

GWAS Discovery of Stress Tolerance Trait Genes in Wheat Crops

Wang Hongpeng, Li Minghua ✉

Biotechnology Research Center, Cuixi Academy of Biotechnology, Zhuji, 311800, Zhejiang, China

✉ Corresponding author: Lileea@gmail.com

Triticeae Genomics and Genetics, 2024, Vol.15, No.1 doi: [10.5376/tgg.2024.15.0003](https://doi.org/10.5376/tgg.2024.15.0003)

Received: 15 Dec., 2024

Accepted: 29 Jan., 2024

Published: 12 Feb., 2024

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Preferred citation for this article:

Wang H.P., and Li M.H., 2024, GWAS discovery of stress tolerance trait genes in wheat crops, *Triticeae Genomics and Genetics*, 15(1): 19-30 (doi: [10.5376/tgg.2024.15.0003](https://doi.org/10.5376/tgg.2024.15.0003))

Abstract Significant progress has been made in research on stress tolerance trait genes of wheat crops, which has greatly promoted the development of crop improvement and food security. Through high-throughput sequencing technology and genome-wide association analysis (GWAS), researchers have successfully identified multiple key genes and regulatory networks related to stress response, especially under stress conditions such as drought, salinity, and low temperature. The application of gene editing technologies, such as CRISPR/Cas9, provides new strategies for precisely improving stress tolerance traits. At the same time, the development of phenomics has deepened the understanding of crop stress response mechanisms. Future research will focus on the application of systems biology methods, integrating multi-omics data to reveal complex stress tolerance mechanisms; using precision breeding technology to precisely operate stress tolerance genes; strengthening research on the interaction between environment and genetics, and exploring the genetic diversity of wild resources. and promote international cooperation and knowledge sharing. These directions will provide a solid scientific basis for cultivating wheat crop varieties with high stress tolerance to cope with climate change and global food security challenges.

Keywords Wheat crops; Stress tolerance traits; Gene editing; Genome-wide association studies (GWAS); System biology

Wheat crops, as one of the important sources of food for mankind, play an irreplaceable role in global agriculture. The wheat family, consisting of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare*), corn (*Zea mays*), rice (*Oryza*), etc., provides major carbohydrates, proteins and other important nutrients to people around the world, is one of the cornerstones of human survival and development. However, the growth and yield of wheat crops are often affected by various adverse factors, such as drought, salinity, high temperature, pests and diseases, etc. These factors will not only directly reduce the yield and quality of the crops, but may also lead to the deterioration of the ecological environment and have a negative impact on the environment. Threats to global food security.

The impact of adversity on the yield and quality of wheat crops is multifaceted. Under adverse conditions, the crop growth cycle is prolonged and the growth rate is slowed down, resulting in insufficient biomass accumulation throughout the growing season. Adversity conditions will destroy the growth and development process of crops and affect key physiological processes such as photosynthesis, nutrient absorption, and material transport, thereby leading to crop growth deformities and dysfunction. In addition, adversity can easily trigger the outbreak of crop diseases and insect pests, making crops more vulnerable. The impact of adversity on the yield and quality of wheat crops is comprehensive and complex, severely restricting the production potential and stability of wheat crops (Zhang et al., 2021).

Facing the challenge of adversity, genetic improvement is considered to be one of the effective ways to improve the adversity tolerance of wheat crops. Genetic improvement can improve the adaptability and resistance of crops to stress by screening and breeding new varieties with stress-resistant traits. In the process of genetic improvement, it is crucial to discover and utilize stress tolerance trait genes in wheat crops. These genes carry genetic information related to stress resistance and can provide an important genetic basis for crop stress resistance. Therefore, in-depth study of wheat crop stress tolerance trait genes and discovery of their molecular mechanisms and functional characteristics have important theoretical and practical significance for improving crops' ability to adapt to stress and enhancing their productivity and stress resistance.

This study will comprehensively review the progress and challenges in the GWAS discovery of stress tolerance trait genes in wheat crops over the past decade, and discuss the current research status and future development directions in this field. Through the analysis and summary of relevant literature and research results, it aims to provide scientific basis and theoretical support for the genetic improvement of stress tolerance of wheat crops and contribute to solving global food security issues.

1 Application of GWAS in the discovery of stress tolerance trait genes in wheat crops

1.1 Principles and methods of GWAS

Genome wide association analysis (GWAS) is to detect the genetic variation (marker) polymorphisms of multiple individuals across the entire genome to obtain genotypes, and then combine the genotypes with observable traits, that is, Phenotype, perform statistical analysis at the population level, screen out the genetic variations (markers) most likely to affect the trait based on statistics or significant *p-values*, and mine genes related to trait variation.

GWAS is a method of identifying genetic variants associated with specific traits or diseases by comparing genotypic and phenotypic data in large sample populations. Researchers need to collect genotype data from large sample populations. This is usually achieved through gene chips or the latest sequencing technology. Genotype data includes the SNPs on the genome of the samples participating in the study (Single Nucleotide Polymorphism) Information.

At the same time, researchers also need to collect phenotypic data on the sample population participating in the study, that is, the manifestation of the trait or disease of interest. These phenotypic data may cover many aspects such as biology, physiology, and behavior. Before conducting GWAS, the collected genotypic and phenotypic data need to be cleaned and quality controlled. This includes steps such as removing missing values, correcting genotype bias, and excluding population structures to ensure the accuracy and reliability of the data (Xia et al., 2019).

Next, the researchers used statistical methods to analyze the association between genotype and phenotype. In GWAS, commonly used correlation analysis methods include chi-square test, linear regression model, logistic regression model, etc. Through these methods, researchers can determine the relationship between genotypic variation and traits or diseases.

