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Research Progress on Traits Improvement of Cotton Plants Based on Comparative Genomics

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Abstract Cotton is a globally significant crop, valued for its fiber and oil, and improving its traits remains a priority for sustainable agriculture. This study examines recent advancements in cotton trait improvement through comparative genomics, emphasizing the utility of genome sequencing, synteny and orthology analyses, and gene expression profiling across cotton species. We discuss how comparative genomics has facilitated the identification of key genes and regulatory elements linked to fiber quality, stress tolerance, and yield-related traits. This study also explores evolutionary insights such as polyploidy, subgenome divergence, and domestication signatures, and presents a case study comparing *Gossypium hirsutum* and *Gossypium barbadense*, revealing critical genomic features that guide breeding programs. Despite notable progress, challenges such as incomplete genomic resources and integration with other omics persist. We conclude that comparative genomics offers powerful tools for trait dissection and cultivar development, and future efforts should focus on multi-omics integration and advanced bioinformatic platforms to fully harness its potential for sustainable cotton improvement.

Keywords Comparative Genomics; Cotton improvement; Trait dissection; Fiber quality; Polyploidy

1 Introduction

Cotton (*Gossypium* spp.) is a very important crop planted worldwide. It is the main source of natural fiber for the textile industry and contributes greatly to the economies of many countries (Fang et al., 2017; Zhang et al., 2019). Due to its large planting area, cotton fiber quality, yield and stress resistance have always been the focus of attention. Improving these traits is critical to meet market demand and achieve sustainable agricultural development (Ma et al., 2021; Joshi et al., 2023).

However, the cotton genome is complex, with both polyploidy and various structural changes, which poses considerable challenges to genetic improvement, but also provides some opportunities (Wang et al., 2022; Manivannan and Amal, 2023). Comparative genomics is a useful method to study the genetic basis of fiber quality, yield and stress resistance in cotton (Li et al., 2022b). Now, with great progress in high-throughput sequencing and genome assembly technologies, it is easier to find quantitative trait loci (QTLs), structural variations, and candidate genes associated with traits (He et al., 2021).

The breeding and evolution of cotton, on the surface, seems to be a step-by-step process driven by humans, but in fact, there are many hidden genetic changes involved. When researchers compare the genomes of different cotton species and varieties, they cannot draw clear conclusions at the beginning. They noticed that in some gene regions, there were traces of selection, gene introgression and even recombination. These changes did not occur in isolation, but connected some key nodes in the long-term evolution of cotton (Shen et al., 2019). Although these differences at the genetic level are not always directly reflected in the traits, they do provide us with a more accurate breeding "roadmap" - not only can we improve a certain trait in a targeted manner, but also optimize multiple aspects at the same time (Li et al., 2024).

This study summarizes the research results of using comparative genomics to improve cotton traits in recent years, focusing on the progress of genome sequencing, the discovery of genes and structural variations related to important agronomic traits, and how to use genomic methods in cotton breeding. By collating and integrating the contents of different studies, this study hopes to show the current level of research, point out the problems that still exist, and put forward some ideas for how to use comparative genomics to improve cotton traits in the future.

2 Comparative Genomics Platforms and Tools for Cotton

2.1 Genome sequencing of cotton species

Today's high-throughput sequencing technology is very advanced, and high-quality reference genomes of many diploid and tetraploid cotton species can be obtained, which lays the foundation for comparative studies and trait improvement (Manivannan and Amal, 2023). Technologies such as long-read sequencing can better assemble complete gene transcripts and help us understand the complex polyploid genome of cotton more clearly. These technologies can also find genes related to fiber development and stress resistance. There are currently some databases, such as CottonGen and CottonFGD, that integrate these genomic information, annotation data, genetic markers, and trait loci, which can be used by researchers and breeders to search for information, analyze or do breeding (Zhu et al., 2017).

