

The Current Situation and Future of Using GWAS Strategies to Accelerate the Improvement of Crop Stress Resistance Traits

Wenzhong Huang ✉

Hainan Provincial Key Laboratory of Crop Molecular Breeding, Sanya, 572025, Hainan, China

✉ Corresponding email: Wenzhonghuang@126.com

Molecular Plant Breeding, 2024, Vol.15, No.2 doi: [10.5376/mpb.2024.15.0007](https://doi.org/10.5376/mpb.2024.15.0007)

Received: 26 Jan., 2024

Accepted: 01 Mar., 2024

Published: 15 Mar., 2024

Copyright © 2024 Huang. This is an open access article published under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Preferred citation for this article:

Huang W.Z., 2024, The current situation and future of using GWAS strategies to accelerate the improvement of crop stress resistance traits, Molecular Plant Breeding, 15(2): 52-62 (doi: [10.5376/mpb.2024.15.0007](https://doi.org/10.5376/mpb.2024.15.0007))

Abstract This study explores the current state and future prospects of accelerating crop resistance trait improvement through Genome-Wide Association Studies (GWAS) strategies. With the rapid development of high-throughput sequencing technology and bioinformatics, GWAS has emerged as a powerful tool for linking DNA variations to important crop traits. This research particularly emphasizes the strategies for integrating multi-omics data, as well as the application of precision breeding and gene editing technologies based on GWAS findings, offering new directions and strategies for the improvement of crop resistance traits. Additionally, the emergence of methods such as Transcriptome-Wide Association Studies (TWAS) provides robust tools for identifying genes associated with complex traits, suggesting a more comprehensive understanding of genomic regulation and genetically regulated genes in the future. These advancements not only propel the scientific research of crop genetic improvement but also provide a solid scientific foundation for the sustainable development of crop production and food safety.

Keywords Genome-wide association studies (GWAS); High-throughput sequencing technology; Bioinformatics; Crop resistance traits; Transcriptome-wide association studies (TWAS)

1 Introduction

The genetic diversity of crops is the basis of breeding efforts, and by identifying beneficial alleles and useful variations in target traits, new varieties can be developed that can meet global agricultural challenges (Alison et al., 2022). In this context, technologies such as whole-genome resequencing, genome excerpts, partial-genome sequencing strategies, and high-density genotyping arrays have enabled large-scale assessment of the genetic diversity of a wide range of species, including major and “orphan” crops (Alison et al., 2022). However, unless these technologies are combined with adaptive and functional improvements in crops, their value will be limited. With the advancement of high-throughput phenotyping technology, the “phenotypic bottleneck” has been overcome, making powerful phenotypic data points that accurately characterize crop agronomic and physiological attributes available (Alison et al., 2022). A growing number of studies are leveraging these scientific advances and data science techniques to reveal the relationship between the genome and phenotype, unlocking the breeding potential of plant genetic resources. Genome-wide association studies (GWAS) and genomic selection (GS) are methods to explore marker-trait associations. The powerful data science method of MTAs can accelerate the rate of genetic gain of crops and shorten the breeding cycle in a cost-effective manner (Xu et al., 2021).

For example, a GWAS analysis of 217 upland cotton varieties revealed genetic variation and candidate genes for traits related to salt tolerance (Rafael et al., 2021). These findings provide breeders with a rich toolbox for developing new varieties. In addition, new methods using new library preparation technologies and single-molecule long-read sequencing technologies (such as PacBio and Oxford Nanopore Technologies) have rapidly developed, making it possible to align sequences from multiple individuals, and due to the read length and be able to characterize missing sequences in the reference genome (Rafael et al., 2021).

Furthermore, the creation of pan-genome references enables the characterization of structural variation in a non-reference-biased manner. Access to multiple reference-quality genome assemblies provides the opportunity for analysis of SVs (structural variation) in crop species, although doing so is problematic in some crop species. Its large and complex genome is costly.

By exploring the application of GWAS in improving crop stress resistance traits, we can clearly see the potential of this strategy in understanding crop genetic diversity, accelerating crop breeding cycles, and developing new varieties that can adapt to changing environments. Future research needs to further explore and optimize GWAS methods to overcome challenges such as large data volumes and high computational costs, while improving the accuracy and applicability of GWAS results. This will help improve crop productivity and sustainability globally, especially in the face of climate change and increased food demand.

2 Basic Concepts of Crop Stress Resistance Traits

2.1 Define stress resistance traits of crops

In contemporary agriculture, the study of crop stress resistance traits is particularly important. These traits enable crops to maintain growth and yield in the face of various biotic and abiotic stresses. With the intensification of global climate change and environmental pressure, it has become particularly urgent to study and improve crop stress resistance traits.

The stress resistance traits of crops can be mainly divided into two categories: biotic stress resistance and abiotic stress resistance. Biological stress resistance includes the resistance to biological pressures such as pathogens and insect pests, while abiotic stress resistance covers the tolerance to environmental stresses such as drought, salinity, cold, and heat stress (Brandes et al., 2020). The basis of these traits is the complex physiological and molecular mechanisms formed by plants in order to adapt to changing environments during the long evolutionary process.

