



RESEARCH ARTICLE

CRISPR in Agricultural Food Crops: Exploring the Significant Contribution of this Beneficial Gene Editing Technique in Food Crops for Yield Improvement

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ABSTRACT

This review paper provides a comprehensive overview of the applications, implications, and future prospects of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 gene editing technology in agriculture, with a particular focus on food crops. Over the past decade, CRISPR has revolutionized agricultural biotechnology, enabling precise modifications for crop improvement, including enhanced yield, nutritional content, disease and pest resistance, and environmental stress tolerance. We examine these developments in detail, citing specific examples of CRISPR-edited crops. We further delve into the technical workings of CRISPR, explaining its mechanisms and associated safety and ethical considerations. This review also addresses significant challenges, including potential off-target effects, regulatory discrepancies, and public acceptance. Notably, we underscore the importance of transparency, engagement, and education in fostering public trust and acceptance of genetically edited crops. Finally, we gaze into the future of CRISPR technology in agriculture, considering emerging trends and technologies such as base editing, prime editing, and multiplex editing. We posit that, while there are challenges to be navigated, CRISPR technology, if handled responsibly and thoughtfully, holds significant promise in contributing to sustainable agriculture and securing our future food supply.

Key words: CRISPR-Cas9, Agricultural Biotechnology, Genetically Edited Crops, Crop Improvement.

INTRODUCTION

Advancements in genetic engineering and biotechnology have revolutionized agriculture and food production over the last few decades, offering promising solutions to some of the most pressing challenges in feeding the growing global population (BABAR et al., 2022). The introduction of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 gene-editing system represents a significant milestone in this progression (Zhu et al., 2020).

The CRISPR-Cas9 system is a naturally occurring defence mechanism found in a wide variety of bacteria. This system, in its natural state, allows bacteria to 'remember' and destroy invading viruses by incorporating bits of the virus's genetic material into their own DNA and using this as a reference for future attacks. When a known virus invades, the CRISPR-Cas9 system produces an RNA molecule that matches the

viral DNA sequence, and this RNA molecule, paired with a Cas9 enzyme, binds to the invading DNA and cuts it, disabling the virus (Chen et al., 2019).

The beauty of the CRISPR-Cas9 system lies in its simplicity and precision. Scientists have repurposed this system to edit genes in a variety of organisms. By providing the CRISPR-Cas9 complex with an RNA guide of their design, they can target and edit virtually any gene of interest. The ease-of-use and affordability of CRISPR-Cas9, compared to previous gene-editing techniques, have led to a broad and rapid uptake of this technology across various fields of research and development (Molla et al., 2020).

Brief History of Genetic Engineering in Agriculture

Genetic engineering in agriculture has been employed for several decades with the goal of improving crop productivity and quality. The first genetically modified crop, the Flavr Savr tomato, which

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had an extended shelf life, was introduced in the U.S. in the 1990s. Since then, the use of genetically modified organisms (GMOs) in agriculture has expanded, encompassing traits such as herbicide tolerance and pest resistance. However, traditional genetic engineering methods often involved the insertion of foreign DNA into crops, raising public and regulatory concerns over potential ecological and health impacts. The advent of CRISPR technology marks a shift from these traditional transgenic methods. CRISPR allows for precise editing of an organism's existing genes without the need for foreign DNA insertion, which could potentially alleviate some concerns associated with GMOs. Nevertheless, the technology is not without its own ethical and regulatory debates, which are crucial to its application in agriculture (Altieri, 2004).

Overview of the CRISPR's Role in Crop Improvement

The role of CRISPR-Cas9 in crop improvement cannot be overstated. By enabling precise genetic modifications, this technology allows scientists to develop crops with desirable traits, such as higher yields, improved nutritional profiles, and increased resistance to pests, diseases, and environmental stresses (Khalid, Tahir, et al., 2021). CRISPR can also facilitate the development of novel crop varieties by introducing beneficial traits from wild or less commonly used species. The role of CRISPR in crop improvement goes beyond just modifying traits. The technology can also be used to better understand plant biology by enabling functional studies of genes, leading to improved breeding strategies. Moreover, CRISPR holds potential for addressing challenges in post-harvest losses and food waste by modifying genes involved in ripening and spoilage (Jaganathan et al., 2018).

