

CREATION OF RESISTANT GENOTYPES AGAINST FUNGAL DISEASES IN WHEAT USING THE CRISPR-CAS 9 METHOD (A REVIEW)

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Abstract. Wheat is one of the main food crops cultivated in the world. Although wheat yield has increased significantly in recent years, wheat production is predicted to face great difficulties due to global climate change and diseases. Pests and diseases cause 20-40% of global food losses and cause 220 billion US dollars in agricultural trade losses every year. Diseases such as wheat rusts, consisting of leaf, stem and stripe rusts, fusarium wilt, etc., are significant problems for farmers worldwide and cause major yield losses in one of the most important staple crops in the world. Currently, targeted genome editing is a rapid technology for generating mutations in candidate genes. CRISPR/Cas9-based genome editing can be effectively used to target genes involved in resistance to biotic and abiotic stress factors. In the research, the pleiotropic effect and susceptibility mechanism of candidate genes in wheat plant resistant samples to various fungal diseases were comparatively studied using CRISPR/Cas9 technology and the advantages and disadvantages of each were identified.

Keywords: Wheat, powdery mildew, CRISPR/Cas9.

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1. Introduction

The importance of the wheat plant and its spread around the world

Wheat is one of the most important food crops in the world (Mammadova *et al.*, 2025). First of all, it is the most important source of carbohydrates, proteins, vitamins, iron and zinc for humans. More than 55% of the world's carbohydrate sources and 20% of the calories from food in the world fall on wheat. Approximately 67% of the wheat produced is used for food purposes, 21% for feed purposes. The remaining small part is used in the industry for the production of paper and cardboard and in recent years, as bioethanol, i.e. fuel (Khalid *et al.*, 2023).

When looking at wheat production in the world and in Azerbaijan, 216 million ha of wheat are planted in the world. In Azerbaijan, this figure is 546.3 thousand ha. The amount of wheat harvested was 765.7 million tons in the world and 1 million 685 thousand tons in Azerbaijan. It should also be noted that compared to 2023, the cultivated area in 2024 increased by 0.6%, but the harvest decreased by 8%.

The diverse climatic conditions of our republic allow for the cultivation of agricultural crops over a wide area (Alizade *et al.*, 2023; Alizade, 2023). Two types of wheat are cultivated in our republic. These are soft and durum wheat. They are, in turn, divided into groups according to their lifestyle. Durum wheats are divided into 3 groups

and soft wheats into 2 groups. Hard wheats: autumn (must be sown in autumn), spring (air temperature must be above 0°C) and semi-autumn. Soft wheats are divided into 2 groups: spring (cultivated in areas with mild winters) and autumn (cultivated in areas with harsh winters) (Mammadov & Ismayilov, 2012).

Main fungal diseases of wheat plants

Plants are exposed to a number of adverse environmental influences during their development (Alizada, 2022; Alizada & Aliyeva, 2024). The occurrence of a number of diseases is inevitable until the ripening stage of wheat, which is the main food source. Among the diseases that seriously affect plants productivity, the most widespread diseases are fungal diseases (Alizada *et al.*, 2024; Amrahov *et al.*, 2024). Infection of fungal diseases is spread through airborne soil and seeds infected with injuries. In most cases, warm and humid weather is a positive factor in the development and spread of fungal diseases. Fungal diseases damage the vegetative parts of plants: roots, stems and leaves, preventing their photosynthesis process and preventing grain ripening. In addition, they also cause direct crop loss by infecting spikes and grains.

Zymoseptoria tritici is a fungal disease that causes spots on wheat leaves and seriously reduces yield by 20%-50%. The formation of spots on the leaves reduces the photosynthesis area. Thus, it directly affects the health of the plant and indirectly prevents the grains from ripening. The spots are observed in mixed shapes, as well as elongated and oval shapes, brown in color. Black dots (pycnidia) are observed inside these spots. Pycnidia participate in the production of fungal spores. The first symptoms are observed at the beginning of winter. The spots are first observed in a chlorotic (yellowed) form on the woody shoots. As the infection spreads, the disease moves upwards and the leaf tissue gradually becomes necrotic with dark brown borders. Grains and spikes are also affected by this disease. As the disease progresses, drying and premature shedding of leaves are observed (Afzal *et al.*, 2022). Humid weather conditions, as well as humidity and rain, have a positive effect on the development of this fungus, as well as its spread by water droplets (pycnidiospores) and wind (ascospores). This fungus survives in the soil and plant debris as well as in plant tissues. This causes the disease to spread again at a later time. The fungus undergoes a latent period of 7-14 days. As the name suggests, no symptoms of infection appear during this period. This makes it difficult to detect and control the disease in the later stages of development.