In response to these stresses, crops exhibit stress resistance mechanisms that include, but are not limited to, regulating growth patterns, stimulating immune responses, enhancing antioxidant systems, adjusting osmotic pressure balance, and initiating processes to repair damaged tissues. In addition, the interaction between plants and their microbial symbionts also plays an important role in improving stress resistance. For example, certain plant bacteria can help crop growth under salt stress conditions.

Systematically improving stress-resistant traits in crops requires not only understanding the fundamentals of these complex mechanisms, but also transferring knowledge gained from model plants to crops and applying this knowledge through modern plant breeding techniques. This requires closer collaboration between researchers, breeders and policymakers to achieve effective translation and application of knowledge.

Regarding future research directions, considering the changing climate and environmental pressure, it is necessary to continue to explore the deep-seated mechanisms of crop stress resistance traits, and at the same time, we must also focus on interdisciplinary and multi-angle research methods to comprehensively improve the stress resistance of crops, ensuring the sustainable development of agricultural production (Thomas and Hückelhoven, 2018).

2.2 Classification of stress resistance traits

As global climate changes and population grows, developing crop varieties with strong stress-resistant traits becomes increasingly important. Through the comprehensive application of traditional breeding technology and modern biotechnology, such as gene editing and transgenic technology, we are expected to improve the stress resistance traits of crops to ensure food security and sustainable agricultural development. However, this process requires interdisciplinary collaboration, including the combined efforts of genetics, biotechnology, ecology and agricultural sciences.

Drought resistance refers to the ability of a crop to maintain growth, development and yield under drought conditions. Improving drought-resistant traits often involves enhancing a plant's water use efficiency, root depth, and leaf transpiration efficiency. For example, through gene editing technology, scientists have successfully improved the root growth of certain crop varieties so that they can more effectively absorb water from deep soil, thus increasing their drought resistance.

Salt tolerance refers to the ability of plants to maintain their growth and development under salt stress conditions. Crops' tolerance to salt can be improved by adjusting salt absorption by roots, increasing salt distribution in the body, and enhancing the balance of salt inside and outside cells. Examples include genetically modified tomatoes

that improve growth performance in high-salt environments by enhancing the elimination mechanism of salt-ionizing molecules.

Cold-resistant traits enable crops to tolerate low temperatures and frost damage. The improvement of these traits usually involves regulating the expression of antifreeze proteins in plants, the stability of cell membranes, and the regulation of plant hormone levels. For example, some rice varieties have been genetically modified to enhance their survival and yield under low temperature conditions.

Disease resistance traits involve the ability of crops to resist attack by pathogens such as bacteria, viruses, fungi and insects. Through traditional breeding and modern biotechnology, researchers have developed a variety of crop varieties that are highly resistant to disease. This includes genetically modified crops, which are endowed with specific resistance genes that effectively protect against attack by specific pathogens.

2.3 Introduction to the complexity and genetic basis of crop stress resistance traits

The improvement of crop stress resistance traits is an important field in agricultural scientific research, especially in the context of global climate change posing increasingly severe challenges to agricultural production. In recent years, advances in genome-wide association studies (GWAS) and molecular biotechnology have provided new tools and methods to reveal the complexity of crop stress resistance traits and their genetic basis.

Crop stress resistance traits, including drought resistance, salt resistance, disease resistance, etc., are complex traits controlled by multiple genes and involve a large number of genes and regulatory networks. For example, the response of plants to salt stress involves the salt hypersensitive (SOS) signaling pathway. The *SOS1*, *SOS2*, and *SOS3* genes in this pathway play a key role in the ability of plant cells to exclude Na⁺ ions and maintain ion homeostasis. Through genetic engineering techniques, such as overexpressing the *SOS2* gene in tomato and overexpressing the *SOS1* gene in tobacco and sugar beet, plants' tolerance to salt stress can be significantly improved (Wang and Chang, 2024).

In addition, under drought stress, plants perform physiological and metabolic regulation by expressing specific genes such as *CYP450*, *ZmNF-YB16*, *ZmbZIP33*, *GNAC*, and *Coda* family genes to enhance resistance or tolerance to drought. These genes are involved in regulating various physiological processes such as photosynthesis, enzyme activity, and plant hormone accumulation in plants (Mores et al., 2021).

In terms of disease resistance, crop resistance to pathogens often involves complex host-pathogen interaction mechanisms. Modern molecular breeding technologies such as marker-assisted selection (MAS), genomic selection (GS) and effector science can effectively identify and utilize genes or quantitative trait loci (QTL) related to disease resistance to accelerate the breeding process of crop disease resistance traits (Nardana et al., 2022).

Research on these complex traits not only reveals the genetic mechanisms of crop stress resistance traits, but also provides new strategies and targets for future crop breeding. For example, precisely changing specific resistance genes through transgenic technology or gene editing technology (such as CRISPR/Cas9) can directly improve the stress resistance of crops while reducing adverse impacts on the environment.