Objective and Structure of the Review

The objective of this review is to provide an in-depth exploration of the significant contributions of CRISPR-Cas9 gene editing in crop improvement, focusing on its role in enhancing yield. We will delve into the specific applications of this revolutionary technology in creating disease and pest-resistant crops, improving nutritional content, enhancing environmental stress tolerance, and reducing post-harvest losses (Khalid, Amjad, et al., 2021).

The review will also address the regulatory, ethical, and public perception aspects of CRISPR-edited crops, as these factors are critical to the technology's adoption and success in agriculture. The review will conclude with a discussion on the future prospects of CRISPR technology in achieving sustainable agricultural practices and food security. This review aims to serve as a valuable resource for researchers, policymakers, and stakeholders in understanding the transformative potential of CRISPR in agriculture (BABAR et al., 2022).

Fundamentals of CRISPR-Cas9

The advent of the CRISPR-Cas9 gene-editing technology has marked a significant turning point in the

field of genetic engineering, bringing with it a degree of precision and ease-of-use that was previously unthinkable. To fully appreciate its application and potential, it is essential to understand the fundamentals of the CRISPR-Cas9 system, its working mechanism, and the considerations regarding its use (Zhang et al., 2021).

Understanding the CRISPR-Cas9 System

CRISPR, an acronym for Clustered Regularly Interspaced Short Palindromic Repeats, and its associated protein, Cas9, comprise a system that bacteria and archaea have long used to fend off invading viruses. Discovered in the early 1990s and further elucidated in the subsequent decades, the CRISPR-Cas9 system is a form of 'adaptive immunity' that allows these microorganisms to recognize and destroy viral genetic material. The system is made up of two key components. The first is the CRISPR array, a section of the bacterial DNA consisting of repeating sequences interspersed with unique 'spacer' sequences derived from previous viral invaders. The second component is the Cas9 protein, a nuclease enzyme capable of cutting DNA.

Mechanism of CRISPR-Cas9 Gene Editing

The CRISPR-Cas9 gene-editing technology harnesses this natural bacterial defense mechanism. In its laboratory use, scientists first transcribe the CRISPR array into a long RNA molecule that is then processed into smaller CRISPR RNAs (crRNAs). Each crRNA carries a sequence matching a specific target gene in the organism to be edited. When a crRNA combines with a trans-activating CRISPR RNA (tracrRNA) and the Cas9 protein, it forms a complex that can locate and bind to the target DNA sequence (Khalid, Tahir, et al., 2021). The Cas9 protein then acts as a pair of molecular scissors, creating a break in the DNA. The organism's own DNA repair machinery repairs the break, but can introduce insertions or deletions that disrupt the gene, or a new DNA sequence can be incorporated (Jin, 2017).

Safety and Ethical Considerations

While the precision and efficiency of the CRISPR-Cas9 system are remarkable, it also presents a number of safety and ethical considerations. One of the foremost concerns is off-target effects, where the Cas9 protein may cut the DNA at unintended sites, potentially leading to undesired genetic alterations (BABAR et al., 2022). While significant strides have been made in reducing off-target effects, the risk is not entirely eliminated. Ethically, the application of CRISPR-Cas9 technology, especially in the field of agriculture, triggers debates on a number of fronts. These range from concerns about the ecological impact of genetically edited organisms to considerations about the ownership, control, and access to this technology. There are also discussions around the social and cultural acceptability of gene-edited foods, which can vary greatly across different societies and cultures (Shinwari et al., 2018).

Potential Limitations and Challenges

Despite the transformative potential of CRISPR-Cas9, several limitations and challenges exist (BABAR et al., 2022). In addition to the aforementioned off-target effects, the efficiency of the system can vary depending on the target organism and the specific genetic modification desired (Khalid, Abdullah, et al., 2021). Another challenge is the delivery of the CRISPR-Cas9 components into the cells of the organism (Bhutta et al., 2023). While various techniques have been developed, the effectiveness of delivery can greatly influence the success of the editing process. Furthermore, the regulatory landscape for CRISPR-edited organisms is complex and varies across countries. This can pose a significant hurdle to the deployment of CRISPR-edited crops, particularly in regions where such regulations are strict or unclear (Rath, 2018).

In summary, while the CRISPR-Cas9 system holds tremendous potential for revolutionizing agriculture, it also poses technical, regulatory, and ethical challenges. Addressing these effectively is crucial for leveraging the full potential of this groundbreaking technology.

