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THE ROLE OF GENOME EDITING IN SOLVING PLANT PROTECTION PROBLEMS

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Abstract. The impact of climate change and the worsening of the phytosanitary status of agricultural crops require the strengthening of agriculture by applying the achievements of biotechnology and genetic engineering. The application of pesticides and breeding strategies, including the implementation of genetically modified organisms (GMOs), although they occupy significant areas, remain insufficient to ensure the resilience and productivity of agricultural crops, creating global ecological problems. The achievements of modern scientific activity in genome editing technologies, in particular CRISPR/Cas9, open up prospects for crop protection through precise genome modifications. The method allows the development of resistant crops against pathogens of diseases, pests and weeds and is applied to eliminate susceptibility genes and modify defense genes, which opens up prospects for advancing crop protection and ensuring food security.

Keywords: ecological agriculture, gene, genome editing, harmful organisms, plant protection

INTRODUCTION

Based on the worsening of the phytosanitary condition of agricultural crops against the background of climate change and the increasing vital role of agriculture in the economy, over the past decade there has been an increase in the role of plant health. Harmful organisms (pathogens of diseases, harmful arthropods and weeds) cause serious economic losses, reduce the yield of the branch by yield losses by 25-40% or completely compromise crop yields [1]. To ensure crop protection against, using scientific and practical achievements, agricultural producers apply an impressive range of procedures and means, including a wide range of chemical preparations.

Taking into account the negative effects of pesticides on non-target entities and the ecological state of the environment, against the background of high economic indicators of chemical means, the scientific community develops and proposes al-

ternative approaches to preserving plant health [2]. The achievements recorded have been the basis for plant improvement by increasing resistance to harmful organisms and increasing the yield and health of plants. Particularly questionable are the results of obtaining and applying genetically modified organisms (GMOs), which by modifying the genetic apparatus of the plant by introducing one or more genes, which encode the genetic information regarding the resistance of agricultural crops to chemical means of protection (herbicides, fungicides, bactericides). Currently, public opinion is reluctant to GMOs, and although impressive areas of genetically modified crops are recorded, evidence of problems caused by their application is becoming more and more frequent [3].

Although discussions around the controversies of these inventions continue, it is still necessary to recognize that transgenic plants have traits that would not have arisen naturally or through conventional crosses. Against the background of the problems caused by the application of GMOs, research is currently expanding in order to apply methods for improving the productivity of crop plants by editing the genome of agricultural crops. New genomic techniques have become particularly relevant and promising, bringing together a group of modern methods by which a plant's DNA can be modified with greater precision and mutational phenomena reduced. Unlike GMOs, these are not based on the introduction of foreign genes from other species, and the modifications are made to the plant's own genetic material. As a result, it becomes possible to acquire the desired qualities that cannot be obtained by traditional breeding methods, but it offers the possibility of developing new plant traits. Plant genome editing allows for precise restructuring of chromosomes, including small insertions, deletions, substitutions and complex rearrangements, such as inversions, duplications and translocations [4].

The complex nature of food security and environmental issues necessitates the application of modern biotechnology capabilities to penetrate the mechanisms established during the evolution of the biosphere, obtaining genome editing levers. In this regard, plant breeding techniques are applied, such as synthetic nucleases: zinc finger nuclease (ZFN), transcription activator-type effector nuclease (TALEN), the CRISPR-Cas9 system, oligonucleotide-directed mutagenesis, cisgenesis and intragenesis, RNA-mediated DNA methylation, which allow the induction of directed changes in the genomes of organisms. The development of new genomic techniques is expanding worldwide, reaching 108 crops belonging to 32 species [2].

The most significant advances in genome editing have been made by the Clustered Regularly Interspaced Short Palindromic Repeats and its associated Cas proteins (CRISPR/Cas9) technique, which demonstrates superior applicability compared to other gene editing techniques based on endonucleases or nucleases. The CRISPR technology, whose potential was first demonstrated by Jennifer Doudna and Emmanuelle

Charpentier in 2012, allows the modification of the genome of any biological entity and was recognized as the invention of the year 2015, ensuring the winning of the Nobel Prize in Chemistry in 2020. CRISPR is a tool that allows the correction of DNA defects and functions like molecular "scissors" that allow the selective cutting of unwanted areas within the genome and their replacement with new DNA fragments [5].