Since GWAS involves a large number of SNP-trait comparisons, multiple testing correction is required to reduce the risk of false-positive results. Commonly used correction methods include Bonferroni correction, FDR (false discovery rate) correction, etc. Researchers need to interpret and functionally annotate discovered associations. This may involve research on the biological function of the gene itself, pathway analysis, expression regulation, etc., to gain a deeper understanding of the relationship between genotypic variation and traits or diseases.

1.2 Current status of GWAS application in wheat crops

GWAS The application of (genome-wide association analysis) in wheat crops has made a series of important progress, providing strong support for revealing the stress tolerance trait genes and related genetic mechanisms of wheat crops. GWAS has been widely used to discover key genes affecting disease resistance and stress resistance traits of wheat crops. In wheat, researchers used GWAS to identify SNP markers and genes related to resistance to important diseases such as stripe rust and head blight, providing important genetic resources for wheat disease resistance breeding.

GWAS has also made important progress in studying the adaptation mechanisms of wheat crops to drought, high temperature, salinity and other stress stresses. By analyzing large-scale genotypic and phenotypic data, researchers discovered multiple candidate genes related to stress response, revealing a complex network of stress response pathways.

For example, Wang et al. (2020) tried to use TASSEL5.0 software to combine the whole panicle germination rates of 207 wheat varieties (lines) with 16 typing genes screened by 90K SNP chips. Genome-wide association

analysis of 686 high-quality SNP markers. Based on the MLM (Q+K) model, when $P \leq 0.001$, the marker is considered to be significantly associated with the trait, and the loci detected in multiple environments are regarded as stably heritable loci (Figure 1). GWAS analysis results showed that a total of 34 significant loci were detected, distributed on wheat chromosomes 3A, 3B, 4A, 4B, 5D, 6A, 6B, 6D, 7B and 7D. A single locus can explain 5.55%~11.63% Phenotypic variation (Wang et al., 2020).

GWAS provides a new way to utilize wheat crop germplasm resources. By analyzing genotypic and phenotypic data of different germplasm resources, researchers can discover useful genes hidden in natural variation, providing new genetic resources and candidate genes for genetic improvement.

With the development of GWAS technology, researchers have begun to combine functional genomics methods to deeply explore the functions and regulatory mechanisms of key genes discovered by GWAS. Through gene expression analysis, transcriptomics, proteomics and other methods, we can reveal the functional connection between genotype and phenotype and further understand the molecular mechanism of stress tolerance traits. The key genetic markers identified by GWAS can be used as high-throughput molecular markers to assist in the genetic improvement of wheat crops. These molecular markers can help breeders select excellent germplasm with target traits, accelerate the breeding process, and improve breeding efficiency (Sun et al., 2017).

The application of GWAS in wheat crops has made significant progress, providing powerful tools and methods for analyzing the genetic basis of wheat crops, revealing the molecular mechanisms of stress tolerance traits, improving crop stress resistance and accelerating the breeding process. With the continuous advancement of technology and the continuous improvement of methods, it is believed that the application of GWAS in the field of genetic improvement of wheat crops will become more extensive and in-depth.

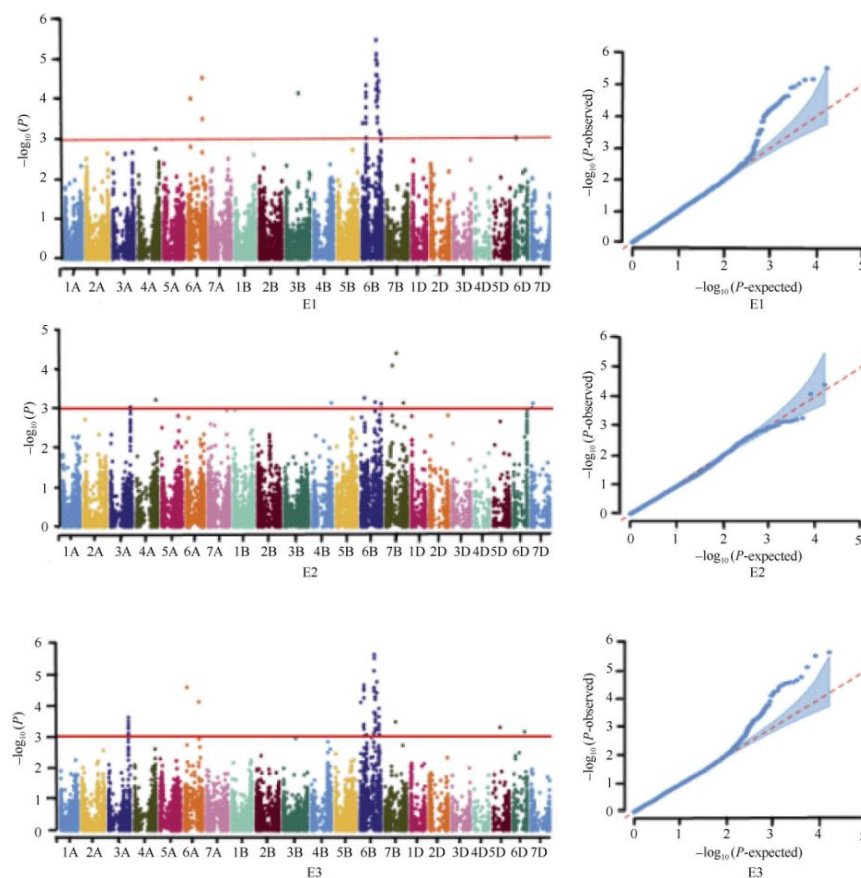


Figure 1 Analysis of panicle germination rates in different environments (Wang et al., 2020)

Note: E1: 2017-2018, Manas; E2: 2018-2019, Manas; E3: Average environment

1.3 Advantages of GWAS in discovering stress tolerance trait genes in wheat crops

GWAS has many advantages in discovering stress tolerance trait genes in wheat crops, making it an important method for studying the stress tolerance of wheat crops. GWAS can be analyzed at the entire genome level, covering all genes in wheat crops, not just specific candidate genes. This means it can reveal the genetic variation associated with stress tolerance traits hidden throughout the genome, thus providing a more comprehensive genetic basis.