A number of measures are taken to combat this disease. The first agrotechnical measures that come to mind are the destruction of infected plants without infecting other plants and crop rotation methods. Biotechnologically more thoughtful measures include planting resistant varieties during planting.

Cereal plants are affected by yellow rust (*Puccinia striiformis* f. sp. *tritici*) and brown rust (*Puccinia triticina*).

Yellow rust is noticeable on the spikes and leaves with its yellow-orange color and elongated shape. During the course of the disease, thin yellow spots appear on the leaves. This disrupts the photosynthesis process in the leaves, the infected leaves dry up and the development is weakened. Unlike Septoria Leaf Spot, this fungus requires cool weather conditions for its development (approximately 10-15°C). Like other fungi, this fungus also requires humidity for its development (more than 90%). By disrupting the photosynthesis process, yellow rust prevents the grains from fully ripening and causes premature leaf fall.

If the disease is observed in the early stages of wheat development, it causes a minimum of 30% to a maximum of 70% yield loss. Since this pathogen has numerous physiological races (subtypes that show multiple genetic differences without plant resistance), it is difficult to obtain pathogen-resistant varieties.

The disease manifests itself when the plants are still in the tillering stage. Wind plays a key role in the spread of the yellow rust fungus. This causes the disease to spread rapidly among plants. Therefore, early detection of this disease is very important.

Puccinia triticina brown rust appears on the surface of the leaves of the plant. It is observed in small, circular or oval shapes in reddish-brown color. Unlike yellow rust, stripes do not form on infected plants, but spread in an irregular shape. The water balance of plants infected with *P. triticina* is disrupted and evaporation increases. Thus, the leaves dry and fall off. Unlike yellow rust, brown rust requires warm weather conditions (approximately 15-22°C) and moderate humidity for its reproduction. If the plant is infected during the grain filling stage, it causes a 10-40% yield loss. *Puccinia triticina* is spread by the wind. It can overwinter in mild climates and when infected with plants of the *Thalictrum* genus.

After harvesting, the fields with plants infected with these diseases should be cleaned and deep plowed. As a method of combating the disease, a crop rotation system should be applied.

Ustilago tritici settles in the awns of wheat plants and multiplies there. In wheat plants infected with the disease, black masses appear on the spikes instead of normal seeds. On these, black powdery stone-like masses contain spores of the fungus (teliospores). The wind causes the spores to spread over long distances and infect other plants. Although *Ustilago tritici* is destroyed when it falls on the soil, when it falls into a warehouse or a series of storage places with infected seeds, it is destroyed for 3-5 years and it can even retain its germination capacity. Plants infected with this fungus cannot be identified in the early stages. There are no visible signs at that time. For the development of the fungus, a relatively low temperature of 5-10°C and a humidity of 40-60% in the middle range are required (He *et al.*, 2023).

Since the main “target” of the fungus is seeds, this disease can cause 100% crop loss. Because it is impossible to obtain a crop from infected seeds. In addition, if infected seeds are intended for sowing the following year, the disease will inevitably spread to subsequent years (Ma *et al.*, 2009).

The use of a rotational cropping system is one of the main measures to prevent the disease. In addition, planting sustainable, i.e. certified varieties is also included in the measures. It is possible to get rid of the disease with thermotherapy. Keeping seeds in water at a temperature of 52°C for 10 minutes destroys the fungus (Milus & Mirlohi 1995).

Septoria spp., especially seen in cereal plants (wheat, oats, barley). Damages the vegetative organs of plants - stems and leaves, directly preventing the photosynthesis process of the plant. This disease is caused by fungi of the genus *Septoria* and is characterized by the formation of yellow, brown, oval and elongated necrotic spots on the leaves (Merrick *et al.*, 2021).