This technology is expected to revolutionize the study and treatment of a wide range of diseases in humans, plants and animals, including cancers and incurable infections, as well as hereditary conditions. Current scientific advances demonstrate that CRISPR is not only an extremely versatile technology, but one that is proving to be precise, increasingly safe to use and promising. Gene editing offers efficiency and precision by reducing research and development and breeding times and for combating rapidly evolving harmful organisms [6].

CRISPR/Cas9, with its site-specific nuclease activity, triggers DNA repair mechanisms in cells, providing a wide range of genetic modifications depending on the repair pathways and the availability of repair templates [7]. Another way in which crop protection can be achieved is by genetically modifying insect pests by disrupting their functional characteristics by preventing mating attempts and introducing body defects in insects.

CRISPR/Cas9 is a genome editing technology that has significantly strengthened the field of genetic manipulation, which has also contributed to the discovery and development of CRISPR-associated enzymes (Cas), the most studied of which are due to their ability to fragment nucleic acid into oligonucleotides in a targeted manner. Significant advantages have been recorded in the exploitation of CRISPR and Cas9, which exploit natural genomic properties developed during millennia of biochemical evolution by archaea and bacteria to precisely edit the genome of an organism. Precise capabilities have been achieved in editing the genome of plants for crop protection by eliminating genes mediated by CRISPR/Cas9 with a high degree of effectiveness in conferring resistance to harmful organisms. For this purpose, several delivery methods are already being applied, such as Agrobacterium-mediated delivery, bombardment delivery, and polyethylene glycol application [8], which ensure a high degree of precision in editing the plant genome aimed at demonstrating resistance to crop pests.

The application of CRISPR/Cas9 opens up significant possibilities for efficient and effective editing of the plant genome. Since the first application of CRISPR/Cas9 in plant genome editing [9], numerous scientific publications have demonstrated the value of the method for various purposes, among which resistance against pathogens, insects, and weeds is particularly valuable. Thus, the CRISPR/Cas9 system as a strategy opens up real prospects for genome editing to protect crops against pathogens, insects, and weeds, contributing to ensuring food security and maintaining environmental conditions.

APPLIED MATERIALS AND METHODS

The forecasting of the development of harmful organisms was carried out using the "AgroExpert" electronic system for determining climatic indicators and their warning [1].

The protocol methods of microbiological and virological research adapted for the development of alternative means of plant protection and cultivation in vivo and in vitro were applied, using the production of biological agents on the surface, in depth and semi-depth [10].

Testing in laboratory conditions, on experimental plots and for the production of microbiological means of plant protection was carried out in randomized repetitions with statistical processing of the results [11].

In order to analyze the prospects for the development of agriculture and in order to reduce the impact of harmful organisms on agricultural crops, the concept of the development of biological preparations for plant control and the implementation of organic agriculture through the application of integrated plant protection was developed.

RESULTS AND DISCUSSIONS

CRISPR/Cas9 in the control of crop pathogens. Phytopathogenic agents (viruses, bacteria, fungi, nematodes) represent significant challenges for agriculture, causing biotic constraints and threats to food security, and the damage caused by them causes significant reductions in the quantity and quality of crops by 20-25%, and under conditions of epiphytic development it exceeds 50% [1].

To solve these problems, genome editing is proposed, which is a set of modern technologies that allow precise modification of the DNA of an organism, by adding, removing or replacing fragments of genetic material, which are assembled in the CRISPR/Cas9 technology, which originates from a natural defense mechanism of bacteria against viruses. During evolution, when bacteria were attacked by viruses, the host cell retained fragments of viral DNA, intercalating them with its own genome and forming structures that act as an immunological memory. Upon a new infection, the bacteria produce RNA segments capable of recognizing the viral DNA, and the Cas9 enzyme intervenes to cut and deactivate it. Using this phenomenon, scientists have adapted this mechanism to edit cellular DNA. In this sense, a small fragment of guide RNA is created, which binds to a target genetic sequence. In combination with the Cas9 enzyme, this RNA directs the system to the exact place in the genome where the intervention is desired. As a result, once the DNA sectioning has occurred, the cell's natural repair mechanisms are used to add, remove or replace genetic segments.