GWAS can provide high-resolution genotype-phenotype correlations and can more accurately locate genes or gene regions related to stress tolerance traits. This helps to accurately identify candidate genes, providing more reliable information for subsequent functional studies and breeding applications.

GWAS usually utilizes genotype and phenotypic data from large-scale sample populations for analysis, so it has high statistical power and reliability. The application of large sample sizes can increase the chance of detecting genetic variants and reduce the occurrence of false positive results, making the results more credible (Sukumaran et al., 2018).

GWAS can utilize diverse germplasm resources, including different varieties, natural germplasm and wild species, to mine potential genetic variations related to stress tolerance traits. This helps to make full use of the genetic diversity of wheat crops and provides a wider range of genetic resources for the genetic improvement of stress tolerance traits.

Key genetic markers identified by GWAS can be used as molecular markers for assisted breeding. These markers can help breeders select excellent germplasm with target traits, accelerate the breeding process, improve breeding efficiency, and achieve precise selection during the genetic improvement process.

Stress tolerance traits are often jointly regulated by multiple genes and have high genetic complexity. GWAS can analyze multiple genotypes and phenotypes simultaneously, reveal the genetic basis and regulatory network of complex traits, and provide deeper insights into understanding stress response mechanisms.

GWAS has unique advantages in discovering stress tolerance trait genes in wheat crops, providing powerful tools and methods for analyzing the genetic basis of stress tolerance traits, accelerating crop genetic improvement, and cultivating adaptive varieties. Genomic selection has great potential in wheat improvement, but actual cases of its application in wheat breeding are currently limited, and the cost of genotyping is also a key constraint for large-scale implementation of genomic selection. In addition, the current genome-wide association studies (GWAS) of wheat use small-scale association panels, insufficient research on complex traits, and few studies focus on multiple traits. These current situations reflect the necessity of conducting overall multi-trait GWAS in a large group (Khan et al., 2022).

2 Research Progress in the Past Decade

2.1 Application cases of GWAS under different types of adversity conditions

In the past decade, GWAS has been widely used to study the coping mechanisms of wheat crops under various stress conditions. For example, using a multilocus GWAS (ML-GWAS) approach, researchers successfully identified genomic regions associated with traits related to grain weight in wheat under rain-fed conditions. This method is particularly effective in dealing with complex traits. For example, in the study of Gahlaut et al. (2021), many traits usually have polygenic control, low heritability, and large genotype \times environment interactions. Their study analyzed 320 spring wheat varieties and revealed multiple new molecular markers and candidate genes related to drought tolerance, providing a powerful tool for marker-assisted selection (MAS) (Figure 2).

For another example, genetic relationship analysis based on markers and pedigrees identified QTL haplotype variation for salt tolerance in Chinese wheat germplasm. Yu et al. (2020) collected 5 More than 000 wheat germplasm resources, and 660 wheat The K SNP chip determined the genotypes of more than 1,500 materials. Subsequently, they selected 307 genetically representative materials, including China's modern main varieties,

core germplasm, local farm varieties, etc., and conducted salt tolerance identification at the bud stage and genome-wide association analysis (GWAS). By analyzing the pedigree, genetic relationships and modern molecular marker information of wheat, combined with the GWAS results of salt tolerance traits, they conducted a traceability analysis of the excellent haplotypes related to salt tolerance in China's wheat germplasm (Figure 3).

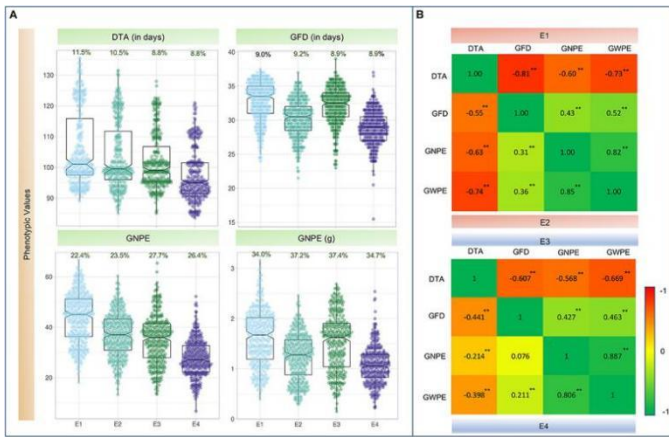


Figure 2 Phenotypic variation of water stress and its impact on grain weight-related traits (Gahlaut et al., 2021)

Note: A: Box plots show the value distribution of four traits in four environments, and for each trait, its coefficient of variation (CV%) is shown on the top of each plot; B: Heat map shows the distribution of values among the four traits. Pearson correlation coefficient (r value), the value above the diagonal line on the left is the r value in the irrigated environment (E1 and E3), the value below the diagonal line on the right represents the r value in the rainfed environment (E2 and E4); **: Significance is $P < 0.001$; DTA: Days to flowering stage; DTM: Maturity date; GFD: Grain filling duration; GNPE: Number of grains per panicle; GWPE: Grain weight per panicle; E1: Density Irrigated Rut (IR); E2: Rainfed Meerut (RF); E3: Irrigated Powarkheda (IR); E4: Rainfed Powarkheda (RF)

