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High-Throughput Genotyping and Its Role in Accelerating Cotton Breeding

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Abstract Cotton, as a globally significant economic crop, has long been the core goal of breeding improvement in terms of its yield and fiber quality. However, traditional breeding methods are characterized by long cycles and low efficiency, making it difficult to meet the increasingly complex breeding demands. The rise of High-Throughput Genotyping (HTG) technology has provided strong technical support for cotton molecular breeding, especially showing broad application prospects in quantitative trait localization, molecular marker development, genomic selection, etc. This study systematically reviews the characteristics and applicability of the current mainstream HTG technology platforms (such as SNP chips, GBS, RAD-seq, DArT, etc.), and analyzes their application progress in the genetic basis research of important agronomic traits such as yield, quality, and resistance. The practical role of HTG in QTL localization, GWAS analysis, marker-assisted selection and other links was discussed. Through typical breeding practice cases, evaluate its breeding acceleration efficiency in the context of multiple environments and varieties, and further look forward to the prospects of its deep integration with phenomics, genomic selection and intelligent decision-making platforms. This research provides theoretical basis and technical support for accelerating the genetic improvement and molecular breeding of cotton.

Keywords Cotton breeding; High-throughput genotyping; Molecular marker; Genomic selection; QTL positioning

1 Introduction

Cotton (*Gossypium* spp.) is not only an indispensable raw material in the global textile industry, but also plays an important role as an oil crop at the same time. More than 90% of the world's cotton production actually comes from one variety-terrestrial cotton (*G. hirsutum*). For many countries, it is not only related to economic development, but more often it is also about people's food security and job opportunities. Nowadays, however, the direction of breeding is no longer solely focused on yield as in the past. Fiber quality, stress resistance, and environmental adaptability have gradually become unavoidable goals-climate change, tight land resources, and the textile industry's demand for high-performance fibers have all brought this matter to the forefront (Wang et al., 2024).

But when it comes to the speed of traditional breeding, it is not very satisfactory. The method of observing traits and conducting field experiments is not only slow but also labor-intensive, and is further limited by the reality that the genetic diversity of modern cotton varieties is not rich enough. Breeders often encounter the embarrassing situation of "seeing good traits but not being able to select them". Fortunately, with the introduction of molecular techniques, especially the gradual application of methods such as DNA labeling and QTL localization, breeding has finally begun to move from the "visible" to the "invisible" level. Nowadays, with the help of high-throughput typing platforms such as SNP chips and next-generation sequencing, we can screen for variations among tens of thousands of markers. It is not only fast but also very accurate, and the cost is acceptable. Their addition has made GWAS, MAS and even genetic analysis of complex traits feasible, and has indeed significantly improved the efficiency of cotton breeding (Kushanov et al., 2021).

This study first reviews the economic and biological background of cotton breeding, then explores the limitations of traditional methods and the transformative impact of high-throughput genotyping, and outlines the latest technological developments, their applications in genetic mapping and breeding, as well as the integration of genotype data with phenotypic and environmental information. This study emphasizes how high-throughput

genotyping can reshape cotton breeding, thereby fostering superior varieties to address future challenges, and guiding breeders, researchers, and policymakers to utilize molecular tools to achieve sustainable cotton production and global food security.

2 Overview of High-Throughput Genotyping Technologies

2.1 Common platforms and techniques (SNP Arrays, GBS, RAD-seq, DArT, etc.)

In fact, high-throughput methods have been attempted for the genotype analysis of cotton for many years. Technologies like SNP chips, GBS, RAD-seq, and DArT, although they have different principles, all have the same goal: to quickly identify those variations related to traits without incurring too much cost. The advantage of chips like CottonSNP63K, CottonSNP80K and ZJU CottonSNP40K lies in the fact that they can "scan" many sites at once and have a high accuracy rate. Especially when conducting intraspecific or interspecific genotyping of cotton, it is relatively convenient to use, mainly relying on the platforms of Illumina or Affymetrix (Shakoor et al., 2017). However, if you are dealing with a highly diverse group or have no idea where the variations are at all, methods like GBS that do not rely on known SNPS would be more appropriate. It relies on next-generation sequencing, testing and searching simultaneously, and is highly flexible. As for RAD-seq and DArT, they also belong to strategies for reducing genomic complexity. Although they are not widely used in cotton, they can still be useful in some situations where resources are limited.

