Review

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Enhancing Crop Yields through CRISPR Technology: A Promising Approach for Sustainable Agriculture

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Review

Enhancing Crop Yields through CRISPR Technology: A Promising Approach for Sustainable Agriculture

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Abstract: Crop yield enhancement is crucial for ensuring food security and meeting the growing global demand for agricultural products. The development of innovative technologies, such as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), has provided new avenues for precise genetic modifications in crops. In this research article, we explore the application of CRISPR technology for crop yield improvement. We discuss the methodology, present recent results, and engage in a comprehensive discussion of the potential benefits, challenges, and future prospects of CRISPR-mediated crop yield enhancement.

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Introduction

Crop yield enhancement is a pressing concern to ensure sustainable agriculture and address global food security challenges (Zhang et al., 2023). Traditional breeding methods have made significant contributions to crop improvement, but they often lack precision and efficiency (Gan & Ling, 2022). The emergence of CRISPR technology has revolutionized the field of genetic engineering, providing unprecedented capabilities for targeted genome editing. By leveraging this revolutionary tool, researchers have begun to unlock the potential to enhance crop yield through targeted modifications of specific genes (Kishchenko et al., 2020). The global population is projected to reach 9.7 billion by 2050 (United Nations, 2015), posing significant challenges to global food production and agricultural sustainability. Increasing crop yields while minimizing the environmental impact of agriculture is crucial to ensure food security for future generations. Traditional breeding methods have made significant contributions to crop improvement, but they often require substantial time and resources. In recent years, the development of molecular tools and techniques has provided new opportunities for targeted crop enhancement.

This article aims to explore the application of CRISPR technology for crop yield enhancement, highlighting the methodology, recent results, and the potential implications for sustainable agriculture and provide an overview of the application of CRISPR technology for crop yield enhancement. CRISPR, a revolutionary gene-editing tool, has gained immense popularity due to its precision, efficiency, and versatility in modifying the genomes of various organisms. In the context of agriculture, CRISPR technology offers promising possibilities for improving crop traits related to yield, disease resistance, abiotic stress tolerance, and nutritional content (Kishchenko et al., 2020; Liu et al., 2021; Movahedi et al., 2023; Wang et al., 2022). By exploring the advancements and challenges associated with CRISPR-mediated crop improvement, this research intends to shed light on the potential of this technology for addressing global food security concerns.

By examining the current state of knowledge and research in this field, this paper aims to contribute to a comprehensive understanding of the application of CRISPR technology for crop yield enhancement. It is hoped that it will serve as a valuable resource for researchers, policymakers, and stakeholders interested in the potential of CRISPR-mediated crop improvement to address the challenges of global food security and sustainable agriculture.

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Methodology

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology has revolutionized genetic engineering and offers promising possibilities for enhancing plant yield. The methodology involved in CRISPR-mediated crop yield enhancement comprises several key steps. Initially, the target gene(s) associated with yield-related traits are identified through comprehensive genetic and functional analyses. The CRISPR system is then employed to introduce precise modifications in the plant genome. This typically involves the delivery of guide RNA (gRNA) and Cas9 protein to the plant cells, which enables the creation of double-strand breaks at the desired genomic loci. Subsequently, the repair mechanisms of the cell can be exploited to introduce specific genetic changes, such as gene knockout, gene insertion, or gene replacement. The edited plant cells are then regenerated into whole plants using tissue culture techniques. Finally, the edited plants are evaluated under controlled conditions to assess the impact of the genetic modifications on yield-related traits (Movahedi et al., 2023).

Here is a general methodology for using CRISPR to improve plant yield:

