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NEW PLANT BREEDING TECHNIQUES TO BOOST RESILIENCE OF FOOD SYSTEM

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SUMMARY

Plant breeding has advanced significantly with the advent of new techniques that boost the resilience of food systems. Modern approaches, such as CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9, RNA (ribonucleic acid) interference, and genome-wide association studies (GWAS), have revolutionized the ability to enhance crop resilience against biotic and abiotic stresses. These technologies enable precise and targeted genetic modifications, facilitating the development of crops that can withstand extreme weather conditions, pests, and diseases. Additionally, novel breeding methods contribute to improved nutritional quality and yield stability, essential for food security against climate change. The integration of high-throughput phenotyping and bioinformatics accelerates the identification and incorporation of desirable traits, ensuring rapid progress in crop improvement. These advancements support sustainable agricultural practices as well as reduce reliance on chemical inputs, promoting environmental health. By fostering genetic diversity and enhancing adaptive capacity, new plant breeding techniques play a crucial role in building resilient food systems capable of enduring and thriving under future challenges.

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Keywords: CRISPR/Cas9, genome editing, climate resilience, drought tolerance, disease resistance

Key findings: New breeding techniques (NBTs), such as CRISPR-Cas9 and genomic selection, have revolutionized plant breeding by enabling precise genetic modifications and accelerating the development of robust crop varieties. These techniques enhance crop resilience to environmental stresses, pests, and diseases, significantly contributing to food security. The integration of NBTs with traditional breeding methods offers a comprehensive approach to developing sustainable and resilient food systems, ensuring stable food supplies to combat climate change and other agricultural challenges.

INTRODUCTION

Food security and resilience are critical challenges in the 21st century, driven by a rapidly growing global population, climate change, and the depletion of natural resources (Mbow *et al.,* 2020). Ensuring everyone has access to sufficient, safe, and nutritious food is essential for maintaining health and well-being, as well as for social and economic stability. The resilience of food systems is equally important, as they can withstand and recover from various shocks and stresses, such as extreme weather events, pest outbreaks, and economic fluctuations. In this context, plant breeding plays a pivotal role in enhancing food security and resilience by developing crop varieties that are more productive, nutritious, and resistant to biotic and abiotic stresses (Ngongolo and Mmbando, 2024).

The traditional methods of plant breeding, which have been applicable for thousands of years, involve selecting and crossbreeding plants with desirable traits. While these methods have been successful in improving crop yields and quality, they have significant limitations, including long development times and limited genetic diversity. New plant breeding techniques (NPBTs) have emerged as powerful tools for crop improvement to address such challenges. NPBTs, such as CRISPR-Cas9, transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and oligonucleotide-directed mutagenesis (ODM), allow for precise and targeted modifications of plant genomes, enabling the introduction of beneficial traits more quickly and accurately than traditional methods (Ahmad *et al.,* 2024).

This document aims to provide a comprehensive overview of NPBTs and their potential to boost food system resilience. It explores the historical context of plant breeding, mechanisms and applications of various NPBTs, and their specific contributions to crop improvement. Additionally, the document will examine case studies of successful NPBT applications, discuss regulatory and public perception issues, and highlight the challenges and future directions in the field. By offering a detailed analysis of NPBTs, this document seeks to underscore their importance in ensuring food security and resilience amidst growing global challenges.

Historical context of plant breeding

Traditional plant breeding methods, which have been operational for millennia, involve the selection and crossbreeding of plants with desirable traits to produce improved offspring (Table 1). This process relies on the natural genetic variation within a species, which is often lengthy and iterative. Breeders select parent plants with specific traits, such as disease resistance, higher yield, or improved nutritional content, and cross them to produce new varieties that combine these traits (Bradshaw, 2017). This method has been instrumental in developing numerous crops, forming the basis of modern agriculture, and contributing significantly to food production and security.

New plant breeding techniques overview

New plant breeding techniques (NPBTs) encompass a range of advanced methods that

Table 1. History of plant breeding and genetics.

