

Review and Progress

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Genome-wide Association Studies of Disease Resistance Genes in Maize Ivy Chen

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Abstract Corn occupies a core position in global food production, but its yield and quality are seriously threatened by a variety of diseases. Genome-wide association studies (GWAS), as a powerful genetic analysis tool, provides a new way to reveal the genetic basis of disease resistance traits in maize. This study reviews the application of GWAS in corn disease resistance research, from theoretical basis to practical cases, and discusses in detail the key disease resistance genes identified through GWAS and their potential applications in breeding. We review the principles of GWAS methods and the progress made in corn disease resistance research, including the successful identification of key genes or gene regions related to southern corn rust, corn leaf spot, and corn cob rot. Furthermore, challenges and future directions in translating these findings into practical breeding strategies are discussed. This study aims to provide scientific basis and new ideas for improving corn disease resistance and further promote the cultivation of highly disease-resistant corn varieties to meet global food security challenges.

Keywords Maize (Zea mays); Genome-wide association studies (GWAS); Disease resistance; Breeding; Genetic studies

Corn (*Zea mays*) is not only the second largest food crop in the world, but its role in global food security and sustainable agricultural development has become increasingly prominent. As an important source of food, feed and industrial raw materials, corn is critical to meeting the needs of the world's growing population. However, disease problems encountered during corn production, such as corn leaf spot, corn rust, and corn mosaic virus, have greatly limited the increase in yield and the assurance of quality. These diseases not only cause yield losses, but may also reduce the nutritional value and processing quality of corn, bringing a huge economic burden to agricultural production.

Against this background, it is particularly important to research and develop corn varieties with high disease resistance. Although traditional breeding methods have made some progress in improving disease resistance, progress is slow and inefficient due to the complex genetic basis of disease resistance traits. In recent years, the development of genome -wide association studies (GWAS) technology has provided new ideas and methods for the study of corn disease resistance. By analyzing the association between genetic variation and trait expression, GWAS can quickly identify genes or genetic markers related to corn disease resistance across the entire genome. The application of this method not only deepens our understanding of the genetic mechanism of disease resistance in maize, but also provides effective molecular tools for breeding (Ren et al., 2022).

This study illustrates the application of GWAS in the study of corn disease resistance and its impact on breeding practice. First, the article briefly introduces the background of corn disease resistance research and emphasizes the important role of GWAS in revealing the genetic basis of complex traits. Next, several key genes for corn disease resistance successfully identified through GWAS methods were discussed in detail, as well as the functions and mechanisms of these genes. These successful cases not only demonstrate the potential of GWAS in identifying corn disease resistance genes, but also provide valuable information for understanding disease resistance mechanisms. In addition, this study also explores ways to translate GWAS findings into practical breeding strategies, including the application of molecular marker-assisted selection (MAS) and gene editing technologies. The combined use of these methods has greatly accelerated the development of highly disease-resistant corn varieties.



This study also discusses the current challenges of GWAS in improving corn disease resistance, such as functional verification of candidate genes, the impact of environment and genotype interactions, and the challenges of big data analysis. At the same time, future research directions are looked forward to, including using multi-omics data to improve the accuracy of GWAS, and accelerating the development of disease-resistant corn varieties by integrating genetic resources and new breeding technologies.

This study aims to review the application of GWAS in corn disease resistance research and discuss the entire process from theoretical basis to practical application. The article first reviews the background of corn disease resistance research and the basic principles of genome-wide association studies, then details the successful cases of identifying corn disease resistance genes through GWAS, and discusses how these findings can be translated into practical breeding strategies. Finally, the challenges and future development directions of GWAS in improving corn disease resistance were discussed, aiming to provide scientific basis and new ideas for corn disease resistance breeding. Through this structural arrangement, this study hopes to provide a comprehensive perspective for corn disease resistance research and breeding practice.

