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CRISPR-Gene Editing in Crops

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CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a tool for editing genes that lets you change DNA in plants very accurately. It was based on a natural way that bacteria protect themselves. CRISPR uses a guide RNA (gRNA) to find a specific DNA sequence and a Cas9 enzyme to cut the DNA at that spot. After that, the cell fixes the cut, which can add, remove, or replace genes. This system has been used to get new germplasma resources through gene-directed mutation because it is easy to use and works well at causing mutations. CRISPR-Cas9 editing can quickly create new germplasm resources to improve important agronomic traits because whole-genome sequencing data and information about gene function for important traits are now available. CRISPR is changing the way we improve crops by making it possible to make precise, quick, and long-lasting changes to their genes to solve problems with food security, climate change, and nutrition. We look at this technology and how it can be used on fruit and vegetable crops in this review. We talk about the problems, the different types that are already out there, the rules that go along with them, and what they could be used for in the future.

Introduction

The world's agricultural systems are under a lot of stress because the population is growing and people need more food. This problem is made worse by climate change, which is causing more extreme weather, changes in the way pests and diseases spread, and less land that can be used for farming (Bibi and Rahman, 2023). These changes put crops at risk and make farming less stable, which makes it harder to make sure that everyone has enough food. While traditional breeding methods have greatly improved agriculture in the past, they are often too slow to keep up with these rapid changes in the environment. Genetic engineering, on the other hand, has had problems with accuracy and public acceptance (Afzal et al., 2023; Ambika et al., 2024). Rice, wheat, maize, and soybeans are staple crops that are the basis of global food security. They are the main source of calories for a large part of the world's population (Morrow et al., 2023). These crops are very important for people to eat, but they are also very important for feeding animals and for use in industry. Climate change, pests, and diseases are making these important crops less productive and less able to withstand stress. To make sure there is enough food for everyone, it is important to improve the yield, nutritional value, and stress tolerance of staple crops. This is especially true as the world's population grows and arable land becomes less available. The development of agricultural technology from selective breeding to advanced genetic tools shows that we are still working to solve these problems. CRISPR/Cas technology has changed the game in agricultural biotechnology by being a revolutionary tool for editing genomes. CRISPR/Cas systems are a revolutionary tool for targeted genome editing that has changed both basic and applied research in agriculture. The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) mechanism was first found in the adaptive immune systems of bacteria and archaea. It uses a guide RNA (gRNA) to direct the Cas (CRISPR-associated) nuclease to a specific DNA sequence, where it makes a precise double-strand break. The cell's natural DNA repair mechanisms then fix this break, which lets scientists make specific changes to the genome.

CRISPR/Cas systems are easier to design, work better, and cost less than earlier genome editing tools like Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs). This makes them very useful for a wide range of crop improvement tasks. Finding the CRISPR/Cas system as a genome editing tool was not only about finding it in bacteria, but also about figuring out how to use it and improve it for use in more complicated organisms. Early studies elucidated the essential functions of crRNA (CRISPR RNA) and tracrRNA (trans-activating crRNA) in directing the Cas9 protein for accurate DNA cleavage, which was instrumental in transforming CRISPR/Cas into a multifaceted genome editing tool (Jung et al., 2024). The process starts with the guide RNA binding to the target DNA sequence to make an RNA-DNA hybrid. This directs the Cas9 protein to the right part of the genome. When Cas9 gets there, it makes a double-strand break, which the cell's natural DNA repair pathways fix. These pathways are either non-homologous end joining (NHEJ) or homology-directed repair (HDR) (Yuan et al., 2024). This in-depth knowledge of how CRISPR/Cas works shows how well it works for making precise and efficient changes to the genome. It is a key technology for improving crop traits and solving global problems like climate change and food insecurity (Raza et al., 2024). CRISPR technology has become a game-changing tool that makes it possible to quickly create new crop varieties with better traits, such as better resistance to biotic and abiotic stresses, higher nutritional value, and more potential for higher yields. Additionally, CRISPR/Cas systems improve agricultural productivity and sustainability because they are simple, flexible, cheap, and acceptable to the public because they can make precise changes without adding foreign DNA (Ali et al., 2023). Recent developments, like prime editing and base editing, have made genome editing even more accurate and useful. This means that it can be used to make more complex genetic changes with fewer unintended effects. These new ideas are making it possible for the next generation of crops to grow well in changing weather and meet the needs of a growing population. This review seeks to furnish a thorough examination of CRISPR/Cas technology in augmenting crop resilience and the productivity of staple grains in the face of climatic adversities. This article discusses how CRISPR/Cas systems could change modern agriculture by looking at the most recent research and technological advances. It would give us a complete picture of how to understand new technologies and find strategic paths for future research and development, which would help make sure that food is safe around the world and that farming is done in a way that is good for the environment.

