

Genome-Wide Association Study of Maize Kernel Quality Related Traits and Their Molecular Mechanisms

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Abstract In recent years, significant progress has been made in revealing the genetic basis and molecular mechanisms underlying maize kernel quality traits, thanks to the development and application of Genome-Wide Association Studies (GWAS). Kernel quality traits in maize, such as starch, protein, and oil content, not only directly affect its nutritional value and processing quality but are also crucial for enhancing food security. This study summarizes the application of GWAS in the study of maize kernel quality traits, including the discovery of key genes and loci, how these genes regulate specific quality traits, and their potential applications in maize breeding. Furthermore, the study discusses the challenges and limitations of GWAS research, as well as future directions, particularly in the application of high-throughput sequencing technologies, precise gene editing, and integration of multi-omics data analysis, aiming to further improve maize quality. By deeply understanding the genetic and molecular mechanisms of maize kernel quality traits, this study highlights the importance and prospects of molecular breeding in the improvement of crop quality.

Keywords Genome-wide association studies (GWAS); Maize; Kernel quality; Genetic basis; Molecular mechanisms; Breeding improvement

As a food crop with the largest planting area and output worldwide, corn is not only one of the main food sources for humans, but also a key raw material for animal feed, bioenergy and various industrial raw materials. Corn occupies an irreplaceable position in global food security and agricultural economy, and its production and quality are directly related to the stability of the food supply chain and human health.

The quality of corn kernels involves many aspects such as the starch, protein, and oil content of the kernels. These quality traits not only determine the nutritional value of corn, but also affect its performance in processing and industrial utilization (Yano et al., 2016). For example, corn with high starch content is suitable for deep processing as an energy plant, while corn with high protein and high oil content is more favored by the food and feed industry. Therefore, improving the quality of corn kernels can not only meet people's demand for food with high nutritional value, but is also of great significance for increasing the economic value of corn.

In recent years, genome-wide association analysis (GWAS), as a powerful genetic research tool (Uffelmann et al., 2021), has shown great potential in the field of crop genetic improvement. By analyzing the association between genetic variation and trait phenotype, GWAS can accurately locate key genes or genetic markers that affect specific traits across the entire genome. This method not only greatly accelerates the pace of crop genetic improvement, but also provides a new perspective for us to understand the genetic mechanisms of complex traits.

Although GWAS has achieved remarkable results in many fields, its application in the study of corn grain quality traits still faces many challenges, such as the complexity of traits, the influence of environmental factors, and the processing of large-scale genetic variation data. Therefore, this study aims to review the latest progress of GWAS in analyzing the molecular mechanisms of corn grain quality traits, and explore the application and potential of GWAS methods in identifying molecular markers of related traits, revealing the molecular pathways of trait formation, and guiding molecular breeding of maize (Schaid et al., 2018).

By summarizing and analyzing the research results in recent years, we hope to better understand the genetic basis of corn grain quality and provide scientific basis for future genetic improvement and variety selection. At the same time, this study will also discuss the limitations of GWAS in current technologies and methods, as well as possible breakthrough directions in future research, aiming to provide reference and inspiration for scholars in the fields of corn quality improvement and genetic research.

1 Overview of Corn Kernel Quality Traits

As one of the important food crops in the world, corn's grain quality traits are directly related to its application value in food, feed and industrial processing (Dai et al., 2007). The quality traits of corn kernels mainly include starch content, protein content, oil content, etc. The level of these traits determines the nutritional value and processing characteristics of corn. As people's demands for health and nutrition continue to increase, attention to corn quality traits is also increasing.

1.1 Classification of quality traits

Starch, protein, and oil are the three main components that make up corn kernels, and each has a significant impact on the energy value and processing potential of corn. Starch is the main component of corn kernels, and its content directly determines the energy value of corn. The level of starch content plays a crucial role in the processing of corn such as alcohol fermentation and saccharification. Generally speaking, the higher the starch content of corn, the greater its value in processing and utilization (Liu, 2004, *Cereal Crops*, 24(5): 258-260.).

