

# Application of Molecular Marker Assisted Selection in Wheat Stress Resistance Breeding

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**Abstract** This study explores the key role of molecular marker assisted selection in wheat stress resistance breeding. Wheat is one of the most important food crops in the world, but it faces challenges from climate change and stress, which affect yield and quality. Molecular marker technology provides a powerful tool for wheat breeding, allowing for more efficient selection of stress resistance genes. This study introduces the importance of wheat as a food crop, as well as the relationship between stress resistance and wheat breeding. Explored different types of DNA markers and their applications in wheat stress resistance breeding, including marker assisted selection, QTL analysis, and gene editing techniques. The study emphasizes the importance of molecular marker strategies and methods to accelerate the identification and breeding of stress resistant genes. Finally, some successful cases of wheat stress resistance breeding were summarized, emphasizing the potential of molecular marker assisted selection and looking forward to future development trends. This study emphasizes the importance of molecular marker technology in wheat stress resistance breeding, providing new hope for food production and food security.

**Keywords** Wheat breeding; Resistance to adversity; Molecular markers; QTL analysis; Gene editing

Wheat (*Triticum aestivum* L.) is one of the most important food crops in the world, occupying a core position in world food production. However, the yield and quality of wheat are often threatened by various adverse factors, such as climate change, pests and diseases, saline alkali soils, and drought. These adverse conditions have had a serious impact on the growth and development of wheat, posing a threat to global food security. Therefore, wheat stress resistance breeding has become a crucial task for current and future food production (Bakala et al., 2021).

Traditional wheat breeding often takes several years to cultivate new varieties, and progress is slow. The introduction of molecular marker assisted selection (MAS) technology has completely changed the pattern of wheat stress resistance breeding. MAS uses genetic markers to identify and select individuals with specific stress resistance genes, thereby improving breeding efficiency. This method enables breeders to screen wheat germplasm resources with the required stress resistance more accurately and quickly.

The goal of this study is to explore in depth the application of molecular marker assisted selection in wheat stress resistance breeding, emphasizing the potential impact of this method on wheat yield and quality, and to provide a detailed introduction to the background and importance of wheat stress resistance, from climate change to soil conditions, from growth environment to pest and disease pressure, highlighting the challenges faced by wheat. Meanwhile, we will discuss how different types of molecular markers, such as marker assisted selection, quantitative trait loci (QTL) analysis, and the latest gene editing techniques, can be applied in wheat stress resistance breeding.

In addition, this study will share some successful cases of wheat stress resistance breeding, which demonstrate the effectiveness of molecular marker assisted selection methods. Finally, we will look forward to the future and explore the challenges that the field of wheat stress resistance breeding may face, such as regulatory and ethical issues, as well as the application prospects of emerging technologies and methods.

## **1 Wheat Breeding and Stress Resistance**

### **1.1 Wheat as an important food crop**

Wheat (*Triticum aestivum* L.), as one of the most important food crops in the world, has always held a core position in human diet. Its extensive cultivation and high yield make it one of the main sources of food for many countries around the world. The importance of wheat is not only reflected in its large-scale production quantity, but also in its significant impact on global food supply, agricultural economy, and food security.

The diversity of wheat is crucial for its adaptation to different climate and soil conditions. Different varieties and subspecies of wheat grow in different regions and adapt to various environments, from temperate to subtropical, from plateaus to plains. This adaptability makes wheat one of the main crops in various ecosystems, thus meeting the food needs of populations in different regions.

Wheat not only plays a crucial role in global food supply, but also serves as a major ingredient in foods such as bread, noodles, grains, and pastries. It is rich in carbohydrates, proteins, vitamins, and minerals, providing humans with abundant nutrition. Therefore, wheat not only plays an important role in agriculture and food industry, but also has a profound impact on global human health and livelihoods.

However, wheat production is often threatened by various adverse factors, including climate change, pests and diseases, declining soil quality, and drought. In order to maintain global food supply, meet human food needs, and cope with constantly changing environmental conditions, wheat breeding and genetic improvement are particularly important. By introducing stress resistance genes and improving the growth and yield of wheat, we can increase its ability to resist various stress conditions (Bajwa et al., 2020).

