Proceedings of the 4th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/60/20241416

CRISPR-Cas9: Revolutionizing Crop Resistance Enhancemement

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Abstract. The security of food has grown to be a serious concern in recent years, due to factors such as environmental changes, pest infestations affecting crop yields and food safety. Traditional breeding methods, such as hybrid breeding, often involve long cycles, consuming substantial human and material resources and constrained by germplasm limitations, significantly hindering the rapid development of animal and plant breeding. In contrast, CRISPR-Cas9 genome editing technology allows precise modification of genetic information inanimals and plants at the molecular level, directly optimizing target traits or imparting new genetic characteristics, demonstrating more direct and efficient breeding advantages compared to traditional methods. The implementation and advancement of the CRISPR-Cas9 genome editing system to increase plant and animal resilience is the main topic of this review, which also discusses its drawbacks and potential applications.

Keywords: CRISPR-Cas9, Gene Editing,Plant Diseases, Abiotic Stress.

1. Introduction

These days, disease assaults and climate variability represent a serious threat to the world's food security and have a considerable influence on growing crops yield. The critical issue of global food security is characterized by problems such as an exponentially increasing population [1], land degradation, water shortage, and climate change, food loss, economic disparities, and political instability. These factors threaten the availability, accessibility, and affordability of food [2]. To improve agricultural output and guarantee food security, crop cultivars with disease and pest resistance have been developed via the use of traditional breeding techniques. Crop resilience has been greatly aided by methods like marker-assisted selection, crossbreeding, mutant breeding, and selective breeding. The primary goals of these strategies are to enhance genetic features that facilitate crops in withstanding biotic challenges, diminish reliance on chemical pesticides, and elevate overall food safety. The advent of the clustered regularly interspaced short palindromic repeat and their associated protein 9 (CRISPR-Cas9) technology represents a significant advancement in this field. This state-of-the-art gene-editing technique allows for accurate alteration of crop genomes so that they can better help plants resist pests and diseases. The integration of cutting-edge technology like as CRISPR-Cas9 with conventional breeding methods is crucial in tackling global food security issues and promoting sustainable farming methods.

With the new gene-editing technique CRISPR-Cas9, scientists may accurately change the DNA of living things. It has drawn a lot of attention because of its simplicity, effectiveness, and adaptability in a variety of biological research fields, and because of its potential applications in medicine and agriculture [3].

CRISPR-Cas9 is capable of identifying and cutting specific sections of DNA by collaborating with a complementary sgRNA sequence, leading to the formation of double-strand fractures. These interruptions initiate cellular repair mechanisms, frequently resulting in alterations at or in close proximity to the locations where the DNA double helix is broken into two separate strands. The CRISPR/Cas9 technique has greatly revolutionized genome editing technologies since its creation and is now being widely used to alter the genetic composition of many crop species.

The purpose of this review is to investigate possible uses of CRISPR-Cas9 technology to improve crop resistance. Traditional breeding techniques might not be able to quickly and effectively satisfy the demands of crop production in light of the problems that global agriculture is experiencing from issues like climate change, soil degradation, and water shortages. Thus, CRISPR-Cas9, a recently developed genetic editing instrument renowned for its accuracy and efficiency, provides fresh approaches to agricultural production. This project aims to provide a thorough comprehension of the relevance and prospective uses of CRISPR-Cas9 technology in modern agriculture to agricultural scientists, decision-makers, and the general public.

2. Applications in crop resistance enhancement

2.1. Disease resistance

Numerous diseases, including bacteria, viruses, and fungi, pose serious obstacles to crop productivity and continue to threaten the world's food security. Enhancing plant resistance to diseases is vital for maximizing agricultural productivity. The fundamental pillars of generally effective methods include crop types that are resistant to pests and diseases, as well as chemical controls. Nevertheless, the development of resistant varieties of agricultural diseases through recombination or mutation, as well as growing apprehensions about the use of pesticides and the effects it has on human health, can rapidly undermine the efficacy of current disease management strategies.

Notably, the CRISPR/Cas system gained recognition as a vital instrument for addressing various agricultural challenges. Among the many plant species, CRISPR/Cas 9 has proven to be very effective in fighting bacterial and fungal infections, viruses, and other illnesses.