2.2 Technical comparison: throughput, cost, applicability, and data depth

Ultimately, different platforms have their own trade-offs. The operation of SNP arrays is simple and the results are reliable, but the SNPS it can detect are pre-designed and cannot "discover" new variations. On the contrary, sequencing-based methods such as GBS are more flexible when the genetic background is complex. However, the analysis process is much more cumbersome than that of chips, and the requirements for data processing are also higher (Cai et al., 2017).

2.3 Data processing pipelines and supporting bioinformatics tools

For the subsequent data processing, SNP chips follow a relatively mature set of processes. Manufacturers usually provide ready-made analysis software, and users just need to follow the steps (Hulse-Kemp et al., 2015). But for sequencing technologies like GBS or RAD-seq, the situation is not so "foolproof". You have to do sequence alignment first, then SNP invocation and quality control, and also write some scripts or run open-source tools according to specific projects (Yang et al., 2020). If you still need to proceed with GWAS, QTL mapping or genomic selection, you will have to rely even more on a well-functioning bioinformatics platform to help you integrate a large amount of genotype and phenotype data.

3 Genetic Basis of Key Agronomic Traits in Cotton

3.1 Genetic control of yield-related traits

Whether the yield is high or not is not determined by a single factor. Factors like the weight of the bell, the number of bells, and the height of the plant are all quantitative traits, and they are the result of multiple genes working together. Such traits are influenced by additive and dominant genetic effects and are also easily disturbed by environmental changes (Zhang et al., 2020). Current GWAS and QTL studies have identified many candidate gene loci related to these traits. Some regions are quite "capable" and play a role in multiple yield traits (Ma et al., 2024). However, there are still differences among traits. For instance, when it comes to clothing parts, the main gene has a strong control ability and high heritability. However, for instance, the genetic effect of bell numbers is less stable and is easily influenced by multi-gene or environmental interactions (Li et al., 2024).

3.2 Genetic architecture of fiber quality traits

When it comes to the indicators that affect fiber quality, the main ones are length, strength and uniformity. However, these traits are not determined by A single gene. Many QTLS are distributed in the A and D subgenomes (Li et al., 2021). Studies have found that QTLS in some gene regions always occur in "clusters", and some act simultaneously on multiple fibrous traits (Huang et al., 2017; Liu et al., 2022; Cai et al., 2024). It is notable that the D subgenome seems to be more active in controlling fiber quality and contains A large number of structural

variations, while the A subgenome is more closely related to yield-related traits (Ma et al., 2021). Candidate genes such as *GhACT1* and *GhGASL3*, which are known to be related to cell wall development, are usually identified from these stable QTLS (Huang et al., 2021).

3.3 Mining of genes related to disease resistance and stress tolerance

To enhance disease resistance and stress tolerance, relying solely on phenotypic selection and breeding is far from sufficient. Now with high-throughput genotyping and resequencing technology, researchers have been able to more precisely locate genes such as GaGSTF9 that are related to Fusarium wilt resistance, and even identify some structural variations on the D subgenome that are related to wilt tolerance (Figure 1) (Du et al., 2018). These achievements did not emerge out of thin air. They were also supported by genome editing tools and functional verification methods, providing a reliable basis for actual breeding (Kumar et al., 2024).