Period/Year	Event	Significance
8000 BCE	Domestication of plants	Beginning of agriculture, selection of wild plants with desirable traits
1600s	First hybridization	Early hybridization techniques such as grafting in China
1865s	Mendel's laws of inheritance	Modern genetics through pea plant experiments
1940s-1960s	Green revolution	Development of high-yielding crop varieties
1980s	Genetic engineering	Direct manipulation of plant genetic material
2000s	GMOs	GMOs with traits like pest resistance
2020s	CRISPR and new breeding techniques	Precise genome editing for traits like climate resilience

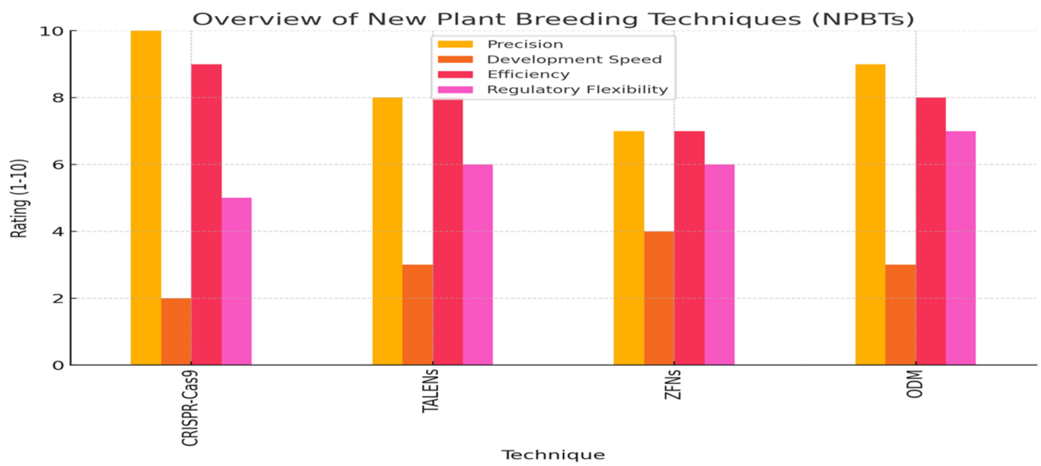

Figure 1. Overview of new plant breeding techniques (NPBTs), including CRISPR-Cas9, TALENs, ZFNs, and ODM. The chart compares these techniques for precision, development speed, efficiency, and regulatory flexibility.

enable precise and targeted modifications to plant genomes. Unlike traditional breeding methods, which rely on crossbreeding and selection based on natural genetic variation, NPBTs utilize molecular biology tools directly to alter DNA sequences. It enabled the introduction, deletion, or modification of specific genes with accuracy. NPBTs' classification can depend on their mechanisms of action based on genome editing techniques, including CRISPR-Cas9, TALENs, and ZFNs, as well as targeted mutagenesis methods like ODM (Figure 1). These techniques represent a significant advancement in plant breeding, offering the potential to develop crop varieties with improved traits more quickly and accurately.

Key NPBTs: CRISPR-Cas9, TALENs, ZFNs, ODM

Among the most prominent NPBTs is CRISPR-Cas9, a revolutionary genome-editing tool that uses a guide RNA to direct the Cas9 enzyme to a specific DNA sequence, where it introduces a double-strand break. It allows for precise genetic modifications, as the cell's natural repair mechanisms introduce mutations or particular changes at the target site. Another key NPBT is TALENs, which employ custom-designed proteins to bind specific DNA sequences and create double-strand breaks, facilitating targeted genetic modifications (Valavanidis, 2016). Similarly, ZFNs use engineered zinc finger proteins to recognize and bind to specific DNA sequences, enabling precise genome editing. The ODM involves the introduction of synthetic oligonucleotides to direct certain nucleotide changes in the plant genome.

CRISPR-Cas9 technology

CRISPR-Cas9 is a ground-breaking genome-editing tool providing precise and targeted modifications to the DNA of living organisms, including plants (Mushtaq *et al.,* 2021). The system comprises a guide RNA (gRNA) that directs the Cas9 enzyme to a specific DNA sequence, where Cas9 creates a double-strand break. This break becomes repaired by the cell's natural mechanisms, either through non-homologous end joining (NHEJ) or homology-directed repair (HDR), resulting in targeted genetic changes. The CRISPR-Cas9 has revolutionized plant breeding by enabling the rapid development of crops with improved traits, such as enhanced disease resistance, increased yield, improved nutritional content, and greater tolerance to environmental stresses (Borrelli *et al.,* 2018; Farooq, 2024). Its precision, efficiency, and versatility make it a powerful tool for addressing the challenges of modern agriculture. Although, it also presents regulatory, technical, and ethical challenges needing careful consideration.