2.1.1. CRISPR/cas9-based resistance to viruses.

Certain plant species have effectively acquired resistance to begomoviruses by employing targeted genome editing with the CRISPR-Cas9 technology. This editing process cuts specific viral sequences that are necessary for replication [4]. For example, the tomato yellow leaf curl virus (TYLCV)'s intergenic region (IR), replication protein (Rep)'s RCRII motif, and capsid protein (CP) were targeted by sgRNA molecules. Tobacco plants received these sgRNAs by overexpressing Cas9 nuclease with a TRV vector. The efficiency of a single sgRNA against various viral strains was demonstrated by its successful reduction of clinical symptoms for the viruses.

In another work, specific alterations to the Wheat dwarf virus (WDV) genome at four different loci were achieved using a CRISPR/Cas9 strategy that used four bespoke sgRNA/Cas9 constructs. Mutant plants with viral infection resistance were produced as a result of this genetic modification.

2.1.2. CRISPR/Cas9-based resistance to fungi.

Fusarium graminearum is the pathogen responsible for the debilitating condition referred to as fusarium head blight, which specifically impacts barley and wheat crops. The mycotoxins generated by F. graminearum infection pose a significant risk to the well-being of both people and livestock. The susceptibility factor to Fusarium graminearum, At2OGO, which was discovered in Arabidopsis thaliana, was subjected to targeted alterations utilizing the CRISPR/Cas9 system. At2OGO knockout mutants were more F. graminearum-resistant [5]. Furthermore, the mutants regained sensitivity when they were complemented with Hv2OGO, a barley gene that is similar to At2OGO. This suggests functional equivalency and the possibility of similar functions in barley susceptibility to fusarium head blight [5]. These findings demonstrate the CRISPR/Cas9 system can improve the resistance of crops with fungi..

2.1.3. CRISPR/Cas9-based resistance to bacteria.

Because phytopathogenic bacteria do not always cause symptoms, controlling them is more difficult than controlling viruses or fungus because there are less effective chemicals available.

A sucrose transporter protein that is encoded by the rice OsSWEET13 S-gene has a negative regulatory function in the plant's defense against infections. For example, the bacterial blight-causing pathogen Xanthomonas oryzae pv. oryzae secretes PthXo2, an effector protein that promotes OsSWEET13 to be expressed and increases sensitivity in rice. As a result, it has been determined that OsSWEET13 is a viable target for editing using CRISPR/Cas9 to create transgenic plants that are resistant. In fact, rice plants that had their OsSWEET13 gene mutated using CRISPR/Cas9 have demonstrated resistance to X. oryzae [6].

Because of CRISPR/Cas9 technology, several plants have been genetically engineered using CRISPR technology, leading to their remarkable resistance to a diverse array of diseases.

2.2. Pest resistance

There isn't much information available right now about genetic engineering being used to provide plants insect resistance. In one prominent case, rice cytochrome P450 CYP71A1, an enzyme that converts tryptamine to serotonin, was confirmed to be inactivated using CRISPR/Cas9 technology [7]. Due to this genetic modification, plants were no longer able to produce serotonin, which resulted in higher salicylic acid (SA) levels and greater resistance against stripped stem borers and brown plant hoppers.

Using a different strategy, six genetic loci linked to tomato productivity and yield in the wild tomato species Solanum pimpinellifolium were targeted using CRISPR-Cas9 [8]. Within a single generation, this genetic intervention improved the quality and production of the modified tomato lines. The process of domesticating this untamed tomato conserved its ability to fight pests while enhancing its agricultural characteristics, even though this research did not particularly focus on genes responsible for resistance (R) or susceptibility (S). Although more testing of the de novo domesticated plants against pests is required to substantiate these benefits, it is anticipated that this innovation would increase productivity.

Furthermore, a mutation that makes the S gene less attractive to aphids makes the plant more resistant. Unlike old chemical or radiation mutagenesis procedures, which might produce mutations in non-target genes alongside the intended target gene, modern mutagenesis techniques now allow targeted alterations inside target genes. CRISPR/Cas9 is now the most advanced and extensively employed approach.