Therefore, as one of the major global food crops, wheat's importance is not only reflected in meeting food needs, but also in its ability to provide solid support for agricultural production, food industry, and human livelihoods. To ensure sustainable production of wheat and global food supply, wheat breeding and genetic improvement will continue to be important areas of scientific research and agricultural practice.

### **1.2 Relationship between stress resistance and wheat breeding**

The relationship between stress resistance and wheat breeding is of great significance. As a major global food crop, wheat production is often threatened by various adverse factors such as climate change, pests and diseases, declining soil quality, and saline alkali land. Therefore, improving the stress resistance of wheat has become an urgent task in breeding to ensure global food supply, agricultural sustainability, and food safety.

Stress resistance breeding aims to cultivate wheat varieties with stronger tolerance to various stress conditions, which can include adaptation to climate change, resistance to pests and diseases, tolerance to saline alkali land, and so on. Through genetic improvement, scientists can introduce genes related to stress resistance to enhance wheat's ability to cope with stress. This not only increases wheat production and yield, but also reduces crop losses (Sun et al., 2020).

The success of stress resistance breeding cannot be achieved without the application of modern biotechnology such as molecular marker assisted selection (MAS). MAS enables breeders to more accurately screen candidate wheat plants with stress resistance, thereby saving time and resources. Meanwhile, it also helps to avoid potential unsuitable offspring in traditional breeding, and the development of this technology has improved breeding efficiency and accelerated the cultivation of stress resistant wheat varieties (Sallam et al., 2019).

Obviously, stress resistance is a core area of wheat breeding, which is directly related to wheat production and global food supply. By introducing genes related to stress resistance and applying modern biotechnology, wheat can better adapt to constantly changing environmental conditions, increase yield, reduce losses, and thus make important contributions to the sustainability of agriculture and global food safety.

### **1.3 The role of molecular marker assisted selection**

Molecular marker assisted selection (MAS) plays an important role in wheat breeding. MAS is a modern

biotechnology tool that uses molecular or genetic markers to predict the genetic characteristics of plant individuals or offspring. In wheat stress resistance breeding, MAS provides many significant advantages, helping to improve varieties and enhance their adaptability to different stress conditions.

MAS can accelerate the breeding process. In traditional breeding, cultivating stress resistant wheat varieties requires years of complex experimentation and observation. With MAS, scientists can identify candidate plants with stress resistance related genes in a shorter period of time, greatly reducing the complex breeding cycle.

MAS provides higher precision and efficiency, and traditional breeding often requires a large number of offspring plants to screen for varieties with stress resistance. This method is very time-consuming and resource intensive. In contrast, MAS can more accurately select plants with target genes through genetic markers, thereby reducing resource input and time costs (Yang et al., 2019).

In addition, MAS also helps to avoid unsuitable offspring. In traditional breeding, the inability to directly observe the genotype of plants may result in offspring that do not meet expectations. MAS helps to avoid these issues and ensure the quality and targeting of offspring by providing genetic information. Most importantly, MAS has brought more choices for wheat breeding. Scientists can select specific stress resistance related genes as needed to create more diverse wheat varieties to adapt to different regions and climate conditions.

Therefore, molecular marker assisted selection provides an efficient, precise, and diverse method for wheat stress resistance breeding. It accelerates the breeding process, improves breeding efficiency and resource utilization, and provides more choices for cultivating wheat varieties that can adapt to different stress conditions. This makes wheat breeding more promising and has the potential to address challenges such as global climate change, increase wheat yield, and ensure food supply.

## **2 Molecular Marker Technology and Applications**

### **2.1 DNA marker types**

In molecular marker assisted selection (MAS), there are various types of DNA markers, each with its unique applications and advantages. Microsatellites are short repetitive sequences in DNA sequences that exhibit length variation between different individuals. This variation can be used to determine differences between individuals, and therefore has a wide range of applications in MAS. Microsatellite markers typically exhibit high polymorphism and are suitable for studying genetic diversity and phylogenetic relationships within species.