1. Target Identification: Identify the specific genes or genetic elements that play a crucial role in determining plant yield. This can involve studying the plant's physiology, metabolism, or genetic pathways associated with yield-related traits.
2. Designing Guide RNAs: Design guide RNAs (gRNAs) that are complementary to the target genes or genetic elements. gRNAs guide the Cas9 protein to the target DNA sequence.
3. Delivery of CRISPR Components: Introduce the CRISPR components into plant cells. This can be done using various techniques, such as Agrobacterium-mediated transformation, biolistic particle delivery, or protoplast transformation.
4. Editing the Plant Genome: Once inside the plant cells, the gRNAs and the Cas9 protein form a complex. The Cas9 protein, guided by the gRNA, recognizes the target DNA sequence and induces a double-strand break (DSB) at that site.
5. DNA Repair Mechanisms: Cells possess inherent DNA repair mechanisms that can repair the DSB. These mechanisms are mainly of two types: non-homologous end joining (NHEJ) and homology-directed repair (HDR).
6. NHEJ-Mediated Mutagenesis: In most cases, the repair occurs through NHEJ, which tends to introduce small insertions or deletions (indels) at the site of the DSB. These indels can disrupt the function of the target gene or genetic element, leading to a loss-of-function mutation.
7. Functional Validation: Confirm the functional impact of the mutations by analyzing the edited plant cells or tissues. This can involve techniques such as PCR, DNA sequencing, gene expression analysis, or phenotypic characterization.
8. Plant Regeneration: Select edited plant cells that carry the desired mutations and regenerate whole plants from them. This step may involve tissue culture techniques, depending on the plant species.
9. Field Trials: Conduct field trials with the edited plants to evaluate their performance and assess the effects of the targeted gene modifications on plant yield. Compare the edited plants with unmodified or wild-type plants under various environmental conditions.
10. Iterative Improvement: Based on the results of the field trials, refine the CRISPR-mediated modifications if necessary. This may involve editing additional genes or fine-tuning the existing modifications to optimize yield-related traits.

It is important to note that the methodology may vary depending on the specific plant species and the target genes involved. Additionally, ethical and regulatory considerations should be taken into account when applying CRISPR technology to enhance plant yield.

Results

Recent studies have demonstrated the successful application of CRISPR technology for crop yield enhancement. For instance, researchers have targeted genes involved in plant architecture, flowering time, and fruit development to improve overall yield. By modifying these genes, researchers have achieved enhanced branching patterns, altered plant height, and synchronized flowering, resulting in increased grain or fruit production. Additionally, CRISPR-mediated

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modifications in genes related to nutrient uptake and utilization have been shown to improve nutrient efficiency and boost crop yields.

Table 1. List of genes targeted to manipulate yield efficiency.

Plants	Gene targeted	Trait	Method	Reference
Maize	ZmPHYC1 ZmPHYC2	flowering time/ plant height	gene knockout & overexpression	Li et al. 2020
Oilseed rape	BnaSDG8.A BnaSDG8.B	plant flowering	gene knockout/down	Jiang et al. 2018
Rice	OsGS3	Grain length	site directed mutagenesis	Usman et al. 2021
Soybean	GmPRR37	flowering time	site directed mutagenesis	Cai et al., 2018; Cai et al., 2020
Rapeseed	BnaSDG8.A and BnaSDG8. C	Early flowering	Small indels	Jiang et al. (2018)
Soyabean	GmE1 GmPRR37 GmPRR3b	Early flowering	Long deletions Small indels Short deletions	Han et al. (2019) Wang et al. (2020) Li et al. (2020)
Tomatoes	SlSP5G	Rapid flowering	Small and long deletions	Soyk et al. (2017
Apple	MdTFL1.1	Early flowering	Small indels located in the target sequence	Charrier et al. (2019)
Rice	OsFTL1-11 OsEhd1 OsHBF1 and OsHBF2 OsVP1	Premature leaf aging, Prolonged vegetative growth, late flowering, higher yield, Early flowering, Speeding-up of germination and reduction of seed dormancy.	Small indels	Ma et al. (2015) Wu et al. (2020) Brambilla et al. (2017) Meng et al. (2018) Jung et al. (2019)
Wheat	TaQsd1	Changed germination rates	Small indels	Abe et al. (2019)
Maize	ZmCCT9	Early flowering	Long deletions	Huang et al. (2018)

Discussion

The application of CRISPR technology in crop yield enhancement offers several potential benefits. Firstly, the precise and targeted modifications enabled by CRISPR allow for faster and more accurate trait improvement compared to traditional breeding methods. Secondly, CRISPR-mediated yield enhancement has the potential to reduce the environmental impact of agriculture by increasing productivity on existing farmland and reducing the need for additional land conversion (Sallam et al., 2023; Wang et al., 2022). Moreover, improved crop yields can contribute to enhanced economic stability for farmers and ensure a stable food supply for growing populations. However, challenges

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such as regulatory frameworks, public acceptance, and potential off-target effects need to be addressed to fully realize the potential of CRISPR in agriculture (Gan & Ling, 2022). CRISPR-mediated genome editing holds promise for improving yield-related traits in major staple crops. For instance, researchers used CRISPR to modify genes involved in flowering time regulation in wheat, enabling the plants to escape frost damage and extend the growing season. In maize, CRISPR has been employed to modify genes associated with plant architecture, leading to increased yield potential (Huang et al. 2018). Additionally, CRISPR technology has been utilized to modify genes involved in seed development and grain size in crops like rice and barley, resulting in improved crop yields (Jung et al. 2019).

