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CRISPR Revolution: Precision Breeding for Enhanced Tea Quality and Disease Resistance

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Abstract CRISPR/Cas genome editing has shown immense potential in agricultural applications, including improving crop quality and disease resistance. CRISPR/Cas9 and its variants have successfully introduced targeted modifications in plant genomes, enhancing traits such as pathogen resistance and nutritional quality. The application of CRISPR technology in tea breeding has already demonstrated promising results, enabling the cultivation of disease-resistant tea plants and improving tea quality through precise genetic modifications. The CRISPR revolution has opened new avenues for precision breeding in tea, providing a powerful and efficient method to enhance tea quality and disease resistance. By leveraging the advanced capabilities of the CRISPR/Cas system, this study seeks to develop tea varieties with improved traits, addressing the challenges of crop quality and disease management in tea production. Future research should focus on optimizing CRISPR technology and addressing potential limitations to fully harness the benefits of this revolutionary technology in tea breeding.

Keywords CRISPR technology; Precision breeding; Tea quality; Disease resistance; Genome editing

1 Introduction

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology, coupled with the CRISPR-associated protein 9 (Cas9), has revolutionized the field of genetic engineering since its discovery as a bacterial adaptive immune system. This technology allows for precise, targeted modifications to the genome, making it a powerful tool for both basic research and applied sciences. The CRISPR/Cas9 system has been widely adopted due to its simplicity, efficiency, and versatility in editing genes across various organisms, including plants (Chen et al., 2019; Veillet et al., 2020; Ahmad et al., 2020). Recent advancements have further enhanced its capabilities, introducing techniques such as base editing and prime editing, which allow for even more precise genetic modifications (Veillet et al., 2020; Zhu et al., 2020; Li et al., 2021).

Precision breeding, particularly through genome editing technologies like CRISPR, is crucial for addressing the challenges faced by modern agriculture. Traditional breeding methods, while effective, are often time-consuming and less precise. In contrast, CRISPR technology enables the rapid development of crops with desirable traits such as enhanced disease resistance, improved yield, and better nutritional quality (Langner et al., 2018; Chen et al., 2019; Zaidi et al., 2020). This is particularly important in the context of global food security, where increasing population and climate change pose significant threats to crop production. By enabling the development of crops that can withstand biotic and abiotic stresses, CRISPR technology holds the promise of creating more resilient and sustainable agricultural systems (Borrelli et al., 2018; Ahmad et al., 2020; Zaidi et al., 2020).

This study explores the current state of CRISPR technology and its applications in plant genome editing, assessing the impact of CRISPR-mediated precision breeding on improving tea quality, including aspects such as flavor, nutritional content, and yield. Additionally, the study investigates the role of CRISPR in cultivating disease-resistant tea varieties, aiming to reduce reliance on chemical pesticides and promote more sustainable agricultural practices. The research aims to identify the challenges and limitations of using CRISPR technology in tea breeding and propose potential solutions to overcome these obstacles. By achieving these objectives, this study seeks to contribute to the application of CRISPR technology in agriculture and provide insights into its potential for revolutionizing tea cultivation.

2 CRISPR Technology in Plant Breeding

2.1 Mechanisms of CRISPR-Cas9

The CRISPR-Cas9 system, derived from a bacterial adaptive immune mechanism, has revolutionized genome editing by enabling precise and targeted modifications in plant genomes. The system relies on the complementarity of a guide RNA (gRNA) to a specific DNA sequence and the endonuclease activity of the Cas9 protein, which introduces double-strand breaks at the target site. These breaks are then repaired by the cell's natural repair mechanisms, either through non-homologous end joining (NHEJ) or homology-directed repair (HDR), leading to targeted gene modifications (Arora and Narula, 2017; Ahmad et al., 2020; Li et al., 2021). Recent advancements have expanded the CRISPR toolbox to include base editing and prime editing, which allow for even more precise nucleotide substitutions without introducing double-strand breaks (Chen et al., 2019; Zhu et al., 2020).

2.2 Applications of CRISPR in plant genomics

CRISPR technology has been widely applied in plant genomics to enhance various traits, including disease resistance, yield, and quality. For instance, CRISPR-Cas9 has been used to develop disease-resistant crops by targeting and modifying susceptibility genes, thereby enhancing resistance to viral, fungal, and bacterial pathogens (Borrelli et al., 2018; Langner et al., 2018). Additionally, CRISPR has been employed to improve crop quality traits such as nutritional content, appearance, and palatability (Liu et al., 2021). The technology has also facilitated the creation of high-throughput mutant libraries and the fine-tuning of gene regulation, further accelerating crop improvement (Chen et al., 2019; Veillet et al., 2020).