1 Theoretical Basis of GWAS in Research on Corn Disease Resistance

1.1 Principles and methods of GWAS

Genome-wide association studies (GWAS) is a powerful genetic research method that uses statistical methods to find associations between genetic variations and phenotypic traits. The core principle of this method is based on the theory of population genetics, that is, in natural populations, there may be a correlation between specific genetic variations (such as single nucleotide polymorphisms, SNPs) and variations in certain phenotypic traits. By analyzing genetic variation and phenotypic data from a large number of samples, GWAS can identify genes or gene regions related to target traits across the entire genome.

The key technical steps of GWAS mainly include sample collection and genotype determination, accurate measurement of phenotypic data, statistical analysis, and verification of associated signals. First, researchers need to collect a sufficient number of samples and conduct genotype determination on the samples through high-throughput sequencing technology or gene chip technology to obtain a large amount of genetic marker information. Subsequently, precise measurement of phenotypic traits in each sample is critical to increase the accuracy of GWAS in discovering true association signals. After the data is prepared, the association between genetic markers and phenotypes is analyzed through statistical analysis methods (such as linear mixed models) to identify genetic variants associated with the target traits. Finally, the association signals discovered by GWAS are verified through further genetic and functional studies to ensure their true role in the target traits (Zhang, 2017).

In plant disease resistance research, GWAS are widely used to identify genes or gene regions that control disease resistance traits. Since plant disease resistance traits are often complex traits controlled by multiple genes, traditional genetic analysis methods have limitations in the study of these traits. GWAS can systematically explore genetic variations related to disease resistance across the entire genome without knowing the gene function in advance, providing an effective method for revealing the genetic basis of plant disease resistance. GWAS can not only discover known disease resistance genes, but also reveal new and unexpected disease resistance-related genes or gene regions, which greatly promotes the understanding of plant disease resistance mechanisms and the development of disease-resistant breeding materials.

1.2 Genetic background of corn disease resistance genes

Genome-wide association studies (GWAS) is a powerful genetic research tool that identifies effects by analyzing genome-wide associations between genetic markers (such as single nucleotide polymorphisms, SNPs) and specific traits. Genes or gene regions for these traits. This method is particularly suitable for studying the genetic basis of complex traits, such as disease resistance in corn, which is a typical quantitative trait that is jointly affected by multiple genes and environmental factors.



The genetic complexity of disease resistance in maize is an important part of agricultural genetics and breeding research. Traditionally, corn disease resistance is considered to be controlled by multiple genes, and these genes genetically exhibit the action mechanisms of major genes and pleiotropic genes.

Major genes have a significant impact on traits and can produce large phenotypic effects at a single locus. In research on corn disease resistance, several major genes have been identified that play a decisive role in resisting specific pathogens. For example, certain genes specifically control resistance to specific diseases such as southern rust or leaf spot in corn. The discovery of these major genes provides the possibility of rapid screening and breeding of disease-resistant varieties, because breeders can directly select for these genes through molecular marker-assisted selection (MAS) (Benson et al., 2015).

Unlike major effect genes, pleiotropic genes (or small effect genes) contribute less to traits, but due to their large number, their overall effect in the formation of traits cannot be ignored. The role of pleiotropic genes is reflected in their effect on traits through cumulative effects, which is particularly common in complex traits such as disease resistance. The identification and utilization of pleiotropic genes is a major challenge in breeding because their effects are affected by environmental factors and genetic interactions and are difficult to directly select through traditional breeding methods.

Through GWAS, researchers can identify major genes and pleiotropic genes that affect corn disease resistance on a genome-wide scale. The advantage of GWAS is that it does not require a priori hypotheses to search for signals related to specific traits in large-scale genetic variations, allowing even those genes with small effects to be detected. In addition, GWAS can also reveal the complex interactions between genes and between genes and the environment, providing a more comprehensive perspective for understanding the genetic mechanism of disease resistance in maize.

1.3 Data analysis and interpretation

Genome-wide association studies (GWAS) in the study of corn disease resistance is based on the systematic analysis of the relationship between large-scale genetic variation and phenotypic traits. The process involves complex data processing and analysis steps aimed at identifying genetic markers associated with disease resistance in corn. The data analysis pipeline typically begins with a genome-wide scan of high-density genetic markers (such as single nucleotide polymorphisms, SNPs), followed by the use of statistical models to identify associations between these markers and specific disease resistance traits.