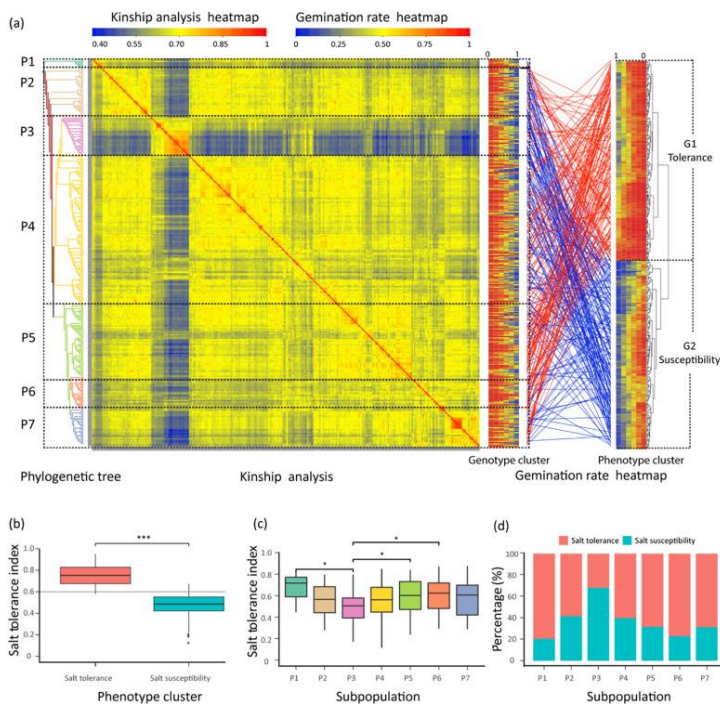


Figure 3 Phenotypic clusters and genetic population structures and their relationships (Yu et al., 2020)

Note: a: Similarity tree and heat map showing phenotypic (left) and genotypic relationships (right) of traits; phenotypic tree is constructed based on correlation matrix; b: Classification by phenotypic tree (salt tolerant and salt sensitive) The two main clusters and their STI boxplots; c: Accessions with $STI > 0.6$ in different subpopulations in the diversity panel; d: Boxplots of sexually transmitted infections in different subpopulations in the diversity panel

Leaf rust is caused by *Puccinia triticina* and can cause wheat yield losses of more than 50%. A large number of leaf rust resistance genes have been located, but due to the rapid mutation of the pathogen, many of the exploited resistance genes have gradually become ineffective. Therefore, finding new resistance genes to improve rust resistance durability is a current research hotspot.

Zhang et al. (2021) used phenotypic data and 35K SNP data of 400 wheat lines (including advanced breeding materials, local varieties and modern varieties), combined with 6 multi-locus GWAS methods, to systematically identify the genetics of leaf rust resistance. This is the first study to use multiple GWAS models to deeply explore the genetic basis of leaf rust resistance in wheat. GWAS detected four loci that are resistant to leaf rust and stripe rust, including the known gene *Lr46/Yr19* located on 1BL. In addition, *QLr-2AL.1/QYr-2AL.1* located on chromosome 2AL also showed stable facultative resistance, and the frequency of this resistance site was relatively high in the tested materials (accounting for 73.5%). However, the predicted effective disease resistance genes *Lr34/Yr18* and *Lr37/Yr17* were not detected in this study. Although the test varieties were tested using molecular markers closely linked to the genes, the results showed that 26 varieties contained *Lr34/Yr18* and 18 varieties contained *Lr37/Yr17*, and the T test results showed that these two genes showed expression in the test materials. Excellent leaf rust and stripe rust resistance. This illustrates the possible disadvantages of GWAS, that is, resistance genes with lower frequency in the population may not be detected, but these genes are likely to be potentially important genes (Figure 4).