These case studies exemplify the successful application of CRISPR technology in crop improvement, showcasing its potential for enhancing disease resistance, abiotic stress tolerance, nutritional content, and yield-related traits. While these examples highlight the achievements of CRISPR-mediated crop enhancement, it is important to note that each crop and trait may present unique challenges and considerations. Further research and development, along with regulatory frameworks, will be crucial in translating these advancements into practical and widely adopted solutions for crop yield enhancement. CRISPR technology offers a versatile platform for modifying crop genomes, allowing for targeted enhancements of various agronomic traits associated with crop yield.

CRISPR technology can be employed to modify genes associated with yield-related traits, such as flowering time, plant architecture, fruit development, and seed yield (Kishchenko et al., 2020). By precisely editing genes involved in plant growth and development, researchers can optimize traits that directly impact crop productivity. For example, altering genes involved in hormone signaling pathways or controlling flowering time can lead to improved crop yields by extending the growing season or optimizing the allocation of resources.

This demonstrates the potential of CRISPR-mediated crop improvement in enhancing yield-related traits. By targeting specific genes associated with disease resistance, abiotic stress tolerance, nutritional content, and yield-related traits, CRISPR technology provides precise control over desired modifications, offering new opportunities for crop yield enhancement.

In the following section, challenges and future prospects of CRISPR-based crop improvement, addressing important considerations for its broader adoption and deployment is discussed;

Challenging and future prospects

1. Challenges and Future Prospects

1.1 Regulatory Frameworks and Public Perception: One of the key challenges associated with CRISPR-based crop improvement is the regulatory landscape surrounding genetically modified organisms (GMOs). The classification of CRISPR-edited crops varies among countries, with some considering them as GMOs and subjecting them to stringent regulations. Establishing clear and science-based regulations that are harmonized globally is crucial to facilitate the development and commercialization of CRISPR-edited crops. Additionally, addressing public perception and providing transparent information about the safety and benefits of CRISPR technology is essential for its wider acceptance and adoption (Gan & Ling, 2022).

1.2 Off-Target Effects and Unintended Consequences: Despite the high precision of CRISPR technology, off-target effects can occur, leading to unintended genetic modifications. Minimizing off-target effects and increasing the specificity of CRISPR systems are ongoing research priorities. Continued advancements in CRISPR technology, such as the development of novel Cas variants and improved sgRNA design strategies, hold promise for reducing off-target effects and enhancing the precision of genome editing (Gan & Ling, 2022; Wang et al., 2022).

1.3 Intellectual Property Rights and Access to CRISPR Technology: The intellectual property landscape surrounding CRISPR technology is complex, with patents held by multiple entities. The availability and accessibility of CRISPR tools and technologies, including the Cas enzymes and sgRNA design tools, can be influenced by intellectual property rights. Ensuring fair and equitable access to CRISPR technology for researchers, breeders, and farmers, particularly in developing countries, is crucial for its widespread adoption and implementation.

1.4 Ethical Considerations and Environmental Impact: CRISPR-mediated crop improvement raises ethical considerations related to the deliberate modification of organisms. Open discussions and ethical frameworks are needed to address concerns regarding unintended consequences, environmental impact, and the potential for unintended effects on non-target organisms. Assessing the long-term ecological and environmental impacts of CRISPR-edited crops is essential to ensure sustainable agricultural practices (Wang et al., 2022).

1.5 Future Directions and Emerging Technologies: The future of CRISPR-based crop improvement holds immense promise. Ongoing research aims to overcome existing challenges and expand the application of CRISPR technology in crop improvement. Further advancements in delivery methods, such as developing efficient gene delivery systems, can enhance the transformation efficiency in different crop species. Additionally, exploring emerging technologies, such as base editing and epigenome editing, could further expand the scope of CRISPR-mediated crop enhancement.

Collaboration between researchers, breeders, policymakers, and stakeholders is critical for the successful implementation of CRISPR technology in agriculture. It is essential to establish platforms for knowledge sharing, capacity building, and public engagement to ensure responsible and sustainable deployment of CRISPR-mediated crop improvement strategies (Wang et al., 2022).

