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GWAS Revealed the Key Genetic Factors Affecting Cotton Fiber Quality

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Abstract Cotton fiber quality is an important factor in determining the economic value of cotton, mainly including fiber length, strength, fineness, maturity and other indicators. Genome-wide association study (GWAS) revealed several key genetic factors affecting cotton fiber quality. It provides an important basis for the application of molecular marker-based assisted breeding and gene editing technology. This study mainly discusses the application of GWAS in revealing the key genetic factors affecting cotton fiber quality, and summarizes the basic principles and methods of GWAS in the study of cotton fiber quality by analyzing the genetic regulation mechanism of cotton fiber development and the history of variety improvement. This study explores the future direction of cotton fiber quality improvement, and emphasizes the importance of in-depth study of genetic factor function and application of new technologies.

Keywords Cotton fiber quality; GWAS; Genetic factor; Molecular marker-assisted breeding; Gene editing

As one of the most important textile raw materials in the world, cotton fiber quality directly affects the quality and market value of cotton textiles. The main indexes of cotton fiber quality include length, strength, fineness, maturity and whiteness. These quality characteristics not only determine the processing performance of cotton and the appearance and feel of finished products, but also affect the economic value and market competitiveness of cotton (Mathangadeera et al., 2020), so improving cotton fiber quality has always been one of the important goals of cotton breeding.

Genetic factors play a decisive role in the formation of cotton fiber quality. The formation of fiber quality is a complex biological process involving the expression and regulation of multiple genes. With the development of molecular biology and genomics technology, people have a deeper understanding of the genetic regulation mechanism of cotton fiber development. Through identification and research of key genetic factors affecting fiber quality, molecular markers can be provided for cotton breeding, so as to achieve more accurate and efficient quality improvement (Ahmed et al., 2018).

Genome-wide association study (GWAS) are a powerful tool for identifying candidate genes or genetic variants in genetic studies of complex traits. It reveals the genetic factors that influence specific traits by analyzing the association between genetic markers and phenotypic traits (Rahimi et al., 2019). In the study of agricultural genetics, GWAS has been widely used in the genetic analysis of crop yield, disease resistance, quality and other traits. For cotton, GWAS has become an important means to reveal the genetic basis of fiber quality. Through GWAS, researchers can identify genetic variants and candidate genes related to fiber quality in the whole genome, which provides important information for revealing the molecular mechanism of fiber quality formation and guiding molecular breeding of cotton.

The improvement of cotton fiber quality is an important goal of cotton breeding, and genetic factors play a key role in the formation of fiber quality. As an effective genetic analysis tool, GWAS has shown great potential in the study of cotton fiber quality. This study will focus on the application of GWAS to reveal the key genetic factors affecting cotton fiber quality and its potential value in cotton breeding.

1 Genetic Basis of Cotton Fiber Quality

1.1 Main indexes of cotton fiber quality

The main indexes of cotton fiber quality include fiber length, fiber strength, fiber fineness, maturity, whiteness, yellowing, and impurity content (Ul-Allah et al., 2021). Fiber length refers to the average length of cotton fiber, usually expressed in inches or millimeters, fiber length is an important indicator affecting textile quality and spinning performance, long fiber usually means higher spinning efficiency and better finished product quality. Fiber strength refers to the ability of cotton fibers to withstand tension without breaking, usually expressed in grams/Denier (g/tex) or grams/foot (g/ft), fiber strength is very important in determining the durability and strength of textiles. Fiber fineness, also known as fiber diameter or linear density and usually expressed in micrograms per inch (μg/in) or denier, affects the ability of fibers to interweave during spinning and weaving, and fine fibers generally produce softer, more delicate fabrics.

Maturity refers to the degree of development of cotton fiber, reflecting the wall thickness and cavity size of the fiber, and insufficient maturity of the fiber will affect the strength and appearance of the textile. Whiteness refers to the color or brightness of the cotton fiber, and high whiteness cotton is easier to dye, producing more vivid colors. The degree of yellowing reflects the color change of cotton fiber, which is usually related to the storage conditions and processing process of the fiber. Impurity content refers to the content of non-fiber impurities in cotton flowers, including leaves, seed debris and dust, etc. The impurity content directly affects the cleanliness of cotton and the efficiency of processing (Ul-Allah et al., 2021). These indexes comprehensively determine the quality of cotton fiber, and then affect the performance and market value of cotton textiles. In the process of cotton breeding and production, it is an important goal to improve the quality of cotton fiber by improving the performance of these indexes.