Protein is another key factor affecting the nutritional value of corn. It not only determines the quality of corn as food and feed, but also affects the quality of corn processed products. Corn with higher protein content is more suitable for the production of high-protein feed and food to meet the market demand for products with high nutritional value.

The oil content mainly affects the energy value of corn and the extraction efficiency and quality of corn oil. The oil in corn kernels is mainly concentrated in the endosperm. The oil content is not only related to the energy value, but also directly affects the preference of different corn varieties in the edible oil processing industry. Typically, corn varieties with higher oil content are more popular in the edible oil processing industry because they can provide higher oil extraction rates and better oil quality.

1.2 Impact of quality traits on corn processing and consumption

The quality traits of corn kernels play a vital role in its processing and consumption. Different quality traits make corn have specific suitability in multiple fields (Zheng et al., 2021): corn with high starch content is most suitable for the production of alcohol and starch; while corn with high protein content is more suitable for the production of high protein Food and feed; at the same time, corn with high oil content is mainly used to extract corn oil.

In the processing industry, the quality traits of corn are key factors that determine product yield and quality. For example, starch content not only directly affects alcohol production and starch extraction rate, but oil content also affects the output and quality of corn oil. Therefore, according to the different needs of processed products, the processing industry has clear requirements for the quality traits of corn.

In the consumer market, the quality traits of corn are also an important criterion for consumer selection (Zeng et al., 2022). Because corn with high protein and oil content has higher nutritional value, this type of corn is favored by health-conscious consumers. In addition, corn varieties with specific quality traits. For example, sweet corn and waxy corn are very popular in the consumer market because of their unique taste and flavor. Sweet corn contains high sugar content and is suitable for eating directly or processing into canned products, frozen products, etc. Because of its unique sticky texture, waxy corn is often used to make traditional delicacies such as glutinous rice cakes and rice dumplings. These special quality corn varieties meet consumer demand for food variety and special taste. These situations collectively reflect that the quality traits of corn kernels not only affect its application in the processing field, but also profoundly affect consumer choices and consumption behaviors.

2 Introduction to Genome-wide Association Analysis (GWAS)

Genome-wide association analysis (GWAS) is a method widely used in genetic research to identify genetic markers that influence a specific trait by analyzing the association between genetic variation in the genome and the trait. The emergence of GWAS marks a new era in genetic research, especially in the field of plant genetics, which provides a powerful tool for analyzing the genetic basis of complex traits.

2.1 Principles of GWAS and its application in plant genetics

The basic principle of GWAS is to use genomic data of a large number of individuals (Uffelmann et al., 2021) to find those single nucleotide polymorphisms (SNPs) that are significantly associated with phenotypic variation of specific traits through statistical analysis. These SNPs may be located in the coding region of the gene or in the regulatory region, affecting gene expression. In plant genetics research, GWAS is widely used to analyze important agronomic traits of crops, such as yield, disease resistance, quality, etc. Compared with traditional genetic analysis methods, GWAS can directly identify genetic variations related to traits across the entire genome without knowing the gene location in advance, greatly improving the efficiency and accuracy of genetic research (Cortes et al., 2021).

Through GWAS, researchers can identify key genetic factors affecting specific traits in natural populations with rich genetic diversity (Figure 1), which is of great significance for the genetic improvement of crops and the cultivation of new varieties. For example, through GWAS analysis, scientists have successfully identified multiple key genes that affect corn grain quality, wheat disease resistance, and rice yield. These findings not only enrich our understanding of the genetic mechanisms of these traits, but also provide information for crop breeding. new target.