Advancements in Crop Resistance Using CRISPR

Crop disease and pest infestations are significant factors that limit agricultural productivity, leading to substantial losses worldwide each year. Given the increasing global food demand and environmental concerns related to the extensive use of pesticides and fungicides, developing resistant crop varieties is of paramount importance. Herein, CRISPR technology provides an innovative, promising approach for achieving this goal (Bhutta et al., 2023).

Enhancing Disease Resistance in Crops

Plant diseases, caused by a variety of pathogens including bacteria, viruses, and fungi, pose a significant threat to global food security. Traditional methods of disease control often involve the use of chemical treatments, which can be environmentally damaging, or conventional breeding, which can be time-consuming and may not always yield the desired results. CRISPR technology is changing this landscape by providing a precise and rapid means to introduce disease resistance traits (Bhutta et al., 2023). By editing specific genes that either control the plant's immune response or are exploited by the pathogen for infection, scientists can engineer crops with enhanced disease resistance. For instance, research has shown that knocking out susceptibility genes (genes that pathogens utilize to infect the host) can confer resistance against diseases such as powdery mildew in wheat and bacterial blight in rice (Borrelli et al., 2018).

Improving Pest Resistance

Just as with diseases, pests also pose a significant challenge to crop yield and quality. Traditional pest control methods often involve chemicals that can have detrimental environmental effects and lead to the

development of pesticide-resistant pests. CRISPR technology offers a potential solution to these challenges by allowing the editing of plant genes to enhance natural pest resistance mechanisms or to introduce new one (Hussain et al., 2018). An excellent example of this is the engineering of the Bt trait in crops. Bt (*Bacillus thuringiensis*) crops are transgenic plants that produce a protein toxic to specific insects, offering built-in pest resistance. While this trait has traditionally been introduced using transgenic methods, CRISPR provides a potential tool for integrating such traits without introducing foreign DNA (Bhutta et al., 2023).

Examples of CRISPR-Edited Resistant Crops

There have been several successful examples of developing disease and pest-resistant crops using CRISPR (Khalid & Amjad, 2018) (Razzaq et al., 2021; Zafar, Mustafa, et al., 2022). One such example is the development of tomato plants resistant to powdery mildew by editing a single susceptibility gene. Similarly, scientists have created wheat resistant to powdery mildew by knocking out susceptibility genes. Another success story involves rice. Scientists have edited the SWEET genes in rice that are exploited by the bacterial blight pathogen, resulting in crops with improved resistance against this devastating disease (Bao et al., 2019).

Future Prospects for Crop Resistance

The future prospects for using CRISPR to enhance crop resistance are vast. As our understanding of plant-pathogen and plant-pest interactions deepens, and as the CRISPR technology itself continues to advance, we can expect to see a growing number of resistant crop varieties (Khalid, Abdullah, et al., 2021). However, alongside these advancements, we must also consider the potential implications and challenges. For instance, just as pests can develop resistance to pesticides, they may also evolve to overcome genetically engineered resistance. This emphasizes the need for ongoing research and the development of crops with multiple resistance traits. Moreover, the regulatory landscape will play a crucial role in the future of CRISPR-edited resistant crops. Regulatory frameworks will need to evolve to accommodate these developments, balancing the need for safety and environmental protection with the potential benefits of these technologies (Razzaq et al., 2021; Zafar, Mustafa, et al., 2022).

In summary, CRISPR represents a powerful tool for enhancing crop resistance to diseases and pests, offering potential solutions to some of the most pressing challenges in agriculture. With continued research and thoughtful regulatory oversight, the coming years promise to bring exciting advancements in this area (Khalid, Abdullah, et al., 2021).

Enhancing Nutritional Content and Quality Using CRISPR

Apart from yield, disease resistance, and pest tolerance, another aspect of crop improvement where

CRISPR technology can make significant contributions in enhancing the nutritional content and quality of crops. The ability to increase or add essential nutrients, vitamins, and minerals can help alleviate global malnutrition, making CRISPR a valuable tool in promoting food and nutritional security (Khalid, Amjad, et al., 2021).

Nutritional Biofortification

Nutritional biofortification involves the genetic modification of crops to increase their nutritional value. Traditionally, this has been accomplished through conventional breeding methods, but these methods can be time-consuming and less precise. With the advent of CRISPR technology, the process of biofortification has been made considerably easier and more efficient (Khalid, Tahir, et al., 2021). By editing specific genes, scientists can increase the content of essential nutrients such as vitamins, minerals, and essential amino acids in crops. This has the potential to help address nutrient deficiencies prevalent in many regions globally. For instance, CRISPR can be used to enhance the iron content in rice or increase the levels of beta-carotene (which the body converts to Vitamin A) in staple crops like bananas or cassava (Zheng et al., 2021).