SNP is a single nucleotide variation in the genome and is currently the most commonly used DNA marker. Due to its richness and wide distribution, SNP markers are used for high-throughput genotyping, providing highly accurate data for MAS. SNP markers are suitable for identifying and screening various stress resistance related genes in wheat.

RAPD (Random amplified polymorphic DNA) and AFLP (Amplified fragment length polymorphism) markers use random primers or specific restriction enzymes to generate polymorphic DNA fragments. They are widely used in MAS that do not require prior knowledge of the target gene or sequence, as they do not rely on previous gene sequence information (Al Tamimi and Al Janabi, 2019).

EST is a DNA fragment obtained from transcripts, which is used to study gene expression. In MAS, EST markers can be used to identify gene expression differences related to stress resistance, which helps to select wheat varieties with higher stress resistance. CNV is a DNA fragment in the genome that undergoes copy number variation, which can affect gene expression and function. Therefore, CNV markers are used in MAS to screen wheat varieties that adapt to different stress conditions. DNA methylation is an epigenetic marker that can affect gene expression. In MAS, studying DNA methylation status can help determine differences in stress resistance among wheat varieties (Wang et al., 2021).

## 2.2 Application of molecular markers in wheat stress resistance breeding

The application of molecular markers in wheat stress resistance breeding is a key and popular technology, which is crucial for improving wheat yield and quality. Wheat is one of the world's major food crops, however, it is often threatened by adverse factors such as drought, high temperature, saline alkali soil, etc. To address these challenges, breeders need to cultivate wheat varieties that are more tolerant to stress, and molecular marker assisted selection (MAS) has become a powerful tool to achieve this goal.

Molecular markers can help identify stress resistance genes related to specific stress conditions, which can come from different wheat varieties or wild parents. With the assistance of molecular markers, these genes can be easily introduced into the target variety to improve its stress resistance. MAS allows breeders to select individuals with target stress resistance genes during the breeding process, which can save time and resources and avoid heavy field trials. Therefore, through molecular markers, wheat breeders can more effectively cultivate stress resistant varieties.

For example, *Puccinia striiformis* f. sp. Tritici (Pst) is one of the fungal diseases that cause widespread, prevalent, and severe damage to wheat stripe rust. Searching for new disease resistant resources and cultivating disease resistant varieties is one of the most cost-effective ways to control the spread of wheat stripe rust. The study utilized resistance gene similarity sequence polymorphism (RGAP) markers Xwgp36, Xwgp43, and Xwgp44 for assisted selection to transfer the wheat stripe rust resistance gene Yr39 to Chuanmai 42, Bainong Aikang 58, Han 6172, and Zhengmai 9023. Through field agronomic trait evaluation and disease resistance identification, a group of resistant families with excellent agronomic traits were selected. Through molecular markers, breeders can selectively improve specific traits of wheat, such as drought tolerance, high temperature tolerance, and salt alkali tolerance. This is expected to improve the stress resistance of wheat, thereby increasing yield and quality (Zheng et al., 2022) (Figure 1).