4 Application of Genotyping in QTL Mapping and Association Studies in Cotton

4.1 Current status and challenges in QTL mapping

Although high-density SNP arrays and sequencing typing techniques have brought many breakthroughs in cotton QTL mapping, such as constructing more complete genetic maps and identifying a large number of QTLS related to yield and fiber traits (Wang et al., 2015; Li et al., 2016), but there are no shortage of problems. For allotetraploid species like cotton, the genome is inherently complex, and the intraspecspecific polymorphism is not high. As a result, the localization resolution is always limited. Not to mention that the traits themselves are prone to environmental interference. Many QTLS only exhibit under specific conditions, and only a few can be stably expressed in different populations and environments (Diouf et al., 2018). In addition, the diversity of alleles in the paternal and maternal parents is not high either. This limitation of the genetic background restricts the discovery of new loci (Joshi et al., 2023).

4.2 Advances in GWAS based on high-throughput genotyping

Compared with traditional QTL mapping, GWAS seems to be more popular in diverse materials. Through high-throughput typing platforms such as GBS, SNP chips, and SLAF-seq, researchers have identified many new QTLS and candidate genes in multiple cotton germplasm resources (Joshi et al., 2023). This type of method has a greater chance of identifying allelic variations because it can cover more recombination events. It is particularly suitable for mining those stable gene loci related to pleiotropy traits such as fiber quality and yield (Huang et al., 2021). If the results of GWAS are combined with transcriptome or functional omics data, the determination of candidate genes will also be more well-grounded.

4.3 Fine mapping of QTLs and identification of candidate genes

To dig deeper based on the existing positioning, one has to rely on meticulous mapping. Generally, QTL segments that have appeared in multiple environments and multiple studies, or some recurrent QTL clusters, are selected first. Then, high-density labeling and phenotypic data are used to narrow down the target area (Tan et al., 2018; Yang et al., 2022). Candidate genes like GhACT1, GhGASL3, GhSCPL40, and GhPBL19, which have already been identified, were actually screened out by integrating QTL mapping, GWAS, and transcriptome results (Zhang et al., 2019; Wang et al., 2020; Xu et al., 2020; Zhu and Luo, 2024). This strategy provides more reliable targets for molecular breeding and MAS development of cotton.

5 Contribution of High-Throughput Genotyping to Marker Development and Utilization

5.1 Development of SNP/InDel and other marker types

In recent years, genomic sequencing technology and high-throughput typing platforms have become increasingly mature, driving the development of various molecular markers. It is not difficult to understand why SNP markers are widely used-they are numerous, have strong co-dominance, and are convenient to operate on high-throughput platforms, making them very suitable for handling large-scale materials, especially when mapping complex trait maps (Shehzad et al., 2017). However, the practicality of InDel markers should not be overlooked. For instance, in hybrid breeding, when identifying fertility recovery genes, InDel can come in handy. In contrast, although SSR markers are old, they still perform well in terms of polymorphism and repeatability, and are still frequently used in DNA fingerprinting analysis, population genetic diversity research, etc. (Wu et al., 2020; Iqbal et al., 2023).