Modifying Crop Taste and Texture

Apart from nutritional content, another aspect of food quality is taste and texture, both of which significantly influence consumer preference and acceptance. Again, CRISPR offers opportunities for improvement in these areas (Khalid & Amjad, 2018). By editing genes that influence properties such as sugar content, acidity, or firmness, the taste and texture of crops can be optimized. For example, CRISPR could be used to modify the genes responsible for the production of organic acids in fruits, altering their sweetness or tartness. Similarly, genes that influence the texture of fruits or vegetables can be edited to improve their mouthfeel or shelf-life (Khan et al., 2023).

Case Studies of Nutritionally Improved Crops

There have already been several successful applications of CRISPR for improving the nutritional content and quality of crops. A notable example is the work done on rice. By using CRISPR to knock out the *OsBADH2* gene, scientists have been able to produce rice with a stronger aroma, a characteristic associated with premium varieties of rice (Khalid, Tahir, et al., 2021). This editing not only enhances the sensory quality of the rice but also its market value. Another example involves the biofortification of bananas with enhanced vitamin A content. By editing genes involved in the biosynthesis of beta-carotene, researchers were able to significantly increase the vitamin A potential in bananas, a staple food in many countries where vitamin A deficiency is prevalent (Kiran, 2020).

Future Directions in Nutritional Improvements

Looking ahead, CRISPR holds significant potential for further enhancing the nutritional content and quality of our food crops. As we gain a deeper understanding of the genetic basis for nutritional content and other quality traits, the possibilities for crop improvement will continue to expand. However, along with these promising prospects come challenges and considerations. The introduction of nutritionally enhanced crops must be accompanied by robust safety assessments to ensure that the modifications do not inadvertently introduce allergens or toxins or negatively impact other aspects of crop health or environmental safety. Furthermore, public acceptance and regulatory approval are critical factors that will shape the future of nutritionally enhanced crops developed using CRISPR. Overall, with its ability to precisely and efficiently edit genes, CRISPR stands to revolutionize the field of nutritional crop improvement, offering new solutions for addressing global malnutrition and improving the quality of our food supply. As with all its applications, the successful deployment of CRISPR for these purposes will depend on responsible use, comprehensive safety assessments, and clear regulatory guidelines.

CRISPR's Role in Boosting Crop Yield

One of the most pressing challenges in global agriculture is the need to increase crop yield to meet the food demand of a growing population. Traditional breeding methods and chemical enhancements have made significant strides, but they are often insufficient, slow, and may have environmental repercussions. Enter CRISPR-Cas9, a revolutionary gene-editing tool that is poised to redefine the paradigm of yield optimization.

Gene Targets for Yield Enhancement

Increasing crop yield through genetic modification often involves targeting genes that control plant architecture, nutrient absorption, and photosynthesis efficiency, among others (Razzaq et al., 2021). For example, genes that control plant height can be edited to create dwarf varieties that are more resistant to lodging (falling over), a major cause of yield loss. Similarly, genes that regulate root structure can be modified to enhance nutrient uptake, while those involved in photosynthesis can be edited to increase the plant's energy production (Mandal et al., 2022).

Success Stories of CRISPR in Yield Improvement

Numerous examples of successful crop yield enhancement using CRISPR technology exist, demonstrating its potential for significant agricultural advancements. In rice, researchers have used CRISPR to create a semi-dwarf variety by editing the 'Green Revolution' gene, *SD1*, resulting in increased yield and improved lodging resistance. Another success story involves the use of CRISPR in modifying the 'Ideal Plant Architecture' (IPA) genes in rice, which led to plants with more tillers (branches), increased grain size, and

ultimately, higher yield. These examples highlight how CRISPR can be used to manipulate plant architecture for yield enhancement. Similarly, scientists have employed CRISPR to optimize the photosynthetic efficiency of tobacco plants. By editing genes involved in photorespiration, a process that reduces the efficiency of photosynthesis, they created plants with significantly higher biomass and up to 40% greater productivity, illustrating the potential of this approach for enhancing yield in food crops (Chaudhary et al., 2022).