1.2 Genetic regulation mechanism of cotton fiber development

The genetic regulation mechanism of cotton fiber development is a complex biological process involving multiple genes and signal transduction pathways. Fiber development can be divided into four main stages: initiation, elongation, secondary wall thickening and maturation. In the fiber initiation stage, a series of transcription factors such as MYB25, MyB25-like and MYB109 play an important role in the differentiation of cotton epidermal cells (Jan et al., 2022). These transcription factors promote the transformation of epidermal cells into fibrocytes by regulating the expression of downstream genes. Plant hormones such as gibberellin (GA) and auxin (IAA) are also involved in the initiation process of fiber, affecting the differentiation and proliferation of fiber cells.

In fiber elongation stage, the cell walls of relaxation related genes such as Expansin (EXP), Xyloglucan endotransglucosylase/hydrolase (XTH) and Cellulose synthase (CesA) are a large number of expressions. These genes are involved in cell wall remodeling, promoting rapid elongation of fibers, and hormones such as gibberellin and auxin continue to play a role in this stage, regulating the rate and duration of elongation of fiber cells. In the secondary wall thickening stage, the growth rate of fiber cells slows down, and the cell wall begins to accumulate a large amount of cellulose, lignin and pectin (Jan et al., 2022). Some transcription factors such as MYB and NAC family members regulate the expression of cellulose synthesis genes at this stage, affecting the thickness and strength of the cell wall. In the fiber maturation stage, the fiber cells stop growing and a series of biochemical changes occur inside the cell, including dehydration and further changes in the cell wall composition, allowing the fiber to acquire its final physical and chemical properties.

1.3 History and present situation of cotton variety improvement

Cotton variety improvement has a long history, which has experienced the evolution from traditional breeding to modern molecular breeding. In the early stages, cotton breeding mainly relied on the experience and selection of farmers, and gradually improved varieties by selectively conserving and planting cotton seeds with good traits. With advances in genetics and breeding science, the beginning of the 20th century marked the arrival of the era of scientific breeding, and breeders began to use techniques such as hybridization and selection to create many high-yielding, high-quality cotton varieties (Shahzad et al., 2022) (Figure 1). The development of hybrid cotton technology brought revolutionary changes to cotton breeding in the middle of the 20th century, and hybrid cotton varieties have higher yields and better disease resistance, greatly improving the economic benefits of cotton.