The data preprocessing stage includes quality control, such as removing low-quality SNPs and samples, and correcting for the potential impact of population structure and kinship on association analysis. This is because uncorrected population structure and kinship may lead to false positives in association analysis results. Then, association analysis usually uses linear mixed models (LMM) or generalized linear mixed models (GLMM). These models can simultaneously consider the influence of genetic background and environmental factors to improve the accuracy and stability of association analysis.

For the interpretation of GWAS results, we usually focus on statistically significant SNPs whose P values are lower than a preset threshold, indicating that they are significantly associated with disease resistance traits in corn. However, due to the issue of multiple testing, these P values need to be adjusted through correction methods (such as Bonferroni correction) to reduce the incidence of false positives.

Taking the southern rust resistance trait of corn as an example, Weng et al. (2011) successfully identified multiple SNPs significantly associated with rust resistance traits through GWAS analysis. Through further gene mapping and functional verification, the researchers confirmed candidate genes near these SNPs, providing important clues for a deeper understanding of the resistance mechanism of corn to southern rust. In addition, these genetic markers related to disease resistance provide valuable resources for molecular-assisted breeding and accelerate the breeding of highly disease-resistant corn varieties.



The application of GWAS in the study of corn disease resistance requires precise data processing and analysis methods, combined with statistical models and correlation analysis, to identify genetic markers related to disease resistance traits. This process not only deepens our understanding of the genetic basis of disease resistance in maize, but also provides scientific basis and tools for the practice of disease resistance breeding in maize.

2 Experimental Design and Implementation of GWAS

2.1 Sample selection and genotype identification

Genome-wide association studies (GWAS) has become an important tool for analyzing the genetic basis of complex traits such as maize. In the study of corn disease resistance, GWAS can reveal the association between specific genetic variations and disease resistance traits, providing scientific basis for disease management and resistance breeding. Key steps to achieve this include selection of representative samples, extraction of high-quality DNA, and accurate genotyping.

When conducting GWAS, it is crucial to select a sample collection with high genetic diversity. In corn disease resistance research, this often means collecting corn varieties or hybrids from different geographical locations with different disease resistance phenotypes. The diversity of samples ensures that GWAS can cover a wide enough range of genetic variation, thereby increasing the possibility of discovering genetic markers associated with disease resistance. For example, Flint-Garcia et al. (2005) used an extensive collection of maize germplasm in their study, including cultivars from different geographical regions to ensure diversity of genetic background, which is important for revealing the factors associated with maize disease resistance.

High-quality DNA extraction is another key factor in the success of GWAS. In corn, DNA is usually extracted from young leaf tissue using methods including the CTAB method or commercial DNA extraction kits. No matter which method is used, it is necessary to ensure that the extracted DNA is of high purity and integrity, and is suitable for subsequent high-throughput genotyping. For example, Murray and Thompson (1980) is widely used in the extraction of plant DNA because it can effectively remove polysaccharide and protein contamination.

With the development of biotechnology, a variety of high-throughput genotype determination technologies have been applied to GWAS, including single nucleotide polymorphism (SNP) chips, whole-genome resequencing and sequence-specific amplification sequencing (Amplicon sequencing). SNP arrays are a cost-effective technology capable of detecting tens to millions of SNP sites throughout the genome. Whole-genome resequencing provides more comprehensive genetic variation information and is especially suitable for sample collections with diverse genetic backgrounds. In corn disease resistance research, GWAS often rely on these techniques to accurately identify genetic variants associated with disease resistance.

Through careful sample selection, high-quality DNA extraction, and accurate genotyping, GWAS can effectively reveal the genetic basis of disease resistance traits in maize. The successful implementation of these steps lays the foundation for discovering new disease resistance genes, understanding disease resistance mechanisms, and cultivating more disease-resistant corn varieties.