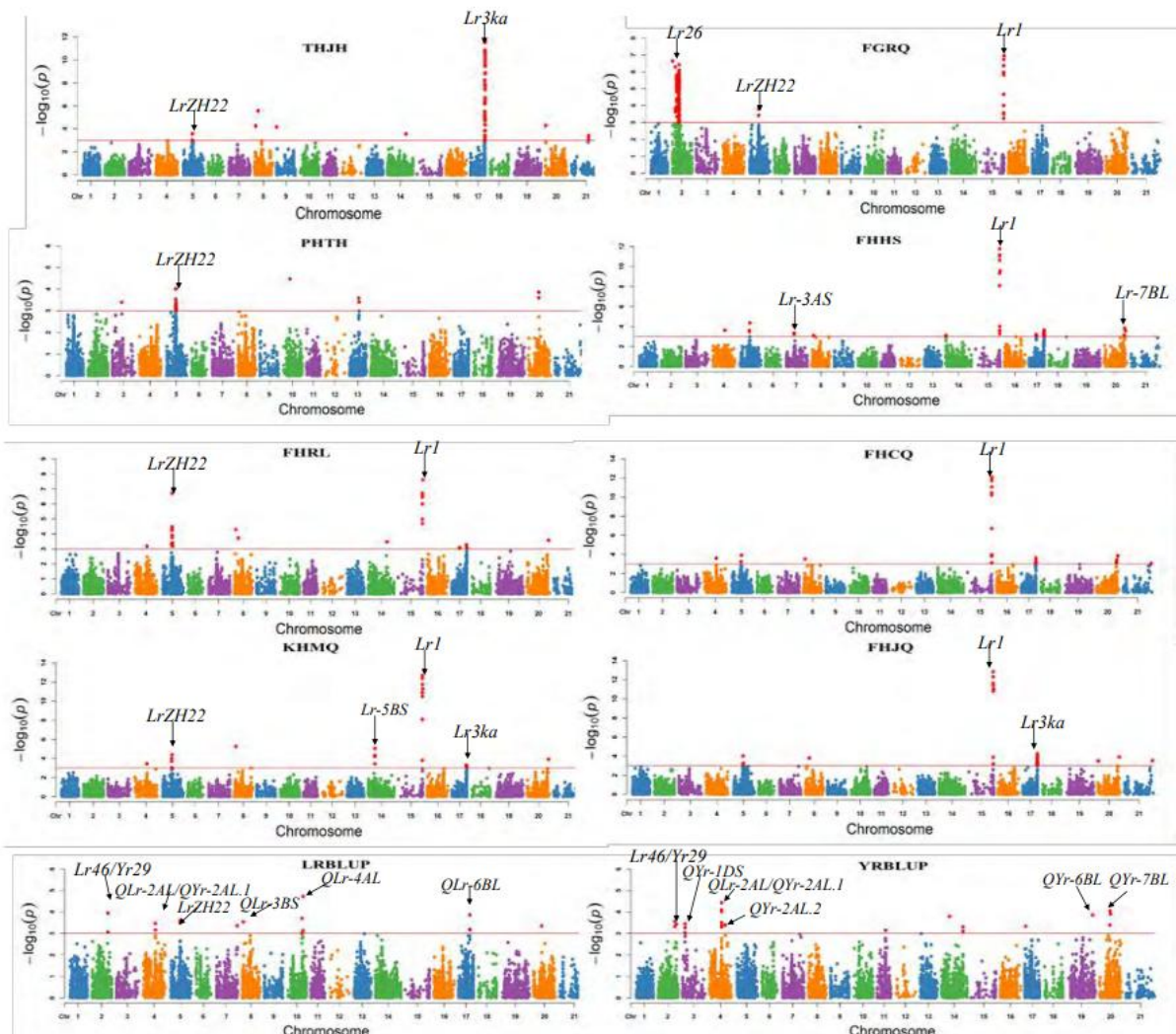


Figure 4 Analysis of genetic loci for resistance to leaf rust (Zhang et al., 2021)

GWAS studies over the past decade have significantly advanced our understanding of the genetic basis of wheat crops under stress conditions. By identifying relevant genetic markers and genes, GWAS provides valuable information for crop improvement, allowing breeders to more effectively select and breed crop varieties adapted to specific environmental conditions. Despite challenges, such as the processing and interpretation of large-scale multi-phenotypic data, GWAS remains a very valuable tool in crop genetic research. With the advancement of genome sequencing technology and the improvement of data analysis methods, it is expected that GWAS will continue to play a key role in the study of crop stress adaptability in the future.

2.2 Discovery and functional analysis of stress tolerance trait genes in wheat crops

In the fields of agricultural science and plant biotechnology, the discovery and functional analysis of stress tolerance trait genes in wheat crops is a key advance. Wheat crops, including wheat, barley and oats, form the basis of global food security, providing essential nutrients and calories to billions of people around the world. However, these crops are often affected by multiple stress factors such as drought, salinity, extreme temperatures and diseases, which significantly reduce yield and quality. Therefore, understanding the genetic basis of the stress response mechanisms of these plants is critical to developing more resilient crop varieties through breeding and genetic engineering.

The discovery of stress tolerance trait genes in wheat crops benefited from advances in genomics, bioinformatics and molecular biology technologies. High-throughput sequencing technology has enabled a comprehensive mapping of the genome of wheat crops, allowing researchers to identify genes and regulatory elements associated with mechanisms to cope with stress. Comparative genomics also plays a key role in identifying conserved genetic elements related to stress tolerance among different species (Xie et al., 2021).

A landmark discovery in this field was the identification of dehydration response element binding protein (DREB) genes that play a key role in resistance to drought and cold stress. These transcription factors regulate the expression of a large number of downstream genes involved in protective responses to dehydration and low temperature. Another important finding was the identification of salt hypersensitivity (SOS) pathway genes, which are critical for maintaining ion balance under salty conditions, allowing plants to withstand high salt levels.

Functional elucidation of these genes involves elucidating their role in plant response to stress and how they enhance stress tolerance. This is often achieved by combining genetic engineering (overexpression or knockout of genes of interest in model plants or wheat crops) and physiological assessment (evaluation of changes in response to stress, growth and yield under stressful conditions).

For example, overexpression of DREB genes in transgenic plants has shown enhanced drought and low temperature tolerance, leading to improved water use efficiency and survival under adverse conditions. Similarly, manipulating genes in the SOS pathway showed increased salt tolerance in genetically modified wheat crops, allowing them to grow in high-salinity soils that would otherwise be unsuitable for cultivation (Su et al., 2018).

The discovery and functional analysis of stress-tolerance trait genes in wheat crops have far-reaching implications for agriculture, especially in the context of climate change and increasing environmental pressure. By integrating these genes into wheat crop varieties through traditional breeding or genetic engineering, crops more resistant to stress factors can be developed to maintain agricultural productivity and food security under changing environmental conditions.