Mechanism of action

CRISPR-Cas9 is a powerful genome-editing tool allowing precise and targeted modifications of DNA. The system consists of two main components: gRNA and the Cas9 enzyme. The gRNA design helps match a specific DNA sequence within the genome. When introduced into a cell, the gRNA directs the Cas9 enzyme to this target sequence. Cas9 then introduces a double-strand break at the specified location in the DNA. The cell's natural repair mechanisms respond to this break in one of two ways: 1) the NHEJ, which often results in random mutations, or 2) HDR, which can introduce specific genetic changes if providing a repair template. This precise targeting and cutting mechanism make CRISPR-Cas9 an invaluable tool for genome editing in plants and other organisms (Singh *et al.,* 2024).

Applications in plant breeding

CRISPR-Cas9 has been widely beneficial in plant breeding for efficient and accurate improvement of crop traits. Its applications span a broad range of objectives, including enhancing resistance to diseases, improving yield and quality traits, and increasing tolerance to abiotic stresses, such as drought and salinity. For example, CRISPR-Cas9 can serve to knock out genes that make plants susceptible to certain diseases or pests, thereby creating resistant varieties (Borrelli *et al.,* 2018). It can also be effective in modifying metabolic pathways to enhance the nutritional content of crops or alter growth characteristics to raise yield. The versatility and precision of CRISPR-Cas9 make it a game-changer in the development of new plant varieties that can meet the challenges of modern agriculture.

Case studies of successful CRISPR-Cas9 applications

One notable example of CRISPR-Cas9's success in plant breeding is the development of rice varieties highly resistant to bacterial blight, a serious disease affecting rice production worldwide (Mishra *et al.,* 2023). By targeting and knocking out specific susceptibility genes in the rice genome, researchers created cultivars that exhibit significant resistance to this disease, thereby improving yield and reducing the need for chemical treatments. Another successful application is the modification of wheat to increase its resistance to powdery mildew. By editing the *Mildew Locus O.* (*MLO*) gene, which has susceptibility relations to this disease, scientists have developed more resilient wheat varieties to powdery mildew attacks. Additionally, CRISPR-Cas9 has been favorably enhancing the nutritional content of crops, such as increasing the beta-carotene levels in rice to combat vitamin A deficiency in developing countries.

Transcription activator-like effector nucleases

The TALENs are a sophisticated genome-editing technology that enables precise modifications of specific DNA sequences in living organisms, including plants. TALENs work by using engineered proteins called transcription activator-like effectors (TALEs) to bind to particular DNA sequences, which are then fused to a nuclease, typically FokI, to introduce double-strand breaks at the targeted sites. These breaks are repaired by the cell's natural mechanisms, leading to targeted genetic changes. TALENs have been effectively helping plant breeding develop crops with desirable traits, such as enhanced disease resistance, improved yield, and better nutritional content (Tyumentseva *et al.,* 2023).

TALENs technology

The TALENs are an advanced genome-editing technology providing precise genetic modifications by targeting specific DNA sequences. TALENs use engineered proteins called TALEs to bind to designated DNA sequences, which then connect to a nuclease, typically FokI, to create double-strand breaks at these sites (Tyumentseva *et al.,* 2023). These breaks become subsequently repaired by the cell's natural mechanisms, resulting in targeted genetic changes. TALENs have been successfully applied in plant breeding to enhance traits, such as disease resistance, yield, and nutritional quality. For example, they have been functional in developing rice varieties resistant to bacterial blight and soybeans with higher oleic acid content (Tyumentseva *et al.,* 2023). While TALENs offer significant benefits for improving crop traits, they also face challenges, including regulatory and public acceptance issues and the need to ensure precision to minimize off-target effects. Despite these challenges, TALENs represent a powerful tool for advancing agricultural biotechnology and creating more resilient and productive crops.

Mechanism of action

TALENs, a genome-editing tool, allow precise modifications to particular DNA sequences. The technology utilizes TALEs, which are proteins engineered to bind to specific sequences of DNA (Teper *et al.,* 2023). These TALEs become fused to a nuclease, typically FokI, which introduces a double-strand break in the DNA at the targeted location. Then, the cell repairs this breakthrough natural mechanism, such as NHEJ or HDR. The ability of TALENs to create targeted double-strand breaks enables accurate genetic modifications, making them a valuable tool for plant breeding and genetic research.