The improvement of crop stress resistance traits is a complex multidisciplinary field involving genetics, molecular biology, physiology and other disciplines. Through the comprehensive use of modern biotechnologies such as genome-wide association studies (GWAS), molecular markers, and genome editing, we can accelerate the research and application of crop stress resistance traits and contribute to ensuring global food security.

3 Introduction to GWAS Strategies

3.1 Basic principles and methods of GWAS

Genome-wide association studies (GWAS) are a method of studying the association between genetic variation and specific traits by searching for frequently occurring variants (usually single nucleotide polymorphisms, SNPs) across the genomes of multiple individuals), to identify genetic markers that may influence specific traits. This

strategy does not rely on a priori genetic knowledge and can reveal the genetic basis of trait formation without knowing the candidate genes.

The basic principle of GWAS is based on the concept of genetic linkage disequilibrium (LD), that is, certain genetic markers (such as SNPs) tend to be inherited together due to limitations of historical recombination events. When a SNP is in the same LD block as a gene that has a greater influence on the trait, the frequency change of the SNP may be associated with the variation of the trait (Mores et al., 2021). By detecting SNPs that are significantly associated with a trait, researchers can determine the underlying genetic basis of the trait and guide subsequent functional research and breeding efforts.

The implementation steps of GWAS usually include sample collection, genotype determination, statistical analysis and result verification. First, a sufficient number of individuals with or without a specific trait are collected as a research sample. Then, high-throughput sequencing or gene chip technology is used to determine the genome-wide SNPs of these samples. Afterwards, statistical methods (such as linear regression analysis) are used to evaluate the strength of the association between each SNP and the trait. Finally, the biological significance of key SNPs was verified through functional experiments.

A typical example of GWAS is its application in the genetics of human disease, such as searching for genetic variants associated with type 2 diabetes. By analyzing genetic data from thousands of patients and healthy controls, the researchers were able to identify multiple SNPs associated with increased disease risk. These findings not only provide new insights into disease mechanisms, but also provide the possibility for the development of new treatments and preventive strategies.

Although GWAS is a powerful tool, it also faces some challenges and limitations. First, due to genetic linkage disequilibrium, the SNPs identified by GWAS may not be causal variants that directly lead to trait variation, but are located within the same LD block as the genes that actually affect the trait. Second, for complex traits, small effects of multiple genes may be difficult to detect. In addition, large-scale GWAS require large amounts of samples and expensive genotyping costs, although the development of high-throughput sequencing technologies is lowering these barriers (Elena and Giménez, 2021).

GWAS provides an effective strategy for analyzing the genetic basis of complex traits and has been widely used in many fields such as human diseases and crop trait improvement. With the advancement of gene sequencing technology and the development of data analysis methods, the accuracy and efficiency of GWAS will be further improved, providing more support for biomedical research and precision agriculture.

3.2 Comparison of GWAS and traditional breeding methods

Genome-wide association studies (GWAS) and traditional breeding methods each have their own unique applications and advantages in crop improvement. Traditional breeding relies on phenotypic observation and selection, using known genetic variations to improve crop traits, such as drought tolerance and disease resistance. This approach relies on the complex interaction of the phenotypic expression of the trait and the genetic background, often requiring multiple generations of selection to ensure stable expression of the desired trait. Although traditional breeding technology has achieved remarkable achievements historically, it has its limitations, especially in improving the efficiency and precision of crop traits (Elena and Giménez, 2021).

With traditional breeding methods, GWAS provides a powerful tool to identify genes or genetic markers that influence traits by analyzing the association between genetic variation in the crop genome and specific traits. GWAS uses molecular markers, such as single nucleotide polymorphisms (SNPs), to explore the connection between genes and traits. The advantage of this method is that it can cover the entire genome and identify precise genetic locations related to traits, thereby providing a scientific basis for molecular breeding of crop traits. Compared with traditional breeding, GWAS has obvious advantages in understanding the genetic basis of traits and improving the accuracy of selection (Elena and Giménez, 2021).

For example, in cotton breeding, one of the challenges faced by conventional breeding is the narrowing of genetic diversity, which limits the potential for crop improvement. Traditional approaches are based on the evaluation and selection of phenotypic variants, relying on visible or measurable traits such as morphological and cytological markers. However, these methods have limited information content and are not sufficient to solve the problem of improving complex traits. In contrast, GWAS uses DNA molecular markers, such as SNPs, to provide more genetic information and reveal genes or regions that control important agronomic traits in crop genomes, thereby accelerating the improvement of crop traits (Figure 1) (Kushanov et al., 2021).