Targeting genes linked to cell wall construction may be another strategy to lessen vulnerability to aphids by preventing aphid-induced cell wall loosening. Some cell wall-related genes, such pectinase and expansin, have been found in earlier research to express more when aphids are present. This suggests that these genes might be targets for RNA interference or CRISPR/Cas9 [9]. Transient RNA interference has verified the existence of a subset of cell wall-related potential susceptibility (S) genes: (1,3;1,4)-β-glucanases, which break down cellulose in plant cell walls. Following D. noxia infection, it was discovered that these RNA sequences were more strongly induced in susceptible wheat lines as opposed to resistant lines. Plant resistance to D. noxia was enhanced by knocking down plant (1,3;1,4)-β-glucanase sequences, suggesting their involvement as aphid-induced susceptibility factors [10].

2.3. Abiotic stress tolerance

Abiotic stressors that affect agricultural productivity worldwide include salt, heat, cold, and drought. Through the control of transcription and cellular processes, plants have developed complex defense systems to withstand and react to various stimuli. Plants that are under extreme stress may have cellular damage, membrane damage, and outward signs of necrosis [11]. Researchers now have more opportunities to study tolerance mechanisms and develop novel characteristics that help them withstand abiotic challenges because to the development of genome-editing technology. Plants that have had their S-genes knocked out using CRISPR have become more resilient to abiotic stressors [12]. Scientists may be able to develop crops that are more stress-tolerant by modifying these genes and learning more about how they relate to stress sensitivity.

2.3.1. Drought tolerance.
Drought and water shortage are major worldwide issues that impact both industrialized and developing nations. Climate change has increased Earth's evaporation, which has made drought stress in plants worse. Specific plant species have undergone genetic modification using the technique known as CRISPR/Cas to augment their capacity to endure dry conditions. Scientists have succeeded in enhancing crop resistance to drought by specifically manipulating specific genes linked to drought-response pathways. For instance, the CRISPR/Cas editing of the SAPK2 gene induces a mutation that improves drought tolerance in Oryza sativa (rice) via modulating the ABA signaling system, where SAPK2 acts as a pivotal mediator [13]. A range of crop species may benefit from increased drought tolerance thanks to CRISPR/Cas gene editing, which focuses on specific genes linked to drought-response pathways, as these examples demonstrate. The goal of these genetic alterations is to reduce crop losses and strengthen plants against drought stress.

2.3.2. Salt tolerance.
Plant cells face substantial difficulties when exposed to salt stress. The CRISPR/Cas gene editing method has successfully improved the salt stress tolerance of several genes. For instance, CRISPR/Cas-mediated alterations that increased OsRAV2 expression (associated with the ABI3/VP1 2 gene) enhanced O. sativa's ability to withstand salt [14]. These illustrations demonstrate how CRISPR/Cas gene editing may target certain genes that are part of the pathways that respond to salt stress in order to make a variety of plant species more tolerant to salt stress, therefore enhancing the plants' overall tolerance and strengthening their resilience to salt stress.

2.3.3. Heat tolerance.
Plants respond to heat stress in many ways. For example, CRISPR/Cas gene editing has targeted several genes associated to heat sensitivity, which aims to improve thermotolerance in plants and clarify their functions in heat tolerant. The CRISPR/Cas method of deleting Heat Stress-Sensitive Albino 1 (HSA1) in tomato mutants resulted in plants with a greater heat stress sensitivity than the wild-type plants, indicating a potential function for HSA1 in heat tolerance [15]. Using CRISPR technology, it was possible to change the expression of BZR1 (Brassinazole-Resistant 1) and create thermostat tolerance in tomatoes. The involvement of BZR1 in the body's reaction to heat stress was highlighted by mutant BZR1 lines that showed decreased production of apoplastic hydrogen peroxide (H2O2) and decreased activation of Respiratory Burst Oxidase Homolog 1 (RBOH1) [16]. These instances demonstrate CRISPR/Cas gene editing's potential to elucidate heat stress-related gene functions and enhance plant thermotolerance. By modifying these genes, one hopes to increase plants' tolerance for high temperatures and reduce the detrimental effects of extreme temperatures on agricultural productivity.

3. Challenges and future prospects
Even though CRISPR-Cas9 has improved agricultural tolerance to abiotic stressors, diseases, and pests significantly, there are still a number of issues and concerns to be addressed.

3.1. Ethical implications of gene editing in agriculture
Gene editing in agriculture presents profound ethical considerations, primarily concerning the alteration of organisms' genetic foundations. These ethical concerns encompass issues of equity, the preservation of biodiversity, and the well-being of future generations. The ability to modify genes raises questions about fairness in access to genetic enhancements and the potential unintended consequences for ecosystems and society at large.