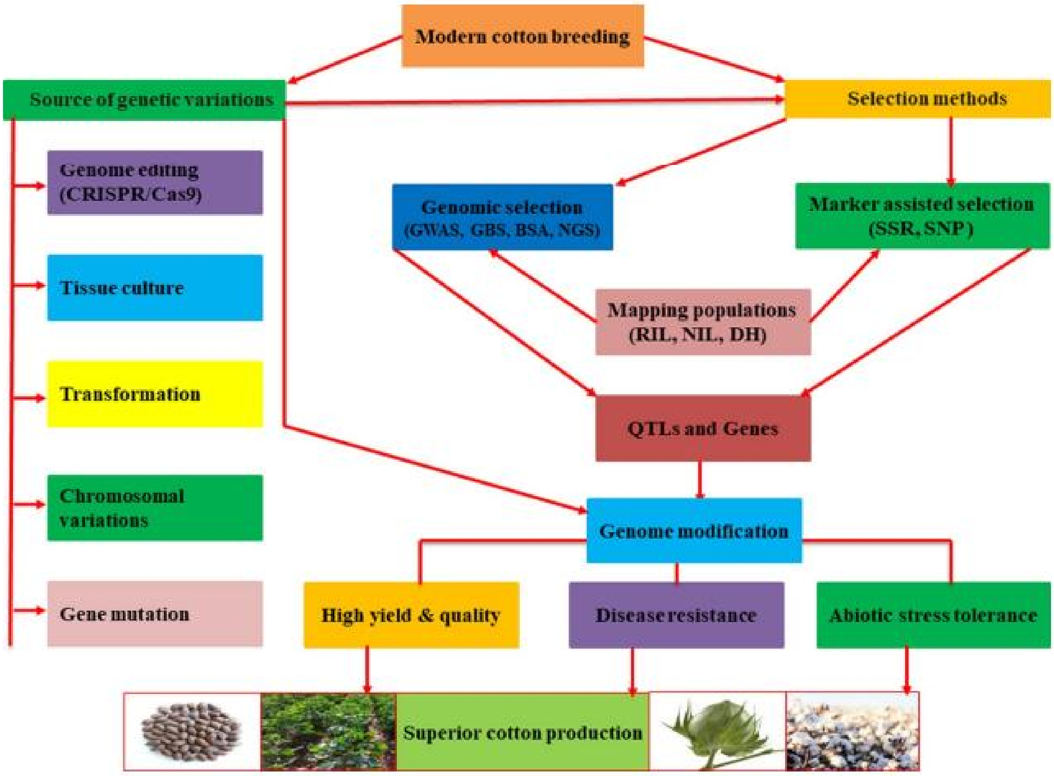

Figure 1 The overall scheme of modern breeding measures to produce superior cotton (Shahzad et al., 2022)

In modern times, advances in molecular biology technology have brought new tools to cotton breeding. Molecular marker-assisted breeding (MAS) uses molecular markers to locate and select genes related to target traits, which improves breeding efficiency and accuracy. The application of transgenic technology allows breeders to directly insert or modify specific genes into the cotton genome to create transgenic cotton varieties with characteristics such as resistance to insects and glyphosate (Zeng et al., 2022). In recent years, the emergence of gene editing technologies such as CRISPR/Cas9 has provided new possibilities for cotton breeding, and targeted improvement of specific traits can be achieved by precisely editing the cotton genome.

2 Application of GWAS in the Study of Cotton Fiber Quality

2.1 Basic principles and methods of GWAS

Genome-wide association study (GWAS) is a powerful method for exploring the relationship between genetic variation and complex traits. The rationale for GWAS is based on population genetics, focusing on genome-wide genetic variation loci such as single nucleotide polymorphisms (SNPs) that may be directly related to variation in traits or linked to genes that affect traits (Uffelmann et al., 2021). When conducting GWAS, it is necessary to consider the effect of population structure (i.e. genetic correlations that exist between different individuals) and linkage imbalances (i.e. non-random associations between genetic variants at certain loci) on the results.

The methodological steps of GWAS include sample collection and genotyping, statistical analysis, calibration of results, and analysis of candidate genes and biological pathways. First, samples of individuals with different phenotypes were collected and genome-wide genotyping was performed on these samples. Subsequently, statistical methods were used to analyze the degree of association between each genetic marker and trait, usually by calculating P-value or other statistical indicators to determine which genetic markers were significantly correlated with trait (Uffelmann et al., 2021). Due to a large number of statistical tests, multiple test correction of the results was required to control the false positive rate. Based on the results of GWAS, candidate genes and biological pathways related to traits can be identified, and the roles and mechanisms of these genes in trait formation can be further studied (Figure 2).