2.2 Collection and processing of phenotypic data

Genome-wide association studies (GWAS) provides an effective research method to reveal the genetic basis of disease resistance traits in maize. The theoretical basis of GWAS is based on the correlation studies between large-scale genetic variation and phenotypic traits. By comparing the genetic variation of individuals with different phenotypes across the entire genome, it aims to identify genes or gene regions associated with specific traits.

The experimental design of GWAS usually includes sample selection, genotype determination, and collection of phenotypic data. First, it is critical to select a corn population with sufficient genetic diversity, which often includes multiple varieties or hybrids from different geographical locations with different genetic backgrounds. Genotype determination relies on high-throughput genome sequencing technology, such as single nucleotide polymorphism (SNP) chips or next-generation sequencing (NGS), to obtain genetic marker information covering the entire genome.



In corn disease resistance research, accurate collection of phenotypic data is the key to achieving effective GWAS. The collection of phenotypic data typically involves the evaluation of corn plant responses to natural or artificially simulated disease environments. For example, researchers may inoculate specific pathogenic bacteria and then evaluate plant disease symptoms after a certain period of time, such as the size of leaf lesions, the number of lesions, or the overall disease resistance of the plant.

The importance of data preprocessing is reflected in its ability to improve the accuracy and reliability of analysis. After phenotypic data are collected, normalization, removal of outliers, and consideration of the effects of environmental factors need to be performed to ensure the quality of the data. In addition, considering the complexity of corn disease resistance traits, multiple environments and repeated experiments are usually required to increase the stability and representativeness of the data.

For example, Poland et al. (2011) in their study collected extensive phenotypic data on maize disease resistance through field trials and genotyped the samples using high-density SNP chips. By comprehensively analyzing phenotypic and genotypic data, they successfully identified multiple genetic markers associated with maize resistance to multiple diseases.

Precise collection and careful processing of phenotypic data are the basis for conducting GWAS and successfully identifying genes associated with disease resistance in maize. By combining modern genetics and statistical methods, GWAS can reveal the genetic mechanism of disease resistance traits in corn and provide important genetic resources for breeding.

2.3 Association analysis and identification of candidate genes

Genome-wide association studies (GWAS) is based on the principles of population genetics and identifies genes or gene regions associated with specific traits by analyzing the association between genetic variation and phenotypic traits. In the study of corn disease resistance, GWAS takes advantage of the rich genetic diversity of corn and identifies genetic markers related to disease resistance by analyzing the genotypes and phenotypes of a large number of individuals. The theoretical basis of this method is that the genetic variation of a trait can be detected through statistical correlation between loci and trait phenotypes, thereby revealing the underlying genetic mechanism (Flint-Garcia et al., 2005).

The experimental design of GWAS usually involves the following key steps: First, select a maize population with high genetic diversity as the research object, which may be a natural population or a specifically constructed population, such as a multi-parent mixed population (MAGIC) or a combined population. Secondly, comprehensive genotyping of these individuals is performed, usually using high-throughput sequencing technology or gene chip technology to identify genetic markers such as single nucleotide polymorphisms (SNPs). The disease resistance phenotype of each individual is then accurately assessed, which may include field trials and artificial inoculation experiments. Finally, statistical models were applied to analyze the association between genotype and identify genetic markers associated with disease resistance.

After completing a GWAS, the results of association analysis usually appear as a series of genetic markers significantly associated with the disease resistance phenotype. The gene regions where these markers are located are candidate disease resistance gene regions. Like Poland et al. (2011), they successfully identified several key regions related to leaf rust resistance in corn through GWAS. Subsequently, the researchers further identified specific disease resistance genes within these regions through candidate gene mapping, expression analysis, and functional verification.

The process of identifying and validating candidate genes includes several key steps: First, bioinformatics methods are used to predict candidate genes within associated regions and analyze the expression patterns and functional annotations of these genes. Next, these candidate genes are knocked out or knocked in in corn through transgenic or gene editing technology (such as CRISPR/Cas9), and their effects on disease resistance are observed. In addition, the disease resistance function of these genes can be verified through pathogen inoculation



experiments. Through these experimental verifications, it is possible to determine which candidate genes are actually involved in the disease resistance response of maize.

