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Role of CRISPR/Cas9 in Crop Improvement

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The global population is anticipated to reach 9.8 billion in 2050. This presents agriculture with the crucial constraint of meeting the growing demand for food while mitigating the impacts of climate change on the environment. Conventional plant breeding has historically been effective, but has struggled with problems such as delayed progress, the possibility of undesirable trait associations, and reliance on subjective observations. Although genetically modified (GM) crops can increase agricultural production and improve food security, there are several challenges and concerns regarding their use, including environmental consequences, public opinion, and ethical considerations. Concerns include the risk of unexpected consequences for organisms that were not targeted and for the environment, as well as major social and ethical disputes over corporate control of the food supply. Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas technology is a gene-editing device that has transformed agriculture, providing precise, and effective techniques to improve plant traits and agricultural practices. It can be applied to produce herbicide-resistant plants, increase yields, improve quality, and increase resistance to diseases and pests. Additionally, CRISPR-Cas enables the creation of plant varieties that are tolerant to drought and salinity, and more resilient and sustainable in several agricultural systems. It has been successfully applied in improving crop quality. For instance, modification of crops can contain less allergens. CRISPR has been utilized to generate gluten-free wheat strands that are suitable for people with coeliac disease. This is a positive sign that the efficiency and productivity of agriculture can also be increased when CRISPR application is embraced.

Rapid advances in sequencing technologies are making more genomic data available on a growing number of crop species, and genome editing techniques can be utilized to precisely alter genes, opening up new avenues for crop improvement. The terms “CRISPR” and “CRISPR-Cas9” are frequently used interchangeably in the context of genome engineering to refer to the various CRISPR-Cas9, CRISPR-Cpf1, CRISPR-Cas12a, etc. Researchers can use these techniques to permanently alter genes in living cells, tissues, organs, or organisms. Approximately 20 crop species have so far harnessed the CRISPR-Cas9 gene-editing technique to achieve a range of traits, such as higher yields and the ability to cope with various biotic and abiotic stress. For instance, the CRISPR-Cas9 system has been utilized in knocking out precise genes critically to biotic or abiotic stress tolerance systems. To ensure food security, sustainable crop development initiatives are needed in the face of increasing intensity of biotic and abiotic stress factors. Abiotic stress has become a serious challenge for agriculture in the context of advancing climate change, leading to production losses that affect the global food supply. According to Rogo *et al.*, plants are able to adapt to normal fluctuations in their environment and continue to grow and develop. The abiotic stress occurs when a plant's responsiveness is seriously affected by non-biological environmental factors such as salt, heat, water scarcity or nutrient imbalances (toxicity or deficiency). When these environmental conditions become severe or persist for long periods of time, it has detrimental effects on plants. Abiotic stress has become a serious challenge for agriculture within the framework of advancing climate change, leading to production losses

that affect the global food supply. The production of disease-resistant crops is severely hindered by biotic stress caused by pathogenic microorganisms. This also contributes to 15% of global food production losses and over 42% of potential yield losses. For instance, in soybeans, 200 pathogens are known to cause destruction to the plants. To ensure food security, the increasing intensity of biotic and abiotic stress factors calls for sustainable crop development initiatives.

CRISPR technology allows for accurate and effective genome editing. Hence, it offers a substantial advantage for improving food security in light of the growing world population and changing climate. Crop yields, nutritional value, and plant resistance to environmental stressors like drought, salt, and pests can all be enhanced when CRISPR-Cas9 technology is applied. More dependable food supplies and higher agricultural output may result from this. Several researchers agree that applications of CRISPR-Cas9 technology in agriculture result in crop improvement. For instance, Li *et al.* employed CRISPR-Cas9 to selectively delete genes related to grain size regulation to increase the yield of rice grains. The capacity to precisely modify plant genomes makes it possible to improve desired characteristics, including stress tolerance, yield, disease resistance, and nutritional value. For instance, Shan *et al.* successfully applied CRISPR-Cas9 in rice, resulting in increased resistance to bacterial blight. In addition, CRISPR-Cas9 has shown the potential to speed up the breeding process. With conventional breeding methods, it often takes several generations to achieve the desired traits incorporated in a breeding material. By introducing targeted genetic changes directly into the plant's germline, CRISPR-Cas9 can drastically reduce the time required for breeding, thereby contributing to solving the problem of global food security. Moreover, this technique offers the possibility of growing nutrient-rich plants that can help prevent malnutrition and improve human health. By examining current limitations and upcoming projections, this chapter aims to demonstrate how CRISPR-CAS technology can be utilized to develop resilient, high-yielding, and nutrient-rich varieties that will ultimately contribute to agricultural sustainability and food security for generations to come.

CRISPR for crop yield improvement and stress resilience

CRISPR technology has transformed agricultural biotechnology internationally. Because of its precise genome editing capacity, it can target genes that govern essential agronomic properties such as crop production potential, biotic resistance, and abiotic stress tolerance. Climate change and rising food demand necessitate the use of the CRISPR-Cas system as a long-term solution for effective photosynthesis improvement, biomass production, and resilience to environmental hazards such as heat, cold, salinity, and drought. CRISPR technology has great potential for increasing crop productivity and tolerance to diverse pressures, making it an effective tool for agricultural enhancement.