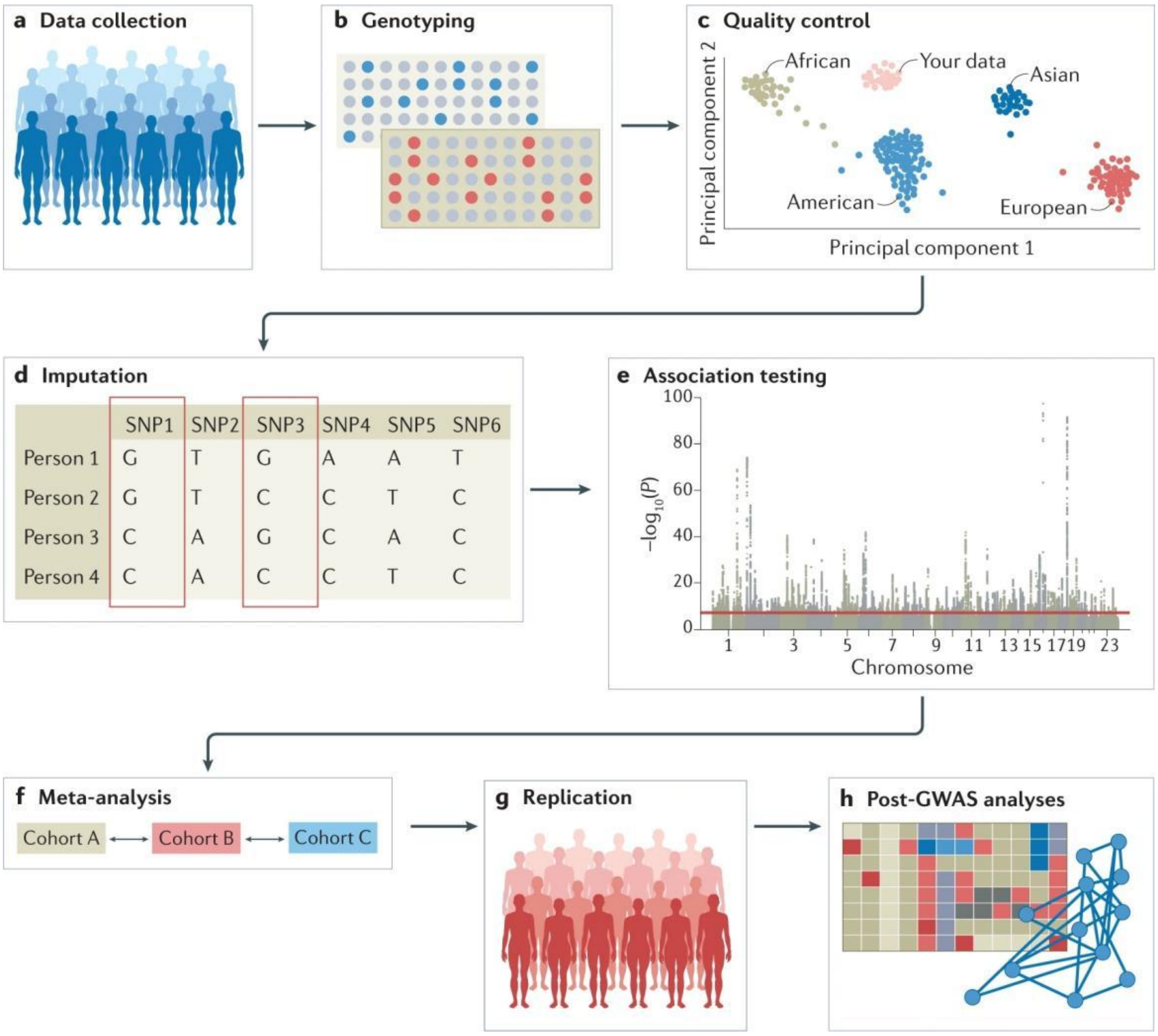

Figure 2 Overview of steps for conducting GWAS (Uffelmann et al., 2021)

2.2 Case study of GWAS related to cotton fiber quality

A GWAS study of 355 *Gossypium hirsutum* L. varieties analyzed five fiber quality traits: fiber length (FL), fiber evenness (FU), fiber fineness (FM), fiber elongation (FE) and fiber strength (FS). The study found significant associations between these traits and identified several genomic regions associated with FL and FS using mixed linear models (MLM). In particular, three genomic regions on chromosome D_t7 and one region on chromosome A_t9 were associated with trait markers for FL, explaining a significant percentage of phenotypic variation in FL. Five associated regions of FS were also identified, distributed on chromosomes A_t4, A_t5, D_t1, D_t4, and D_t7 (Su et al., 2016).

Another study focused on cotton multi-parent high generation hybridization (MAGIC) populations, highlighting genetic factors that control fiber quality traits. Two model methods based on unit point GWAS (sGWAS) and Haplotype GWAS (hGWAS) were used to analyze the genetic basis of fiber quality traits such as fiber elongation (FE), length (FL), strength (FS), evenness (UI) and fineness value (MIC). MAGIC populations show successful random mating with no significant structure and moderate linkage disequilibrium (LD), making them suitable for GWAS studies (Wang et al., 2022).

2.3 Validation and functional analysis of GWAS results

In genome-wide association study (GWAS), once genetic markers associated with specific traits are identified, validation and functional analysis are required to ensure the accuracy and biological significance of the results. Results verification methods include revalidation of the found association sites using a different sample population from the GWAS analysis to confirm that the association of these sites is real and not due to chance factors (Yu et al., 2019). The GWAS experiment was repeated under different environmental conditions, in different years, or using different experimental designs to test the stability of the association results. For some important associated loci, the biological functions of these loci can be further confirmed by gene cloning and functional verification experiments. For example, the effects of specific gene variants on traits can be tested by gene knockout or overexpression experiments.