2.2 Synteny and orthology analysis

Not all genes are preserved in evolution, but some regions can persist between different cotton varieties. At this time, synteny and orthology analysis are very important. They can help us see which gene regions remain relatively stable and connect the genetic relationships between varieties. Databases like CottonGen provide many practical tools. Not only can you view genetic maps, but you can also directly compare genes to see which are "relatives" to each other (Yu et al., 2013). Of course, this kind of analysis is not just for the sake of looking at the map. The more practical significance is that we can use it to find key genes that may be related to traits, and sometimes even transfer existing trait information. Digging down along these clues can further clarify the evolution of the cotton genome and the genetic loci related to some important agronomic traits (Yu et al., 2015).

2.3 Comparative gene expression and regulation

Comparative gene expression studies use a large amount of transcriptome data from different cotton tissues and varieties. With this data, researchers can see which genes are controlling the regulatory network of important cotton traits (Yang et al., 2022b). Now, some databases, such as CottonMD and CottonFGD, have collected a lot of transcriptome, epigenomic and metabolome data (Figure 1). These data can help us analyze how genes are expressed and see which regulatory factors are related to trait changes. With the help of these resources, we can more easily find key genes and regulatory pathways, which is very helpful for trait improvement and functional research (Ashraf et al., 2018).

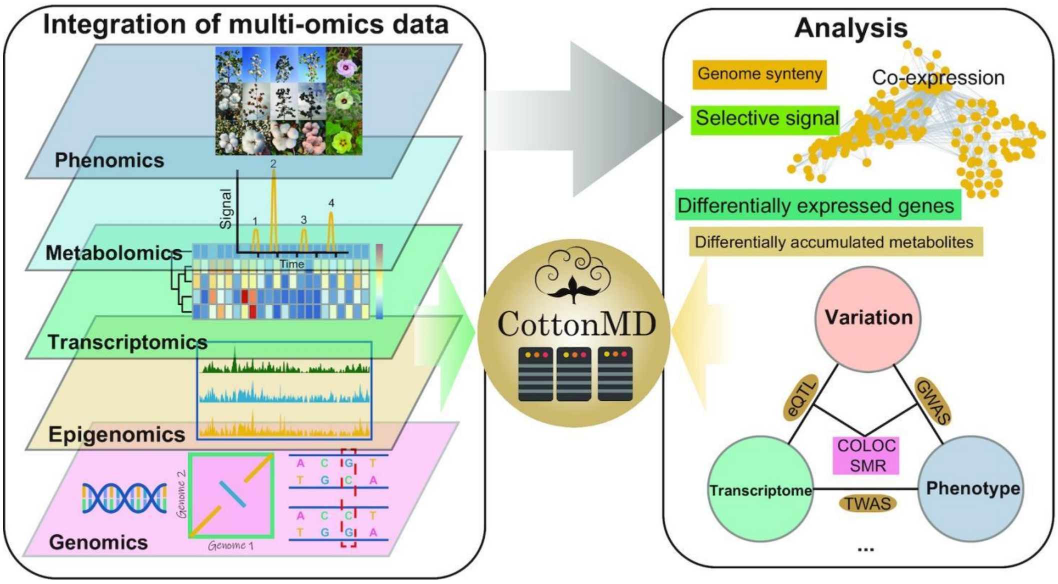

Figure 1 Overview of CottonMD. Construction pipeline of CottonMD through integration of multi-omics data (Adopted from Yang et al., 2022b)

3 Trait Improvements Facilitated by Comparative Genomics

3.1 Fiber quality traits

It is actually quite difficult to improve the fiber quality of cotton by traditional methods, especially when facing traits such as fiber length, strength, uniformity, and elongation. What was the difficulty in the past? To a large extent, it was because we didn't know where the genes behind them were. But now it is different. Comparative genomics has broken this situation (Naoumkina and Kim, 2023). Relying on high-quality reference genomes and GWAS (genome-wide association analysis), researchers have found many QTLs and SNPs related to high-quality fibers (Wang et al., 2018a). These findings not only make marker-assisted selection more directional, but also make breeding more accurate. By the way, after adding transcriptome and gene network analysis, the accuracy of the prediction model has been significantly improved, especially in indicators such as elongation and strength, the improvement can reach 5% (Khalilisamani et al., 2024). Breeders can therefore understand more clearly how fibers develop and breed targeted varieties more quickly (Li et al., 2018; Ijaz et al., 2019).