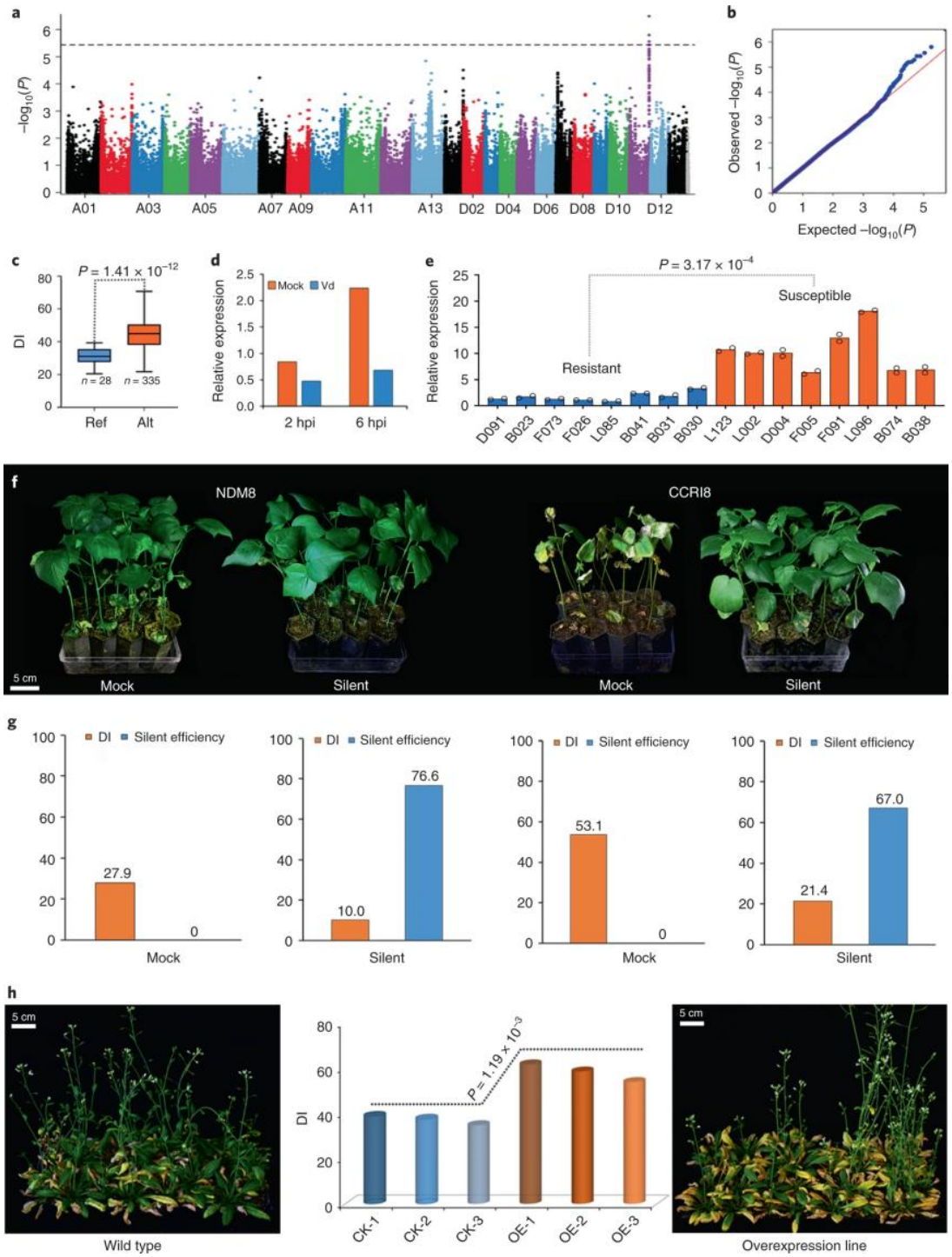

Figure 1 Identification of the causal gene *GhNCS* related to VW resistance on chromosome Dt11 (Adopted from Ma et al., 2021)

Image caption: a, Manhattan plot. Dashed line represents the significance threshold ($-\log_{10}(P) = 5.44$). We performed statistical analysis with a two-tailed Wald test. b, Quantile-quantile plot. c, Boxplot for DI on the basis of structural variation (D11: 69329075). In the box plots, the center line denotes the median, box limits are the upper and lower quartiles and whiskers mark the range of the data; n indicates the number of accessions with the same genotype. The difference significance was analyzed by two-tailed *t*-test. d, Expression level of *GhNCS* in resistant variety ND601 inoculated with Vd LX2-1. e, qRT-PCR analysis of GhNCS in eight resistant and eight susceptible varieties under Vd stress. Ghhistone3b was used as an internal control. The data were analyzed from the total of 16 varieties and expressed as the mean from two experiments. The difference significance was analyzed by two-tailed *t*-test. f, Silencing of *GhNCS* in tolerant variety NDM8 and susceptible variety CCRI8 led to obviously increased resistance compared with the mock. Scale bar, 5 cm. g, For each independent virus-induced gene silencing experiment, 35 cotton seedlings with higher silent efficiency were used for VW disease resistance detection. h, *GhNCS* overexpressed in Arabidopsis made transgenic plants highly susceptible compared with the wild type. Scale bar, 5 cm (Adopted from Ma et al., 2021)

