technology in agriculture due to its ability to induce precise genetic changes. While widely recognized for direct crop modifications such as enhancing yield, pest resistance, and stress tolerance, CRISPR-Cas9 offers broader potential applications within agricultural systems. This tool's versatility extends to engineering the soil microbiome, where modifying microbial communities could enhance nutrient availability and reduce dependence on chemical fertilizers [1]. CRISPR-based gene drives have shown promise in pest and disease control targeting specific pest populations, potentially reducing pesticide usage, and limiting ecological impact. Additionally, CRISPR-Cas9 can be used to improve livestock feed efficiency by enhancing nutrient absorption or reducing methane emissions, thus contributing to lower greenhouse gas emissions from agriculture. However, these innovative applications raise ethical and regulatory challenges, including concerns about ecological risks, public acceptance, and the potential for off-target effects. This review explores these emerging applications, examines specific research advancements, and discusses the regulatory and ethical considerations that accompany CRISPR-Cas9's broader adoption in agriculture, emphasizing the need for continued research to ensure safe and sustainable implementation [2].

Novel Uses in Soil Microbiome Engineering

Enhancing soil health through microbiome editing

The soil microbiome, comprising a diverse community of bacteria, fungi, and other microorganisms, is critical for soil health. These organisms drive processes like nutrient cycling, organic matter decomposition, and disease suppression. By using CRISPR-Cas9 to edit genes within soil microorganisms, researchers aim to enhance specific functions, such as nitrogen fixation, which allows plants to utilize atmospheric nitrogen more efficiently and reduces reliance on synthetic fertilizers. For instance, studies have shown that editing nitrogenase-related genes in soil bacteria could increase nitrogen bioavailability in plants, potentially reducing the need for nitrogen-based fertilizers and enhancing sustainable crop production [3]. Additionally, CRISPR-Cas9 could be used to boost the production of plant growth-promoting compounds in soil microbes, further supporting plant resilience and yield. This targeted approach to microbiome engineering may also suppress soilborne pathogens, thereby reducing crop losses and dependency on chemical treatments. However, the practical application of CRISPR-edited microbes in open soil environments requires a thorough assessment to prevent unintended ecological impacts and ensure the stability of these engineered traits [4].

Targeting soil pathogens

Soilborne pathogens pose a significant threat to agricultural productivity, often leading to crop diseases that are difficult to control. CRISPR-Cas9 offers a novel approach to controlling these pathogens by targeting genes that enhance their virulence or enable survival in soil environments. For example, researchers have demonstrated that CRISPR-Cas9 can be used to deactivate pathogenicity-related genes in fungi like

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Fusarium species, which cause wilt diseases in various crops. By precisely editing these genes, CRISPR-Cas9 can reduce the pathogen's ability to infect host plants, potentially lowering disease incidence and improving crop yields without relying on chemical fungicides [5]. However, applying gene-editing tools in open soil presents challenges, such as the potential for horizontal gene transfer, where gene edits could unintentionally spread to non-target organisms, potentially altering native microbial communities. Additionally, ensuring gene stability in natural soil environments requires further investigation, as environmental factors like pH, temperature, and microbial competition may impact the persistence and effectiveness of these edited traits. Furthermore, careful regulatory oversight and containment strategies are essential to prevent unintended ecological impacts and maintain ecosystem balance [6].

Applications in Pest Management and Disease Resistance

Gene editing for insect pest control

Insect pests represent a major challenge in agriculture, often requiring chemical pesticides that have negative environmental impacts. CRISPR-Cas9 has introduced the possibility of managing pests through gene-editing techniques like gene drives, which spread specific genetic modifications throughout pest populations. For example, by targeting reproductive genes in pests such as mosquitoes or aphids, researchers can effectively reduce pest populations. A recent study demonstrated that gene drives could significantly lower pest populations over several generations, potentially reducing the need for chemical pesticides [7]. Recent field trials have shown that modified pest populations can decrease by up to 70% within four generations when specific reproductive genes are targeted. Additionally, these genetic modifications can be designed to be species-specific, making them safer for beneficial insects like bees and natural predators that help control other pests [8].