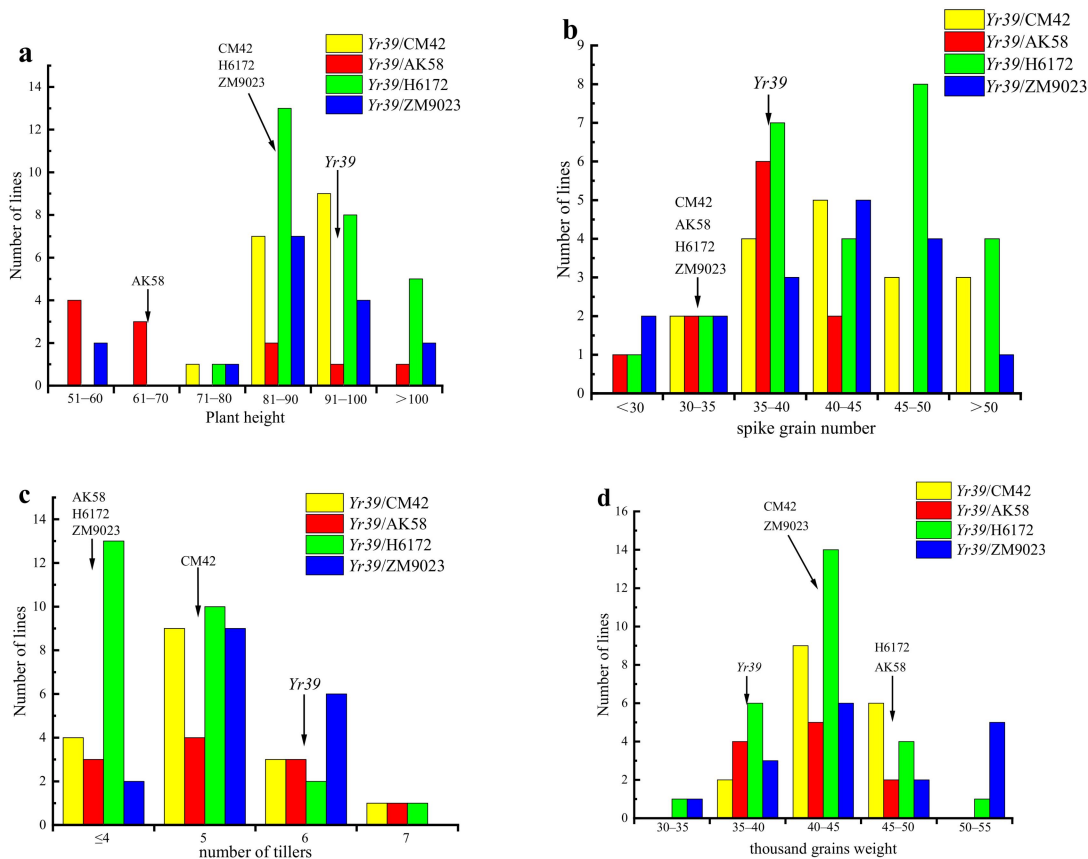


Figure 1 Frequency distribution of 71 backcross offspring lines in four hybrid combinations (Zheng et al., 2022)

Note: a: Plant height; b: Spike grain number; c: Number of tillers; d: Thousand grain weight

Molecular markers play a crucial role in wheat stress resistance breeding, providing an efficient, precise, and sustainable approach to help breeders cultivate wheat varieties that adapt to different stress conditions, thereby contributing to global food security. The continuous development and innovation in this field will continue to drive more success in wheat stress resistance breeding.

### **2.3 Current development of technology and tools**

The field of wheat stress resistance breeding has made remarkable progress in the development of technology and tools, providing wheat breeders with more choices to effectively respond to constantly changing climate and environmental conditions. With the complete interpretation of the wheat genome, researchers can gain a deeper understanding of the genetic mechanisms of wheat, which provides a foundation for the identification and utilization of stress resistance genes. The advancement of molecular marker technology enables breeders to screen and select wheat varieties with target genes more quickly and accurately, thereby improving breeding efficiency.

The continuous development of bioinformatics tools enables researchers to process and analyze large-scale genetic data, including wheat genome data, expression profiles, and metabolomics data. These tools help reveal the expression and regulatory mechanisms of stress resistance related genes, accelerating the research progress of stress resistance breeding.

CRISPR-Cas9 and other gene editing techniques have brought new hope for wheat stress resistance breeding. By precisely editing the wheat genome, researchers can directly modify target genes to improve wheat's adaptability to adversity. The potential of this technology is constantly being demonstrated, providing broad space for innovative breeding strategies (Li et al., 2021).