In conclusion, while CRISPR technology holds tremendous potential for enhancing crop yield, addressing global food security, and improving agricultural sustainability, several challenges need to be addressed. Overcoming regulatory hurdles, improving precision, ensuring equitable access to technology, addressing ethical concerns, and fostering public acceptance are crucial for the future prospects of CRISPR-based crop improvement. With continued research, technological advancements, and collaborative efforts, CRISPR has the potential to revolutionize agriculture and contribute to a more sustainable and food-secure future.

Conclusion

CRISPR technology holds great promise for crop yield enhancement, offering precise and efficient methods for genetic modifications in crops. The application of CRISPR-mediated genetic engineering has shown positive results in improving various yield-related traits in crops, ultimately leading to enhanced productivity. However, further research and development, along with proper regulatory oversight, are necessary to ensure the safe and responsible deployment of CRISPR-edited crops. By leveraging the potential of CRISPR technology, we can pave the way for sustainable agriculture and meet the increasing global demands for food production.

References

Abe F, Haque E, Hisano H, Tanaka T, Kamiya Y, Mikami M, Kawaura K, Endo M, Onishi K, Hayashi T, Sato K (2019) Genome-edited triple-recessive mutation alters seed dormancy in wheat. Cell Rep 28:1362-1369.e4. https://doi.org/ 10.1016/j.celrep.2019.06.090

Brambilla V, Martignago D, Goretti D, Cerise M, Somssich M, de Rosa M, Galbiati F, Shrestha R, Lazzaro F, Simon R, Fornara F (2017) Antagonistic transcription factor complexes modulate the floral transition in rice. Plant Cell 29:2801. https://doi. org/10.1105/tpc.17.00645

Cai Y, Chen L, Liu X, Guo C, Sun S, Wu C, Jiang B, Han T, Hou W (2018) CRISPR/Cas9-mediated targeted mutagenesis of GmFT2a delays flowering time in soya bean. Plant Biotechnol J 16:176–185. https://doi.org/10.1111/pbi.12758

Cai Y, Chen L, Zhang Y, Yuan S, Su Q, Sun S, Wu C, Yao W, Han T, Hou W (2020) Target base editing in soybean using a modified CRISPR/Cas9 system. Plant Biotechnol J. https:// doi.org/10.1111/pbi.13386

Charrier A, Vergne E, Dousset N, Richer A, Petiteau A, Chevreau E (2019) Efficient targeted mutagenesis in apple and first-time edition of pear using the CRISPR-Cas9 system. Front Plant Sci 10:40. https://doi.org/10.3389/fpls.2019.00040

Gan, W. C., & Ling, A. P. K. (2022). CRISPR/Cas9 in plant biotechnology: applications and challenges. In *Biotechnologia* (Vol. 103, Issue 1, pp. 81–93). Termedia Publishing House Ltd. https://doi.org/10.5114/bta.2022.113919

Han J, Guo B, Guo Y, Zhang B, Wang X, Qiu L-J (2019) Creation of early flowering germplasm of soybean by CRISPR/Cas9 technology. Front Plant Sci 10:1446. https://doi.org/10. 3389/fpls.2019.01446

Huang C, Sun H, Xu D, Chen Q, Liang Y, Wang X, Xu G, Tian J, Wang C, Li D, Wu L, Yang X, Jin W, Doebley JF, Tian F (2018) ZmCCT9 enhances maize adaptation to higher latitudes. Proc Natl Acad Sci U S A 115:E334–E341. https://doi.org/10. 1073/pnas.1718058115

Jiang L, Li D, Jin L, Ruan Y, Shen W-H, Liu C (2018) Histone lysine methyltransferases BnaSDG8.A and BnaSDG8.C are involved in the floral transition in Brassica napus. Plant J 95:672–685. https://doi.org/10.1111/tpj.13978

Jung Y, Lee H, Bae S, Kim J, Kim D, Kim H, Nam K, Nogoy FM, Yongbo D, Kang K (2019) Acquisition of seed dormancy breaking in rice (Oryza sativa L.) via CRISPR/Cas9-targeted mutagenesis of OsVP1 gene. Plant Biotechnol Rep 13:511– 520. https://doi.org/10.1007/s11816-019-00580-x

Kishchenko, O., Zhou, Y., Jatayev, S., Shavrukov, Y., & Borisjuk, N. (2020). Gene editing applications to modulate crop flowering time and seed dormancy. In *aBIOTECH* (Vol. 1, Issue 4, pp. 233–245). Springer. https://doi.org/10.1007/s42994-020-00032-z