Another promising application is targeting genes responsible for pesticide resistance. By suppressing resistance-related genes in pests like aphids and weevils, CRISPR-Cas9 can make them more susceptible to existing pesticides, thereby improving pest control efficiency. However, ethical and ecological concerns arise from releasing gene drives into natural ecosystems, as these alterations may unintentionally impact non-target species or disrupt ecological balances. Studies have shown that combining gene drive approaches with traditional pest management methods can provide better control while reducing the risk of unexpected ecological effects. Furthermore, researchers are developing methods to limit gene drive spread to specific geographical areas, which could help address concerns about the uncontrolled spread of modified genes in pest populations [9].

Enhancing disease resistance in crops and livestock

CRISPR-Cas9 is also being applied to improve disease resistance in associated agricultural organisms, particularly in livestock. One application involves editing genes in livestock that make animals susceptible to specific viral infections. For example, researchers have successfully used CRISPR-Cas9 to deactivate genes linked to susceptibility to porcine reproductive and respiratory syndrome (PRRS), a viral disease that impacts pig production globally. By targeting viral entry receptors on host cells, CRISPR-Cas9 can reduce the incidence of infection without relying on extensive vaccination efforts, which can be costly and difficult to implement on a large scale [10]. Recent studies have shown that CRISPR-edited pigs demonstrate up to 85% increased resistance to PRRS virus infection compared to non-edited controls. Additionally, these genetic modifications appear to be stable across multiple generations, suggesting a long-term solution for disease management in pig farming [11]. In addition to livestock applications, CRISPR-Cas9 has the potential for engineering symbiotic organisms that interact with crops to increase resistance to bacterial or fungal infections. For instance, gene-editing in beneficial microbes could help plants resist diseases by promoting pathogen resistance at the rhizosphere (root zone) level, though these applications are still experimental and require further testing for stability and effectiveness in the field. Research has shown that edited symbiotic bacteria can enhance plant defense responses by up to 60% against common soil pathogens when compared to unmodified bacterial strains. Furthermore, these modified microbes have demonstrated the ability to colonize plant roots more effectively, leading to better distribution of disease protection throughout the root system [12].

Potential for Improving Livestock Feed Digestibility

Engineering digestive microbes in livestock

Feed digestibility and efficiency are critical factors in livestock production, influencing both productivity and environmental impact. CRISPR-Cas9 can be used to modify the gut microbiome of ruminant animals, such as cows and sheep, to improve nutrient absorption and reduce methane emissions, a potent greenhouse gas produced during digestion. By editing specific genes in gut bacteria responsible for breaking down complex carbohydrates, researchers have shown it is possible to enhance nutrient absorption, thereby improving feed efficiency and lowering methane emissions. Recent trials have shown that modified gut bacteria can survive and establish stable populations within the rumen environment for up to six months after introduction. Studies have also demonstrated that these modifications can lead to a 15% reduction in feed costs while maintaining optimal growth rates in cattle [13].

A recent study demonstrated that modifying the rumen microbiome in cattle could increase fiber digestion by up to 20%, potentially reducing methane emissions and feed costs. However, the introduction of gene-edited microbes into animal microbiomes raises concerns about ecological impacts and gene transfer risks, particularly if modified bacteria were to spread beyond the target host. Long-term monitoring studies have shown that modified bacteria tend to stay within the host animal's digestive system, with minimal detection in manure after 48 hours. Additionally, researchers are developing containment strategies, such as nutrient-dependent growth requirements, to ensure that modified bacteria cannot survive outside the target host environment [14].

Gene editing in forage crops to enhance digestibility

An alternative approach to improving livestock feed efficiency involves directly editing forage crops, such as alfalfa or maize, to enhance their digestibility. CRISPR-Cas9 has been used to reduce lignin content in these crops, making them easier for livestock to digest. Lower lignin levels improve the accessibility

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of cellulose and other nutrients, which can increase feed conversion rates and reduce the amount of feed required. This also contributes to lower methane emissions, as more digestible feed produces less undigested material that can ferment in the rumen. Research has shown that CRISPR-edited forage crops can reduce feed requirements by up to 15% while maintaining the same growth rates in cattle. Studies have also demonstrated that these modified crops maintain their structural integrity in the field, despite having reduced lignin content [15].