The first experiments with this technology were carried out on animals and plants, especially in the agricultural and industrial fields. In plant science, the method has been used to increase plant resistance to phytosanitary agents and to adapt agricultural crops to climate change and harsh habitat conditions. CRISPR/Cas9 technology opens up prospects for introducing artificial mutations, which modify the target gene to resemble a type resistant to disease pathogens, which have evolved in relationships with pathogens. Current research is aimed at creating conditions for excluding mutations and nucleotide variations, which would affect plant viability and productivity [12]. The results recorded mitigate yield losses and ensure increased productivity and sustainability of agriculture.

The use of CRISPR/Cas9 to enhance crop resistance to phytopathogenic viruses. Of significant resonance are the results of the application of CRISPR/Cas9 to introduce genetic modifications in alleles associated with viral resistance by applying the 4E initiation factor, which has an important role in the life cycle of RNA viruses [13]. The application of the eIF protein in the initiation of protein translation by recognizing and interacting with mRNA structures and ribosomes can prevent viral proliferation and host infection, which confers viral resistance to various plants. Thus, CRISPR/Cas9 technology can also be used for direct targeting of the viral genome, offering a promising approach for generating virus resistance [14].

CRISPR/Cas9 technology offers a powerful strategy to confer durable resistance against viral pathogens in crops. The method was used to edit the AC2 and AC3 genes of the mosaic virus genome, which play crucial roles in gene activation, viral pathogenicity, and suppression of viral gene silencing. CRISPR/dCas9 can serve as a DNA-binding protein to effectively block the promoter region of target genes, thereby preventing virus replication and reducing viral infection rates [15]. By harnessing Cas9 complexes with transcription ejector complexes, the researchers induced transcriptional interference at the promoter regions of target genes. This interference disrupts the binding or elongation of RNA polymerase, further inhibiting virus replication and reducing the spread of viral infection in plant tissues. Activities to highlight the potential of CRISPR/Cas9-mediated transcriptional interference for the development of virus-resistant crops and improving plant defense mechanisms against viral pathogens are becoming current.

Gene editing to enhance plant resistance to fungal diseases. CRISPR/Cas9 technology offers possibilities for developing plants resistant to pathogenic fungi by targeting genes associated with susceptibility to fungal diseases. The method has been demonstrated to be effective in engineering crops resistant to fungal pathogens by targeting genes involved in plant-pathogen interactions. By eliminating the gene of the powdery mildew resistance locus, the method led to resistance against the pathogen in various crops [16]. As a result, the resistance locus encodes proteins that make

plants susceptible to the pathogenic fungi, and mutations encoding the loss of this function lead to resistance to the studied disease.

The recorded results emphasize the potential of CRISPR/Cas9 in creating fungal-resistant crops by targeting key genes involved in plant-fungus interactions. The method has demonstrated high efficacy in conferring resistance to agricultural crops by targeting specific genes involved in plant defense mechanisms. Mutations induced in the ethylene response factor gene have been shown to enhance resistance to rice blastoma [17]. The factor serves as a negative regulator of pathogen resistance, and frameshift mutations in this gene contribute to increased resistance to rice pathogens.

The disruptions provided by the CRISPR/Cas9 method led to increased synthesis of salicylic acid, which as a signaling molecule is involved in plant defense responses against pathogens. High levels of salicylic acid contribute to strengthening the plant's immune response, thus improving its ability to resist infections with crop pathogens.