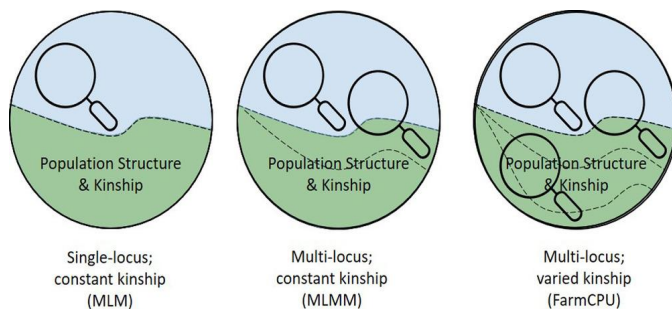


Figure 1 Different genome-wide association study methods ask different questions

2.2 Comparison of GWAS and traditional genetic marker association studies

Although GWAS has shown its advantages over traditional genetic marker association studies in many aspects (Brachi et al., 2011), there are also certain complementarities between the two in practical applications. Traditional genetic marker association studies, such as linkage analysis, usually look for markers related to traits by analyzing genetic data of specific groups (such as families or inbred lines) in a limited genetic background. This method relies on genetic linkage and is therefore somewhat limited in positioning accuracy, especially in the study of complex traits.

In contrast, because GWAS is conducted in a wide range of natural populations, it can take advantage of the large number of genetic recombination events accumulated in natural populations to accurately locate genetic variations associated with traits across the entire genome. In addition, GWAS can also reveal the interaction of multiple genes in the formation of traits, making it possible to understand the genetic complexity of traits. However, GWAS also has its limitations, such as the high requirements on population structure and degree of association, and the so-called "missing heritability" problem, that is, GWAS sometimes cannot explain all genetic variation.

3 Application of GWAS in the Study of Corn Grain Quality Traits

Genome-wide association analysis (GWAS), as a powerful genetic research tool, has been widely used in the study of corn grain quality traits. It provides an efficient method to reveal the genetic basis that affects corn quality traits by analyzing the association between genetic variation and trait phenotypes.

3.1 Overview of GWAS research methods

The basic process of GWAS includes sample collection, genotype sequencing, and application of statistical analysis methods (Wen et al., 2014). First, researchers collect genetically diverse populations of corn, which may come from different geographic locations or have different genetic backgrounds. Next, these samples are analyzed for genome-wide genetic variation through high-throughput genotype sequencing technology, usually focusing on single nucleotide polymorphisms (SNPs). Finally, sophisticated statistical analysis methods, such as linear mixed models, were used to identify genetic markers significantly associated with corn kernel quality traits.

3.2 Review of recent GWAS research results on corn grain quality traits

In the past few years, GWAS has made a series of important discoveries in corn kernel quality traits. Researchers have successfully identified multiple genes or genetic loci that are significantly related to quality traits such as starch content, protein content, and oil content. These findings not only increase our understanding of the genetic control mechanisms of corn quality traits, but also provide valuable molecular markers for future corn variety improvement (Guo et al., 2019).

The functions of genes or loci discovered through GWAS research include enzyme genes that affect the starch synthesis pathway, factors involved in protein synthesis and regulation, and key genes that regulate oil metabolism. For example, variations in certain enzyme genes directly affect the biosynthetic pathway of starch, thereby changing the starch content of grains; while variations in certain transcription factors or regulatory genes may affect key links in the protein synthesis pathway, thereby regulating grain Protein content (Guo et al., 2023). How these specific genetic variations finely regulate the quality traits of corn kernels requires further functional verification and mechanism research.

3.3 Limitations and challenges of GWAS studies

Although GWAS has made remarkable achievements in revealing the genetic basis of corn grain quality traits, this method also has some limitations and challenges. GWAS requires large-scale sample data to ensure the accuracy of statistical analysis, which places higher requirements on sample collection and genotype sequencing. Due to the complexity of environmental factors and genetic background, the association between genetic markers and traits discovered by GWAS may have a certain degree of volatility and needs to be verified under different genetic backgrounds and environmental conditions. In addition, although GWAS can identify genetic loci associated with traits, further biological verification and mechanism research are needed to deeply understand the specific functions of these loci and their role in trait formation.

As an efficient genetic research method, GWAS has made significant progress in the study of corn grain quality traits. Through future research, we are expected to gain a deeper understanding of the genetic mechanisms underlying the formation of corn kernel quality and use this knowledge to promote the improvement and optimization of corn varieties.