Field studies have shown promising results, with edited alfalfa exhibiting improved digestibility and nutrient availability. However, further field testing is necessary to assess the stability of these traits in variable environmental conditions, as well as any potential impacts on livestock health and productivity over long-term feeding trials. Initial data from multi-year studies indicates that animals fed with modified forage crops show no negative health effects and maintain normal growth patterns over multiple generations. Additionally, economic analyses suggest that using these modified crops could reduce feed costs by up to 20% while improving overall farm productivity [16].

Regulatory Considerations and Implementation Challenges

Regulatory frameworks for CRISPR-Cas9 applications

The regulatory environment for CRISPR-Cas9 applications in agriculture is complex and varies significantly by region, impacting the adoption of this technology. In many countries, gene-edited organisms are subject to stringent regulatory frameworks similar to those for genetically modified organisms (GMOs). For instance, the European Union considers most CRISPR-edited organisms as GMOs, requiring extensive risk assessment and approval processes. In contrast, the United States has taken a more lenient approach when no foreign DNA is introduced, allowing some CRISPR-modified crops to bypass traditional GMO regulations. These regulatory discrepancies create barriers to international trade and complicate the commercialization of CRISPR-based agricultural products. Recent studies show that countries with more flexible regulations have seen a 30% increase in CRISPR-related agricultural research and development projects over the past three years. Additionally, several developing nations are now creating their regulatory frameworks specifically for gene-edited crops, focusing on balancing innovation with safety concerns and public acceptance [17].

Ethical and ecological concerns

CRISPR-based applications, particularly those involving gene drives or microbiome engineering, raise ecological and ethical concerns. Gene drives, for example, could have unintended consequences if they spread beyond target pest populations, potentially affecting non-target species and disrupting ecological balances. There is also concern about the long-term impacts of releasing gene-edited microbes into open environments, as they could transfer edited genes to native microbial populations, altering soil or gut ecosystems in unpredictable ways [18]. Public perception of gene-edited organisms in agriculture also affects regulatory policies, as ethical considerations and consumer acceptance play significant roles in shaping policy. Recent surveys indicate that public understanding and acceptance of CRISPR technology varies widely, with only 40% of consumers showing awareness of the difference between traditional GMOs and CRISPR-edited organisms. Furthermore, ongoing research is focused on developing containment strategies and reversible gene drives to address these ecological concerns, with promising results in laboratory settings showing up to 95% control over gene spread in test populations [19].

Practical implementation challenges

Alongside regulatory and ethical considerations, the practical application of CRISPR-Cas9 in agriculture faces various obstacles. The high costs of development, the requirement for specialized knowledge, and access to cutting-edge laboratory facilities restrict the availability of CRISPR-Cas9 technologies for smaller farmers. Additionally, although CRISPR-Cas9 is known for its precision, concerns about off-target effects persist, requiring comprehensive testing to confirm that modifications do not lead to undesired consequences or traits. Transitioning CRISPR-Cas9 applications from controlled settings to actual field conditions necessitates further research to improve stability, efficiency, and cost-effectiveness within real-world agricultural systems [20].

Conclusion

CRISPR-Cas9 presents significant potential for advancing agriculture beyond conventional crop modification. Its use in engineering soil microbiomes, controlling pests, enhancing disease resistance, and improving livestock feed could lead to more sustainable and productive agricultural practices. Nevertheless, hurdles related to regulation, ethical issues, and challenges in implementation need to be tackled before these applications can gain widespread acceptance. Future studies should concentrate on increasing the precision of gene editing, assessing ecological effects, and aligning international regulatory standards to promote the use of CRISPR-Cas9. By overcoming these obstacles, CRISPR-Cas9 can be a crucial instrument in realizing sustainable agricultural objectives.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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