2.3 Advantages over traditional breeding methods

CRISPR technology offers several advantages over traditional breeding methods. Traditional breeding is often time-consuming and imprecise, relying on the natural occurrence of beneficial mutations and extensive backcrossing to eliminate undesirable traits. In contrast, CRISPR allows for precise, targeted modifications, significantly reducing the time required to develop new crop varieties (Arora and Narula, 2017; Ahmad et al., 2020). Moreover, CRISPR can introduce specific traits without the need for transgenes, resulting in non-GMO crops that are more acceptable to consumers and regulatory bodies (Langner et al., 2018; Ahmad et al., 2020). The versatility and efficiency of CRISPR make it a powerful tool for modern plant breeding, enabling the rapid development of crops with enhanced traits and improved resilience to environmental stresses (Schindele et al., 2018; Li et al., 2021).

3 Enhancing Tea Quality with CRISPR

3.1 Target traits for quality improvement

The quality of tea is primarily determined by its flavor, aroma, and nutritional content. These traits are influenced by various biochemical compounds such as catechins, theaflavins, and volatile aromatic compounds. Enhancing these traits through genetic modification can significantly improve the overall quality of tea. For instance, increasing the levels of catechins and theaflavins can enhance the health benefits and taste profile of tea, while modifying the biosynthesis pathways of volatile compounds can improve its aroma (Liu et al., 2021).

3.2 Gene editing strategies for quality traits

CRISPR/Cas9 technology offers a precise and efficient method for editing genes associated with quality traits in tea plants. This technology allows for targeted modifications in the genome, enabling the enhancement of specific traits without introducing foreign DNA. Strategies include the use of base-editing tools for targeted nucleotide substitutions and the development of DNA-free methods to avoid transgenic modifications (Chen et al., 2019). Additionally, CRISPR can be used to fine-tune gene regulation, thereby optimizing the expression of genes involved in the biosynthesis of quality-related compounds (Chen et al., 2019; Liu et al., 2021).

3.3 Case studies of quality enhancement

Several studies have demonstrated the successful use of CRISPR/Cas9 in improving the quality of tea plants. For example, Li et al. (2023) studied the application of CRISPR/Cas9 gene editing in the tea tree genome, identified 248 million potential editing sites, and found five PAM types (AGG, TGG, CGG, GGG, NGG). Through bioinformatics analysis, the researchers revealed the distribution and characterization of these loci in the tea tree genome, particularly their importance in secondary metabolism and amino acid biosynthesis pathways (Figure 1). The results show that CRISPR/Cas9 technology can provide a powerful tool for molecular breeding of tea plants, and help to improve the quality and yield of tea.

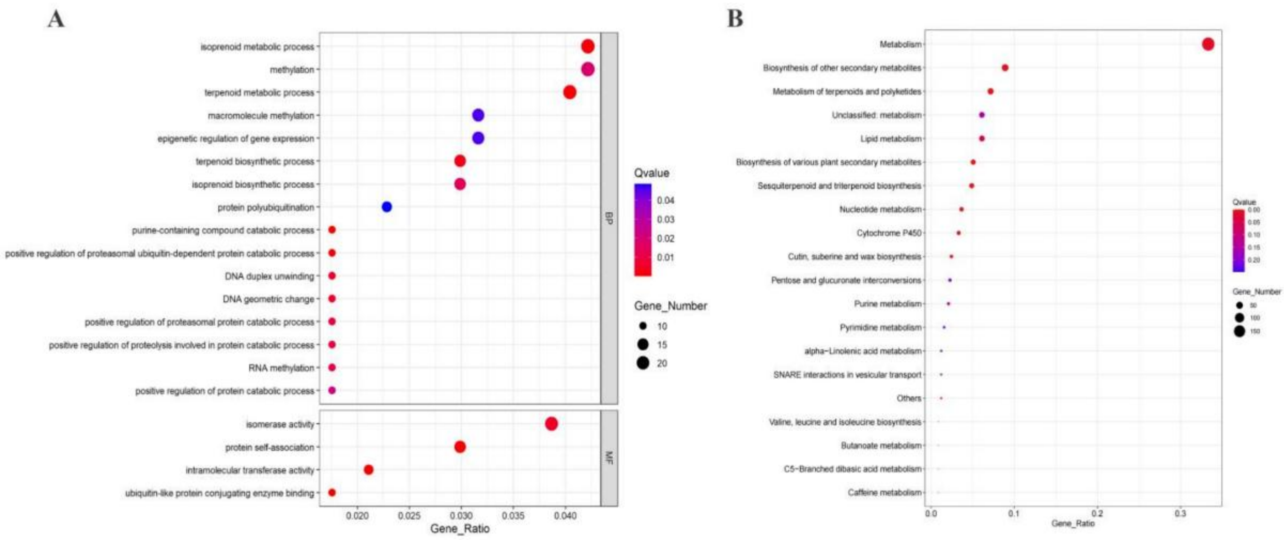

Figure 1 Functional analysis of genes in specific CRISPR-edited regions in 15 chromosomes (Adopted from Li et al., 2023)