Applications in plant breeding

TALENs have their wide adoption in plant breeding to introduce desirable traits with high precision. They help develop crops with enhanced resistance to diseases, improved yield, and better nutritional quality (Nerkar *et al.,* 2022). For example, TALENs can knock out genes conferring susceptibility to diseases, thus creating disease-resistant varieties. They can also be useful to modify genes involved in metabolic pathways to increase the nutritional content of crops, such as enhancing vitamin or protein levels.

Case studies of successful TALENs applications

Several successful applications of TALENs in plant breeding highlight their potential. One notable example is the development of disease-resistant rice varieties (Oliva *et al.*, 2024). Researchers used TALENs to target and disrupt the susceptibility gene *OsSWEET14*, resulting in rice plants with increased resistance to bacterial blight, a major threat to rice production. Another example is the use of TALENs to improve the oil content of soybeans. By editing the *FAD2* gene, involved in fatty acid biosynthesis, scientists have created soybean varieties with higher oleic acid content, offering health benefits and improved oil stability. Additionally, TALENs have been employed to develop tomato plants with enhanced tolerance to high temperatures by targeting the *SLN1* gene, thereby improving fruit set and yield under heat stress conditions (Singh *et al.,* 2024).

Zinc finger nucleases

The ZFNs are a remarkable genome-editing technology, enabling precise alterations of specific DNA sequences within the genomes of living organisms (Singh *et al.,* 2024). The ZFNs comprised two main parts: a DNA-binding domain made up of engineered zinc finger proteins that recognize and bind to specific DNA sequences, and a nuclease domain, typically derived from the FokI enzyme, that introduces double-strand breaks at these target sites. The cell's natural repair mechanisms then fix these breaks, either through NHEJ or HDR, leading to targeted genetic modifications. The use of ZFNs has been progressive in plant breeding to develop crops with improved traits, such as enhanced resistance to diseases, increased yield, and better tolerance to environmental stresses (Singh *et al.,* 2024). For example, ZFNs have been successful in creating herbicide-resistant maize and disease-resistant wheat.

Mechanism of action

The ZFNs are a genome-editing technique that ensures accurate modifications to specific DNA sequences within an organism's genome (Ahmad *et al.,* 2024). The ZFNs consist of two main components: a DNA-binding domain composed of engineered zinc finger proteins that recognize particular DNA sequences and a nuclease domain, typically derived from the FokI enzyme, which introduces a double-strand break at the target site. Each zinc finger protein's design helps in binding to a unique triplet of nucleotides, and linking multiple zinc fingers together can help recognize longer DNA sequences. The nuclease domain is only active when it dimerizes, which ensures the cutting of DNA at the precise target site. Once the double-strand break appears, the cell's natural repair mechanisms, such as NHEJ or HDR, fix the break, allowing the insertion, deletion, or modification of genes at the target location (Singh *et al.,* 2024).

Case studies of successful ZFNs applications

One notable application of ZFNs in plant breeding is the development of herbicide-resistant maize. Researchers used ZFNs to target and modify the acetolactate synthase (ALS) gene, which is involved in the synthesis of amino acids and is a common target for herbicides (Bhuyan *et al.,* 2023). By introducing specific mutations into the ALS gene, scientists created maize plants that are resistant to certain herbicides, allowing for more effective weed management (Table 2). Another successful application is the use of ZFNs to create disease-resistant wheat. In this case, ZFNs were used to knock out the MLO gene, which is associated with susceptibility to powdery mildew. The resulting wheat plants exhibited increased resistance to this fungal disease, reducing the need for chemical fungicides.

Oligonucleotide-directed mutagenesis

The ODM is a distinct genome-editing technique that uses synthetic oligonucleotides to introduce specific mutations into the DNA sequence of an organism (Mishra *et al.,* 2023). These oligonucleotides are short, single-stranded pieces of DNA or RNA designed to be nearly identical to the target sequence, except for the desired mutation. When introduced into the cell, the oligonucleotide binds to the target DNA, and the cell's natural repair mechanisms recognize the mismatch, incorporating the desired mutation during the repair process. This method allows for targeted genetic modifications without introducing foreign DNA (Mishra *et al.,* 2023). The ODM has been benefitting plant breeding to create crops with desirable traits, such as herbicide resistance, improved yield, and enhanced nutritional content.

Table 2. ZFNs role in advancing genome editing across various organisms and applications.