Traditional breeding methods still play a crucial role, but modern technology provides more possibilities for precision breeding. Breeders can selectively select parents and design mating plans based on the genetic information of wheat varieties to improve stress resistance. This helps to reduce the breeding cycle and cultivate new varieties faster. Modern agriculture also benefits from advanced environmental monitoring technologies and big data analysis, which can monitor meteorological, soil, and other environmental parameters in real-time, helping decision-makers better manage farmland and take timely measures to mitigate the adverse effects of adversity on wheat yield.

In short, wheat stress resistance breeding is ushering in a technological revolution. The continuous development of technology and tools provides wheat breeders with more choices and opportunities to cultivate new varieties that are more adaptable to diverse adverse conditions, thereby helping to ensure the stability of global food supply. In the future, with further innovation and application of these technologies, we can expect more exciting breakthroughs to meet the growing demand for food.

## **3 Molecular Marking Strategies and Methods**

### **3.1 QTL analysis and associated genetics**

QTL (Quantitative trait locus) analysis is a method of identifying gene regions associated with specific traits by measuring the association between quantitative traits and molecular markers. In the study of wheat stress resistance, QTL analysis usually involves large-scale correlation studies between genetic markers and phenotype data. By establishing genetic maps and analyzing genetic marker data, researchers can identify QTLs related to stress resistance. These QTLs can include genes related to drought resistance, disease resistance, salt tolerance, etc.

Association genetics is a method of identifying related genes by analyzing the relationship between genetic diversity and phenotypic differences in natural populations. In wheat, this typically involves collecting wheat samples from different geographic populations and analyzing the correlation between their genetic data and phenotypic characteristics. This method can help identify genes related to stress resistance, especially those that function under natural conditions.

The application of these two methods in wheat stress resistance breeding has achieved important results, and researchers have successfully identified and utilized multiple QTLs and associated genes to improve wheat stress resistance. The advantage of these methods is that they can screen and breed for various adverse conditions, enabling wheat to exhibit better traits in different environments (Kumar et al., 2021).

It is not difficult to find that QTL analysis and associated genetics are important tools in wheat stress resistance breeding. They help to decipher the molecular mechanisms of stress resistance and provide strong support for cultivating wheat varieties that are more adaptable to changing environmental conditions. The continuous development and innovation of these methods will further accelerate the process of wheat stress resistance breeding, and are expected to make important contributions to global food security.

### **3.2 Markup based selection method**

The marker based selection method is a widely used strategy in wheat stress resistance breeding, which fully utilizes the information of molecular markers to select and improve wheat varieties. This method achieves targeted improvement of stress resistance by analyzing the relationship between molecular markers related to stress resistance and the genetic background of wheat varieties.

In order to apply marker based selection, researchers first need to identify genetic markers associated with the target stress resistance trait. This can be identified through methods such as genetic mapping, QTL analysis, and association genetics. These markers are typically located in the wheat genome and are associated with specific stress resistance traits such as drought resistance, disease resistance, and salt tolerance (Kumar et al., 2020).

Once appropriate genetic markers are identified, researchers can conduct marker phenotype association analysis to determine the degree of association between these markers and specific stress resistance traits. This helps to screen out the most promising candidate markers for subsequent selection and breeding. In the breeding process, marker assisted selection methods allow researchers to directly select wheat individuals or varieties with target stress resistance traits based on molecular marker information. This helps improve breeding efficiency and reduces heavy field trials.

With the development of high-throughput molecular marker technologies, such as SNP (single nucleotide polymorphism) markers, the screening and application of molecular markers have become more efficient and accurate. These new technologies enable researchers to conduct genetic analysis on a larger scale to better understand the genetic substrates underlying wheat stress resistance.

The marker based selection method helps to enrich the genetic resource pool of wheat. By identifying and preserving genetic variations related to stress resistance, researchers can better maintain and improve the stress resistance of wheat. With the continuous development of molecular biology and bioinformatics, marker based selection methods will continue to provide powerful tools for wheat stress resistance breeding. Future research will delve deeper into the molecular mechanisms of wheat to better adapt to ever-changing environmental conditions and contribute to global food security.