Image caption: (A): Gene ontology (GO) enrichment analysis showed that genes in these regions were mainly involved in the biological processes of terpenoid biosynthesis, gene expression regulation and methylation. (B): KEGG enrichment analysis showed that these genes are closely related to secondary metabolites and amino acid biosynthesis pathways, and these results suggest that genes in specific CRISPR-edited regions play key roles in important physiological and biochemical processes of tea plants, providing potential molecular targets for genetic improvement of tea plants (Adapted from Li et al., 2023)

4 Disease Resistance in Tea Plants

4.1 Common diseases affecting tea plants

The tea plant (*Camellia sinensis*) is susceptible to various diseases that can significantly impact its yield and quality. Some of the most prevalent diseases include blister blight, gray blight, and red root rot. Blister blight is caused by a fungus, leading to the formation of blisters on young leaves, severely affecting photosynthesis and plant growth. Gray blight, caused by the fungus Pseudopestalotiopsis, produces gray spots on the leaves, resulting in defoliation and reduced plant vigor. Red root rot, caused by the soil-borne pathogen *Poria hypobrunnea*, affects the roots, leading to plant wilting and death. Additionally, while not a disease, the tea mosquito bug (*Helopeltis theivora*) is a significant pest that causes damage similar to disease symptoms, including leaf spots and dieback.

4.2 CRISPR strategies for developing disease-resistant varieties

The advent of CRISPR/Cas9 technology has revolutionized the development of disease-resistant crops, including tea plants. CRISPR/Cas9 allows for precise genome editing, enabling the introduction of disease resistance traits without the need for traditional breeding methods, which are often time-consuming and less precise.

One common strategy involves knocking out susceptibility (*S*) genes that make plants vulnerable to pathogens. For instance, CRISPR/Cas9 can be used to disrupt these genes, thereby enhancing resistance to fungal, bacterial, and viral diseases (Borrelli et al., 2018; Langner et al., 2018; Ahmad et al., 2020). Another approach is to enhance the plant's innate immune response by editing genes involved in pathogen recognition and defense signaling pathways. This can lead to a more robust and rapid response to pathogen attacks (Veillet et al., 2020; Zaidi et al., 2020; Wang et al., 2022).

Furthermore, CRISPR/Cas9 can be employed in a way that does not leave any foreign DNA in the plant genome, addressing public concerns about genetically modified organisms (GMOs). This is particularly important for crops like tea, which are consumed directly (Chandrasekaran et al., 2016; Chen et al., 2019).

4.3 Case studies: successful implementations

Some case studies have demonstrated the successful application of CRISPR/Cas9 in cultivating disease-resistant plants, serving as models for tea plant improvement. Researchers used CRISPR/Cas9 technology to target the eIF4E gene, developing virus-resistant cucumber plants. The resulting plants exhibited immunity to multiple viruses without any transgenic elements, highlighting the potential for similar strategies in tea plants (Figure 2) (Chandrasekaran et al., 2016). CRISPR/Cas9 has also been used to confer resistance to fungal pathogens in crops such as rice and tomatoes by editing susceptibility genes. These improvements significantly reduced disease incidence and increased crop yields (Figure 3) (Borrelli et al., 2018; Paul et al., 2021).