3.2. Regulatory challenges and public perception

Navigating the regulatory landscape for gene-edited crops involves complex challenges. Regulatory bodies face the task of balancing safety and efficacy assessments with fostering innovation in agricultural biotechnology. Public perception of gene editing varies widely, influenced by concerns over food safety, environmental impact, and ethical boundaries. Building public trust requires transparent communication and robust regulatory frameworks.

3.3. Potential environmental impacts and concerns

The environmental implications of gene editing in agriculture are multifaceted. While promising benefits such as pest resistance and improved crop yields are anticipated, potential risks include unintended ecological disruptions, altered biodiversity patterns, and gene flow to wild populations. Addressing these concerns necessitates rigorous environmental monitoring, risk assessment protocols, and sustainable agricultural practices to mitigate adverse impacts.

3.4. Future prospects of CRISPR/Cas9 technology

By making it easier to swiftly and accurately introduce the required features than with conventional breeding, new plant breeding techniques provide researchers with new opportunities. The application of CRISPR/Cas9 is a noteworthy development in plant breeding and agricultural development for numerous crops. This technique holds promise for developing crop varieties that can better adapt to their environment, addressing the longstanding demand for improved environmental adaption. These include photo-thermosensitivity, biological nitrogen fixation, biofortification, and efficient production of biofuels.

Recently, it has been used on several plant species to enhance other economically significant traits, alleviate both living organism-related and non-living organism-related pressures, and increase crop productivity. It is widely used in staple crops globally and has the potential to address issues of hunger and poverty by ensuring food security for the growing global population. Furthermore, as a significant portion of the work conducted is in the first phase and requires enhancement, this technique requires several modifications to maximize its effectiveness in achieving the objective. An innovative application of this technology is enhancing the disease resistance of agricultural plants by adding a wide array of resistances.

Recent developments in CRISPR-Cas9 research are fostering new trends in enhancing crop traits, particularly through integration with other biotechnological approaches. This synergy allows for precise genome editing combined with traits from traditional breeding or complementary technologies like RNA interference. These integrations not only expedite trait development but also broaden the scope of possible improvements, from disease resistance to nutritional enhancement. With promising prospects for widespread adoption and commercialization, CRISPR-Cas9 offers a pathway to swiftly engineer crops with improved yields and resilience against environmental stressors. Its transformative impact on crop resistance enhancement is unmistakable, as it enables targeted modifications to specific genes responsible for stress tolerance. Looking ahead, these advancements hold considerable potential for promoting sustainable agriculture by addressing global food security challenges through tailored, resilient crop varieties.

4. Conclusion

CRISPR/Cas9 technology has inspired innovative applications in crop improvement and accelerated breeding processes, thus demonstrating its status as the most reliable and innovative technology in the field of agriculture and opening up new frontiers in plant genome editing. Looking ahead, scientific research is expected to focus on the diverse applications of crop enhancement through the use of gene editing technologies, such as production enhancement, nutritional value optimization, abiotic and biotic adversity resistance enhancement, quality improvement, and strengthening of other economic traits. The maintenance of the global food supply will also be ensured by in-depth research and implementation of CRISPR/Cas9 and its derivatives in the aforementioned domains, which will also establish a solid basis for the sustainable growth of contemporary agriculture. and concurrently ensure the abundance and safety of the world's food supply.

References

[1] Lenaerts B., Collard B. C. Y., and Demont M. (2019). Review: Improving global food security through accelerated plant breeding. Plant Sci, 287, 110207. https://dx.doi.org/10.1016/j.plantsci.2019.110207

[2] Lobell D. B., Gourdji S. M. (2012). The influence of climate change on global crop productivity. Plant Physiol, 160 (4), 1686-1697. https://dx.doi.org/10.1104/pp.112.208298

[3] Li T., Yang Y., Qi H., Cui W., Zhang L., Fu X., He X., Liu M., Li P. F., and Yu T. (2023). CRISPR/Cas9 therapeutics: progress and prospects. Signal Transduct Target Ther, 8 (1), 36. https://dx.doi.org/10.1038/s41392-023-01309-7

[4] Ali Z., Abulfaraj A., Idris A., Ali S., Tashkandi M., and Mahfouz M. M. (2015). CRISPR/Cas9-mediated viral interference in plants. Genome Biol, 16, 238. https://dx.doi.org/10.1186/s13059-015-0799-6