Application	Organism/Cell Type	Description	Reference
Gene disruption	*Drosophila melanogaster* (fruit fly)	Targeted mutations	Carroll (2011)
Gene knockout models	Rats (*Rattus norvegicus*)	Gene function and human diseases	Carroll (2011)
HIV resistance	Human CD4⁺ T cells	ZFNs were employed to disrupt the *CCR5* gene in human T cells	Wang and Cannon (2016)
Crop trait improvement	Maize (*Zea mays*)	ZFNs enabled precise genome modifications in maize	Carroll (2011)
Disease modeling	Human-induced Pluripotent Stem Cells (hiPSCs)	ZFNs were utilized to introduce specific mutations into hiPSCs	Carroll (2011)
Functional genomics	Zebrafish (*Danio rerio*)	ZFNs were applied to disrupt specific genes in zebrafish	Zhu *et al.* (2011)
Gene therapy research	Human cells	ZFNs have been explored as tools for gene therapy	Carroll (2011)
Transgenic animal creation	Mice (*Mus musculus*)	ZFNs were used to create transgenic mice with specific gene alterations	Carroll (2011)
Biotechnological applications	Various plant species	ZFNs have been employed to modify plant genomes	Carroll (2011)
Therapeutic development	Human Hematopoietic Stem/Progenitor Cells	ZFNs have been used to edit genes in human stem cells	Wang and Cannon (2016)

Mechanism of action

The ODM, a precise genome-editing technique, involves the use of synthetic oligonucleotides to introduce specific mutations in an organism's DNA sequence. These synthetic oligonucleotides are short, single-stranded pieces of DNA or RNA designed to complement the target sequence, except for the desired mutation (Mishra *et al.,* 2023). Upon introduction into the cell, the oligonucleotide attaches to the target DNA sequence through base pairing. The cell's natural repair mechanisms recognize the mismatch between the oligonucleotide and the genomic DNA, leading to the incorporation of a desired mutation during the repair process. This method enables the precise editing of specific nucleotides without introducing foreign DNA, making it a highly targeted and efficient approach to genome editing.

Applications in plant breeding

The ODM application in plant breeding has helped create crops with desirable traits by introducing specific and targeted genetic changes. This technique can be beneficial to develop herbicide-resistant plants, improve crop yield, and enhance resistance to diseases and environmental stresses. For instance, ODM can introduce mutations that confer resistance to particular herbicides, allowing farmers to manage weeds more effectively without harming the crop (Mishra *et al.,* 2023). Additionally, ODM can be effective in enriching nutritional qualities by modifying genes involved in the biosynthesis of vitamins, minerals, and other essential nutrients. The precision of ODM allows for the development of improved crop varieties while maintaining the integrity of the plant's genome, which can be advantageous for regulatory approval and public acceptance.

Case studies of successful ODM applications

One successful application of ODM is the creation of herbicide-resistant oilseed rape (canola). Researchers used ODM to introduce a specific point mutation in the *ALS* gene, which confers resistance to ALS-inhibiting herbicides (Yadav *et al.,* 2023). This modification enables farmers to control weeds more effectively, leading to increased crop productivity. Another example is the use of ODM to improve the nutritional content of wheat by altering the genes involved in the biosynthesis of essential amino acids, resulting in wheat varieties with enhanced nutritional profiles. These case studies demonstrate the potential of ODM to address key agricultural challenges and improve crop performance through precise genetic modifications.

Applications of NPBTs in crop improvement

The applications of NPBTs in crop improvement are vast and transformative, offering precise and efficient methods to enhance crop traits. The NPBTs, such as CRISPR-Cas9, TALENs, ZFNs, and ODM, enable targeted genetic modifications, significantly improving crop yield, quality, and resilience. These technologies have been aiding in developing disease-resistant varieties, reducing the need for chemical pesticides, and increasing agricultural sustainability. They also enable the creation of crops with improved tolerance to abiotic stresses, such as drought, salinity, and extreme temperatures, which is crucial in battling climate change. Furthermore, NPBTs have been suitable to enrich the nutritional content of crops, such as increasing the levels of essential vitamins and minerals and addressing malnutrition and health issues in developing countries (Singh et al., 2024). The ability to introduce specific traits rapidly and accurately makes NPBTs invaluable for addressing global food security challenges, ensuring a stable and nutritious food supply in a changing world. Figure 2 depicts the utilization of NPBTs for augmenting crop development.