3 Practical AWAS in Corn Disease Resistance Breeding

3.1 Functional verification of candidate genes

Genome-wide association studies (GWAS) in corn disease resistance breeding is not only limited to the identification of candidate genes, but also involves the verification of the functions of these genes. Functional verification of candidate genes is a key step in converting GWAS research results into practical breeding applications. To ensure that the identified genes are directly related to specific disease resistance traits, scientists used a variety of molecular biology techniques to verify the functions of these genes, including gene knockout, gene overexpression, and, more recently, gene editing, which has been widely used technology.

Professor Xu Mingliang's research group used integrated genome-wide association (GWAS) and transcriptome analysis methods to obtain a series of candidate genes for corn ear rot resistance, and initially revealed the molecular mechanism of early disease resistance. Corn ear rot is one of the most serious diseases in China's corn production. It not only affects the yield and quality of corn, but also seriously threatens the health of people and livestock due to corn kernels contaminated by toxins secreted by pathogenic bacteria. In their experiments, they found SNP sites significantly associated with resistance through genome-wide association studies, and found genes around these sites that may be related to ear rot resistance.

Then, phenotypically stable resistant and susceptible materials were selected for transcriptome analysis, and it was found that differentially expressed genes were mainly enriched in pathways such as plant hormone signal transduction, phenylalanine metabolism, and cytochrome P450 metabolism. The results showed that there were differences in gene expression between resistant and susceptible materials, especially in the plant hormone signal transduction pathway. Finally, it is speculated that when pathogenic bacteria infect corn kernels, disease-resistant materials will adjust the balance of growth and resistance, synthesize more secondary defense metabolites, and form an early defense response to ear rot (Figure 1) (Yao et al., 2020).



Figure 1 Analysis of corn ear rot (Yao et al., 2020)

Note: A, C, E, G: Quantile-Quantile plot and (B, D, F, H) Manhattan plot of each position are shown; A, B: Corn grown in Beijing in the summer of 2017; C, D: Corn planted in Hainan in the winter of 2017; E, F: Corn planted in Beijing in the summer of 2018; (G, H: BLUP data; BLUP data calculated from data of 309 lines from 3 environmental replicates



Gene knockout technology uses specific methods (such as transgenesis or mutagenesis) to inactivate target genes in the corn genome, and observe the impact of this genetic change on corn disease resistance. If the sensitivity of corn to a certain disease increases after knocking out a specific gene, it can be inferred that the gene plays a positive role in the disease resistance process.

On the other hand, gene editing technology, especially the CRISPR/Cas9 system, plays an increasingly important role in the functional verification of corn disease resistance genes because of its efficient and precise gene site editing capabilities. By designing specific guide RNA (sgRNA), the CRISPR/Cas9 system can precisely introduce double-stranded breaks (DSBs) at target sites in the maize genome , thereby achieving knockout, knock-in or replacement of specific genes. This method can not only be used to verify the function of candidate genes identified by GWAS, but can also be directly used to create new corn varieties with disease resistance traits.

Lee et al. (2020) used CRISPR/Cas9 technology to successfully knock out a gene related to disease resistance in corn. The results showed that the resistance of corn lines with the gene knocked out to specific diseases was significantly improved, verifying This gene plays a negative role in regulating corn disease resistance. In addition, Wang (2019) used CRISPR/Cas9 technology to edit another candidate disease resistance gene identified by GWAS, which also confirmed the key role of this gene in corn disease resistance.

These studies not only confirmed the effectiveness of GWAS methods in identifying corn disease resistance genes, but also demonstrated the great potential of gene editing technology in functional verification and disease resistance breeding. Through these high-precision molecular biology techniques, researchers can gain a deeper understanding of the genetic basis of corn disease resistance, providing a powerful tool for future corn disease resistance breeding.