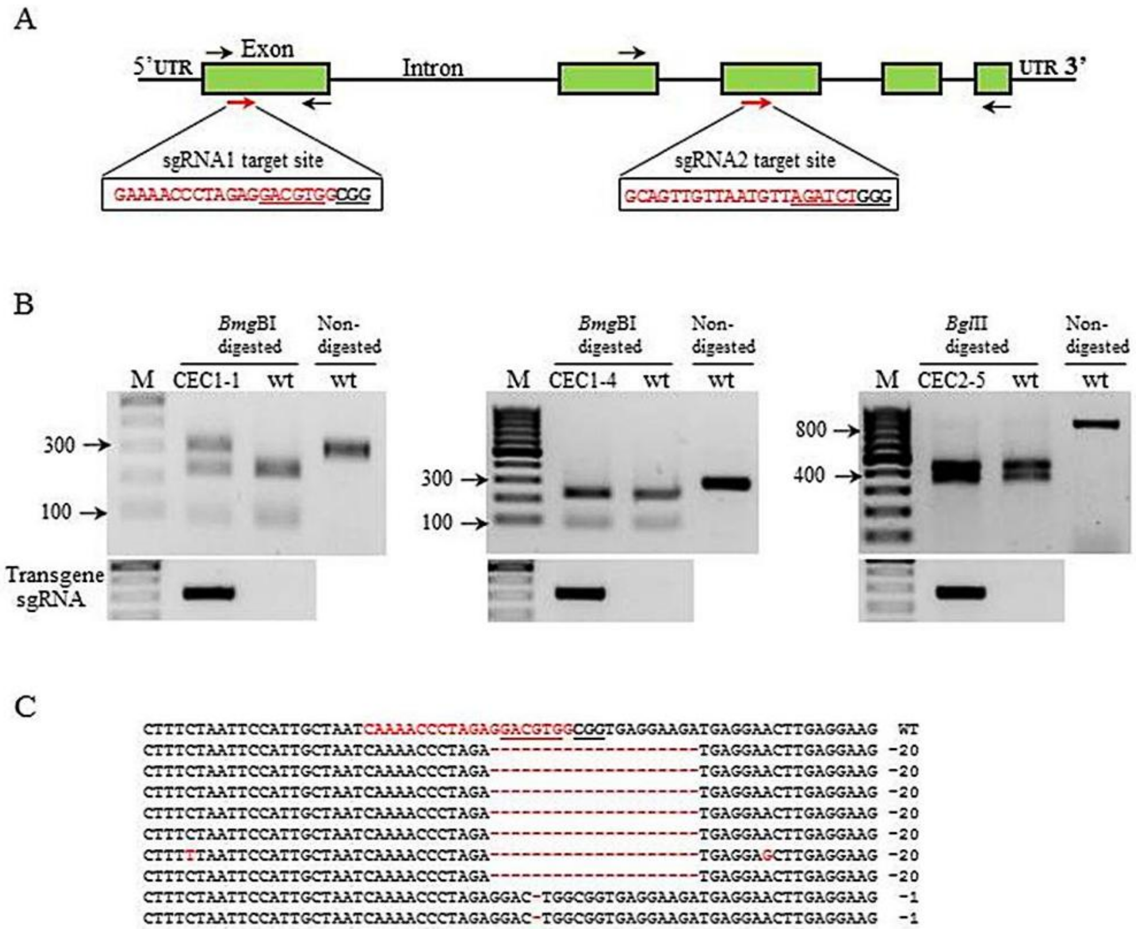

Figure 2 Gene editing of *eIF4E* mediated by CRISPR/Cas9 in transgenic cucumber plants (Adopted from Chandrasekaran et al., 2016)

Image caption: (A) Schematic representation of the cucumber *eIF4E* genomic map and the sgRNA1 and sgRNA2 target sites (red arrows). The target sequence is shown in red letters together with the restriction site (underlined), and the protospacer adjacent motif (PAM) is marked in bold underlined letters. The black arrows indicate the primers flanking the target sites used to detect the mutations. (B) Restriction analysis of T0 polymerase chain reaction (PCR) fragments of CEC-1, CEC1-4 and CEC2-5. (C) Alignment of nine colony sequences from the undigested fragment of line 1 with the wild-type (wt) genome sequence. DNA deletions are shown by red dashes and deletion sizes (nucleotides) are marked on the right side of the sequence (Adopted from Chandrasekaran et al., 2016)