Challenges in Yield Optimization

Despite the potential of CRISPR technology for yield enhancement, several challenges remain. First, the genetic basis of yield is often complex, involving multiple genes that interact in intricate ways. While we have made significant progress in understanding these networks, much remains to be learned. Second, while enhancing one trait, such as yield, it's important to ensure that other important traits, such as disease resistance or stress tolerance, are not adversely affected. This is an area where the precision of CRISPR can be beneficial, but it requires a comprehensive understanding of the interplay between different plant traits. Third, there are potential off-target effects and unintended consequences of gene editing to consider. While significant advancements have been made in improving the specificity of CRISPR, it remains a concern that needs to be carefully managed (Zittersteijn et al., 2021).

Future of High-Yielding CRISPR Crops

Looking forward, the role of CRISPR in creating high-yielding crops is poised to become increasingly significant. As our understanding of the genetic basis of yield and our ability to manipulate genes with precision continue to improve, the potential for yield enhancement using CRISPR will grow. However, for these potential benefits to be realized, several key considerations need to be addressed. The development of high-yielding CRISPR crops will need to be accompanied by rigorous safety and efficacy testing. Moreover, a balanced and science-based regulatory framework is needed, one that encourages innovation while ensuring safety and considering ethical implications. Public acceptance and understanding of gene-edited crops will also be crucial. It's important to communicate the benefits and risks of these technologies clearly and transparently to foster public trust and acceptance. In conclusion, CRISPR technology holds tremendous potential for enhancing crop yield, a crucial aspect of meeting global food demand. As we continue to advance our knowledge and techniques in this exciting field, the prospects for sustainable and efficient agricultural production will continue to expand.

Environmental Stress Tolerance Induced by CRISPR

Agriculture, as a largely climate-dependent endeavor, is continually challenged by environmental stressors such as drought, salinity, and extreme

temperatures. With climate change intensifying these stressors, the need for crops that can withstand such conditions has never been more critical. CRISPR-Cas9 technology has emerged as a promising tool to engineer crops with improved resilience to these environmental stressors.

Drought Resistance

Water scarcity and periodic droughts are major factors that limit crop productivity worldwide. CRISPR technology offers an innovative means to confer drought resistance in crops. The ability to manipulate specific genes associated with the plant's response to water stress can lead to enhanced drought tolerance. For example, genes related to stomatal regulation (the plant's pores involved in gas exchange) or those involved in the plant's hormonal responses to drought can be targeted. There have been significant breakthroughs in this area. For instance, using CRISPR, scientists have edited the stomatal development genes in tomato plants, resulting in reduced stomatal density and enhanced drought tolerance. Similarly, modification of the ARGOS8 gene in maize has shown improved grain yield under water-limited conditions, highlighting the potential of this technology in creating drought-resistant crops (Joshi et al., 2020).

Salt Tolerance

Soil salinization is another significant environmental stressor affecting agricultural productivity, especially in irrigated lands (Manan et al., 2022; Zafar, Razzaq, et al., 2022; Zafar, Rehman, et al., 2022). High soil salinity impairs plant growth and reduces crop yield. CRISPR can be used to enhance crop salt tolerance by editing genes involved in sodium transport or those that mediate the plant's stress response to high salinity. An example of this is the successful editing of the rice SOS1 gene (a sodium transporter) using CRISPR, which resulted in plants with enhanced salt tolerance. This opens up the possibility of cultivating crops in areas previously considered unsuitable due to high salinity (Ganie et al., 2021).

Temperature Resistance

Temperature extremes, both high and low, can significantly impair crop growth and productivity. Using CRISPR technology, scientists can manipulate genes involved in the plant's thermal stress response, creating crops that can better withstand temperature extremes. For instance, in tomatoes, the modification of the SELF PRUNING 5G (SP5G) gene using CRISPR resulted in a variety that could maintain fruit set under high temperatures, a trait of significant importance in the face of global warming (Ganie et al., 2021).

Progress and Challenges in Developing Stress-Resistant Crops

The examples mentioned above underscore the potential of CRISPR technology in developing crops with enhanced tolerance to environmental stressors. However, despite these advancements, challenges

remain. Firstly, the genetic basis of stress tolerance is often complex, involving multiple genes and intricate regulatory networks. Therefore, understanding these networks fully is crucial for the effective use of CRISPR in this context.