Gene editing to increase plant resistance to bacterial infections. Starting from the expansion of the distribution area and the intensity of the development of bacterial pathogens, the use of CRISPR/Cas9 contributes substantially to the development of plants with resistance to these infections. In this regard, the modification of gene promoters is applied, which increase resistance to various pathogens of agricultural crops. The main effector of the transcription activator type in pathogens of the genus *Xanthomonas* binds specifically to the effector binding element in the gene promoter. If the binding of these elements activates gene expression, accelerating the manifestation of the disease, then mutations within this complex reduce or even eliminate the activity of the promoter and increase the resistance of the crop plant [18].

By using CRISPR/Cas9 to disrupt the gene of the bacterial agent, plants obtain increased tolerance to pathogens of bacterial origin. As an example of the elimination of susceptible genes, a study was conducted to eliminate the gene encoding a protein in apple (*Malus domesticus*) [19], which plays a key role in the infection mechanism of *Erwinia amylovora*. This technique highlights the potential of CRISPR/Cas9 to target specific genes and regulatory elements, thus providing an effective strategy to enhance plant resistance against bacterial pathogens.

CRISPR/Cas9 can enhance resistance to bacterial blight pathogens in tomato by editing repressor proteins of the JAZ domain [20]. The JAZ protein is essential for stimulating stomatal opening, which facilitates bacterial colonization of leaves. Editing the JAZ gene by CRISPR/Cas9 prevents stomatal reopening, thus preventing bacterial colonization. These approaches highlight the role of CRISPR/Cas9 in the development of disease-resistant crops by targeting genes involved in susceptibility

and enhancing the natural defense mechanisms of agricultural crops. By precisely editing specific genes, CRISPR/Cas9 can significantly improve plant resistance to various bacterial infections, contributing to sustainable agricultural practices and increasing crop yields.

The potential of CRISPR/Cas9 for crop protection against insect pests. Phytophagous insects, which feed on the vegetative and generative components of plants, pose a threat to agricultural development. Insects are the most diverse group of arthropods and cause serious damage [21], causing annual losses of 20–25% of global agricultural production and playing a crucial role as vectors for the transmission of various plant disease pathogens. This necessitates genome editing to reduce the damage caused by insect pests [22].

The application of the method has opened up promising possibilities for modifying the biochemical composition of different organs of insect pests by using mutations in various harmful phenotypes, such as abnormal gonads, deformed segments, embryonic lethality, which cause the manifestation of male and female infertility [23]. Similarly, it has been shown that the elimination of proteins that bind sex pheromones in some insect pests, such as *Spodoptera litura*, changes the perception of pheromones by females, thus preventing mating attempts [24]. This demonstrates the capabilities of CRISPR/Cas9 to influence genes that encode the biosynthesis of proteins that determine insect development.

Based on the growing interest in crop resistance as an indicator of plant health, scientists in the field of CRISPR/Cas9 application have recorded results in modifying the chemical composition of insects, such as the achievements recorded in the amplification of the generation of salicylic acid, which is like an insect repellent [25] and also acts as an immune signal, which opens up perspectives oriented towards the development of pest-resistant crops, thus reducing dependence on chemical means. This expands the possibilities of using the method for population modification by spreading a genetic variant that eliminates a harmful trait.

The extension of the application of the method achieves impressive results have been applied to gene induction systems, including meiotic induction, such as transposons, as DNA sequences that change their position from one place to another within the genome of the same cell, stopping embryonic dominance by maternal effect [26]. Thus, a CRISPR/Cas9-mediated gene induction construct was developed for *Plutella xylostella*, which significantly increased the efficiency of the genetic impulse, which opens up real prospects for controlling the development of the pest and maintaining the state of the environment.

CRISPR/Cas9 Crop Protection Capabilities for Weed Control. Among the entities that affect crop development, weeds represent one of the biotic limiters on agricultural yield [1, 2], manifesting themselves as competitors for essential resourc-

es. Weeds serve as hosts for various insects, viruses, pathogens of different origins and devastate native habitats, disrupting ecosystems.