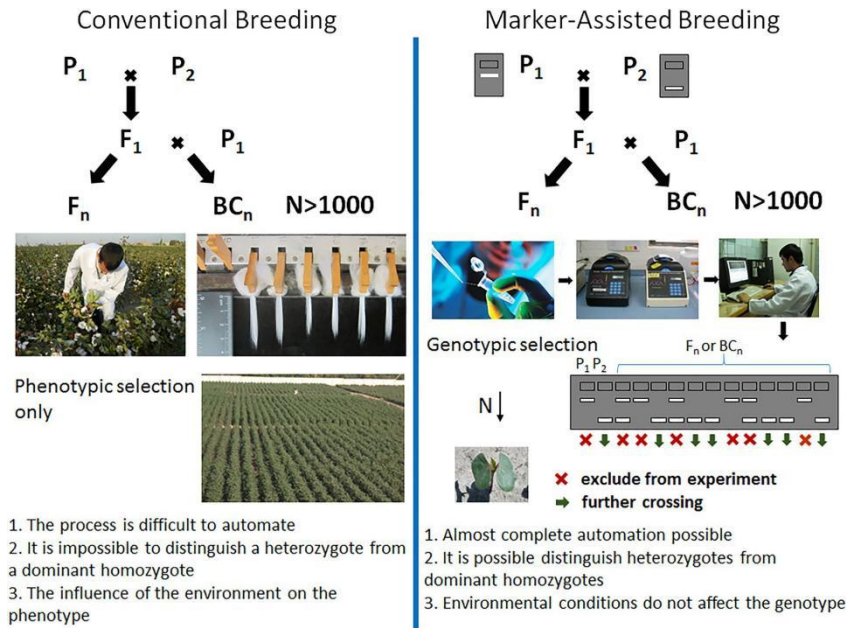


Figure 1 Comparison of marker-assisted selection with traditional breeding, P1 and P2-parental genotypes, F1-first generation cross, Fn-hybrid progeny obtained from the first generation by self-pollination and BCn-backcross generation (Adopted from Kushanov et al., 2021)

Compared with traditional breeding methods, GWAS provides an efficient and precise way to identify and utilize genetic variation that affects crop traits. By using GWAS strategies, breeders can more effectively utilize crop genetic resources, accelerate the improvement of crop stress resistance traits, and meet the needs of agricultural production. Although GWAS has significant advantages, combining the advantages of traditional breeding methods with the accuracy of GWAS, using a multi-strategy integrated breeding approach will be a key approach to future crop improvement.

3.3 Advantages and limitations of GWAS in crop genetic research

When exploring the advantages and limitations of genome-wide association studies (GWAS) in crop genetic research, we found that GWAS provides a powerful tool that can reveal the genetic basis of complex traits. GWAS identify genotypes associated with specific traits by testing the genomes of multiple individuals in a population for genetic variation. Although this method has made important progress in revealing the genetic mechanisms of crop stress resistance traits, there are also some limitations that need to be noted.

With traditional QTL (quantitative trait loci) mapping, GWAS provide higher resolution and can detect historical recombination events, thereby revealing genomic regions associated with numerous physiological, agronomic and adaptive traits. This is especially obvious in plants, such as corn, rice, soybean, sesame, etc., where GWAS has been successfully used to study complex traits such as plant height, flowering time, seed number, stress resistance traits, and grain yield (Figure 2) (Berhe et al., 2021).

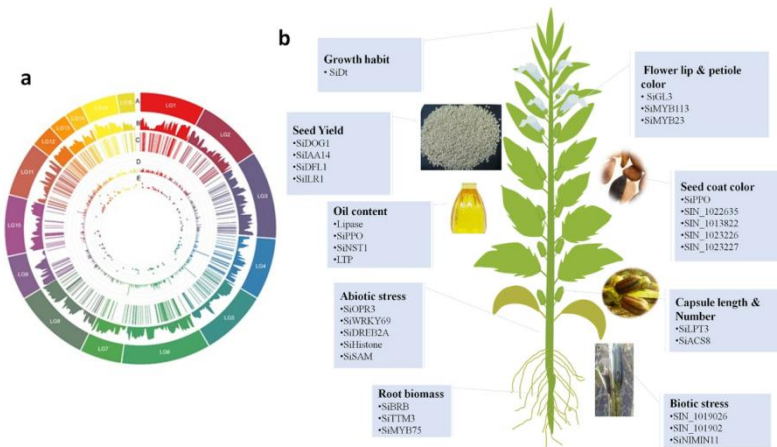


Figure 2 Application of GWAS in sesame (Adopted from Berhe et al., 2021)

Image caption: a: Circos diagram summarizes the genetic findings of important agronomic traits of sesame; A: Pseudomolecule (LG); B: Gene density; C: QTL position; D: $-\log(p)$ of peak SNP; E: Pleiotropic QTL; b: Schematic showing potential candidate genes associated with important agronomic traits of sesame discovered to date (Adopted from Berhe et al., 2021)

GWAS can exploit historical recombination events in populations and provide higher allele frequencies and comprehensive genome coverage, making it a powerful tool for exploring genetic diversity and the genetic basis of complex traits (Korte and Farlow, 2013).

The power of GWAS is highly dependent on sample size. In some cases, even hundreds of samples may not be sufficient to discover small effect sites. Furthermore, genetic heterogeneity, whereby samples from different geographical locations may have different genetic variants that affect the trait due to local adaptation, reduces the ability to discover the association of each variant with the trait.

Rare variants may be affected by strong or complete association with many nonpathogenic rare variants, such that one pathogenic locus may confer many synthetic associations. This demonstrates the importance of considering rare variants when conducting GWAS, but also exposes the limitations of identifying pathogenic variants through existing microarray datasets.