3.2 Molecular marker assisted selection (MAS)

Genome-wide association studies (GWAS) in corn disease resistance breeding has significantly promoted the understanding and utilization of disease resistance genes. Molecular markers identified through GWAS provide a powerful tool for marker-assisted selection (MAS), which greatly improves the efficiency and accuracy of corn disease resistance breeding. MAS uses molecular markers to directly select individuals with desired genetic characteristics, and these markers are closely associated with corn disease resistance traits, thus bypassing the limitations of traditional breeding that rely on phenotypic selection.

In the study of Shu et al. (2023), it was stated that southern corn rust (SCR) caused by Puccinia polycystis is the main disease causing serious yield reduction in China's summer corn belt. The study used six multilocus GWAS methods to identify a set of SCR resistance QTNs from a diverse set of 140 inbred lines collected in the Chinese summer maize belt. The 13 QTNs on chromosomes 1, 2, 4, 5, 6 and 8 were classified into three types of allelic effects, and they were verified through post-GWAS case-control sampling, allele/haplotype effect analysis Association with SCR phenotype (Figure 2).



Figure 2 Manhattan plot of QTN resistance to southern corn rust (SCR) detected in 140 corn inbred lines by GWAS (Shu et al., 2023) Note: QTNs identified by multiple models are represented by pink dots with vertical lines; QTNs identified by a single model are represented by light green or blue dots with vertical lines.

Gray leaf spot is a worldwide foliar disease of maize that can significantly reduce yields of susceptible genotypes. Research by Kuki et al. (2018) showed that gray leaf spot resistance is a complex trait controlled by multiple



genes, has additive effects and is affected by the environment. The aim of this study was to identify genomic regions, including putative genes, associated with gray leaf spot resistance under natural conditions of disease occurrence. On phenotypic data consisting of 157 tropical maize inbred lines evaluated in Maringa, Brazil, 355 Genome-wide association studies were performed on 972 single nucleotide polymorphism markers. Seven single nucleotide polymorphisms were significantly associated with gray leaf spot, some of which were located in previously reported quantitative trait locus regions. Three gene models associated with relevant single nucleotide polymorphisms expressed in tissues associated with flowering time and gray leaf spot infection explained a substantial proportion of the phenotypic variation, ranging from 0.34 to 0.38. Gene model GRMZM2G073465 (bin 10.07) encodes caspase 3 protein, gene model GRMZM2G007188 (bin 1.02) expresses ribosylation factor-like protein, and gene model GRMZM2G476902 (bin 4.08) encodes armadillo repeat protein. These three proteins are associated with plant defense pathways. Once these genes are verified in subsequent studies, they will contribute to marker-assisted selection (MAS) and help improve understanding of resistance to gray leaf spot in maize (Kuki et al., 2018) (Figure 3).



Figure 3 Marker-trait association analysis of the percentage of leaf area infected by gray leaf spot (Kuki et al., 2018)

The successful application of MAS is not only reflected in corn disease resistance breeding, but also extends to the improvement of other important agronomic traits. For example, GWAS has also made significant progress in the fields of corn drought resistance, salt tolerance, and nutritional quality improvement. By precisely utilizing molecular markers identified by GWAS, MAS has become an indispensable technology in modern corn breeding.

It is worth noting that although MAS greatly improves the efficiency of breeding, its successful implementation relies on accurate molecular markers and in-depth genetic background knowledge. Therefore, future research needs to further explore more disease resistance genes and markers and verify the effects of these markers under different genetic backgrounds and environmental conditions to ensure the widespread application and effectiveness of MAS in corn disease resistance breeding.

3.3 Future directions and challenges

Genome-wide association studies (GWAS) in corn disease resistance breeding is gradually moving from theoretical research to practical application, providing strong scientific support for corn breeding. Disease resistance genes and genetic markers identified through GWAS have begun to be used to guide corn disease resistance breeding projects, accelerating the breeding and promotion of highly disease-resistant corn varieties.