Secondly, off-target effects and potential unintended consequences of gene editing pose challenges. For instance, enhancing one trait may negatively affect another. Therefore, comprehensive assessment and monitoring of edited plants are necessary.

Thirdly, the regulatory landscape of genetically modified crops varies widely across countries, and this poses challenges for the deployment of CRISPR-edited crops. Regulations must strike a balance between ensuring safety and environmental protection, and promoting innovation and development.

Lastly, public acceptance of genetically edited crops will play a significant role in their successful deployment. Therefore, public engagement and transparent communication about the benefits and risks of these technologies are crucial.

Despite these challenges, CRISPR holds immense potential for developing stress-resistant crops, which will be increasingly necessary in our changing climate. Continued research and development, responsible regulation, and public engagement are key to harnessing this potential for the future of sustainable agriculture (Ganie et al., 2021).

Regulatory, Ethical, and Public Perceptions of CRISPR-Edited Crops

As CRISPR-Cas9 technology continues to revolutionize agricultural biotechnology, the wider societal implications of these advancements cannot be overlooked. This includes the regulatory, ethical, and public perception aspects of CRISPR-edited crops, which are critical to the successful and responsible deployment of these technologies.

Current Regulatory Framework for GMOs

The regulatory landscape for genetically modified organisms (GMOs) and genome-edited crops varies significantly across the world, with some countries adopting a more precautionary approach while others are more permissive. In the United States, for instance, the USDA has taken the position that most CRISPR-edited plants will not be regulated as GMOs, provided they could have been developed through traditional breeding methods. On the other hand, the European Union has taken a more cautious approach, ruling that crops modified using gene editing, including CRISPR, should be subject to the same stringent regulations as GMOs. This diversity in regulatory frameworks can pose challenges for the international trade of CRISPR-edited crops and products derived from them. Therefore, there is a need for international consensus on a balanced, science-based regulatory framework that ensures safety, promotes innovation, and facilitates trade (Hundleby & Harwood, 2019).

Ethical Considerations in Genome Editing of Food Crops

CRISPR-Cas9 technology also raises several ethical questions. While the tool has the potential to address critical issues such as food security and environmental sustainability, there are concerns about 'playing God', altering nature, and potential unintended consequences. One concern is the notion of "gene drives," alterations that ensure a specific trait is always passed on to the next generation, potentially leading to species-wide changes. While this could have positive implications for pest control or disease resistance, the potential for irreversible changes to ecosystems raises significant ethical concerns. Moreover, concerns exist about the concentration of power and control over the global food system if a handful of corporations control this technology. Therefore, it is crucial to ensure equitable access and benefit-sharing (Rath, 2018).

Public Perception and Acceptance of CRISPR-Edited Crops

Public perception and acceptance are key to the successful deployment of CRISPR-edited crops. Past experiences with GMOs have shown that public opposition can significantly hinder the adoption of these technologies, regardless of their potential benefits. While early surveys suggest that the public may be more accepting of gene editing compared to GMOs, especially if the modifications could be achieved via conventional breeding, there is still significant public concern and a lack of understanding about these technologies (Escajedo San-Epifanio et al., 2023).

The Role of Transparency and Education

The importance of transparency and education in shaping public perception and acceptance of CRISPR-edited crops cannot be overstated. It is crucial for scientists, regulators, and industry to communicate openly and transparently about what gene editing is, how it differs from traditional genetic modification, and what its potential benefits and risks are. Furthermore, the engagement should not be one-sided; public concerns and values should be actively incorporated into decision-making processes about the development and deployment of these technologies. Education initiatives, starting from school level, can help foster a deeper understanding of the science behind CRISPR and the ethical and societal implications of its use. These efforts will contribute to a more informed public discourse on gene editing and can help build public trust and acceptance (Ahmad et al., 2021).

In conclusion, while CRISPR-Cas9 technology offers immense potential for improving our food crops, realizing this potential will require careful navigation of the regulatory, ethical, and societal implications. A balanced and transparent approach that promotes scientific innovation, ensures safety, respects ethical considerations, and values public opinion will be key to harnessing the benefits of this revolutionary technology for our food systems.

Conclusion and Future Prospects

The advent of CRISPR-Cas9 technology has triggered a new era in agricultural biotechnology, providing unprecedented possibilities for crop improvement. As we review its impacts and gaze into the future, it is clear that CRISPR holds immense promise, but its successful application also requires careful navigation through various challenges.