Future research in this area may focus on the discovery of new stress tolerance trait genes and their regulatory networks, using emerging technologies such as CRISPR/Cas genome editing and systems biology approaches. Furthermore, understanding the interactions between different stress response pathways and developing strategies to simultaneously enhance tolerance to multiple stressors will be critical to developing crops that can thrive in increasingly challenging agricultural environments.

2.3 Identification and verification of candidate genes for stress tolerance trait genes

In modern agricultural science, the identification and verification of candidate genes for stress tolerance trait genes are key steps to improve crops' ability to withstand stress. This process involves not only the discovery of potential stress-resistant genes but also the verification of the functions of these genes through a series of experiments. As climate change and environmental stress intensify, it is particularly important to develop crop varieties that can withstand stresses such as drought, salinity, temperature extremes, and disease.

Identification of candidate genes for stress tolerance traits first requires an in-depth understanding of the molecular mechanisms of crop responses to stress. Through tissue-specific expression analysis, transcriptomics, and proteomics studies, scientists are able to identify genes whose expression levels are significantly altered under stress conditions. In addition, using comparative genomics methods, researchers can also search for conserved stress tolerance genes in different species, which may have adapted to specific environmental stresses during evolution (Oyiga et al., 2017).

Subsequently, through bioinformatics tools and databases, such as NCBI, PlantGDB, and Ensembl Plants, further bioinformatics analysis of these candidate genes can be performed, including prediction of gene structure, functional domains, and the signal transduction pathways they may participate in. These analyses help screen out the most promising candidate genes for subsequent validation.

Once candidate genes are identified, the next step is to confirm the role of these genes in plant stress tolerance through functional validation. This typically involves gene overexpression or knockout (gene silencing) experiments and subsequent assessment of phenotypic, physiological and biochemical properties.

Gene overexpression experiments usually use genetic transformation methods to introduce target genes into model plants (such as *Arabidopsis thaliana*) or target crops. These transgenic plants are then exposed to corresponding stresses under laboratory or field conditions to assess whether enhanced gene expression improves the plants' stress tolerance. In contrast, gene knockout or silencing experiments reduce or eliminate the expression of a target gene through specific technologies (such as RNA interference or CRISPR/Cas9 gene editing) and observe how this change affects the plant's response to stress.

In addition, functional verification of candidate genes needs to be performed at the cellular and molecular levels. For example, by analyzing the activity of genes encoding proteins in *in vitro* experiments, or using fluorescence microscopy to observe the localization of proteins in cells, and exploring interactions between proteins through bimolecular fluorescence complementation experiments. These experiments help reveal how candidate genes regulate physiological and molecular responses in plants in response to external stresses.

3 Challenges and Future Prospects

3.1 Interaction between genes and environment

The interaction between genes and the environment is an important topic in biology and genetics research, especially in understanding the expression and variation of biological traits. This interaction reflects the complex process of how an organism's genetic properties are affected by the environment in which it grows. In the fields of agricultural production and human health, research on the interaction between genes and the environment provides an important theoretical basis for improving crop yields, disease prevention and treatment.

Gene-environment interaction refers to the process of interaction between an individual's genetic background and external environmental factors. This interaction can affect the individual's phenotype, that is, the observable traits. Specifically, a certain genetic variant may lead to different phenotypic outcomes under different environmental conditions, and vice versa, the same environmental factor may have different effects in different genetic backgrounds (Saeideh et al., 2018).

In the field of plant science, the study of gene-environment interactions helps scientists understand the adaptability and yield performance of crops in different ecosystems. Under drought conditions, some crop

varieties exhibit better drought tolerance, which is often attributed to the interaction of specific genetic variants with environmental stress. By identifying these key genetic markers, breeders can develop crop varieties better suited to drought conditions.

3.2 Overcoming limitations in GWAS studies

Genome-wide association studies (GWAS) have become a powerful tool to reveal the genetic basis behind complex traits, but there are also some limitations in the research process. These limitations include, but are not limited to, multiple testing issues, genetic heterogeneity, neglect of environmental factors, difficulty in detecting rare variants, and the presence of “genetic dark matter.” In response to these challenges, the scientific community has begun to adopt multiple strategies to overcome or mitigate the limitations of GWAS to improve its efficiency and accuracy in revealing complex genetic traits.

GWAS involves the simultaneous testing of thousands of single nucleotide polymorphisms (SNPs), which introduces the risk of false positives. To control this risk, researchers used strict statistical correction methods, such as Bonferroni correction and false discovery rate (FDR) control, to adjust the significance threshold. In addition, there are more advanced statistical methods, such as Bayesian methods and mixture models, that can handle multiple comparison problems more efficiently while taking into account the influence of genetic background.

Genetic heterogeneity refers to the phenomenon that different genetic variants lead to the same phenotype in different individuals, which brings challenges to the interpretation of GWAS. To address this issue, researchers began performing stratified GWAS analyzes to group samples based on factors such as genetic background, subtypes of phenotypes, or environmental exposures. In addition, the use of meta-analysis methods that integrate data from multiple independent cohorts can improve statistical power and thereby identify common and specific genetic markers in different populations (Qaseem et al., 2017).

GWAS usually focus on the influence of genetic factors on phenotype and ignore the role of environmental factors. In order to overcome this limitation, some studies have begun to use environment and gene-environment interaction GWAS (G×E GWAS) to consider both genetic and environmental factors in the analysis. In this way, researchers can gain a more complete understanding of the mechanisms by which phenotypes are formed, especially for traits where environmental factors play an important role.