Functional analysis mainly includes bioinformatics analysis, gene expression analysis and protein interaction analysis. Bioinformatics analysis is the annotation and functional prediction of genes near associated sites using bioinformatics tools to reveal the role of these genes in biological processes. Gene expression analysis is to analyze the expression patterns of genes near the associated sites through real-time quantitative PCR, transcriptome sequencing and other technologies (Yu et al., 2019) to determine whether these genes are expressed in related biological processes. Protein interaction analysis is the study of the interactions of proteins encoded by genes near association sites with other proteins to explore their role in cell signaling or metabolic pathways. Through these methods, the reliability of GWAS results can be improved, and the molecular mechanism behind the associated loci can be revealed, providing valuable information for breeding and gene function research.

3 Key Genetic Factors Revealed by GWAS

3.1 Genetic factors affecting cotton fiber length and strength

Cotton fiber length and strength are important indexes of fiber quality, which are affected by many genetic factors. The genetic factors related to fiber length include GhFL1, GhXTH1 and GhMYB25. GhFL1 is a gene related to fiber length, which is involved in regulating the elongation process of fiber cells. GhXTH1 encodes xylan endoglucosidase/hydrolase (Naoumkina et al., 2019), which is involved in the remodeling of fiber cell wall and affects the elongation of fiber. GhMYB25 transcription factor is involved in the regulation of initiation and elongation of fibers.

The genetic factors related to fiber strength mainly include GhCESA, GhPEPC and GhEXP. GhCESA family encodes cellulose synthase, which affects cellulose biosynthesis and thus fiber strength (Wang et al., 2009). GhPEPC gene is involved in carbon metabolism of fiber secondary wall, while GHEXP-encoded dilator protein is involved in the relaxation and elongation of fiber cell wall. These genetic factors affect the length and strength of cotton fiber by influencing the growth and development of fiber cells and the synthesis and reconstruction of cell wall. Understanding the mechanism of these genetic factors is of great significance for guiding the improvement of cotton varieties and improving fiber quality.

3.2 Genetic factors affecting fiber fineness and maturity of cotton

Cotton fiber fineness and maturity are important quality indexes, which are affected by many genetic factors. The genetic factors related to fiber fineness included Sucrose synthase gene (GhSU) and 3-ketoyl-CoA synthase gene (GhKCS). GhSU is involved in carbohydrate metabolism in fiber cell walls, while GhKCS encodes 3-ketoyl-CoA synthetase involved in fatty acid biosynthesis, all of which affect fiber fineness (Farooq et al., 2015).

The genetic factors related to fiber maturity include ethylene synthetase gene (GhACO1) and cellulose alcohol addition enzyme gene (GhPAG). Ethylene is one of the plant hormones that affect the maturity of fiber. GhACO1 is involved in the biosynthesis of ethylene, while GhPAG is involved in the biosynthesis of cellulose. These factors affect the maturity of fiber (Farooq et al., 2015). Understanding the mechanism of these genetic factors is of great significance for guiding the improvement of cotton varieties and improving fiber quality.

3.3 Other genetic factors affecting cotton fiber quality

In addition to genetic factors affecting cotton fiber length, strength, fineness and maturity, there are other genetic factors that affect cotton fiber quality (Farooq et al., 2015). For example, the GhHB gene family contains several homologous proteins, which are involved in the growth and development of cotton fibers. WRKY transcription factor family (GhWRKY) plays an important role in plant growth and development and response to stress, some of which are related to cotton fiber quality. Growth hormone response factor (GhARF) plays a key role in regulating the development of cotton fiber and affects the quality characteristics of cotton fiber.

GhFATM is involved in fatty acid metabolism in the secondary wall of cotton fiber and affected the fiber quality. Auxin up-regulated small molecule RNA (GhSAUR) regulates the elongation of cotton fiber and affects the fiber quality. These genetic factors jointly determine the quality characteristics of cotton fiber by participating in the growth and development of fiber cells, metabolic pathways and hormone signaling, etc. Understanding the mechanism of these genetic factors is of great significance for further understanding the formation mechanism of cotton fiber quality and guiding cotton breeding.