As in leaf spot disease, black dots are observed inside the spots in *Ustilago* disease. Their function is the same as in *Septoria tritici* disease. The development of the disease, like the development of the *Septoria* fungus, starts from the lower leaves. As the fungus spreads to the plant, it causes the leaves to dry out completely and fall prematurely. Thus, the

photosynthesis process is disrupted and the yield is seriously reduced by 30-50%. In addition, the quality of the wheat also deteriorates (Hafeez *et al.*, 2021).

Septoria spp. Rainy, humid and windy weather plays a positive role in the spread of fungal infection. As in the case of the disease, seeds are also infected in this disease, so seeds and plant residues also play a role in the spread of the fungus. The temperature of 15-25°C is the most favorable temperature for fungal infection.

The main methods of infection control are the destruction of residues and deep plowing methods. Fungicides belonging to the triazole and strobilurin groups are widely used (Jørgensen *et al.*, 2014).

Erysiphe graminis (now known as Blumeria graminis) mainly causes disease in cereal crops (wheat, barley, rye). It severely reduces yield by 10-30%, on average by 25% and in some cases by up to 50%. This fungus, which is usually widespread in hot and dry climates, multiplies faster in cool weather with 85% humidity. The optimum temperature for the development of the fungus is 15-22°C and 60-80% humidity (Gorash *et al.*, 2021).

Mildew disease can only develop in living plant tissues. In the initial stage of infection, yellow spots appear on the leaves. In the advanced stages of the disease, the leaves are covered with a layer of whitish powdery powder. This white powder carries the spores of the fungus. As we have mentioned in other fungal diseases, the disease starts from the lower leaves during this disease. Then the disease spreads to the upper leaves. The disease begins in the development of birch from “young” leaves and continues to develop throughout the entire vegetation period of the plant (Ghimire *et al.*, 2020).

CRISPR-Cas 9-based transformation procedure and plants to which it is applied

In nature, CRISPR-Cas exists as a microbial adaptive immune system that uses RNA-guided nucleases to cut foreign genetic elements such as bacterial phages. Biotechnologists have adapted this system to enable gene editing applications in various species, including plants. More than 30 different CRISPR-Cas systems exist naturally, but most gene editing applications are carried out with CRISPR-Cas9-based systems (Yuan *et al.*, 2024).

Advances in molecular biology have led to the development of different technologies such as the CRISPR-Cas9 system and thus the development of biotechnology in plants. This process, which is naturally present in bacteria and is called a defense system, has allowed its application to plants in agronomically important areas such as disease, stress resistance and increased yield.

The application of the CRISPR-Cas9 Transformation Procedure to plants is as follows:

In the initial stage, the target gene is selected. Then, the guide RNA is “designed” according to this gene. At this time, off-target effects are minimized.

The next stage is the preparation of the vector. The gene parts encoding gRNA and Cas9 are cloned into plasmid vectors as T-DNA-containing binary systems. There are various methods of vector transformation. These are *Agrobacterium tumefaciens*, Biolistic and Protoplast transfection methods.

Transformed cells are selected with the help of markers and placed in a medium rich in hormones (auxin/cytokinin) to transform into plants.

There are several assays available to confirm this editing. These are PCR, Sanger sequencing and qRT-PCR analysis methods.

Some plants where the method has been applied and its application purpose:

This method has been applied to transfer drought resistance genes and edit genes involved in gluten biosynthesis in wheat (*Triticum aestivum* L.) (Wang *et al.*, 2014).

It has been applied to Blast and Bacterial Blight disease, as well as salt tolerance genes in rice (*Oryza sativa*) (Poddar *et al.*, 2023).

It has been used to improve fiber quality and modify oil content in cotton, soybean and flax plants (Jørgensen *et al.*, 2014).

Genes have been introduced in tomato (*Solanum lycopersicum*) to increase resistance to viral diseases and increase product shelf life (Jin *et al.*, 2022).