Enhancing yield and productivity

The NPBTs, such as CRISPR-Cas9, TALENs, ZFNs, and ODM, offer unparalleled accuracy in editing the genomes of crops to enhance yield and productivity (Mbinda, 2024). By targeting specific genes that regulate plant growth and development, these techniques can increase photosynthetic efficiency, optimize resource allocation, and boost reproductive traits. For example, CRISPR-Cas9 has been effective in knocking out genes that limit the number of flowers or seeds a plant can produce, resulting in higher yields (Sojka et al., 2024). TALENs and ZFNs can similarly modify genes involved in growth hormone pathways to produce larger and more robust plants. These modifications increase the amount of food produced per hectare and improve the efficiency of resource use, contributing to more sustainable agricultural practices.

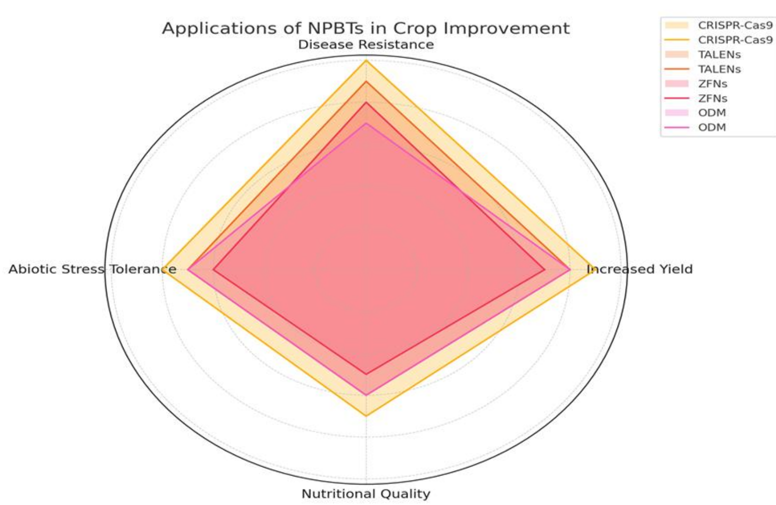

Figure 2. Applications of new plant breeding techniques (NPBTs) in crop improvement. It compares CRISPR-Cas9, TALENs, ZFNs, and ODM for their effectiveness in increasing yield, disease resistance, abiotic stress tolerance, and nutritional quality.

Improving resistance to pests and diseases

The NPBTs are instrumental in developing crops with resistance to pests and diseases, reducing the need for chemical pesticides and mitigating crop losses. Techniques, viz., CRISPR-Cas9, allow a distinct deletion or alteration of genes that confer susceptibility to diseases (Asif *et al.,* 2024). For instance, scientists have used CRISPR-Cas9 to edit the genome of rice to bestow resistance to bacterial blight by disabling specific susceptibility genes. Similarly, TALENs have been able to develop tomato plants resilient to bacterial spot disease. These genetic modifications enhance the plant's innate immune system, enabling it to fend off pathogens more effectively.

Developing tolerance to abiotic stresses

Abiotic stresses, such as drought, salinity, and extreme temperatures, pose significant challenges to agricultural productivity. The NPBTs enable the development of crops that can withstand these harsh conditions by targeting genes involved in stress response pathways (Saeed *et al.,* 2024). For example, CRISPR-Cas9 has been beneficial in enhancing drought tolerance in maize by editing genes associated with water-use efficiency and root architecture (Saeed *et al.,* 2024). Similarly, researchers have used TALENs to develop wheat varieties with improved salt tolerance by modifying genes involved in ion transport and osmotic balance.

Enhancing nutritional quality and shelf-life

Improving the nutritional quality and shelf life of crops is another critical application of NPBTs. Techniques, i.e., CRISPR-Cas9 and ODM, can be useful in raising the levels of essential vitamins, minerals, and other nutrients in crops (Saeed *et al.,* 2024). For instance, the use of CRISPR-Cas9 has been increasing the beta-carotene content in rice, commonly known as "Golden Rice," to address vitamin A deficiency in developing countries (Satyanarayana, 2024). Similarly, NPBTs can be effective in boosting the protein content in staple crops like maize and wheat, providing better nutritional value. In addition to nutritional enhancements, NPBTs can improve the shelf life of fruits and vegetables by targeting genes responsible for ripening and spoilage. For example, CRISPR-Cas9 has helped effectively delay the ripening process in tomatoes by editing genes involved in ethylene production, extending their storage life and reducing food waste (Nie et al., 2024).