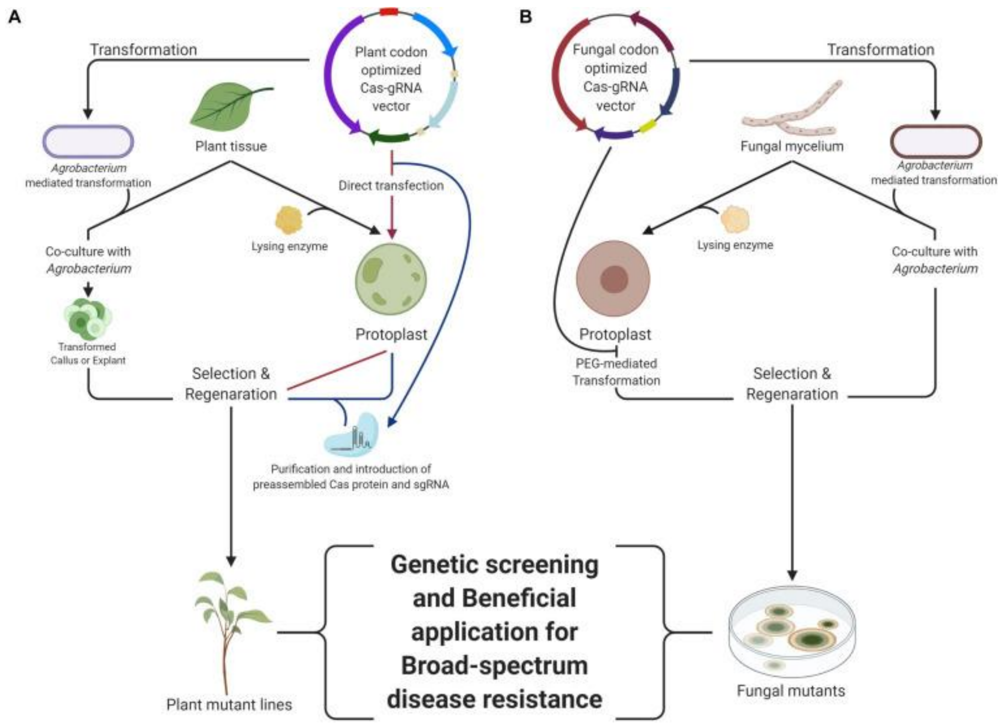

Figure 3 The workflow of CRISPR/Cas system in the plant (A) and fungi (B) (Adopted from Paul et al., 2021)

Image caption: The *Agrobacterium*-mediated transformation is the common method for genomic modification in plants, including the CRISPR/Cas system delivery. The CRISPR/Cas system is being inserted into the plant genome. The ribonucleoprotein (RNP) delivery into plant protoplast generates no trace except targeted modification in desired loci in the genome. They can be delivered by direct transfection or introduction of a preassembled CRISPR/Cas system. The Polyethylene glycol, PEG-mediated transformation is the common method for genomic modification in fungi, which resembles direct transfection of binary vector to plant protoplast. The Agrobacterium-mediated transformation is also optional in the fungal genome modification. With these transformation methods, generation of broad-spectrum resistance can be performed by using each or both mutants (Adopted from Paul et al., 2021)

Advancements in CRISPR technology have enabled crops to resist various pathogens. For example, CRISPR/Cas9 has been used to develop plants with enhanced resistance to both biotic (pathogens) and abiotic (environmental) stresses, which could be highly beneficial for tea cultivation under different growing conditions (Rönspies et al., 2020; Wang et al., 2022). Although there are few direct studies on tea plants in the literature, it is foreseeable that CRISPR technology also has great potential in disease resistance and improvement of tea plants through the wide application of gene editing technology. The technology enables rapid and precise editing of the tea plant genome, enabling improvement of pathogen resistance and other important agronomic traits (Sun et al., 2019)

5 Ethical and Regulatory Considerations

5.1 Ethical implications of CRISPR use in agriculture

The application of CRISPR technology in agriculture raises several ethical concerns. One primary issue is the potential for unintended consequences on ecosystems and biodiversity. The precise nature of CRISPR allows for targeted genetic modifications, but the long-term effects of these changes are not fully understood. There is a risk that edited genes could spread to wild relatives of crops, potentially disrupting natural ecosystems (Chen et al., 2019; Zhu et al., 2020; Sampath et al., 2023). Additionally, the use of CRISPR in agriculture may exacerbate existing inequalities in the food system. Wealthier nations and large agribusinesses are more likely to have access to this technology, potentially widening the gap between developed and developing countries in terms of agricultural productivity and food security (Veillet et al., 2020; Zaidi et al., 2020; Tang et al., 2023). Ethical considerations also extend to the welfare of farmers, who may become dependent on patented CRISPR-modified seeds, leading to issues of seed sovereignty and economic exploitation (Sampath et al., 2023; Gupta et al., 2023).

5.2 Global regulatory landscape

The regulatory landscape for CRISPR technology in agriculture varies significantly across the globe. In the United States, the regulatory framework is relatively permissive, with the USDA stating that it does not regulate plants that could otherwise have been developed through traditional breeding techniques (Nerkar et al., 2022). In contrast, the European Union has taken a more cautious approach, classifying CRISPR-edited organisms as genetically modified organisms (GMOs) and subjecting them to stringent regulations (Gupta et al., 2023; Sampath et al., 2023). Countries like China and Brazil are actively investing in CRISPR research and have developed regulatory frameworks that support the use of this technology in agriculture, albeit with varying degrees of oversight (Chen et al., 2019; Zhu et al., 2020). The lack of a unified global regulatory framework poses challenges for the international trade of CRISPR-modified crops, as differing regulations can lead to trade barriers and market access issues (Zaidi et al., 2020; Tang et al., 2023).

5.3 Public perception and acceptance

Public perception and acceptance of CRISPR technology in agriculture are critical factors that influence its adoption. While the scientific community largely supports the use of CRISPR for its potential to enhance crop quality and disease resistance, public opinion is more divided (Langner et al., 2018; Veillet et al., 2020; Sampath et al., 2023). Concerns about the safety and ethical implications of gene editing contribute to skepticism and resistance among consumers. Misinformation and lack of understanding about the technology further exacerbate these concerns (Ahmad et al., 2020; Zaidi et al., 2020). Effective communication and public engagement are essential to address these issues and build trust. Transparency in the development and regulation of CRISPR-modified crops, along with clear labeling, can help improve public acceptance (Zhu et al., 2020; Tang et al., 2023). Additionally, involving stakeholders, including farmers, consumers, and advocacy groups, in the decision-making process can foster a more inclusive and informed dialogue about the benefits and risks of CRISPR technology in agriculture (Chen et al., 2019; Gupta et al., 2023).