Liu, Q., Yang, F., Zhang, J., Liu, H., Rahman, S., Islam, S., Ma, W., & She, M. (2021). Application of crispr/cas9 in crop quality improvement. In *International Journal of Molecular Sciences* (Vol. 22, Issue 8). MDPI. https://doi.org/10.3390/ijms22084206

Li Q., Wu G., Zhao Y., Wang B., Zhao B., Kong D., Wei H., Chen C., Wang H. (2020) CRISPR/Cas9-mediated knockout and overexpression studies reveal a role of maize phytochrome C in regulating flowering time and plant height. Plant Biotechnol. J. 18(12): 2520–2532. http://doi.org/ 10.1111/pbi.13429

Ma X, Zhang Q, Zhu Q, Liu W, Chen Y, Qiu R, Wang B, Yang Z, Li H, Lin Y, Xie Y, Shen R, Chen S, Wang Z, Chen Y, Guo J, Chen L, Zhao X, Dong Z, Liu Y-G (2015) A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. Mol Plant 8:1274–1284. https:// doi.org/10.1016/j.molp.2015.04.007

Meng S, Xu P, Zhang Y, Wang H, Cao L, Cheng S (2018) CRISPR/ Cas9-mediated editing of GS3 to improve flowering time in japonica Rice. Chin J Rice Sci 32(2): 119–127. https://doi. org/10.16819/j.1001-7216.2018.7112 (In Chinese with English abstract)

Movahedi, A., Wei, H., Kadkhodaei, S., Sun, W., Zhuge, Q., Yang, L., & Xu, C. (2023). CRISPR-mediated genome editing in poplar issued by efficient transformation. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1159615

Sallam, A., Alqudah, A. M., Baenziger, P. S., & Rasheed, A. (2023). Genetic Validation and its Role in Crop Improvement. *Frontiers in Genetics*. https://doi.org/10.3389/978-2-83250-431-4

Soyk S, Müller NA, Park SJ, Schmalenbach I, Jiang K, Hayama R, Zhang L, Van Eck J, Jiménez-Gómez JM, Lippman ZB (2017) Variation in the flowering gene SELF PRUNING 5G promotes day-neutrality and early yield in tomato. Nat Genet 49:162– 168. https://doi.org/10.1038/ng.3733

United Nations. (2015). *World_Population_Projections_Press_Release*.

Usman B., Zhao N., Nawaz G., Qin B., Liu F., Liu Y., Li R. (2021) CRISPR/Cas9 guided mutagenesis of grain size 3 confers increased rice (Oryza sativa L.) grain length by regulating cysteine proteinase inhibitor and ubiquitin related proteins. Int. J. Mol. Sci. 22(6): 1–19. http:// doi.org/10.3390/IJMS22063225

Wang L, Sun S, Wu T, Liu L, Sun X, Cai Y, Li J, Jia H, Yuan S, Chen L, Jiang B, Wu C, Hou W, Han T (2020) Natural variation and CRISPR/Cas9-mediated mutation in GmPRR37 affect photoperiodic flowering and contribute to regional adaptation of soybean. Plant Biotechnol J. https://doi.org/10.1111/pbi. 13346

Wang, Y., Zafar, N., Ali, Q., Manghwar, H., Wang, G., Yu, L., Ding, X., Ding, F., Hong, N., Wang, G., & Jin, S. (2022). CRISPR/Cas Genome Editing Technologies for Plant Improvement against Biotic and Abiotic Stresses: Advances, Limitations, and Future Perspectives. In *Cells* (Vol. 11, Issue 23). MDPI. https://doi.org/10.3390/cells11233928

Wu M, Liu H, Lin Y, Chen J, Fu Y, Luo J, Zhang Z, Liang K, Chen S, Wang F (2020) In-frame and frame-shift editing of the Ehd1 gene to develop japonica rice with prolonged basic vegetative growth periods. Front Plant Sci 11:307. https://doi.org/10. 3389/fpls.2020.00307

Zhang, F., Neik, T. X., Thomas, W. J. W., & Batley, J. (2023). CRISPR-Based Genome Editing Tools: An Accelerator in Crop Breeding for a Changing Future. In *International journal of molecular sciences* (Vol. 24, Issue 10). NLM (Medline). https://doi.org/10.3390/ijms24108623**Disclaimer/Publisher's Note:** The statements, opinions