Development of photosynthetic efficiency and biomass production with CRISPR

CRISPR-Cas9 technology offers great potential for improving photosynthesis and biomass production in crops. This allows researchers to focus on genes related to light yield, CO₂ fixation, and other photosynthetic processes, resulting in increased yields and enhanced efficiency. The productivity and photosynthetic efficiency of plants can be improved by CRISPR-Cas9 technology. CRISPR-Cas9 is a gene-editing technique that enables genetic engineers to disable or alter the function of certain genes in the photosynthetic pathway. According to Li *et al.*, photosynthesis is a sequence of complex reactions that can be optimized by editing critical genes that regulate these activities. Light is necessary for photosynthesis. Improving a plant's ability to utilize light can increase photosynthetic efficiency and crop yield. Genes involved in light uptake, such as the Light Harvesting Complex Protein B1 (Lhcb1), can be modified to improve the absorption of certain wavelengths. The Lhcb1 genes encode important components of the protein complexes that bind chlorophyll and collect light in the thylakoid membrane, which are critical for light uptake during photosynthesis. Targeted modifications of these proteins can improve their

ability to convert light energy into chemical energy. This has been shown to promote plant growth and is particularly useful in locations where plants grow in low light or under controlled conditions.

Rubisco is an important enzyme in the Calvin cycle, although it is inefficient because it is often converted to oxygen instead of carbon dioxide. This process of oxygen fixation, known as photorespiration, leads to a decrease in energy efficiency. Biotechnology can be utilized in various ways to reduce photorespiration: Scientists have applied CRISPR-Cas9 to modify genes such as RBCS (RuBisCo small subunit genes) to improve the selectivity of Rubisco for carbon dioxide or alter its active site to increase catalytic efficiency. To overcome the inefficiency of RuBisCO, CRISPR-Cas9 can be employed to improve the carbon dioxide-concentrating processes of C4 plants, for example, by modifying genes such as PEPC (phosphoenolpyruvate carboxylase). These changes increase the concentration of carbon dioxide near RuBisCO and reduce oxygen competition and photorespiration. CRISPR-Cas9 has been shown to alter genes involved in the photorespiratory system, such as GOX1 (glycolate oxidase), reducing energy waste. Alternatively, genes that enable more efficient recycling of photorespiratory waste can be optimized to direct them into productive metabolic pathways. The stomata of a plant regulate the loss of water and the uptake of carbon dioxide. There are several genes (e.g., *EPF1* and *EPF2*) that regulate stomata density of a which could increase water efficiency while maintaining photosynthesis. The approaches listed above are just a few examples of how biotechnology can be used to modify photosynthesis. Crop yield is the outcome of a comprehensive system. Photosynthesis supplies the carbon and energy essential for the entire system; however, this interaction is nonlinear and influenced by various factors, including growth, leaf, and canopy shape and design, source-sink dynamics, and the impact of photosynthesis within a field crop. In summary, photosynthesis is key to crop yield and any gene involved in photosynthesis can be altered with CRISPR-Cas9 to improve its efficiency, leading to improved photosynthesis and food production.

Application of CRISPR technology to alleviate abiotic stress

The response to abiotic stress is a complicated quantitative trait that is difficult to control since it is regulated by several genes and is therefore difficult to manage. In the last few years, the application of CRISPR-Cas9 technology for beleaguered gene editing in plants has developed dramatically. CRISPR-P, among the most recent technologies, is a web-based platform that enables the creation of sgRNAs in over 20 diverse plant species. Furthermore, the creation of multiple vectors and instruments for gene editing of plants with CRISPR-Cas9 has increased accessibility for useful research. CRISPR-Cas9 type II technology has facilitated accurate site-specific modifications in various plant species, including major plants. For example, CRISPR-Cas9 technology targeting *OsDERF1*, *OsERF922*, and *OsRMC* in rice has shown the ability to generate stable lines with enhanced resistance to abiotic stress, especially drought. In soybean, the application of CRISPR/Cas9 to the *GmMYB118*, *GmDrbza*, and *GmDrbzb* genes has been planned to generate genome-edited varieties that exhibit increased drought and salt tolerance. In wheat, genes such as *TaDREB2*, *TaDREB3*, *TaHAG1*, and *TaALs* are key targets for the creation of varieties with increased tolerance to drought, salinity, and herbicide resistance. Furthermore, the creation of several vectors and tools for CRISPR-Cas9-based gene editing of plants has increased accessibility for useful research. These developments have validated CRISPR-Cas9 technology for genetic modification, transcriptome control, the development of stress-tolerant plants, and molecular exploration of response to multigenic stresses. Additionally, Wu *et al.* pointed out that *Brassica napus* can be modified using CRISPR/Cas9 technology to increase drought and herbicide tolerance by modifying the genes *BnaA6.RGA*, and *BnAls*. Genes like *ZmARGOS8*, *ZmALS1*, *ZmALSZ*, and *ZmTMS5* have been used to create genome-edited lines of maize that are more tolerant to drought, herbicides, and extremely high temperatures. transcription factor *OsMYB30* and *OsJAZ9* bind to the promoter regions of *BMY* genes and thus impair *BMY* function. Researchers created cold-tolerant rice varieties by