Case studies of NPBTs in crop resilience

Case studies of NPBTs in crop resilience highlight their transformative impact on agriculture. For example, CRISPR-Cas9 has been helpful in developing rice varieties with strengthened resistance to bacterial blight by targeting and disabling specific susceptibility genes, significantly reducing crop losses and the need for chemical treatments (Tao *et al.,* 2021). Another case involves the use of TALENs to produce wheat varieties resistant to powdery mildew by editing the *MLO* gene, which confers vulnerability to the disease. This genetic modification has resulted in wheat crops that are more robust and require fewer fungicides. Additionally, researchers have employed ZFNs to create herbicide-resistant maize by introducing mutations in the *ALS* gene, enabling farmers to control weeds more effectively without harming the crop. These case studies demonstrate how NPBTs can heighten crop resilience to biotic and abiotic stresses, contributing to more sustainable agricultural practices and improved food security.

Detailed analysis of specific crops improved through NPBTs

The application of NPBTs has notably improved various crops, intensifying their resilience and productivity. For instance, CRISPR-Cas9 has been benefitting the development of rice varieties with enhanced resistance to bacterial blight by targeting and disabling susceptibility

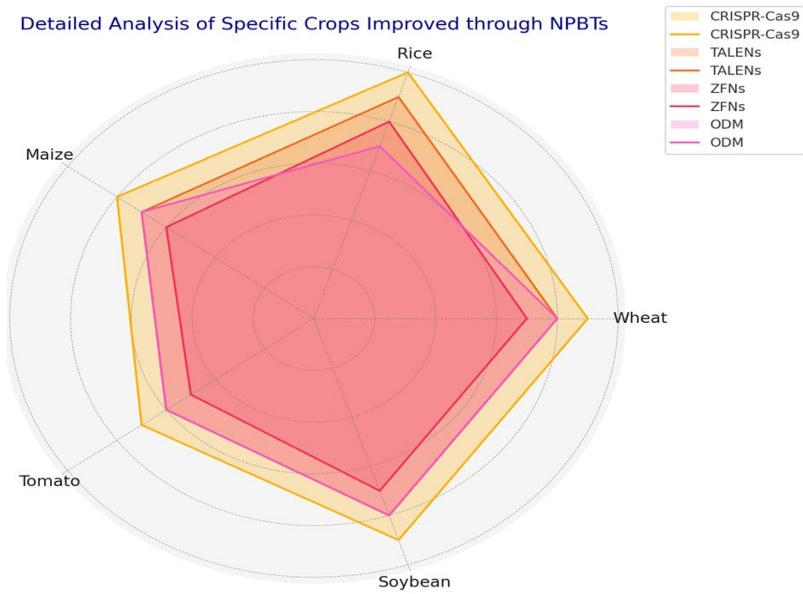

Figure 3. Radar graph providing a detailed analysis of specific crops improved through new plant breeding techniques (NPBTs). The graph compares the effectiveness of CRISPR-Cas9, TALENs, ZFNs, and ODM in improving wheat, rice, maize, tomato, and soybean.

genes, such as *OsSWEET14*, resulting in higher yields and reduced reliance on chemical pesticides (Figure 3). The TALENs have been functional in producing wheat varieties resistant to powdery mildew by editing the *MLO* gene, which has led to more stable and increased yields with less need for fungicides (Wang *et al.,* 2014). Additionally, ZFNs have been desirable in creating herbicide-resistant maize by introducing particular mutations in the *ALS* gene, allowing for more effective weed control and improved crop management. These genetic advancements boost food security by ensuring more stable crop yields, as well as promote sustainable agricultural practices by lowering the dependency on chemical inputs and minimizing environmental pollution (Wang *et al.,* 2014).

Rice: enhanced resistance to bacterial blight

Using CRISPR-Cas9, scientists have developed rice varieties with enhanced resistance to bacterial blight, a critical disease that drastically reduces yield (Zhou *et al.,* 2022). By targeting and disabling susceptibility genes, such as *OsSWEET14*, researchers have created rice plants that are less prone to infection. This genetic improvement ensures higher yields and also lessens the dependency on chemical pesticides, promoting a healthier environment.

Wheat: resistance to powdery mildew

TALENs' application has been helping produce wheat varieties resistant to powdery mildew, a fungal disease that affects wheat crops worldwide (Aroge *et al.,* 2024). By editing the *MLO* gene, researchers have developed wheat plants that can withstand this disease, leading to more stable and increased yields. This advancement reduces the need for fungicides, lowering production costs and minimizing environmental pollution.