Main Body

Recent improvements in CRISPR technology have made genome editing much more specific and effective, which is very important for agricultural uses. Prime editing and base editing are new ways to make precise changes to genes that are very important. Prime editing uses CRISPR-Cas9 and a reverse transcriptase to fix up to 89% of known genetic variants. This lets you directly edit target DNA sequences. Research has shown that it works to make rice more resistant to disease by fixing certain point mutations without breaking double strands (Gupta et al., 2023). On the other hand, base editing makes it possible to change one DNA base into another directly and permanently, which makes point mutations more accurate. Applications encompass the modification of flavour profiles in peas and tomatoes, as well as the enhancement of cold tolerance in soybeans through the alteration of genes involved in fatty acid desaturation and cold response pathways (Nizampatnam et al., 2024). New CRISPR-associated proteins like Cas12 and Cas13 add to the tools that agricultural biotechnology can use. Cas12 is good for multiplex editing because it lets you change multiple traits at once. For example, it can help soybeans have multiple disease resistance genes (Sun et al., 2024). Cas13d is a very strong way to interfere with multiple RNA viruses in potato crops. This makes it a useful tool in the ongoing efforts to improve agricultural productivity and sustainability (Zhan et al., 2023). For genome editing in plants to work, CRISPR components must be delivered quickly and easily. Recent methodologies encompass nanoparticle-mediated delivery, which safeguards CRISPR components from degradation and augments cellular uptake, thereby significantly enhancing trait improvement in maize

(Chakraborty et al., 2023; Yau et al., 2024). Viral vectors, utilising inherent viral infection processes, have demonstrated enhanced efficacy and safety in transient expression initiatives, such as the induction of virus resistance traits in tobacco and tomato (Jogam et al., 2023; Wang et al., 2024e). The ribonucleoprotein (RNP) complex delivery method sends CRISPR parts directly as proteins and RNA, which lowers the chance of unwanted effects. This method has worked well in crops like wheat to make them more resistant to disease and increase their yield (Poddar et al., 2023). Improved computational tools for accurate guide RNA design and the creation of high-fidelity Cas variants show less off-target activity (Zhang et al., 2023b). In wheat, high-fidelity Cas9 variants have been used to lower the number of unintended mutations and make the plants more resistant to drought. These improvements make genetic edits safer and also make CRISPR more useful for creating crops that can survive in changing climates. The ongoing improvement of CRISPR technologies, such as new ways to deliver and edit them, is making it possible for agriculture to make big changes, as shown in Table 1. Researchers are making these tools more accurate and useful, which opens up new ways to use CRISPR and helps create agricultural systems that are stronger, more productive, and more sustainable. The use of CRISPR/Cas technology in farming has a lot of promise for making grain crops more resistant to different types of abiotic and biotic stress (Yadav et al., 2023). This section examines how recent progress in CRISPR/Cas technology has improved the resilience of grain crops to these stresses, thereby promoting sustainable agricultural productivity amid climate change and other environmental challenges. CRISPR/Cas genome editing has evolved into a sophisticated technique for enhancing crop growth, development, and stress responses, as demonstrated in. In this context, we examined recent progress in CRISPR-mediated crop enhancement under abiotic and biotic stresses, as well as enhancements in various growth-related traits.

Improving Crop Yield

CRISPR/Cas technology provides novel avenues to augment crop yield by directly targeting genes that govern plant growth and development. Editing the OsAPL gene that helps move nutrients around has been shown to increase rice yield (Zhang et al., 2024a). Targeting genes that are involved in making chlorophyll and capturing light, like the OsSXK1 gene in rice, has made photosynthesis more efficient, which has led to higher photosynthetic rates and more grain yield (Zheng et al., 2021). Editing genes that help plants take in and use nutrients, like the ARE genes in barley or wheat, also makes plants use nitrogen more efficiently and gives them higher yields when nitrogen levels are low (Karunarathne et al., 2022). Recent research has shown that CRISPR/Cas technology can improve yield-related traits in a number of crops. Editing the DEP1 gene in rice has resulted in the creation of semi-dwarf varieties characterised by enhanced lodging resistance and increased grain yield (Zhang et al., 2023a).