3.2 Stress tolerance traits

Not all varieties can survive adversities such as drought, pests and diseases, especially when extreme climates become more frequent. At this time, comparative genomics is particularly critical. It allows us to identify genes and regulatory pathways involved in stress resistance earlier, and these findings provide important clues for breeding "tough" cotton varieties (Yang et al., 2022a). Of course, identification alone is not enough. To really "use" these genes, we have to rely on tools like CRISPR/Cas9. Combining them with comparative genomics, we can make more precise modifications to stress resistance-related genes without having to select them slowly from generation to generation (Kumar et al., 2024). At present, the climate is unstable and the market pressure is high. Whoever can stabilize production will have the upper hand - so these technologies are not just "available", but truly "necessary".

3.3 Yield-related traits

Comparative genomics is also helpful in improving cotton yield. Through this type of technology, researchers have found QTLs and candidate genes related to lint yield, kernel index, and oil content (Li et al., 2024). Some prediction models that integrate genomic information and pedigree data also have good prediction results for these yield traits, which makes selection in breeding more efficient (Li et al., 2022b). Through GWAS and QTL mapping, people have also discovered some new regions and candidate genes that are related to fiber yield and its related factors (Joshi et al., 2023). These methods can improve multiple yield-related traits at the same time, thereby increasing the overall value and total yield of cotton.

4 Evolutionary Insights into Cotton Genomes

4.1 Polyploidy and subgenome divergence

Polyploidization has been a key process in cotton evolution, which has given cotton a complex genome containing multiple subgenomes. In the process of changing from diploid to tetraploid, the chromatin structure has undergone significant changes, such as the conversion of region A and region B, and the rearrangement of TAD (topologically associated domain). These changes affect the expression and regulation of genes (Wang et al., 2018b). Comparative analysis found that events such as deploidization or the formation of new tetraploids make the genome less stable, which may lead to the loss of some genes, inversion or translocation of DNA, and asymmetric evolution between the two subgenomes (Wang et al., 2016; Pan et al., 2020). These changes make the A subgenome and the D subgenome somewhat different in regulatory function, among which the A subgenome is more prone to structural changes and gene loss. Despite these changes, the number and arrangement order of most genes remain relatively stable. At the same time, transposon exchanges between different subgenomes and the expansion of gene families are also driving new evolutionary changes.

4.2 Domestication signatures in cotton genomes

Domestication has had a significant impact on the cotton genome, especially in cultivated allotetraploid cotton. By comparing the entire genome, researchers have found many expression changes, structural variations, and gene family expansions that only occur in certain species, which are important reasons for the differences between upland cotton (*Gossypium hirsutum*) and sea island cotton (*Gossypium barbadense*) (Hu et al., 2019). Positively selected genes (PSGs) related to fiber improvement are mainly concentrated in the A subgenome, while genes related to stress resistance are mostly found in the D subgenome (Zhang et al., 2015). During fiber development, some genes show similar expression changes and form co-expression networks, indicating that selection and domestication have affected regulatory pathways (Chen et al., 2020). These marks left by domestication are very valuable targets for future breeding programs that want to improve fiber quality and stress resistance.

4.3 Introgression and hybridization

Gene introgression and hybridization are also critical to the evolution and breeding of cotton. By constructing gene introgression populations, such as introducing useful chromosome segments from *G. barbadense* into *G. hirsutum*, researchers can more easily find quantitative trait loci (QTLs) related to high-quality fibers (Wang et al., 2018a). Some wild cotton relatives also provide many useful genes that can cope with environmental problems. Their genes can be introgressed into cultivated cotton, not only breaking through the recombination barrier, but also bringing new favorable alleles (Peng et al., 2022). These processes can increase the genetic diversity of cotton and improve its ability to adapt to the environment, which will help to continuously breed better cotton varieties.