4 Molecular Mechanisms of Corn Kernel Quality Traits

As one of the important food crops in the world, corn's grain quality traits directly affect the nutritional value and processing characteristics of the grain. In recent years, with the advancement of molecular biology technology, scientists have made remarkable achievements in the study of the molecular mechanisms of corn grain quality traits, especially the discovery of key genes, the analysis of gene expression regulatory mechanisms, and the application of these findings in breeding (Table 1).

Table 1 Correlation coefficients between maize quality shape and main agronomic traits

Project	Yield per plant	100 grain weight	Spike length	Ear diameter	Ear row number	Kernels per row	Height	Reproductive period
Crude protein	-0.635*	-0.789**	0.063	0.167	-0.972**	0.777*	0.09	0.766**
Crude fat	-0.636*	0.697*	-0.184	0.160	0.160	-0.906**	0.782**	0.809**
Total starch	0.555	0.135	0.799**	0.228	0.581*	0.618*	0.100	0.229

Note: *: Significant level; **: 0.01 Significant level

4.1 Key genes and regulatory networks revealed

The formation of corn kernel quality traits, such as starch content, protein content and oil content, is determined by a complex gene regulatory network (Liu et al., 2016). Through genome-wide association analysis (GWAS) and functional genomics research, scientists have identified multiple key genes that affect corn grain quality traits. For example, genes involved in starch biosynthesis, AGPase, GBSS, etc., play a vital role in the starch synthesis pathway. In terms of protein content regulation, the discovery of the Opaque-2 (O2) gene marks an in-depth understanding of the regulatory mechanism of corn grain protein synthesis. In terms of regulating oil content, genes such as DGAT and FAD2 are involved in the biosynthesis and metabolism of oil.

These key genes and their interactions form a complex regulatory network that finely regulates the quality of corn kernels. By in-depth studying the composition and function of this regulatory network, scientists can better understand the formation mechanism of corn grain quality traits.

4.2 Gene expression regulation and its impact on quality formation

The regulation of gene expression is a key link that affects the formation of corn grain quality traits (Xiong and Huang, 2022). Factors such as gene transcription level, mRNA stability and translation efficiency can affect the final protein expression, thereby affecting the quality traits of the grain. For example, the *Opaque-2* (O2) gene, as a transcription factor, affects the protein content and composition of corn kernels by regulating the expression of a series of downstream genes.

In addition, the influence of environmental factors on gene expression cannot be ignored. Environmental factors such as temperature, light, and soil nutritional status can indirectly affect the quality traits of corn grains by affecting gene expression patterns. Therefore, the formation of corn kernel quality traits is a complex process influenced by genetic factors and environmental factors.

4.3 Examples of applications of molecular mechanisms in breeding

Based on the understanding of the molecular mechanisms of corn grain quality traits (Li et al., 2017), scientists have applied this knowledge in breeding practice and developed multiple new corn varieties with excellent quality. For example, using molecular marker-assisted selection (MAS) technology to select for specific key genes for quality traits can significantly improve the efficiency and accuracy of breeding.

In addition, through gene editing technologies such as CRISPR/Cas9, scientists can precisely change key genes that affect quality traits, such as increasing the protein content of grains by editing the *Opaque-2* (O2) gene. These breeding technologies based on molecular mechanisms not only speed up the breeding process of excellent varieties, but also provide a powerful tool for the continuous improvement of corn quality (Wang et al., 2023).

Research on the molecular mechanisms of corn grain quality traits provides an important scientific basis for our in-depth understanding of the genetic basis of quality formation. It also provides effective strategies and methods for genetic improvement and variety optimization of corn (Figure 2). With the continuous development of molecular biology technology, the field of corn quality improvement will show greater potential and prospects in the future.

5 Future Research Directions and Prospects

With the rapid development of molecular biology and genetics technology, the research on corn quality traits is in a period of unprecedented development opportunities. Future research directions will not only continue to deepen the understanding of the molecular mechanisms of corn quality traits, but also explore new technologies and methods in order to achieve greater progress in improving corn quality.