REFERENCES

- Ahmad, A., Ghouri, M. Z., Munawar, N., Ismail, M., Ashraf, S., & Aftab, S. O. (2021). Regulatory, ethical, and social aspects of CRISPR crops. *CRISPR Crops: The Future of Food Security*, 261-287.
- Altieri, M. A. (2004). *Genetic engineering in agriculture: the myths, environmental risks, and alternatives*. Food First Books.
- BABAR, M., NAWAZ, M., SHAHANI, A., KHALID, M., LATIF, A., KANWAL, K., IJAZ, M., MAQSOOD, Z., AMJAD, I., & KHAN, A. (2022). GENOMIC ASSISTED CROP BREEDING APPROACHES FOR DESIGNING FUTURE CROPS TO COMBAT FOOD PRODUCTION CHALLENGES. *Biological and Clinical Sciences Research Journal*, 2022(1).
- Bao, A., Burritt, D. J., Chen, H., Zhou, X., Cao, D., & Tran, L.-S. P. (2019). The CRISPR/Cas9 system and its applications in crop genome editing. *Critical reviews in biotechnology*, 39(3), 321-336.
- Bhutta, M. A., Bibi, A., Ahmad, N. H., Kanwal, S., Amjad, Z., Farooq, U., Khalid, M. N., & Nayab, S. F. (2023). Molecular Mechanisms of Photoinhibition in Plants: A Review. *Sarhad Journal of Agriculture*, 39(230).
- Borrelli, V. M., Brambilla, V., Rogowsky, P., Marocco, A., & Lanubile, A. (2018). The enhancement of plant disease resistance using CRISPR/Cas9 technology. *Frontiers in Plant Science*, 9, 1245.
- Chaudhary, M., Mukherjee, T. K., Singh, R., Gupta, M., Goyal, S., Singhal, P., Kumar, R., Bhusal, N., & Sharma, P. (2022). CRISPR/Cas technology for improving nutritional values in the agricultural sector: an update. *Molecular Biology Reports*, 49(7), 7101-7110.
- Chen, K., Wang, Y., Zhang, R., Zhang, H., & Gao, C. (2019). CRISPR/Cas genome editing and precision plant breeding in agriculture. *Annual review of plant biology*, 70, 667-697.
- Escajedo San-Epifanio, L., Filibi, I., Lasa López, A., Puigdomènech, P., & Uncetabarrenechea Larrabe, J. (2023). Possible EU futures for CRISPR-edited plants: Little margin for optimism? *Frontiers in Plant Science*, 14, 803.
- Ganie, S. A., Wani, S. H., Henry, R., & Hensel, G. (2021). Improving rice salt tolerance by precision breeding in a new era. *Current Opinion in Plant Biology*, 60, 101996.
- Hundleby, P. A., & Harwood, W. A. (2019). Impacts of the EU GMO regulatory framework for plant genome editing. *Food and energy security*, 8(2), e00161.
- Hussain, B., Lucas, S. J., & Budak, H. (2018). CRISPR/Cas9 in plants: at play in the genome and at work for crop improvement. *Briefings in functional genomics*, 17(5), 319-328.
- Jaganathan, D., Ramasamy, K., Sellamuthu, G., Jayabalan, S., & Venkataraman, G. (2018). CRISPR for crop improvement: an update review. *Frontiers in Plant Science*, 9, 985.
- Jin, Z. (2017). The molecular mechanism of CRISPR/Cas9 system and its application in gene therapy of human diseases. *Microreviews in Cell and Molecular Biology*, 3.
- Joshi, R. K., Bharat, S. S., & Mishra, R. (2020). Engineering drought tolerance in plants through CRISPR/Cas genome editing. *3 Biotech*, 10(9), 400.
- Khalid, M. N., Abdullah, A., Ijaz, Z., Naheed, N., Hamad, A., Sheir, M. A., Shabir, F., Parveen, K., & Khan, M. D. (2021). Application and Potential Use of Advanced Bioinformatics Techniques in Agriculture and Animal Sciences. *Ind. J. Pure App. Biosci*, 9(3), 237-246.
- Khalid, M. N., Amjad, I., Nyain, M. V., Saleem, M. S., Asif, M., Ammar, A., & Rasheed, Z. (2021). A review: tilling technique strategy for cereal crop development. *International Journal of Applied Chemical and Biological Sciences*, 2(5), 8-15.