Although traditional single-site model analysis is common in GWAS, it has limitations in addressing multiple testing, historical genotype effects, and pleiotropic effects. In contrast, multilocus models offer a more efficient approach that more accurately captures allelic diversity, optimizes the use of high-density marker data, and increases the power of phenotype-interaction discovery, thereby reducing multiplexing. Testing issues (Korte and Farlow, 2013).

Selecting an appropriate genetic model is a challenge in GWAS studies. Single-locus and multi-locus models each have advantages and disadvantages, and which model to choose depends on the specific traits and goals of the study. Integrating single-locus and multi-locus models may improve the power and validity of complex trait association analysis (Korte and Farlow, 2013).

4 Current Status of Using GWAS to Accelerate the Improvement of Crop Stress Resistance Traits

4.1 Application of GWAS in improving crop stress resistance traits in recent years

Genome-wide association study (GWAS) strategies in improving crop stress resistance traits has achieved remarkable results, especially in traits such as drought resistance and salt resistance. GWAS methods provide a powerful tool for understanding the genetic basis of traits by comprehensively scanning the genomes of large numbers of individuals to identify genetic variants associated with specific traits. The following is an analysis of some successful cases using GWAS strategies to improve crop stress resistance traits.

First of all, it needs to be emphasized that the successful application of GWAS in improving crop stress resistance traits often requires large sample sizes and precise phenotypic definition of complex traits. For example, GWAS studies of Arabidopsis (*Arabidopsis*) have shown that although most analyzes used only a few hundred individuals, for some specific traits meaningful results can be obtained even with fewer than 100 cultivars, suggesting that these traits may be determined solely by A few loci control and explain a large amount of phenotypic variation.

For crops, the application range of GWAS has been very wide, from cotton (*Gossypium hirsutum*), Japanese apricot (*Prunus mume*), corn (*Zea mays*), rape (*Brassica napus*), rice (*Oryza sativa*) to soybean (*Glycine max*). In many crops, GWAS has helped to analyze the genetic basis of their stress resistance traits. For example, the GWAS study of rice involved 163 479 association analyzes and 461 traits (Joshi et al., 2023). This shows that GWAS provides a large amount of genetic information and molecular markers in analyzing the genetic basis of stress resistance traits in rice.

Synthetic associations due to correlation (Haile et al., 2023). These challenges need to be overcome through a comprehensive consideration of sample design, statistical analysis methods, and subsequent biological validation.

In the future, with the development of high-throughput sequencing technology and the improvement of bioinformatics analysis methods, research on using GWAS strategies to accelerate the improvement of crop stress resistance traits will become more in-depth and precise. For example, data sets containing more complete genome sequences will allow researchers to more efficiently identify causal variants associated with traits, even if these variants are in linkage disequilibrium (LD) over long distances.

In recent years, genome-wide association study (GWAS) strategies have made significant progress in improving crop stress resistance traits. The GWAS method reveals the resistance mechanisms of various crops to adverse stress by analyzing the association between genetic variation and phenotypic characteristics. This method is particularly suitable for genetic studies of complex traits because it can reveal the role of small-effect genes that are often difficult to detect in traditional breeding methods.

4.2 Analysis of successful cases

Over the past few years, genome-wide association studies (GWAS) have significantly advanced our understanding of the genetic mechanisms of crop stress resistance traits. By combining modern genetics, bioinformatics and statistical methods, GWAS enables scientists to identify loci associated with specific traits, thereby accelerating the process of crop improvement.

In a GWAS study on sesame (*Sesamum indicum*), researchers developed haplotype-based models and multi-locus models to overcome the limitations of single-locus model analysis. By considering associations between multiple genetic markers, these models allow studies to more accurately capture allelic diversity and optimize the use of high-density marker data. The results show that the multi-locus GWAS model is more effective than the single-locus model, and can discover multiple genes/loci controlling complex traits, reducing the false positive rate, and does not require Bonferroni correction for multiple tests, thus potentially avoiding Missing important points. This progress not only improves the quality and depth of association results, but also excels in the efficiency and ability to detect marker-trait associations in different plant species (Figure 3) (Berhe et al., 2021).

Upland cotton (*Gossypium hirsutum*) reveals genetic variants and candidate genes associated with salt stress tolerance. The study considered the population structure and relative kinship matrix through a mixed linear model, conducted a GWAS analysis, and found 25 loci that were significantly associated with three salt tolerance-related traits in the 2016 and 2017 data sets. These loci included 27 significant SNPs, distributed on multiple chromosomes. Through these correlation analyses, the researchers discovered some genes that may be related to salt stress response, such as genes encoding protein kinases and genes encoding aquaporins. These genes may play an important role in salt stress tolerance in cotton. effect. In addition, this study also conducted preliminary functional verification of candidate genes through gene expression pattern analysis, providing important information for the future use of molecular breeding technology to improve cotton's salt stress tolerance (Xu et al., 2021).