[5] Low Y. C., Lawton M. A., and Di R. (2020). Validation of barley 2OGO gene as a functional orthologue of Arabidopsis DMR6 gene in Fusarium head blight susceptibility. Sci Rep, 10 (1), 9935. https://dx.doi.org/10.1038/s41598-020-67006-5

[6] Zhou J., Peng Z., Long J., Sosso D., Liu B., Eom J. S., Huang S., Liu S., Vera Cruz C., Frommer W. B., White F. F., and Yang B. (2015). Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice. Plant J, 82 (4), 632-643. https://dx.doi.org/10.1111/tpj.12838

[7] Lu H. P., Luo T., Fu H. W., Wang L., Tan Y. Y., Huang J. Z., Wang Q., Ye G. Y., Gatehouse A. M. R., Lou Y. G., and Shu Q. Y. (2018). Resistance of rice to insect pests mediated by suppression of serotonin biosynthesis. Nat Plants, 4 (6), 338-344. https://dx.doi.org/10.1038/s41477-018-0152-7

[8] Zsögön A., Čermák T., Naves E. R., Notini M. M., Edel K. H., Weinl S., Freschi L., Voytas D. F., Kudla J., and Peres L. E. P. (2018). De novo domestication of wild tomato using genome editing. Nat Biotechnol, https://dx.doi.org/10.1038/nbt.4272

[9] Reddy S. K., Weng Y., Rudd J. C., Akhunova A., and Liu S. (2013). Transcriptomics of induced defense responses to greenbug aphid feeding in near isogenic wheat lines. Plant Science, 212, 26-36. https://dx.doi.org/https://doi.org/10.1016/j.plantsci.2013.08.002

[10] Anderson V. A., Haley S. D., Peairs F. B., van Eck L., Leach J. E., and Lapitan N. L. (2014). Virus-induced gene silencing suggests (1,3;1,4)-β-glucanase is a susceptibility factor in the compatible russian wheat aphid-wheat interaction. Mol Plant Microbe Interact, 27 (9), 913-922. https://dx.doi.org/10.1094/mpmi-05-13-0141-r

[11] Polle A. (2001). Dissecting the superoxide dismutase-ascorbate-glutathione-pathway in chloroplasts by metabolic modeling. Computer simulations as a step towards flux analysis. Plant Physiol, 126 (1), 445-462. https://dx.doi.org/10.1104/pp.126.1.445

[12] Zafar S. A., Zaidi S. S., Gaba Y., Singla-Pareek S. L., Dhankher O. P., Li X., Mansoor S., and Pareek A. (2020). Engineering abiotic stress tolerance via CRISPR/ Cas-mediated genome editing. J Exp Bot, 71 (2), 470-479. https://dx.doi.org/10.1093/jxb/erz476

[13] Lou D., Wang H., Liang G., and Yu D. (2017). OsSAPK2 Confers Abscisic Acid Sensitivity and Tolerance to Drought Stress in Rice. Front Plant Sci, 8, 993. https://dx.doi.org/10.3389/fpls.2017.00993

[14] Duan Y. B., Li J., Qin R. Y., Xu R. F., Li H., Yang Y. C., Ma H., Li L., Wei P. C., and Yang J. B. (2016). Identification of a regulatory element responsible for salt induction of rice OsRAV2 through ex situ and in situ promoter analysis. Plant Mol Biol, 90 (1-2), 49-62. https://dx.doi.org/10.1007/s11103-015-0393-z

[15] Qiu Z., Kang S., He L., Zhao J., Zhang S., Hu J., Zeng D., Zhang G., Dong G., Gao Z., Ren D., Chen G., Guo L., Qian Q., and Zhu L. (2018). The newly identified heat-stress sensitive albino

DOI: 10.54254/2753-8818/60/20241416

1 gene affects chloroplast development in rice. Plant Sci, 267, 168-179. https://dx.doi.org/10.1016/j.plantsci.2017.11.015

[16] Yin Y., Qin K., Song X., Zhang Q., Zhou Y., Xia X., and Yu J. (2018). BZR1 Transcription Factor Regulates Heat Stress Tolerance Through FERONIA Receptor-Like Kinase-Mediated Reactive Oxygen Species Signaling in Tomato. Plant Cell Physiol, 59 (11), 2239-2254. https://dx.doi.org/10.1093/pcp/pcy146