5 Case Study: Comparative Genomics of *G. hirsutum* and *G. barbadense*

5.1 Background of the study region and cultivar focus

Upland cotton (*Gossypium hirsutum*) and sea island cotton (*Gossypium barbadense*) are the two most widely planted allotetraploid cotton varieties. These two cottons are widely used because of their natural fiber yield. Upland cotton has high yield and strong adaptability, and can be grown in various environments; while sea island cotton is particularly popular because of its very good fiber quality (Figure2) (Hu et al., 2019). In recent years, scientists have successfully completed the assembly of high-quality reference genomes of these two varieties, TM-1 (upland cotton) and 3-79 (sea island cotton). These achievements provide an important basis for subsequent genetic comparison and breeding research.

5.2 Key comparative findings

Comparative analysis of the genomes of upland cotton and sea island cotton revealed many structural differences. For example, large-scale inversions close to centromeres appeared on multiple chromosomes, and there were obvious differences in the centromere region (Meng et al., 2023). The two cottons differ in the expression of some genes, the number of gene families, and gene structure, and these differences are the key to their respective evolution and differentiation (Chang et al., 2023).

In particular, the location and sequence changes of centromeres played an important role in the formation of species. In addition, comparison of mitochondrial genomes showed that there were also obvious differences in the number of genes and repeat sequences between the two (Tang et al., 2015). Through the analysis of QTLs and gene families such as R2R3-MYB, researchers found common and unique gene loci related to fiber quality, yield, and stress resistance of the two cottons (Said et al., 2015).

5.3 Outcomes and breeding implications

These high-quality genome and comparative research results have greatly accelerated the discovery of quantitative trait loci (QTLs) and key genes related to fiber quality and yield in cotton (Wang et al., 2019). Researchers used gene introgression lines to introduce some high-quality chromosome fragments of sea island cotton into upland cotton, which can more accurately locate QTLs that control high-quality fiber traits, and also found some stable sites that improve fiber quality and yield at the same time (Wang et al., 2018a). Now, scientists are also using whole-genome introgression analysis and kmer genotyping methods to further identify useful alleles and fragments that are helpful for multiple traits, which will provide great help for future marker-assisted selection and target trait combinations (Li et al., 2022a). These technological advances provide new methods for combining the high-yield advantages of upland cotton with the high-quality fibers of sea island cotton, and are also expected to improve the economic benefits and agricultural value of cotton.