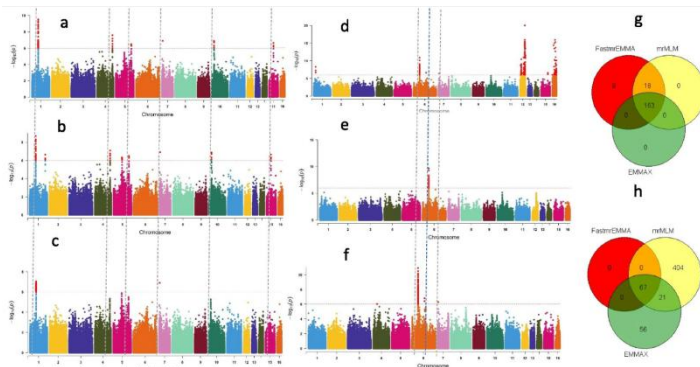


Figure 3 Application of new statistical multi-locus model in sesame (Adopted from Berhe et al., 2021)

Another example is the whole-genome resequencing, diversity analysis and stress resistance trait analysis of 77 grape root stock genotypes. GWAS analysis revealed SNP loci and genes related to grape root stock resistance to various stresses such as root nodule nematodes, salinity, drought, cold and flooding. In particular, the study found that genes related to salt stress are distributed on multiple chromosomes. These genes include multiple cysteine-rich receptor-like protein kinase genes and the sodium/hydrogen exchanger 2 gene located on chromosome 5. These findings provide important clues for further exploring the tolerance mechanism of grapes to salt stress. In addition, for cold tolerance traits, the study identified loci related to the C-repeat binding factor 1 gene, which is the main regulator of plant cold resistance mechanisms. These results are of great significance for improving the stress resistance traits of grapes (Figure 4) (Wang et al., 2024).

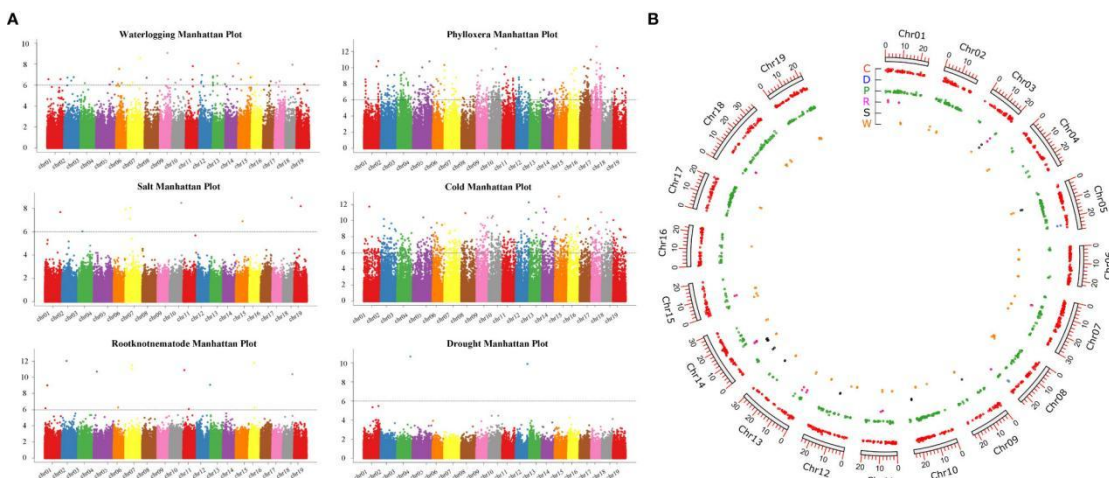


Figure 4 Genome-wide association analysis of six resistance traits (Adopted from Wang et al., 2024)

Advances in bioinformatics have provided key technologies for processing and analyzing high-throughput sequencing data. As the amount of data increases, the development of bioinformatics tools and algorithms makes it possible to extract useful information from complex data. This includes preprocessing of data, identification and annotation of variants, and sophisticated statistical analysis to reveal associations between genetic variants and specific traits.

In the future, with the continued advancement of high-throughput sequencing technology and the continuous innovation of bioinformatics methods, we expect that GWAS will be able to more accurately identify and explain the genetic basis that affects complex diseases and traits. This will include utilizing larger sample sizes, more comprehensive coverage of genetic variation, and integrated analysis of multi-omics data (such as transcriptome, proteome, etc.) to gain a deeper understanding of the molecular mechanisms of disease and promote personalized medicine. and the development of precision treatments.

Over the past few decades, genome-wide association studies (GWAS) have become an important tool to reveal the genetic mechanisms of crop stress resistance traits. GWAS has revealed the genetic basis of disease resistance and stress tolerance traits in many crops by searching genome-wide for markers associated with specific traits. Below, we discuss the contribution of GWAS in this field through specific examples.

4.3 Discuss the contribution of GWAS in revealing the genetic mechanism of stress resistance traits

A clear example is the use of GWAS analysis to reveal genetic variation and candidate genes for traits related to salt tolerance in cotton (*Gossypium hirsutum*). By analyzing the genetic structure and linkage disequilibrium (LD) distribution of 217 cotton varieties, the researchers identified multiple significant association sites related to salt tolerance. This work detected 25 significant associations ($-\log_{10}p > 4$) in the 2016 and 2017 datasets, covering 27 significant single nucleotide polymorphisms (SNPs) located on different chromosomes, the phenotypic variation rate (PVE) explained by each quantitative trait locus (QTL) ranges from 1.29% to 7.00%. These results not only provide molecular markers for the improvement of salt tolerance in cotton, but also reveal the complex genetic basis of this trait.