6 Economic Impact

6.1 Potential economic benefits of CRISPR-edited tea

The application of CRISPR technology in tea breeding holds significant potential for economic benefits. By enabling precise genetic modifications, CRISPR can enhance tea quality and disease resistance, leading to increased yield and reduced losses due to pathogens. This can result in higher profitability for tea producers and a more stable supply chain. For instance, CRISPR has been shown to improve disease resistance in various crops, which can be directly translated to tea plants, reducing the need for chemical treatments and lowering production costs (Chen et al., 2019; Ahmad et al., 2020; Veillet et al., 2020). Additionally, the ability to develop high-quality tea varieties with desirable traits such as improved flavor and nutritional content can cater to premium markets, further boosting economic returns (Zhu et al., 2020; Wang et al., 2022).

6.2 Market demand and consumer trends

The market demand for high-quality, disease-resistant tea is on the rise, driven by consumer preferences for healthier and more sustainable products. CRISPR-edited tea can meet these demands by offering enhanced quality and reduced pesticide use, aligning with the growing trend towards organic and environmentally friendly products (Langner et al., 2018; Zaidi et al., 2020). Furthermore, the precision of CRISPR technology allows for the development of tea varieties with specific traits that appeal to niche markets, such as teas with unique flavors or increased health benefits, which can command higher prices and attract discerning consumers (Zhu et al., 2020; Lyzenga et al., 2021). The ability to rapidly respond to market trends by developing new tea varieties can provide a competitive edge to producers and drive market growth.

6.3 Challenges in commercial adoption

Despite the promising economic benefits, several challenges must be addressed for the commercial adoption of CRISPR-edited tea. Regulatory hurdles are a significant barrier, as the approval process for genetically edited crops varies widely across different countries and can be time-consuming and costly (Tripathi et al., 2020; Liu et al., 2022). Public perception and acceptance of genetically edited products also play a crucial role; there is a need for transparent communication and education to build consumer trust and acceptance (Veillet et al., 2020; Ahmad et al., 2020). Additionally, the initial investment in CRISPR technology and the development of new tea varieties can be substantial, which may be a deterrent for smaller producers (Chen et al., 2019; Wang et al., 2022). Addressing these challenges through supportive policies, public engagement, and collaborative efforts between researchers and industry stakeholders is essential for the successful commercialization of CRISPR-edited tea.

7 Future Research Directions

7.1 Emerging areas of CRISPR research in tea

The application of CRISPR technology in tea breeding is still in its nascent stages, but several emerging areas show great promise. One such area is the development of disease-resistant tea plants. CRISPR/Cas9 has been successfully used to create disease-resistant crops in other plants, and similar strategies could be applied to tea to combat pathogens that threaten tea production (Langner et al., 2018; Ahmad et al., 2020; Veillet et al., 2020). Another promising area is the enhancement of tea quality through the precise editing of genes responsible for flavor, aroma, and nutritional content. This could lead to the production of tea varieties with superior qualities tailored to consumer preferences (Chen et al., 2019; Zhang et al., 2019; Zaidi et al., 2020). Additionally, CRISPR technology could be employed to improve the resilience of tea plants to climate change by editing genes associated with stress tolerance (Rönspies et al., 2020; Zaidi et al., 2020).

7.2 Technological advancements and innovations

Recent advancements in CRISPR technology have significantly expanded its potential applications in plant breeding. The development of prime editing and base-editing tools allows for more precise and efficient genome modifications, which can be particularly useful in fine-tuning tea plant traits (Chen et al., 2019; Veillet et al., 2020). Innovations in delivery systems, such as DNA-free methods, have also improved the efficiency and safety of CRISPR applications in plants (Chen et al., 2019; Nidhi et al., 2021). Furthermore, the integration of high-throughput mutant libraries and advanced screening techniques can accelerate the identification of beneficial genetic modifications in tea plants (Chen et al., 2019; Zhang et al., 2019). These technological advancements will be crucial in overcoming current limitations and enhancing the effectiveness of CRISPR-based tea breeding programs.

7.3 Integration with other breeding technologies

The integration of CRISPR technology with other breeding methods holds significant potential for the development of superior tea varieties. Combining CRISPR with traditional breeding techniques can help in stacking multiple desirable traits, such as disease resistance, improved quality, and stress tolerance, into a single tea variety (Ahmad et al., 2020; Rönspies et al., 2020). Additionally, the use of CRISPR in conjunction with other genome editing technologies, such as TALENs and ZFNs, can provide complementary approaches for achieving complex genetic modifications (Borrelli et al., 2018). The integration of CRISPR with genomic selection and marker-assisted breeding can further enhance the precision and efficiency of tea breeding programs (Langner et al., 2018; Chen et al., 2019). By leveraging the strengths of multiple breeding technologies, researchers can develop tea varieties that meet the demands of both producers and consumers.