To solve these problems, CRISPR/Cas9 technology addresses different pathways for herbicide-resistant plant breeding. This allows the development of genetically modified herbicide-resistant plants and the selective elimination of unwanted weeds, contributing to sustainable agricultural practices. For example, the herbicide glyphosate inhibits the biosynthesis of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase in plants, disrupting the shikimate pathway and causing plant death [27]. A more conventional approach is to transfer the gene from the Agrobacterium strain to crops such as potatoes. The bacterial enzyme from Agrobacterium has a small modification that prevents glyphosate from binding, thus allowing the plant to continue producing amino acids even in the presence of glyphosate.

These methods illustrate how CRISPR/Cas9 can be used to create herbicide-resistant crops by precisely modifying specific genes or regulatory elements. This approach not only offers an alternative to traditional genetic engineering methods, but also offers a more targeted and efficient way to enhance herbicide resistance in crops. Over the past eight decades, the constant use of herbicides has led to the widespread evolution of herbicide resistance in numerous weed species [28]. Therefore, the CRISPR/Cas9 gene drive has emerged as a new genetic control strategy in weed management. As an alternative to herbicides, genetic engineering does not present safety concerns related to exposure to hazardous chemicals that can lead to the lack of disruption of agricultural ecosystems, as well as the low cost of managing invasive plants and the need for minimal human intervention [29].

Regulations on genome-edited crops. Starting from the need to solve phytosanitary problems and considering the similarities and role of genome-edited crops compared to GMOs, it has become necessary to develop and approve national, regional and international legislation [30]. It is necessary to mention that the former fundamentally differ from genetically modified organisms in that they do not introduce foreign DNA, but only modify existing genes. In this regard, the European Union often regulates genome-edited crops under strict GMO legislation, although European legislation is in the process of reform [31].

Regulations on genome-edited crops vary significantly from one country to another, reflecting different public perceptions and agricultural policies. In the US, the regulation of genome-edited crops is overseen primarily by three agencies, which recognize that genome-edited crops are produced through conventional breeding and are not subject to the same regulations as GMOs, but emphasize the need for safety assessment if the edited crop introduces a novel protein with serious nutritional implications. The European Union has a stricter approach to regulating genome-edited crops, and they are regulated similarly to GMOs. China regulates genome-edited

crops through agricultural legislation, which is constantly evolving as China invests heavily in biotechnology research. It is worth noting that in most countries, in addition to assessing human health and food safety, environmental safety is also monitored.

Analyzing the current situation, against the backdrop of worsening phytosanitary and environmental conditions, as regulatory frameworks evolve, it becomes evident that the commercialization of CRISPR/Cas9-edited crops is expected to be accelerated by companies concerned with their promotion and ensuring plant health [32].

CONCLUSIONS

Starting from the need to solve phytosanitary problems and against the background of achievements in the field of genetic engineering and biotechnology, it becomes obvious that the potential of genome-edited crops in the protection of agricultural crops is current, vast, and promising, which increases hopes for achieving resistance and productivity of agricultural crops.

CRISPR/Cas9 technology has established itself at the forefront of these innovative trends, ensuring high efficiency in genome editing. One of the key future prospects is the integration of CRISPR/Cas9 with other emerging technologies, such as synthetic biology and bioinformatics, offering various solutions for maintaining plant health. By combining CRISPR/Cas9 with advanced data analysis and modeling techniques to expand the range of measures and means of plant protection, it has become possible to predict the results oriented to genetic modifications, as well as optimize editing strategies for the effectiveness required by agricultural operators. This allows the development of crops resistant to phytosanitary agents and adapted to specific environmental conditions and that can adapt to abiotic stresses that have become very common.

Starting from practical needs, the use of CRISPR/Cas9 to improve plant nutritional profiles by precisely editing genes involved in metabolic pathways becomes current and necessary, which will open up prospects for applying the capabilities of these technologies.

Against the background of the problems addressed and in the conditions of fluctuation of decisions approved by decision-makers, it becomes rational for scientists and stakeholders to collaborate to ensure the safety and efficacy of genome-edited crops. Future research should focus on identifying and validating target genes involved in responses to abiotic stress and on developing methods and elaborating efficient editing tools to increase crop resistance and ensure plant health.

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