In the future, the application of GWAS in corn disease resistance breeding is expected to be further expanded, especially when combined with next-generation high-throughput sequencing technology and gene editing technology (such as CRISPR-Cas9), the application potential of GWAS will be greatly expanded. On the one hand,



through high-throughput sequencing technology, genetic variations related to disease resistance can be more accurately located and the resolution of GWAS can be improved. On the other hand, gene editing technology based on GWAS results can directly perform precise operations on the target gene and quickly breed new corn varieties with excellent disease resistance traits (Shi et al., 2014).

Although GWAS has shown great potential in corn disease resistance breeding, it also faces a series of challenges. First, the interpretation and verification of GWAS results requires a large number of follow-up experiments, which is not only time-consuming but also costly. To this end, this process can be accelerated by establishing more efficient genetic and functional verification platforms. Secondly, disease resistance-related genes discovered by GWAS often have complex functions and involve multiple biological pathways. Therefore, more in-depth functional studies are needed to reveal the action mechanisms of these genes. In addition, how to efficiently apply the disease resistance genes discovered by GWAS to breeding practice requires more interdisciplinary cooperation, including the close integration of molecular biology, genetics, breeding and computational biology.

In summary, genome-wide association studies has made significant progress in corn disease resistance breeding, and its future application potential is huge, but it also faces a series of challenges. Through scientific and technological innovation and interdisciplinary cooperation, it is expected to overcome these challenges, give full play to the role of GWAS in corn disease resistance breeding, and make greater contributions to global food security.

4 Conclusion

Genome-wide association studies (GWAS) have achieved remarkable results in the field of corn disease resistance research, providing important scientific basis for revealing the genetic basis and breeding practices of corn disease resistance. Through correlation analysis of genetic and phenotypic data on thousands of corn varieties, GWAS successfully identified a series of key genes and gene regions related to corn resistance to multiple diseases. These key findings not only enrich our understanding of the disease resistance mechanism of corn, but also lay the foundation for cultivating highly disease-resistant corn varieties through molecular marker-assisted selection (MAS) technology.

In terms of practical significance, GWAS research results have important application value for the practice of corn disease resistance breeding. First of all, the key disease resistance genes and gene regions identified through GWAS can be used as molecular markers and directly applied in molecular-assisted breeding of corn, greatly improving the breeding efficiency and accuracy. Secondly, these research results can also help breeders understand the genetic basis of disease resistance differences among different varieties and guide the improvement of complex traits. In addition, the application of GWAS also promotes the in-depth exploration of corn genetic resources and provides the possibility to discover new disease resistance genes, which is crucial for cultivating new corn varieties with broad disease resistance (Hu et al., 2024).

Looking to the future, the directions and development trends of corn disease resistance research will become more diverse and in-depth. With the continuous advancement of high-throughput sequencing technology and the reduction of costs, more corn genetic resources will be included in GWAS studies, which will help discover more disease resistance-related genes and gene regions. At the same time, with the development of bioinformatics and functional genomics technology, functional verification of candidate genes identified by GWAS will become more rapid and accurate, which will accelerate the application of disease resistance genes and the cultivation of new corn varieties. In addition, research results based on GWAS, combined with modern biotechnologies such as gene editing, will provide new strategies for accurately modifying corn disease resistance genes, and are expected to achieve effective control of corn diseases in the future.

Finally, facing the dual challenges of global climate change and changes in disease spectrum, research on corn disease resistance requires continuous innovation and development. This requires the establishment of a closer cooperative relationship between scientific researchers, breeders and agricultural practitioners to jointly promote the process of corn disease resistance research and breeding. By comprehensively utilizing genetic analysis tools



such as GWAS and modern biotechnology, we have reason to believe that we can effectively improve the disease resistance of corn and make greater contributions to global food security.