GWAS have traditionally focused on detecting more frequent variants and have less ability to detect rare variants. With the development of next-generation sequencing technology, whole-genome sequencing (WGS) and whole-exome sequencing (WES) provide the possibility to detect rare variants. These techniques allow researchers to identify rare variants genome-wide and assess their contribution to complex traits.

Even in successful GWAS, often only a small portion of the contribution of genetic variation to the phenotype can be explained, which is known as the “genetic dark matter” problem. To uncover this hidden genetic information, scientists have used a variety of strategies, including expanding sample sizes, using more complex genetic models, and exploring epigenetic variations and changes in gene expression levels. In addition, by integrating GWAS data with other large-scale bioinformatics databases, such as gene expression maps and protein interaction networks, researchers can discover new genetic-phenotype associations to gain a deeper understanding of the nature of genetic dark matter.

3.3 Future directions and research priorities

Although GWAS has successfully identified many markers related to important agronomic traits, many results still require further refined mapping and functional verification. Future research will focus on narrowing down the candidate gene region and determining the specific functional genes and their mechanisms of action through cell and molecular biology experiments. In addition, it will become the norm to use gene editing technologies such as CRISPR/Cas9 to verify the functions of candidate genes discovered by GWAS, which will help deepen our understanding of the genetic basis of wheat crops.

With the development of omics technologies such as transcriptomics, proteomics, and metabolomics, future GWAS studies will increasingly use multi-omics data integration analysis. By combining GWAS results with other omics data, the complex relationships between gene expression regulatory networks, protein interactions, and metabolic pathways can be revealed. This integrated analysis will help reveal the complex genetic mechanisms affecting important traits and provide a more comprehensive scientific basis for molecular design breeding of wheat crops.

The trait expression of wheat crops is significantly affected by environmental factors. Therefore, future GWAS research will pay more attention to the impact of environmental factors and their interaction with genetic factors. By repeating GWAS experiments under different environmental conditions, genetic variants that are significant in a specific environment can be identified. In addition, conducting GWAS studies on gene-environment interactions and identifying key genes that can regulate environmental adaptability is of great significance for cultivating wheat varieties that adapt to climate change (Saeideh et al., 2022).

While most GWAS studies focus on major wheat crops, such as wheat and barley, future studies will focus more on minor and wild wheat germplasm. These resources contain rich genetic diversity and are of great value for identifying new genes for stress tolerance, high yield and quality traits. Through methods such as GWAS, these potential genetic resources can be mined to provide new genetic resources for the improvement and innovative breeding of wheat crops.

The future of wheat crop GWAS research will also rely on the strengthening of data sharing and interdisciplinary collaboration. The era of big data requires researchers to share GWAS data sets, including genetic variation information, phenotypic data and environmental parameters, etc. This will promote the reuse of data and accelerate the process of genetic discovery. At the same time, close cooperation among multiple disciplines such as bioinformatics, statistics, genetics, and crop science will provide stronger support for solving the genetic basis of complex traits.

As a bridge connecting genetic variation and phenotypic traits, GWAS plays an increasingly important role in wheat crop research. By continuously deepening the understanding of genetic mechanisms, utilizing new technologies and methods, and strengthening data sharing and interdisciplinary cooperation, future GWAS research will make greater contributions to the genetic improvement and sustainable production of wheat crops.

4 Conclusion

In the past decade, significant progress has been made in the field of genetic research on stress tolerance traits in wheat crops. These research results not only enhance our understanding of the mechanisms of wheat crops coping with stress, but also provide new strategies and tools for breeding, helping to breed more robust crop varieties.

Through high-throughput sequencing technology and genome-wide association studies (GWAS), scientists have successfully identified a large number of key genes and regulatory networks related to stress responses such as drought, salinity, and low temperature. For example, transcription factor families such as DREB and WRKY play a central role in regulating the stress response of wheat crops.

Using molecular marker technology, researchers can quickly identify and exploit genetic variations associated with stress tolerance, accelerating the process of molecular breeding for stress-resistant traits. This not only improves breeding efficiency but also increases breeding accuracy. The development of genome editing technologies such as CRISPR/Cas9 has made it possible to precisely modify wheat crop genomes, making direct modification of stress tolerance trait genes a reality. The application of these technologies has greatly promoted the improvement of wheat crop traits (Sun et al., 2017).

With the development of remote sensing technology and automated phenotyping platforms, phenomic research has provided a new perspective for the study of stress responses of wheat crops. By precisely quantifying crop

growth status and stress responses, researchers can better understand the relationship between genotype and phenotype. Future research will use more systems biology methods to build a comprehensive regulatory network for wheat crop stress tolerance traits by integrating multi-omics data such as genomics, transcriptomics, and proteomics. This will help to deeply understand the stress tolerance mechanism and provide a scientific basis for precision breeding. Using the latest genome editing technology to precisely manipulate stress tolerance genes in wheat crops will be an important direction in future breeding. This includes not just the knockout or knock-in of genes, but also the precise regulation of the underlying gene regulatory mechanisms.

Future research will pay more attention to the interaction between environmental factors and genetic factors, especially how to maintain or enhance crop stress tolerance under changing climate conditions. This includes using advanced ecological models and big data analysis techniques to predict crop performance under different environmental conditions. Exploring and utilizing the genetic diversity of wild relatives of wheat crops will be an important way to enhance crop stress tolerance in the future. Wild germplasm resources contain many unique stress-tolerant genes. Introducing these genes into cultivated varieties through modern molecular breeding technology will greatly broaden the adaptability of crops.