8 Concluding Remarks

The CRISPR/Cas9 technology has significantly advanced the field of plant breeding, particularly in enhancing disease resistance and improving crop quality. This revolutionary genome editing tool allows for precise and targeted modifications, making it possible to develop disease-resistant cultivars with greater efficiency and accuracy compared to traditional breeding methods. The application of CRISPR/Cas9 in tea breeding has shown promising results in creating varieties that are not only resistant to pathogens but also exhibit improved quality traits, which is crucial for both yield and market value. Additionally, the development of new CRISPR-based techniques such as base editing and prime editing further enhances the precision and scope of genetic modifications, opening new avenues for crop improvement.

Despite the remarkable progress, several challenges remain in the application of CRISPR/Cas9 technology in tea breeding. One of the primary challenges is the off-target effects, which can lead to unintended genetic modifications and potential ecological risks. Additionally, regulatory hurdles and public acceptance of genetically edited crops pose significant barriers to the widespread adoption of this technology. However, these challenges also present opportunities for further research and development. Advances in delivery systems, such as DNA-free methods, and improvements in editing specificity can mitigate off-target effects and enhance the safety of CRISPR applications. Moreover, the integration of CRISPR technology with other biotechnological tools and breeding strategies can accelerate the development of superior tea cultivars with enhanced disease resistance and quality traits.

The future of CRISPR technology in tea breeding looks promising, with the potential to revolutionize the industry by producing high-quality, disease-resistant varieties. As the technology continues to evolve, it is expected to become more efficient, precise, and accessible, thereby overcoming current limitations and expanding its applications. Collaborative efforts among researchers, breeders, and policymakers will be essential to address regulatory and public acceptance issues, ensuring that the benefits of CRISPR technology are realized in a sustainable and socially responsible manner. The successful integration of CRISPR/Cas9 in tea breeding will contribute to the sustainability and resilience of the tea industry, meeting the growing demand for high-quality tea while safeguarding against diseases and environmental challenges.

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Conflict of Interest Disclosure
The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Reference
Ahmad S., Wei X., Sheng Z., Hu P., and Tang S., 2020, CRISPR/Cas9 for development of disease resistance in plants: recent progress, limitations and future prospects, Briefings in Functional Genomics, 19(1): 26-39.
https://doi.org/10.1093/bfgp/elz041

Arora L., and Narula A., 2017, Gene editing and crop improvement using CRISPR-Cas9 system, Frontiers in Plant Science, 8: 1932.
https://doi.org/10.3389/fpls.2017.01932

Borrelli V., Brambilla V., Rogowsky P., Marocco A., and Lanubile A., 2018, The enhancement of plant disease resistance using CRISPR/Cas9 technology, Frontiers in Plant Science, 9: 1245.
https://doi.org/10.3389/fpls.2018.01245

Chandrasekaran J., Brumin M., Wolf D., Leibman D., Klap C., Pearlsman M., Sherman A., Arazi T., and Gal-On A., 2016, Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology, Molecular Plant Pathology, 17(7): 1140-1153.
https://doi.org/10.1111/mpp.12375

Chen K., Wang Y., Zhang R., Zhang H., and Gao C., 2019, CRISPR/Cas genome editing and precision plant breeding in agriculture, Annual Review of Plant Biology, 70: 667-697.
https://doi.org/10.1146/annurev-arplant-050718-100049

Gupta A., Mishra G., Yadav H., Gupta R., Singh A., Singh J., and Singh P., 2023, Enhancing crop resilience through CRISPR/Cas9-mediated development of disease-resistant cultivars, International Journal of Environment and Climate Change, 13(10): 2773-2783.
https://doi.org/10.9734/ijecc/2023/v13i102942

Langner T., Kamoun S., and Belhaj K., 2018, CRISPR crops: plant genome editing toward disease resistance, Annual Review of Phytopathology, 56: 479-512.
https://doi.org/10.1146/annurev-phyto-080417-050158

Li C., Brant E., Budak H., and Zhang B., 2021, CRISPR/Cas: a Nobel Prize award-winning precise genome editing technology for gene therapy and crop improvement, Journal of Zhejiang University-Science B, 22: 253-284.
https://doi.org/10.1631/jzus.B2100009

Li H., Song K., Li B., Zhang X., Wang D., Dong S., and Yang L., 2023, CRISPR/Cas9 editing sites identification and multi-elements association analysis in *Camellia sinensis*, Int. J. Mol. Sci., 24(20): 15317.
https://doi.org/10.3390/ijms242015317