- Khalid, M. N., Tahir, M. H., Murtaza, A., Murad, M., Abdullah, A., Hundal, S. D., Zahid, M. K., & Saleem, F. (2021). Application and Potential Use of Advanced Biotechnology Techniques in Agriculture and Zoology. *Ind. J. Pure App. Biosci*, 9(2), 284-296.
- Khalid, M., & Amjad, I. (2018). The application of mutagenesis in plant breeding under climate change. *Bulletin of Biological and Allied Sciences Research*, 2018(1), 15-15.
- Khan, A., Pudhuvai, B., Shrestha, A., Mishra, A. K., Shah, M. P., Koul, B., & Dey, N. (2023). CRISPR-mediated iron and folate biofortification in crops: advances and perspectives. *Biotechnology and Genetic Engineering Reviews*, 1-31.
- Kiran, K. (2020). Advanced Approaches for Biofortification. *Advances in Agri-Food Biotechnology*, 29-55.
- Manan, A., Zafar, M. M., Ren, M., Khurshid, M., Sahar, A., Rehman, A., Firdous, H., Youlu, Y., Razzaq, A., & Shakeel, A. (2022). Genetic analysis of biochemical, fiber yield and quality traits of upland cotton under high-temperature. *Plant Production Science*, 25(1), 105-119.
- Mandal, S., Ghorai, M., Anand, U., Roy, D., Kant, N., Mishra, T., Mane, A. B., Jha, N. K., Lal, M. K., & Tiwari, R. K. (2022). Cytokinins: A genetic target for increasing yield potential in the CRISPR era. *Frontiers in Genetics*, 13, 883930.
- Molla, K. A., Karmakar, S., & Islam, M. T. (2020). Wide horizons of CRISPR-Cas-derived technologies for basic biology, agriculture, and medicine. *CRISPR-Cas methods*, 1-23.
- Rath, J. (2018). Safety and security risks of CRISPR/Cas9. *Ethics Dumping: Case Studies from North-South Research Collaborations*, 107-113.
- Razzaq, A., Ali, A., Zafar, M. M., Nawaz, A., Xiaoying, D., Pengtao, L., Qun, G., Ashraf, M., Ren, M., & Gong, W. (2021). Pyramiding of cry toxins and methanol producing genes to increase insect resistance in cotton. *GM crops & food*, 12(1), 382-395.
- Shinwari, Z. K., Tanveer, F., & Khalil, A. T. (2018). Ethical issues regarding CRISPR mediated genome editing. *Current issues in molecular biology*, 26(1), 103-110.
- Zafar, M. M., Mustafa, G., Shoukat, F., Idrees, A., Ali, A., Sharif, F., Shakeel, A., Mo, H., Youlu, Y., & Ali, Q. (2022). Heterologous expression of cry3Bb1 and cry3 genes for enhanced resistance against insect pests in cotton. *Scientific Reports*, 12(1), 10878.
- Zafar, M. M., Razzaq, A., Farooq, M. A., Rehman, A., Firdous, H., Shakeel, A., Mo, H., Ren, M., Ashraf, M., & Youlu, Y. (2022). Genetic variation studies of ionic and within boll yield components in cotton (*Gossypium Hirsutum* L.) Under salt stress. *Journal of Natural Fibers*, 19(8), 3063-3082.
- Zafar, M. M., Rehman, A., Razzaq, A., Parvaiz, A., Mustafa, G., Sharif, F., Mo, H., Youlu, Y., Shakeel, A., & Ren, M. (2022). Genome-wide characterization and expression analysis of Erf gene family in cotton. *BMC Plant Biology*, 22(1), 134.

- Zhang, D., Zhang, Z., Unver, T., & Zhang, B. (2021). CRISPR/Cas: A powerful tool for gene function study and crop improvement. *Journal of Advanced Research*, 29, 207-221.
- Zheng, X., Kuijter, H. N., & Al-Babili, S. (2021). Carotenoid biofortification of crops in the CRISPR era. *Trends in Biotechnology*, 39(9), 857-860.
- Zhu, H., Li, C., & Gao, C. (2020). Applications of CRISPR–Cas in agriculture and plant biotechnology. *Nature Reviews Molecular Cell Biology*, 21(11), 661-677.
- Zittersteijn, H. A., Gonçalves, M. A., & Hoeben, R. C. (2021). A primer to gene therapy: Progress, prospects and problems. *Journal of Inherited Metabolic Disease*, 44(1), 54-71.