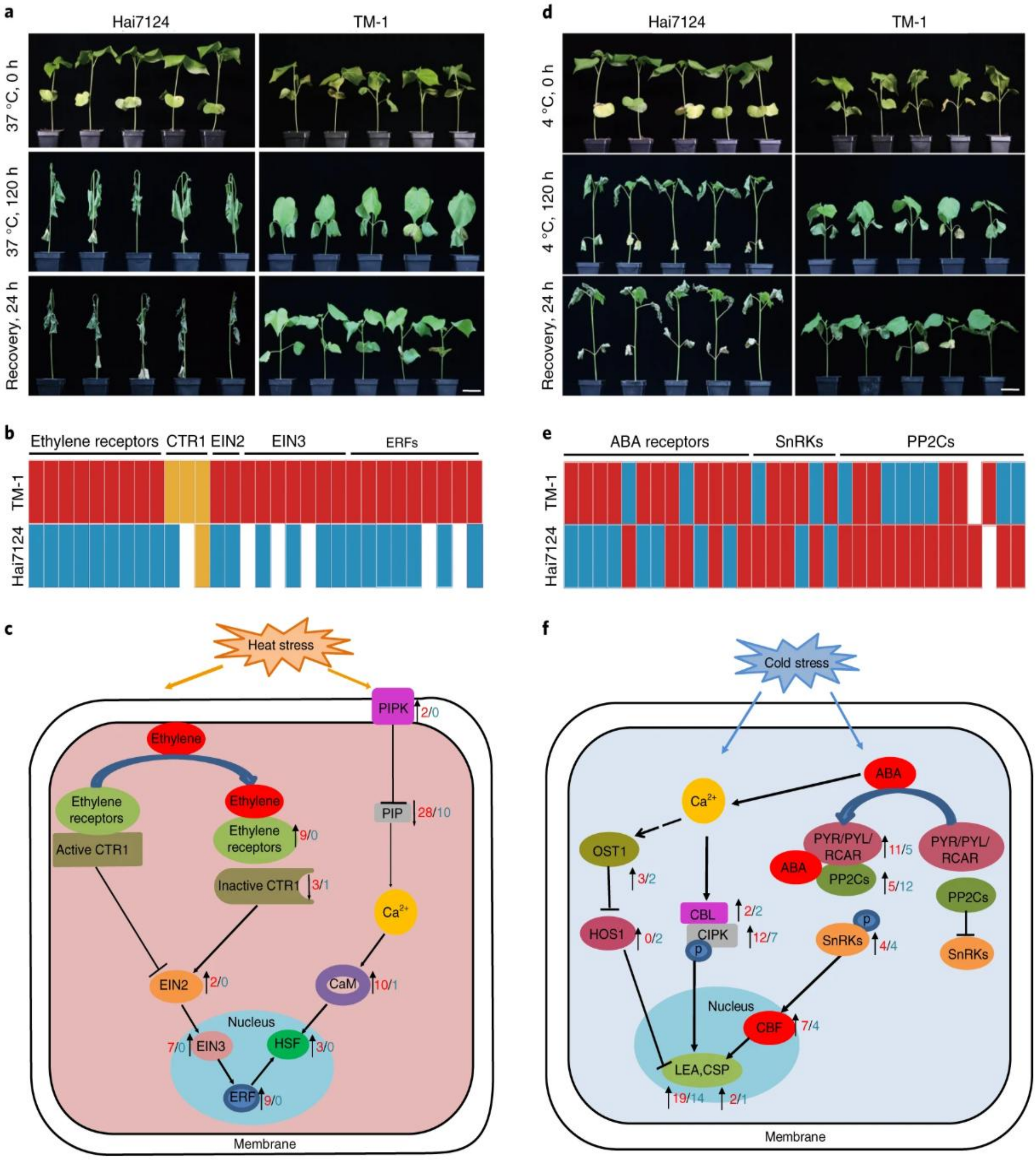

Figure 2 Models depicting the molecular basis of heat and cold tolerance in TM-1 (Adopted from Hu et al., 2019)

Image caption: a,d, Phenotypic comparison of TM-1 and Hai7124 cotton seedlings in response to heat (a) and cold (d) stress. Photographs were taken under normal conditions or for seedlings subjected to heat treatment at 37 °C or cold treatment at 4 °C for 120 h followed by recovery for 24 h. Scale bars, 10 cm. b,e, Heat maps showing differentially expressed genes involved in the ethylene signaling pathway for heat stress (b) and the ABA signaling pathway for cold stress (e) in TM-1 and Hai7124. Red, upregulation; yellow, downregulation; blue, no difference in expression; white, absence of an orthologous gene. c, A proposed model showing that the ethylene and plasma membrane signaling pathways cooperatively enhance heat tolerance in *G. hirsutum*. These pathways work together to maintain high transcriptional activities under heat stress, whereas no difference in expression is observed in *G. barbadense*. f, Diagram of the cold-responsive transcriptional network in cotton. The ABA and calcium signaling pathways were activated in *G. hirsutum*, whereas only a small portion of the differentially expressed genes were activated in *G. barbadense*. Upward- and downward-pointing arrows indicate up- and downregulated genes; red and blue numbers correspond to the number of differently expressed genes in TM-1 and Hai7124, respectively (Adopted from Hu et al., 2019)

6 Challenges and Future Directions

6.1 Limitations of current comparative genomics approaches

Although comparative genomics of cotton has made a lot of progress, there are still many problems. The polyploid genome of cotton is large and complex, with many repeated genes and similar sequences, which makes it difficult to assemble, annotate and study the function of the genome (Yang et al., 2020). At present, it is still not easy to accurately determine the function of a gene, and the speed is not as fast as genome sequencing. On the one hand, this is because a more refined reference genome is needed, and on the other hand, the diversity materials are not rich enough. In addition, current breeding methods rely more on varieties with a narrow genetic background, which may affect future yield increases and may miss some new useful genes (Kumar et al., 2024). There are also some technical problems, such as the CRISPR/Cas system sometimes has off-target effects, and the design of guide RNA also needs to be optimized, which limits its use in actual breeding.