Liu Q., Yang F., Zhang J., Liu H., Rahman S., Islam S., Ma W., and She M., 2021, Application of CRISPR/Cas9 in crop quality improvement, International Journal of Molecular Sciences, 22(8): 4206.
https://doi.org/10.3390/ijms22084206

Liu Z., Wu T., Xiang G., Wang H., Wang B., Feng Z., Mu Y., and Li K., 2022, Enhancing animal disease resistance, production efficiency, and welfare through precise genome editing, International Journal of Molecular Sciences, 23(13): 7331.
https://doi.org/10.3390/ijms23137331

Lyzenga W., Pozniak C., and Kagale S., 2021, Advanced domestication: harnessing the precision of gene editing in crop breeding, Plant Biotechnology Journal, 19: 660-670.
https://doi.org/10.1111/pbi.13576

Nerkar G., Devarumath S., Purankar M., Kumar A., Valarmathi R., Devarumath R., and Appunu C., 2022, Advances in crop breeding through precision genome editing, Frontiers in Genetics, 13: 880-195.
https://doi.org/10.3389/fgene.2022.880195

Nidhi S., Anand U., Oleksák P., Tripathi P., Lal J., Thomas G., Kuča K., and Tripathi V., 2021, Novel CRISPR–Cas systems: an updated review of the current achievements, applications, and future research perspectives, International Journal of Molecular Sciences, 22(7): 3327.
https://doi.org/10.3390/ijms22073327

Paul N., Park S., Liu H., Choi S., Ma J., MacCready J., Chilvers M., and Sang H., 2021, Plant and fungal genome editing to enhance plant disease resistance using the CRISPR/Cas9 system, Frontiers in Plant Science, 12: 700925.
https://doi.org/10.3389/fpls.2021.700925

Rönspies M., Schindele P., and Puchta H., 2020, CRISPR/Cas-mediated chromosome engineering: opening up a new avenue for plant breeding, Journal of Experimental Botany, 72(2): 177-183.
https://doi.org/10.1093/jxb/eraa463

Sampath V., Rangarajan N., Sharanappa C.H., Deori M., Veeraragavan M., Ghodake B., and Kaushal K., 2023, Advancing crop improvement through CRISPR technology in precision agriculture trends-a review, International Journal of Environment and Climate Change, 13(11): 4683-4694.
https://doi.org/10.9734/ijecc/2023/v13i113647

Schindele P., Wolter F., and Puchta H., 2018, Transforming plant biology and breeding with CRISPR/Cas9, Cas12 and Cas13, FEBS Letters, 592(12): 1954-1967.
https://doi.org/10.1002/1873-3468.13073

Sun L., Ke F., Nie Z., Wang P., and Xu J., 2019, Citrus genetic engineering for disease resistance: past, present and future, International Journal of Molecular Sciences, 20(21): 5256.
https://doi.org/10.3390/ijms20215256

Tang Q., Wang X., Jin X., Peng J., Zhang H., and Wang Y., 2023, CRISPR/Cas technology revolutionizes crop breeding, Plants, 12(17): 3119.
https://doi.org/10.3390/plants12173119

Tripathi L., Ntui V., and Tripathi J., 2020, CRISPR/Cas9-based genome editing of banana for disease resistance, Current Opinion in Plant Biology, 56: 118-126.
https://doi.org/10.1016/j.pbi.2020.05.003

Veillet F., Durand M., Kroj T., Cesari S., and Gallois J., 2020, Precision breeding made real with CRISPR: illustration through genetic resistance to pathogens, Plant Communications, 1(5): 100102.
https://doi.org/10.1016/j.xplc.2020.100102

Wang Y., Zafar N., Ali Q., Manghwar H., Wang G., Yu L., Ding X., Ding F., Hong N., Wang G., and Jin S., 2022, CRISPR/Cas genome editing technologies for plant improvement against biotic and abiotic stresses: advances, limitations, and future perspectives, Cells, 11(23): 3928.
https://doi.org/10.3390/cells11233928

Zaidi S., Mahas A., Vanderschuren H., and Mahfouz M., 2020, Engineering crops of the future: CRISPR approaches to develop climate-resilient and disease-resistant plants, Genome Biology, 21: 289.
https://doi.org/10.1186/s13059-020-02204-y

Zhang Y., Malzahn A., Sretenovic S., and Qi Y., 2019, The emerging and uncultivated potential of CRISPR technology in plant science, Nature Plants, 5: 778-794.
https://doi.org/10.1038/s41477-019-0461-5

Zhu H., Li C., and Gao C., 2020, Applications of CRISPR-Cas in agriculture and plant biotechnology, Nature Reviews Molecular Cell Biology, 21: 661-677.
https://doi.org/10.1038/s41580-020-00288-9