In studies of maize (*Zea mays*), GWAS were used to identify candidate genes associated with disease resistance. A landmark study identifies 18 new genes associated with head rust resistance. In addition, for other disease resistance, such as sheath blight, gray leaf spot and Fumei virus resistance, important association loci and candidate genes were also identified through GWAS. These studies not only reveal the genetic mechanisms of crop disease resistance traits, but also provide valuable molecular markers for breeding programs (Xu et al., 2021).

Rice (*Oryza sativa*) is the staple food of more than half of the world's population. For rice, fungal, bacterial and viral diseases are the main limiting factors. Using GWAS methods, researchers identified and verified genomic regions that are resistant to diseases such as bacterial stripe and rice blast. For example, an analysis of 56 important QTLs/genomic regions in different rice blast strains identified one region as the Pik allele, which showed resistance to all three strains.

Through these examples, we can see the significant contribution of GWAS in revealing the genetic mechanisms of crop stress resistance traits. These studies not only increase our understanding of crop genetic diversity and resistance mechanisms, but also provide valuable resources for breeding and crop improvement. Although there are some challenges, such as the demand for data volume and quality, the difficulty of elucidating the genetic structure of complex traits, and the challenges of biological validation and functional analysis of the results.

5 Future Outlook

In the past few years, high-throughput sequencing technology (HTS) and bioinformatics have made great progress in crop genetic improvement, especially in their application in genome-wide association studies (GWAS). These technologies not only improve the efficiency of genotype identification, but also promote research on improving crop stress resistance traits (Pavan et al., 2020). The development of GWAS allows us to link DNA variants to phenotypes of interest, thereby mapping genomic locations associated with economically important traits, including yield, resistance to biotic and abiotic stresses, and quality.

In recent years, combined with high-density single nucleotide polymorphism (SNP) chips and DNA sequencing technology, the genotype space of multiple crops has been deeply explored, which has strengthened the role of GWAS in understanding the relationship between phenotype and genotype. importance. For example, the association of acid phosphatase activities of 280 mustard genotypes under different phosphorus levels was evaluated using a GWAS method, thus laying a solid foundation for using marker-assisted selection to improve the phosphorus efficiency of Indian mustard (Li and Marylyn, 2022).

In addition, statistical methods that integrate GWAS results with functional genomic data (such as gene expression or chromatin activity profiles) are designed to identify cell types relevant to complex diseases, facilitating the translation from genomic discovery to functional understanding based on transcriptome breadth Association study

(TWAS) is a gene-based association method that studies the association between genetically regulated gene expression and complex diseases or traits (Pavan et al., 2020). The development of TWAS provides a powerful tool for the identification of genes related to complex traits, and it is expected that with the development of single-cell sequencing, chromosome conformation capture, gene editing technology and multiplex reporter analysis, we will have insights into genome regulation and genetic regulation of genes. A more comprehensive understanding (Li and Marylyn, 2022).

In the future, with the continuous advancement of high-throughput sequencing technology and bioinformatics, we expect that there will be more strategies to integrate multi-omics data (such as transcriptome, proteome, etc.), thereby providing more accurate information for improving crop stress resistance traits. genetic basis. In addition, the application of precision breeding and gene editing technology will accelerate the improvement of crop traits based on the findings of GWAS, especially in the research and application of resistance traits. These developments will promote new directions and trends in the improvement of crop stress resistance traits, and ultimately achieve sustainable development of crop production and food security.

Acknowledgments

The author would like to appreciate two anonymous peer reviewers for their feedback on the manuscript

Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

- Alison R.B., Chen C., and Nunzio D.A., 2022, Editorial: genome wide association studies and genomic selection for crop improvement in the era of big data, *Front. Genet.*, 13: 873060.
<https://doi.org/10.3389/fgene.2022.873060>
PMid:35669194 PMCID:PMC9164124
- Berhe M., Dossa K., You J., Mboup P.A., Diallo I.N., Diouf D., Zhang X., and Wang L., 2021, Genome-wide association study and its applications in the non-model crop *Sesamum indicum*, *BMC Plant Biol.*, 21(1): 283.
<https://doi.org/10.1186/s12870-021-03046-x>
PMid:34157965 PMCID:PMC8218510
- Brandes N., Linial N., and Linial M., 2020, PWAS: proteome-wide association study-linking genes and phenotypes by functional variation in proteins, *Genome Biol.*, 21(1): 173.
<https://doi.org/10.1186/s13059-020-02089-x>
PMid:32665031 PMCID:PMC7386203
- Elena B., and Giménez E., 2021, Modern approaches for the genetic improvement of rice, wheat and maize for abiotic constraints-related traits: a comparative overview, *Agronomy*, 11(2): 376.
<https://doi.org/10.3390/agronomy11020376>
- Haile J.K., Sertse D., N'Diaye A., Klymiuk V., Wiebe K., Ruan Y., Chawla H.S., Henriquez M.A., Wang L., Kutcher H.R., Steiner B., Buerstmayr H., and Pozniak C.J., 2023, Multi-locus genome-wide association studies reveal the genetic architecture of *Fusarium* head blight resistance in durum wheat, *Front. Plant Sci.*, 14: 1182548.
<https://doi.org/10.3389/fpls.2023.1182548>
PMid:37900749 PMCID:PMC10601657
- Joshi A., Yang S.Y., Song H.G., Min J., and Lee J.H., 2023, Genetic databases and gene editing tools for enhancing crop resistance against abiotic stress, *Biology*, 12(11): 1400.
<https://doi.org/10.3390/biology12111400>
PMid:37997999 PMCID:PMC10669554
- Korte A., and Farlow A., 2013, The advantages and limitations of trait analysis with GWAS: a review, *Plant Methods*, 9: 29.
<https://doi.org/10.1186/1746-4811-9-29>
PMid:23876160 PMCID:PMC3750305
- Kushanov F.N., Turaev O.S., Ernazarova D.K., Gapparov B.M., Oripova B.B., Kudratova M.K., Rafeva F.U., Khalikov K.K., Erjigitov D.S., Khidirov M.T., Kholova M.D., Khusenov N.N., Amanboyeva R.S., Saha S., Yu J.Z., and Abdurakhmonov I.Y., 2021, Genetic diversity, QTL mapping, and marker-assisted selection technology in cotton (*Gossypium* spp.), *Front. Plant Sci.*, 12: 779386.

- Li B.L., and Marylyn D.R., 2021, From GWAS to gene: Transcriptome-wide association studies and other methods to functionally understand GWAS discoveries, *Front. Genet.*, 12: 713230.
<https://doi.org/10.3389/fgene.2021.713230>
- Mores A., Borrelli G.M., Laidò G., Petruzzino G., Pecchioni N., Amoroso L.G.M., Desiderio F., Mazzucotelli E., Mastrangelo A.M., and Marone D., 2021, Genomic approaches to identify molecular bases of crop resistance to diseases and to develop future breeding strategies, *Int. J. Mol. Sci.*, 22(11): 5423.
<https://doi.org/10.3390/ijms22115423>
PMid:34063853 PMCID:PMC8196592
- Nardana E., Shen G.X., and Zhang H., 2022, Genetic manipulation for abiotic stress resistance traits in crops, *Front. Plant Sci.*, 21: 2022.
- Pavan S., Delvento C., Ricciardi L., Lotti C., Ciani E., and D'Agostino N., 2020, Recommendations for choosing the genotyping method and best practices for quality control in crop genome-wide association studies, *Front. Genet.*, 11: 447.
<https://doi.org/10.3389/fgene.2020.00447>
PMid:32587600 PMCID:PMC7299185
- Rafael D.C., Qiu Y.J., Ou S.J., Matthew B.H., and Candice N.H., 2021, How the pan-genome is changing crop genomics and improvement, *Genome Biology*, 22(1): 3.
- Thomas D.H., and Hüchelhoven R., 2018, Biotic and abiotic stress responses in crop plants, *Agronomy*, 8(11): 267.
<https://doi.org/10.3390/agronomy8110267>
- Wang L., and Chang C., 2024, Stomatal improvement for crop stress resistance, *Journal of Experimental Botany*, 75(7): 1823-1833.
<https://doi.org/10.1093/jxb/erad477>
PMid:38006251
- Wang P.P., Zhao F.H., Zheng T., Liu Z.J., Ji X.H., Zhang Z.C., Pervaiz T., Shangguan L.F., and Fang J.G., 2023, Whole-genome re-sequencing, diversity analysis, and stress-resistance analysis of 77 grape rootstock genotypes, *Front. Plant Sci.*, 14: 1102695.
<https://doi.org/10.3389/fpls.2023.1102695>
PMid:36844076 PMCID:PMC9947647
- Xu P., Guo Q., and Meng S., 2021, Genome-wide association analysis reveals genetic variations and candidate genes associated with salt tolerance related traits in *Gossypium hirsutum*, *BMC Genomics*, 22(1): 26.
<https://doi.org/10.1186/s12864-020-07321-3>
PMid:33407102 PMCID:PMC7789578
- Xu P., Guo Q., Meng S., Zhang X.G., Xu Z.Z., Guo W.Z., and Shen X.L., 2021, Genome-wide association analysis reveals genetic variations and candidate genes associated with salt tolerance related traits in *Gossypium hirsutum*, *BMC Genomics*, 26: 21.
<https://doi.org/10.21203/rs.3.rs-66236/v1>

Disclaimer/Publisher's Note

The statements, opinions, and data contained in all publications are solely those of the individual authors and contributors and do not represent the views of the publishing house and/or its editors. The publisher and/or its editors disclaim all responsibility for any harm or damage to persons or property that may result from the application of ideas, methods, instructions, or products discussed in the content. Publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