It can be seen that marker based selection methods have become an important strategy for wheat stress resistance breeding. It provides researchers with more precise and efficient breeding methods, which is expected to provide more opportunities for improving wheat stress resistance and variety innovation.

### **3.3 Gene editing technology**

Gene editing technology is an advanced method that has emerged in wheat stress resistance breeding, providing a revolutionary way to change the genetic characteristics of wheat. This technology enables researchers to directly intervene in the wheat genome to achieve modification and precise control of specific genes. The basic principle of gene editing technology involves the use of specific proteins, such as CRISPR-Cas9, ZFN (zinc finger nuclease), or TALEN (zinc finger nuclease), to cut and repair DNA double strands. This enables researchers to insert, delete, or replace specific DNA sequences in the wheat genome (Cao et al., 2021).

In order to apply the research results to disease resistance breeding, researchers used traditional breeding methods to crossbreed the Tamlo R32 mutant with the main wheat variety in China, and introduced disease resistant traits into the main wheat variety through several generations of backcrossing. More importantly, using CRISPR genome editing technology, corresponding gene mutations can be directly created in the main wheat varieties. In just 2-3 months, wheat germplasms with broad-spectrum powdery mildew resistance and unaffected growth and yield were successfully obtained in multiple main wheat varieties. Compared to traditional breeding methods, genome editing breeding greatly shortens the breeding process. This study is an important progress in wheat breeding for resistance to powdery mildew, fully demonstrating the enormous application prospects of genome editing in modern agricultural production, and providing new strategies and technical routes for cultivating disease resistant and high-yield crop varieties (Li et al., 2022) (Figure 2).