disrupting *OsMYB30* using CRISPR-Cas. The CRISPR-Cas9 technique was used to modify the salt and drought tolerance gene (DST) in the indica rice cv. MTU1010. As a result of increased leaf area and decreased stomatal density, water storage, and light consumption are enhanced; the mutant lines with a 366-bp deletion in the coding sequence were tolerant to salt, osmotic, and drought stressors. Researchers have increased drought tolerance in maize by using CRISPR-Cas9 to interrupt the auxin-regulated gene Involved in Organ Size 8 (*ARGOS8*). Sensitivity of tomato to heat stress is increased by CRISPR-based editing of mitogen-activated protein kinase 3 (*SIMAPK3*) and agamous-like 6 (*SIAGL6*) orthologs, while sensitivity to salt stress is increased by ADP-ribosylation factor 4 (*SIARF4*). According to Bouzroud *et al.*, these CRISPR-edited mutants showed enhanced agronomic characteristics and tolerance to abiotic stress factors. This demonstrates the potential of CRISPR-Cas in contributing to food security in reducing stresses imposed by drought, salinity, among others.

Improving disease resistance and pest control through CRISPR technology

Plant pathogens, including bacteria, fungi, and viruses, trigger several plant diseases that severely affected production, while herbivores like insects further decrease production through direct damage and as vectors for disease. The capacity of plants to cope with various biotic stress factors will be negatively impacted by climate change. Direct crop losses due to biotic stress factors are expected to be 20–40%. Consequently, R gene methods do not necessarily lead to long-lasting broad-spectrum resistance and instead tend to encourage the development of resistance in the target pathogen. An alternative strategy is to use CRISPR-Cas to target susceptibility genes in a plant's genome. But in most cases, R genes are pathogen-specific and unnecessary avirulence genes. Hence, R gene strategies do not necessarily lead to long-lasting broad-spectrum resistance and instead tend to promote the development of resistance in the target pathogen. The use of CRISPR-Cas to target susceptibility genes in a plant's genome is an alternative tactic.

The pathogen (*Magnaporthe oryzae*) produces blast disease, which affects rice production worldwide. By deleting specific susceptible genes in rice like *OsERF92231* and Pi21, rice genotypes resistant to rice hypertrophy have been generated. Likewise, the use of CRISPR-Cas to target the Mildew Locus O (MLO) gene has improved the resistance of wheat to powdery mildew, a disease caused by the fungus *Podosphaera xanthii*.

Biofortification of crops using CRISPR

Enriching food crops with nutrients and enhancing the nutritional value of food are the safest, most effective, and sustainable approaches to comprehensively address protracted hidden hunger among the majority of the population in the least developed countries. Various mechanisms and approaches have been developed, adopted, and deployed to enrich the nutritional content of globally prevalent staple foods such as maize, sweet potatoes, wheat, rice, and beans with essential micronutrients like zinc, iron, and vitamins. Meanwhile, these basic steps have the economic viability to ensure the bioavailability of adequate nutrients, particularly the essential micronutrients that are in limited supply from crops in their natural state. Some of these fortification techniques include agronomic improvement of crop quality through proper soil nutrient management, conventional breeding by crossing desirable individuals to transfer and improve quality, and genetic engineering through gene editing, gene knock-in and knockout, and other sophisticated genetic manipulation systems.

Conclusions

The aim of using numerous techniques to develop safe and affordable crops to satisfy the world's growing food needs can be fraught with difficulties. A critical component will be the application of modern crop improvement methods. Compared to traditional breeding techniques, scientists can use sophisticated breeding techniques to quickly modify genes and introduce the required gene into the genome. CRISPR technology is a potent tool for gene editing with great promise for improving crops and addressing challenges relating to global food security. The CRISPR-Cas9 technique has been refined in recent decades as a flexible,

targeted technique for modifying genes found in many species, including plants. Application of this method to improve the quality, productivity, and disease resistance of crops could therefore be an important area of research in the future. By improving pest, disease, and nutritional traits, this technology increases the resilience of crops to environmental stressors and climate change. It offers the opportunity to achieve high yields, reduce dependence on fertilizers, and increase the resilience of plants. In the last decade, it has been used widely in a variety of crops to conduct useful research, counteract stress-related responses, and improve key agronomic traits. Through knockdown, knock-in, replacement, point mutation, gene regulation, base editing, and other locus-specific modifications, the CRISPR/Cas9 technique has enhanced fundamental research in both plant science and the agricultural sector. Moreover, improving the specificity and accuracy of CRISPR-Cas9 editing is also an important area for future studies. Efforts are being made to reduce unwanted genomic alterations, improve the effectiveness of homology-directed repair (HDR), and reduce the consequences of off-target interventions.