Maize herbicide resistance

The use of ZFNs has been effective in creating maize varieties resistant to ALS-inhibiting herbicides. By introducing specific mutations in the *ALS* gene, scientists have produced maize plants that can survive herbicide application, allowing farmers to control weeds more effectively. This trait improves crop management and productivity, contributing to more efficient agricultural practices.

Impact on food security and resilience

The improvements in these crops through NPBTs have a profound impact on food security and resilience. Enhanced disease resistance in rice and wheat ensures more stable yields, reducing the risk of crop failure and increasing food availability. Herbicide-resistant maize provides more effective weed control, leading to higher productivity and lower production costs. These advancements contribute to a more resilient agricultural system, capable of withstanding various biotic and abiotic stresses, thereby ensuring a stable food supply in the face of climate change and other challenges.

Emerging technologies and advancements

Emerging technologies and advancements in the field of NPBTs promise to further boost the precision, efficiency, and scope of crop genetic improvement. One such advancement is the development of CRISPR-Cas variants with improved specificity and reduced off-target effects. Innovations, such as CRISPR-Cas12 and CRISPR-Cas13, expand the toolbox for genome editing by targeting different types of genetic material, including RNA (Nie *et al.*, 2024). Additionally, progress in base-editing and prime-editing techniques allows for more precise modifications at the nucleotide level, enabling targeted changes without introducing double-strand breaks in the DNA. These technologies will minimize unintended mutations and improve the overall safety and efficacy of NPBTs.

Integration of NPBTs with other biotechnological approaches

Integrating NPBTs with other biotechnological approaches can remarkably enhance their capabilities and applications. For instance, combining genome editing with synthetic biology can enable the design of crops with complex, multi-gene traits tailored to specific agricultural needs. Synthetic biology approaches can be beneficial in constructing novel biosynthetic pathways and improving traits, such as nutritional content, stress tolerance, and disease resistance. Machine learning and artificial intelligence can further optimize NPBT applications by analyzing large datasets to predict gene function and identify optimal target sites for editing. Moreover, evolutions in high-throughput phenotyping and omics technologies (genomics, transcriptomics, proteomics, and metabolomics) can provide comprehensive insights into plant responses to genetic modifications, facilitating the development of more resilient and productive crops.

Future research directions

Future research in plant breeding will focus on integrating advanced genome editing tools, such as CRISPR/Cas9, ZFNs, and TALENs, to develop crops with enhanced resilience to environmental stresses (drought and salinity), pests, and diseases, while ensuring greater precision and reduced off-target effects. A significant emphasis will center on developing climate-resilient crops by identifying and incorporating genes responsible for stress tolerance, supported by high-throughput phenotyping technologies for accurate trait selection. Omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, will deepen the understanding of complex plant stress responses, enabling more targeted breeding strategies. Advanced techniques like marker-assisted selection (MAS) and genomic selection (GS) will streamline the breeding process. Meanwhile, speed breeding methods will shorten crop development cycles.

Future prospects

The prospects of plant breeding techniques hold immense potential to revolutionize global food systems by promoting sustainable agricultural practices. Enhanced breeding strategies will have crops that require fewer inputs, such as water, fertilizers, and pesticides, reducing the environmental footprint of agriculture. Climate-resilient crops will play a critical role in ensuring global food security, especially in regions vulnerable to climate change, by thriving under adverse conditions and stabilizing food production. Efforts to diversify crops will promote the use of underutilized but nutrient-rich species, broadening the agricultural base and improving ecosystem resilience. Future breeding programs will also prioritize consumer preferences, enriching traits such as flavor, nutritional value, and shelf life to meet market demands.

CONCLUSIONS

NPBTs, such as CRISPR-Cas9, TALENs, ZFNs, and ODM, revolutionize crop improvement by enabling precise genetic modifications for enhanced yield, disease resistance, stress tolerance, and nutritional quality. These techniques help stabilize yields, reduce chemical inputs, and promote sustainable agriculture. Despite varying global regulations and public perception challenges, NPBTs are vital for ensuring food security amid climate change and population growth. Collaborative efforts and regulatory harmonization are crucial factors in addressing ethical and societal concerns in fostering trust and accessibility. The future of plant breeding lies in integrating NPBTs with other biotechnological innovations to create resilient, nutritious crops.

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