6.2 Integration with other omics and breeding tools

Comparative genomics alone is far from enough. Especially when faced with complex traits that are easily affected by multiple factors, it is difficult to see the whole picture by studying gene structure alone. Sometimes, phenotypic changes are not a problem with the gene sequence itself, but the "unspoken rules" at the regulatory level. This requires the use of other "omics" together - such as transcriptome, metabolome, proteome, and epigenetic data (Yang et al., 2022a). Although these contents look more complicated when put together, the advantage is that the prediction model can be made more accurate and key pathways and functional genes can be targeted more specifically (Billings et al., 2022). Of course, no matter how much data there is, it is difficult to implement without breeding methods. In order to turn this information into truly useful varieties, it is necessary to combine rapid breeding, high-throughput phenotyping technology, and pan-genomic research. Especially in the current context of the continuous compression of genetic diversity, collecting different germplasm resources may be the only way for us to find those "lost genetic fragments" (Khalilisamani et al., 2024). This is not addition, but a necessary condition.

6.3 Future perspectives and research needs

What should we do next? The direction is not difficult to guess. The foundation needs to be filled first, for example, the genome assembly can be more refined, and the data coverage of resequencing still has a lot of room for improvement. At present, many germplasm materials have not been included in the big data platform. If this gap is not filled, the subsequent analysis will not be able to achieve a "global perspective". In addition, the functional verification step can no longer be delayed - we can't wait for generations of experiments to verify, and the development of a set of high-throughput, fast and effective functional omics tools is the current priority (Ashraf et al., 2018). However, it is not realistic to rely on a single breakthrough. Omics must be interoperable, and breeding technology must be upgraded. This involves the update of tools such as CRISPR, such as improving specificity, reducing off-targets, and editing multiple targets at once (Khan et al., 2023). If we want these studies to not just stay in papers, we must promote the construction of a sharing mechanism - data can be interoperable, resources can be used interchangeably, and ideas can be jointly built. In this way, the public breeding platform will have a real chance to take off, and the sustainable development of cotton will not just be a vision written in a report (Sreedasyam et al., 2024).

7 Concluding Remarks

In the past, our understanding of cotton often stayed at the level of trait performance and breeding experience. Now it is different. With the emergence of comparative genomics, many things began to have an "inside perspective". After the reference genome, pan-genome, and various variant databases were established, the genetic diversity that was originally "hidden" during the domestication or selection process was slowly found. Indeed, not every variation is important, but some newly discovered sites, missing variants, and potential candidate genes are gradually rewriting our understanding of the control of cotton traits. Methods such as whole-genome association analysis, gene introgression maps, and functional verification have made many things that used to rely on guesses have a hammer.

Of course, these studies cannot be implemented by "omics" alone. What really promotes the emergence of "super cotton" varieties is the deep combination of comparative genomics and breeding technology. These new cottons show not only longer fibers or higher yields, but also better resistance to challenges such as drought, pests and diseases. As for the genetic basis behind it, pan-genome and large-scale genetic surveys provide many new clues. Some key gene markers and favorable alleles have begun to be identified, opening up a new situation for germplasm improvement. At a time when the cotton industry is facing climate pressure and sustainable development pressure, these technologies are not icing on the cake, but a rigid need.

It can be said that this stage is a new starting point for cotton research and breeding in the true sense. Comparative genomics is not just a toolkit, but more like a central system that connects traditional breeding with modern technology. Next, what needs to be done is not "whether it is useful" but "how well it is used" - genome assembly must be more accurate, and the integration between multi-omics must also keep up. Breeding methods can no longer follow the old path, and we must dare to try new paths. And for these ideas to be truly implemented, no one can bypass the collaboration between scientific research teams, data sharing and communication mechanisms. Only in this way will these technologies not remain in papers, but actually land in cotton fields and support the future of cotton.

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Conflict of Interest Disclosure

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

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