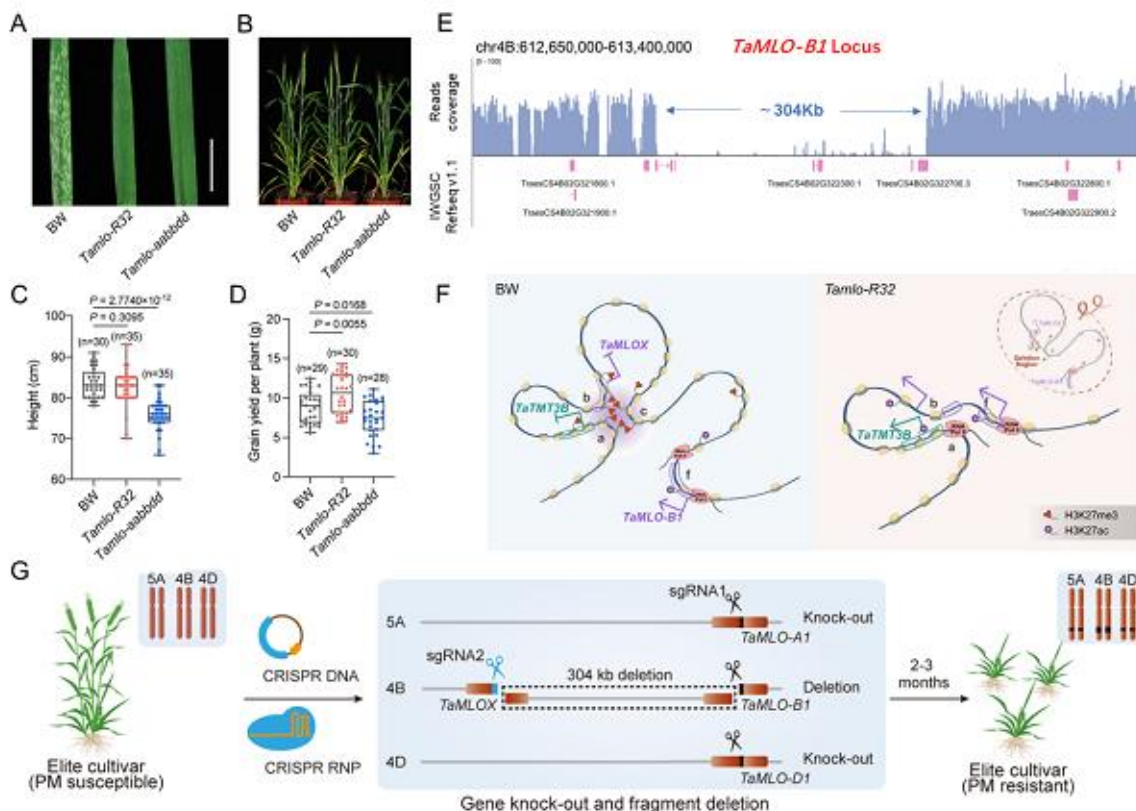


Figure 2 Genome editing mediated chromosomal rearrangement to obtain high-yield wheat resistant to powdery mildew (Li et al., 2022)

Note: A~D: Tamlo R32 is resistant to powdery mildew and has no growth defects; E: Tamlo R32 generates a 304Kb large fragment deletion near TaMLO-B1 Locus; F: Schematic diagram of TaTMT3B expression regulation; G: Rapid acquisition of mutant wheat germplasm through gene editing

A major advantage of gene editing technology is its highly precise targeting, which means researchers can select specific genes to edit to adjust or enhance the stress resistance of wheat. Gene editing technology provides an opportunity to increase the stress tolerance of wheat. By editing genes related to drought resistance, disease resistance, salt tolerance, and other stress resistance traits, wheat can better adapt to harsh environmental conditions.

Traditional breeding methods typically require multiple generations of mating and selection to obtain varieties with target traits. Gene editing technology has accelerated this process, allowing specific gene changes to be achieved within a generation. Gene editing technology also helps to expand the genetic resources of wheat by creating new genetic variations and enriching the genetic diversity of wheat's stress resistance.

Although gene editing technology provides new opportunities for wheat stress resistance breeding, it is also accompanied by ethical and regulatory challenges. Ensuring the safety, traceability, and sustainability of the technology is an important direction for future research. Gene editing technology is gradually changing the face of wheat stress resistance breeding. Although it still faces some technical and ethical issues, this method provides unprecedented opportunities for genetic improvement in wheat and is expected to help solve global food security issues.

#### **4 Outlook**

Breeding has always been an important research direction in the field of genetic improvement of food crops. The application of modern biotechnology such as molecular marker assisted selection and gene editing technology has made significant progress, but still faces some challenges and ethical issues. The future breeding of wheat for stress resistance will rely more on the integration of molecular marker assisted selection, gene editing, and other biotechnology. The combined application of these technologies is expected to provide more genetic resources and methods to improve the stress resistance of wheat, such as drought resistance, disease resistance, and salt tolerance.

With the continuous development of gene editing technology, the future direction will be more precise. This means that specific genes can be edited more precisely to improve the stress resistance of wheat. Improvements in technologies such as CRISPR-Cas9 will provide more efficient editing tools. Through gene editing and selection, new genetic variations can be created to expand the genetic diversity of wheat. This helps wheat better adapt to the constantly changing climate and pest pressures (Kumar et al., 2021).

The widespread application of gene editing technology will make ethical and regulatory issues an important issue. Ensuring the safety and traceability of technology, and formulating relevant regulations to balance the relationship between innovation and food safety will be key challenges in the future. Due to wheat being one of the world's major food crops, future wheat stress resistance breeding requires international cooperation. Sharing genomic information, genetic resources, and technological experience will help promote the development of this field more widely. Future wheat stress resistance breeding should not only pursue high yield, but also focus on sustainability. This means that while improving resilience, ecological balance, resource utilization efficiency, and agricultural sustainability should also be considered (Hossain et al., 2021).

Looking ahead, the future of wheat stress resistance breeding is full of hope, and the application of modern biotechnology provides new ways to improve wheat stress resistance, which is expected to help solve global food security issues. However, the ethical and regulatory issues that come with it need to be carefully weighed and resolved. Global cooperation and sustainable agriculture principles will be key elements for future wheat stress resistance breeding to ensure its success and benefit global society.

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