4 Strategies for Improving Cotton Fiber Quality

4.1 Molecular marker-assisted breeding based on GWAS results

The improvement of cotton fiber quality is one of the important goals of cotton breeding, aiming at improving the quality indexes of fiber such as length, strength, fineness and maturity. In recent years, with the development of molecular biology techniques, marker-assisted breeding (MAS) based on genome-wide association study (GWAS) has become one of the important strategies for cotton fiber quality improvement.

GWAS was able to identify genome-wide genetic markers associated with cotton fiber quality, providing precise molecular markers for MAS. Through MAS, breeders can accurately select individuals carrying favorable alleles at an early stage of breeding, thereby speeding up the breeding process and improving the efficiency and accuracy of breeding (Kushanov et al., 2021). For example, genetic markers associated with fiber length found through GWAS can be used to screen cotton varieties with longer fibers.

MAS can also be combined with traditional breeding methods to use molecular markers for early screening of hybrid offspring, retaining only those individuals with desirable fiber quality characteristics for subsequent breeding and selection. This method, which combines traditional and molecular breeding, can not only improve the efficiency of breeding, but also increase the accuracy of breeding, so as to improve cotton fiber quality more effectively (Kushanov et al., 2021).

Molecular marker-assisted breeding based on GWAS results is one of the important strategies for improving cotton fiber quality. With the continuous progress of molecular biology technology and the accumulation of genetic information, MAS will be applied more and more widely in cotton breeding, providing a powerful tool for improving cotton fiber quality.

4.2 Application of gene editing technology in cotton fiber quality improvement

The improvement of cotton fiber quality is an important goal of cotton breeding, aiming at improving the quality indexes of fiber such as length, strength, fineness and maturity. In recent years, gene editing technology, especially the CRISPR/Cas9 system, has become an important tool for cotton fiber quality improvement. Gene editing techniques can precisely introduce specific mutations into the cotton genome that alter the expression or function of the relevant genes, thereby affecting fiber quality (Zulqurnain et al., 2023). For example, researchers can use gene editing technology to knock out or knock down negative genes that affect fiber quality, or enhance the expression of positive genes to improve fiber quality.

Gene editing technology can also be used to create new cotton varieties with specific fiber quality traits. By precisely editing key genes in the cotton genome, targeted improvement of fiber length, strength and other traits can be achieved, thus breeding new cotton varieties with excellent fiber quality (Ahmar et al., 2020). The application of gene editing technology in cotton fiber quality improvement provides a new strategy and method for cotton breeding. With the continuous development and improvement of gene editing technology, it is expected to play a more important role in cotton fiber quality improvement in the future.

5 Outlook

In the future, genome-wide association study (GWAS) will be more widely and deeply applied to reveal the key genetic factors affecting cotton fiber quality. As high-throughput sequencing technology advances and costs decrease, larger samples and higher density of genetic markers will be used for GWAS, improving the accuracy and reliability of studies. Integrated analysis combining multiple omics data, such as transcriptomic, proteomic and metabolomic data, will also become a trend, contributing to a more comprehensive understanding of how genetic factors affect the formation mechanism of cotton fiber quality.

Molecular marker-assisted breeding (MAS) based on GWAS results will be more widely used in cotton fiber quality improvement. By accurately locating genetic markers related to fiber quality, breeders can more effectively select individuals with superior fiber quality traits, thus accelerating the improvement process of cotton varieties. The development of gene editing technologies, such as CRISPR/Cas9, will also provide new tools for precision breeding based on GWAS results, enabling targeted modifications of specific genetic factors to improve cotton fiber quality more precisely.

With the development of bioinformatics and computational biology technologies, big data analytics and machine learning methods will play a more important role in GWAS, which help to process and analyze large-scale genetic data and identify complex genetic networks and regulatory pathways related to cotton fiber quality. By constructing more accurate genetic and phenotypic data models, the analytical ability of GWAS can be improved, and more genetic factors affecting cotton fiber quality can be revealed.

With the advancement of technology and the innovation of research methods, GWAS will have greater breakthroughs in revealing the key genetic factors affecting the quality of cotton fiber. These results not only help to understand the genetic basis of cotton fiber quality, but also provide important guidance for cotton variety improvement and fiber quality improvement. Precision breeding and gene editing technology based on GWAS results will play a more important role in cotton fiber quality improvement.

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