In view of the global and challenging nature of wheat crop research, strengthening international cooperation and sharing research results, data and technology will be crucial to meeting global food security challenges. Through international cooperation projects and platforms, the progress of research on stress tolerance traits of wheat crops can be accelerated. Important progress has been made in the field of genetic research on stress tolerance traits of wheat crops in the past decade. In the future, more precise and systematic research methods will be used to further explore and utilize the stress tolerance of wheat crops to meet the challenges faced by global food production.

References

- Gahlaut V., Jaiswal V., Harindra S., Arun K.J., and Pushpendra K., 2021, Multi-Locus GWAS for grain weight-related traits under rain-fed conditions in common wheat (*Triticum aestivum* L.), *Front. Plant Sci.*, 12: 758631.
<https://doi.org/10.3389/fpls.2021.758631>
PMid:34745191 PMCID:PMC8568012
- Oyiga B.C., Sharma R.C., Baum M., Ogonnaya F.C., Leon J., and Ballvora A., 2018, Allelic variations and differential expressions detected at quantitative trait loci for salt stress tolerance in wheat, *Plant Cell Environ.*, 41: 919-935.
<https://doi.org/10.1111/pce.12898>
PMid:28044314
- Qaseem M.F., Qureshi R., and Shaheen H., 2019, Genome-wide association analyzes for yield and yield-related traits in bread wheat (*Triticum aestivum* L.) under pre-anthesis combined heat and drought stress in field conditions, *PLoS One*, 14(3): e0213407.
<https://doi.org/10.1371/journal.pone.0213407>
PMid:30883588 PMCID:PMC6422278
- Saeideh J., Mohammad RB, Mansour O., Ali RA, Hadi A. and Pär KI, 2022, Genome-Wide association study (GWAS) and genome prediction of seedling salt tolerance in bread wheat (*Triticum aestivum* L.), *BMC Plant Biology*, 22: 581.
<https://doi.org/10.1186/s12870-022-03936-8>
PMid:36513980 PMCID:PMC9746167
- Su Q.N., Zhang X.L., Zhang W., Zhang N., Song L.Q., Liu L., Xue X., Liu G.T., Liu J.J., Meng D.Y., Zhi L.Y., Ji J., Zhao X.Q., Yang C.L., Tong Y.P., Liu Z.Y., and Li J.M., 2018, QTL detection for kernel size and weight in bread wheat (*Triticum aestivum* L.) using a high-density SNP and SSR-based linkage map, *Front Plant Sci.*, 9: 1484.
<https://doi.org/10.3389/fpls.2018.01484>
PMid:30364249 PMCID:PMC6193082
- Sukumaran S., Reynolds M.P., and Sansaloni C., 2018, Genome-wide association analyzes identify QTL hotspots for yield and component traits in durum wheat grown under yield potential, drought, and heat stress environments, *Frontiers in Plant Science*, 9: 81.
<https://doi.org/10.3389/fpls.2018.00081>
PMid:29467776 PMCID:PMC5808252
- Sun C., Zhang F., Yan X., Zhang X., Dong Z., Cui D., and Chen F., 2017, Genome-wide association study for 13 agronomic traits reveals distribution of superior alleles in bread wheat from the Yellow and Huai Valley of China, *Plant Biotechnol J.*, 15: 953-969.
<https://doi.org/10.1111/pbi.12690>
PMid:28055148 PMCID:PMC5506658

- Wang X.B., Panfeng Guan P.F., Xin M.M., Yongfa Wang Y.F., Chen X.Y., Zhao A.J., Liu M.S., Li H.G., Zhang M.Y., Lu L.H., Zhang J.B., Ni Z.F., Yao Y.Y., Hu Z.R., Peng H.R., and Sun Q.X., 2020, Genome-wide association study identifies QTL for thousand grain weight in winter wheat under normal-and late-sown stressed environments, 134: 143-157.
<https://doi.org/10.1007/s00122-020-03687-w>
PMid:33030571
- Xia H., Luo Z., Xiong J., Ma X.S., Lou Q.J., Wei H.B., Qiu J., Yang H., Liu G.L., Fan L.J., Chen L., and Luo L.J., 2019, Bi-directional selection in upland rice leads to its adaptive differentiation from lowland rice in drought resistance and productivity, Mol. Plant, 12: 170-184.
<https://doi.org/10.1016/j.molp.2018.12.011>
PMid:30584948
- Xie L., RenY., Zhang X.Z., Zhang Z.H., Shi S.B., and Geng H.W., 2021, Genome-wide association study of pre-harvest sprouting traits in wheat, Acta Agronomica Sinica, 47(10): 1891-1902.
<https://doi.org/10.3724/SP.J.1006.2021.01078>
- Yu S., Wu J., Wang M., Shic W.M., Xiad G.M., Jiae J.Z., Kanga Z.S., and Hana D.J., 2020, Haplotype variations in QTL for salt tolerance in Chinese wheat accessions identified by marker-based and pedigree-based kinship analyses, The Crop Journal, 8(6): 1011-1024.
<https://doi.org/10.1016/j.cj.2020.03.007>
- Zeng Khan M.I., Kainat Z., and Maqbool S., 2022, Genome-wide association for heat tolerance at seedling stage in historical spring wheat cultivars, Frontiers in Plant Science, 13: 972481.
<https://doi.org/10.3389/fpls.2022.972481>
PMid:36092407 PMCID:PMC9453861
- Zhang P.P., Li Z.F., and Liu D.Q., 2021, Genome wide association mapping for leaf and stripe rust resistance in 268 wheat accessions using the 90K SNP Array, Theor. Appl. Genet, 10(6): e0129580.
<https://doi.org/10.1007/s00122-021-03769-3>
PMid:33492413



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