Improving Crop Quality

CRISPR/Cas9 technology has made agricultural biotechnology much better by allowing for precise genome editing that can improve the safety, taste, texture, shelf life, and industrial use of many crops. Researchers have used CRISPR/Cas9 to change the CYP79D1 gene in cassava. This has greatly reduced the amount of cyanogenic glycosides, which lowers the risk of cyanide poisoning and makes this important crop safer without affecting its agronomic performance (Juma et al., 2022). The technology has been used in rice to make it smell better by changing the OsBADH2 gene, which makes more 2-acetyl-1-pyrroline (2-AP), a compound that gives off a pleasant smell that people like (Tian et al., 2023). The gbss gene in potatoes has been changed by CRISPR/Cas9, which makes amylose-free starch that has a smoother texture. This is very useful in both cooking and industrial processes. The technology has also been very important in making different crops last longer by targeting genes that are involved in the ripening process, like those that control ethylene production. This slows down the ripening process, lowers post-harvest losses, and makes the crops more economically viable. Also, in barley, CRISPR/Cas9 has been used to make the grains harder by changing the Hina gene. This makes the grains better for industrial use because they have a higher hardness index, but it also makes the grains thinner and heavier (Jiang et al., 2022). Also, in potatoes, targeting the FtsZ1 gene has led to the creation of lines with bigger starch granules. This has greatly increased the viscosity of starch paste, making these potatoes better for certain industrial processes, all without changing the plant's overall phenotype or nutritional quality. These different uses of CRISPR/Cas9 show how it could change the way crops are grown by allowing for changes that meet consumer needs, improve safety, and meet specific industrial needs while still making sure that farming practices are sustainable and profitable.

Nutrition Enhancement

One of the main goals of agricultural biotechnology is to fix nutritional deficiencies by biofortifying crops. CRISPR/Cas technology is a key part of making this happen. For example, CRISPR/Cas has been used to raise the pro-vitamin A content in rice, which is an important step in fighting vitamin A deficiency in people who eat a lot of rice (Maiti and Banik, 2023). Biofortification seeks to enhance the nutrient density of crops, thereby augmenting their nutritional value. Researchers changed the genes that make pro-vitamin A to make "Golden Rice," which has more beta-carotene (Dong et al., 2020; Datta et al., 2021). People have also used RISPR/Cas technology to make crops richer in minerals. Genes like OsNAS have been changed in rice and wheat to raise the levels of iron and zinc. This fixes micronutrient deficiencies that can cause anaemia and make the immune system work less well. In maize, targeting the PSY1, Crtl, and LCYB genes has also increased the production of pro-vitamin A, which has led to the creation of "Golden Maize." The technology also helps crops have more amino acids. CRISPR/Cas has been used to boost the levels of important amino acids and vitamins in cassava, which has greatly improved the nutritional value of this important crop (Otun et al., 2023). Editing genes that are involved in lysine biosynthesis has made maize have more lysine, which is a common problem with cereal grains (Hasan, 2024). Another important goal of CRISPR/Cas technology is to make crops' proteins better. For instance, mutagenesis aimed at the OsAAP6 and OsAAP10 genes in rice can lower the protein content of the grains, which makes the crop taste better and cook better (Wang et al., 2020). By targeting the GmIPK1 gene, CRISPR/Cas technology has been able to lower the levels of antinutritional substances like phytic acid in soybeans. This has made iron and zinc more available to the body, which has improved the overall nutritional quality of the soybeans (Song et al., 2022).

Conclusion

The fast development of CRISPR/Cas technologies has brought about a new era of precision agriculture. These technologies allow for targeted genetic changes that make crops stronger, more productive, higher quality, and more nutritious. New techniques like prime editing and base editing have made genome editing much more precise and useful. They let you fix genetic variants and fine-tune traits without causing double-strand breaks. Adding Cas12 and Cas13 proteins to the CRISPR toolkit and using new delivery methods like nanoparticle-mediated systems and ribonucleoprotein complexes has made these tools even more useful for a wider range of crop species.

These improvements have been very helpful in solving important problems in agriculture, such as making plants more resistant to disease and stress, improving traits related to yield, and making food safer and better. CRISPR/Cas technology has also become a powerful tool for biofortification. It helps fight global nutritional deficiencies by adding important vitamins, minerals, and amino acids to crops.

As computational tools and high-fidelity Cas variants keep improving the accuracy and safety of genome edits, CRISPR/Cas is ready to change the way we farm in a way that is good for the environment. These technologies are not only meeting current needs, but they are also laying the groundwork for a more secure and fair global food system by allowing the creation of crops that can withstand climate change, have more nutrients, and are useful in industry.

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