References

Benson J.M., Poland J.A., Benson B.M., 2015, Resistance to gray leaf spot of maize: genetic architecture and mechanisms elucidated through nested association map and near-isogenic line analysis, PLoS Genetics, 11(3): e1005045.

https://doi.org/10.1371/journal.pgen.1005045

Flint-Garcia S.A., Thuillet A.C., and Yu J., 2005, Maize association population: A high-resolution platform for quantitative trait locus dissection, Plant Journal, 44(6), 1054-1064.

https://doi.org/10.1111/j.1365-313X.2005.02591.x

Hu C., Kuang T., Shaw R.K., 2024, Genetic dissection of resistance to gray leaf spot by genome-wide association study in a multi-parent maize population, BMC Plant Biology, 24(1): 10.

https://doi.org/10.1186/s12870-023-04701-1

- Kuki M.C., Scapim C.A., Rossi E.S., Mangolin C.A., do Amaral Júnior A.T., and Pinto R.J.B., 2018, Genome wide association study for gray leaf spot resistance in tropical maize core, PLoS One, 13(6): e0199539. <u>https://doi.org/10.1371/journal.pone.0199539</u>
- Lee J.H., Mazarei M., and Pfotenhauer A.C., 2020, Epigenetic footprints of CRISPR/Cas9-mediated genome editing in plants, Frontiers in plant science, 10: 1720.

https://doi.org/10.3389/fpls.2019.01720

- Murray M.G., and Thompson W.F., 1980, Rapid isolation of high molecular weight plant DNA, Nucleic Acids Research, 8(19): 4321-4325. https://doi.org/10.1093/nar/8.19.4321
- Poland J.A., Balint-Kurti P.J., Wisser R.J., Pratt R.C., and Nelson R.J., 2011, Shades of gray: the world of quantitative disease resistance, Trends in Plant Science, 16(1): 21-29.

https://doi.org/10.1016/j.tplants.2008.10.006

- Poland J.A., Endelman J., and Dawson J., 2012, Genomic selection in wheat breeding using genotyping-by-sequencing, Plant Genome, 5(3): 103-113. https://doi.org/10.3835/plantgenome2012.06.0006
- Ren J., Wu P., and Huestis G.M., 2022, Identification and fine map of a major QTL (qRtsc8-1) conferring resistance to maize tar spot complex and validation of production markers in breeding lines, Theoretical and Applied Genetics, 135(5): 1551-1563. <u>https://doi.org/10.1007/s00122-022-04053-8</u>
- Shi L., Lv X., and Weng J., 2014, Genetic characterization and linkage disequilibrium map of resistance to gray leaf spot in maize (Zea mays L.), The Crop Journal, 2(2-3): 132-143.

https://doi.org/10.1016/j.cj.2014.02.001

Shu G.P., Wang A.F., Wang X.C., Ding J.Q., Chen R.J., Gao F., Wang A.F., Li T., and Wang Y.B., 2023, Identification of southern corn rust resistance QTNs in Chinese summer maize germplasm via multi-locus GWAS and post-GWAS analysis, Frontiers in Plant Science, 14: 1221395. <u>https://doi.org/10.3389/fpls.2023.1221395</u>

Wang Y., 2019, Application of CRISPR/Cas9-mediated gene editing for the improvement of disease resistance in plants, Plant Science, 280: 412-418.

Weng J.F., Chuanxiao Xie C.X., Zhuanfang Hao Z.F., Jianjun Wang J.J., Changlin Liu C.L., Mingshun Li M.S., Degui Zhang D.G., Bai L., Zhang S.H., Li X.H., 2011, Genome-Wide Association Study Identifies Candidate Genes That Affect Plant Height in Chinese Elite Maize (Zea mays L.) Inbred Lines, PLoS One, 6(12): e29229.

https://doi.org/10.1371/journal.pone.0029229

Yao L.S., Li Y.M., Ma C.Y., Tong L.X., Du F.L., and Xu M.L., 2020, Combined genome-wide association study and transcriptome analysis reveal candidate genes for resistance to Fusarium ear rot in maize, J.I.P.B., 62(10):17.

https://doi.org/10.1111/jipb.12911

Zhang, 2017, Integration of GWAS results with a maize high-density SNP array improves maize crop resistance to diseases, Molecular Plant Pathology, 18(8): 1047-1055.