5.1 Further improvements in GWAS technology and methods

Although genome-wide association analysis (GWAS) has become an important tool for revealing the genetic basis of complex traits in crops such as maize, its accuracy and efficiency still need to be improved. Future research needs to focus on further improving GWAS technology and methods, such as improving the analytical capabilities

of GWAS by developing more efficient statistical analysis models, improving the accuracy and reducing costs of genotype sequencing technology, and optimizing sample selection and design. In addition, using artificial intelligence and machine learning technology to process and analyze large-scale genetic data is also an important direction for future GWAS research.

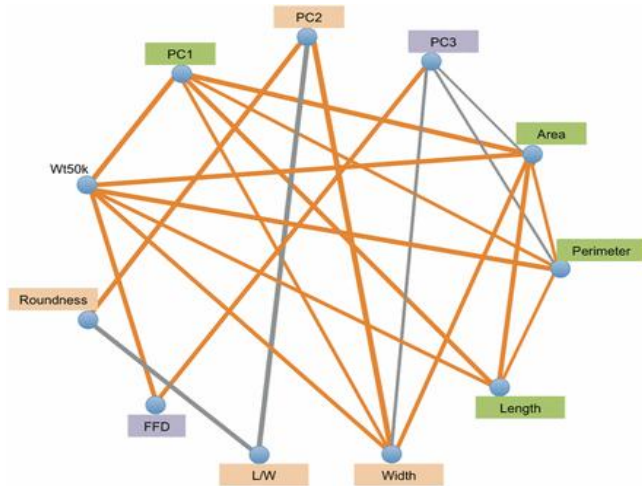


Figure 2 Pleiotropy for kernel traits in maize-teosinte NILs populations

Note: NIL: Infiltration system; PC: Principal component; QTL: Quantitative trait loci; Wt50k: Weighing 50 pills

5.2 The application prospects of multi-omics data integration analysis in revealing the molecular mechanisms of complex traits

With the development of multi-omics technologies such as transcriptomics, proteomics, and metabolomics, integrated analysis of these different levels of biological information has become an important means to reveal the genetic mechanisms of complex traits. In the study of corn quality traits, by integrating multi-omics data such as GWAS, transcriptome, and metabolome, we can more comprehensively understand the formation mechanism of quality traits, which is especially valuable in analyzing the complex regulatory network between genes and traits. . In the future, establishing a more effective multi-omics data integration and analysis platform will help discover new genetic resources and regulatory pathways, and provide more accurate molecular markers for improving corn quality.

5.3 The potential of precision breeding in improving corn quality

Precision breeding technologies, especially gene editing technologies such as CRISPR/Cas9, provide new strategies for improving corn quality traits (Hao et al., 2018). Compared with traditional breeding methods, gene editing technology can accurately modify target genes without introducing exogenous DNA, thereby quickly obtaining the desired quality traits. In the future, through more in-depth research on gene functions and optimization of editing technology, the efficiency and safety of precision breeding will be further improved. At the same time, combined with the key genes and regulatory networks discovered by GWAS and multi-omics data analysis, precision breeding is expected to play a greater role in improving corn quality and provide strong support to meet human needs for high-quality corn.

5.4 The importance of molecular mechanisms for future corn improvement

Although GWAS has made a lot of progress in the study of corn quality traits, in order to achieve fundamental improvements in corn quality, in-depth research on the molecular mechanisms of the formation of these traits is still needed. Future research needs to focus on revealing the functions of key genes and their mechanisms in the formation of corn quality, which is of vital significance for accurately regulating corn quality traits and cultivating new varieties that are more suitable for market demand. In addition, with the development of precision breeding technologies such as gene editing, an in-depth understanding of molecular mechanisms will allow us to more precisely manipulate these key genes to achieve targeted improvements in corn quality.

The application of GWAS in the study of corn grain quality traits has made important progress, providing valuable genetic information and molecular tools for corn quality improvement. However, to achieve sustained and fundamental improvements in corn quality, we still need to continue to study the molecular mechanisms of quality traits in depth, and at the same time, combine modern biotechnology to continuously explore and develop new breeding strategies. In the future, with the continuous progress of science and technology, I believe we can overcome the current challenges and make greater contributions to global food security and sustainable development.

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Conflict of Interest Disclosure

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

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