Genome editing approaches to various fungal diseases in wheat

S gene examples of powdery mildew. The first S gene knockout in wheat using genome editing was TaMLO, which increased resistance to powdery mildew. TALEN-mediated knockout of all three TaMLO homologues showed strong resistance, but resulted in reduced growth and yield, limiting its use in plant breeding. CRISPR/Cas9 knockouts a mutant line with a 304 kilobase deletion in TaMLO-B1 was generated, which was resistant and highly productive. At the same time, CRISPR/Cas9 knockout of *TaEDR1* homeologs generated moderately resistant genotypes. Similarly, knockout of all *TaMPK1* homeologs was found to increase resistance to powdery mildew. Among the *TaMPK1* homeologs, the D-genome contributed the most to susceptibility and the unequal roles that homeologs play in susceptibility were noted (Li *et al.*, 2022).

S genes for rust disease. Knockout of *TaMPK1* by CRISPR/Cas9 was found to increase resistance not only to powdery mildew but also to stripe rust, another pathogen of wheat. In another study, knockout of *TaGW2* and *TaWRKY19* by CRISPR/Cas9 was also reported to increase resistance to leaf and stripe rust in wheat, respectively. In addition to resistance to multiple pathogen races, race-nonspecific resistance has also been achieved by knockouts of the wheat S gene. For example, knockouts of the *TaCIPK14* gene were found to increase resistance to five-stripe rust race without reducing yield in field conditions. Similarly, knockouts of the *TaPsIPK1* gene were found to increase resistance to Chinese stripe rust epidemic in field trials without affecting yield. Knockout of the *TaPsIPK1* gene was also found to improve resistance to leaf rust, but was not effective against root rust. This highlights the importance of testing S gene knockouts against multiple pathogens to detect broad-spectrum resistance potential (He *et al.*, 2023; Liu *et al.*, 2024; Wang *et al.*, 2022).

Examples of S genes for other fungal diseases of Fusarium

Fhb1 is the most effective source of stable and broad-spectrum resistance to FHB (Fusarium head blight) in wheat. Loss of function of *TaHRC-3B* has been identified as a major determinant of Fhb1-mediated resistance and is classified as S gene-mediated resistance. Consequently, knockout of *TaHRC-3B* via CRISPR/Cas9 offers a faster alternative for introducing this locus into wheat varieties, improving FHB resistance. Similarly, knockout of all six copies of the *TaNFXL1* gene, which is associated with susceptibility to the deoxynivalenol toxin, has also been shown to enhance FHB resistance (Cuthbert *et al.*, 2006; Su *et al.*, 2019).

Other fungal pathogens such as *Septoria nodorum* leaf spot and black spot cause disease in wheat by producing necrotrophic effectors such as ToxA, which interact with the *TaTsn1* gene. Loss-of-function mutants of the *TaTsn1* gene were found to be sensitive to ToxA and resistant to *Septoria nodorum* blotch, confirming that this gene is an S gene. In another study, CRISPR/Cas9-mediated silencing of *TaTsn1* increased wheat plants'

susceptibility to ToxA and similar resistance to this fungal pathogen was observed in EMS mutants. Similarly, CRISPR/Cas9-mediated knockout of *TaSnn5*, which is targeted by the necrotrophic effector Tox5, resulted in lines with increased resistance to *Septoria nodorum* blotch and insensitivity to Tox5 (Poddar *et al.*, 2023).

2. Conclusion

Identification of genes involved in stress resistance, their knock-in or knock-out using rapid genome editing technologies, as well as the creation of genetically stable knock-down mutants and their application in breeding programs for the creation of disease-resistant varieties in wheat are of high relevance. CRISPR-Cas9 is the most effective and fastest way to make changes in plants. This method is also the most accurate technology for eliminating problems in plants on a global scale. To date, the main application of genome editing for disease resistance has been the targeted knockout of S genes, which improve resistance to many major diseases of wheat. In future studies, combining the knocked-out S genes in a single organism will create conditions for the creation of varieties more resistant to various pathogens. At the same time, the creation of elite lines against several pathogens by identifying, editing and combining R and S genes that are not subject to negative pleiotropic effects into a single genotype remains one of the important issues. The determination of disease genes in wheat using CRISPR-Cas9 was discussed in the diploma thesis and the results were obtained based on local and foreign literature. It was found that editing the *TaMKP1* and *TaGW2q* genes, which have a positive pleiotropic effect, was more effective in increasing resistance to rust diseases. At the same time, a locus with a multigenic structure that provides resistance to a broad spectrum of rust diseases was identified.

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