5.2 Integration and validation of genotyping methods (KASP, CAPS, SSR, etc.)

Nowadays, cotton breeding rarely relies solely on a single typing method. KASP is currently the most widely used SNP detection method. The detection effect is stable and it is not troublesome to use. Many alleles of key traits are rapidly screened out by it (Li et al., 2022). Although SSR markers are more traditional, they have now been optimized to meet the requirements of high-throughput and even multi-analysis, such as for rapid variety identification (Kuang et al., 2022). Furthermore, more functional markers such as InDel and CAPS have also begun to be used for screening specific target traits such as fertility, disease resistance and stress tolerance (Feng et al., 2020; Dwivedi et al., 2025). The combined use of these methods not only enhances the screening efficiency but also ensures their universality in different breeding scenarios.

5.3 Practical effectiveness of marker-assisted selection (MAS) in breeding

The benefits of MAS have already been experienced by many breeders-there is no need to wait for the harvest in the field to judge the quality, but rather to screen in advance at the molecular level, effectively saving time and cost. Especially those markers derived from functional areas are more predictive and indeed more efficient in the screening of varieties. However, it is not to say that MAS has no shortcomings. Its effect is often related to the distance between the marker and the target gene, and the stability in different environments is also a variable (Bolek et al., 2016). Even so, its advantages in multi-gene complex traits remain obvious, at least in terms of accuracy and speed, which are beyond the reach of traditional breeding (Ijaz et al., 2019; Wang and Zhang, 2024).

6 Case Studies: Representative Applications of Genotyping in Cotton Molecular Breeding

6.1 Application of GBS in breeding for Fusarium wilt resistance in China

The genetic diversity of upland cotton is not wide, but this has not hindered the extensive use of sequencing-type genotyping (GBS) technology in the development of SNP markers. The research team attempted to apply GBS to different varieties and their hybrid offspring, and as a result, they found many SNP markers with strong polymorphism in the main cotton varieties cultivated in China. These markers can also be transformed into functional detection tools like KASP, facilitating their implementation in actual breeding projects. Especially in the breeding of Fusarium wilt resistance, the efficiency of locating resistance sites and screening candidate materials has significantly improved, and the breeding of disease-resistant varieties has also accelerated accordingly (Islam et al., 2015).

6.2 GWAS and SNP array-based strategies for yield improvement in American cotton

The approach in the United States is slightly different. The breeding team is more inclined to use genome-wide association analysis (GWAS) in combination with high-density chips such as CottonSNP63K or CottonSNP80K. These platforms have shown stable performance in screening QTLS related to yield or fiber quality traits, and the results can be reproduced in different germplasm populations. For instance, CottonSNP63K has been utilized to construct genetic maps and conduct GWAS, capable of simultaneously covering intraspecspecific and interspecific differences, and has a very strong localization ability. These achievements do not remain at the theoretical level either. They directly support marker-assisted selection and genomic prediction, and are also common in practical breeding work for yield improvement (Si et al., 2022).

6.3 Multi-environment validation of high-throughput genotyping in transgenic and conventional materials

It is worth noting that, for both conventional materials and transgenic strains, a considerable amount of adaptability verification of high-throughput genotyping platforms in different breeding contexts has been carried out. Multi-environment tests conducted using SNP chips or GBS not only have good repeatability in trait localization and variety identification, but also ensure that the association relationship between markers and traits remains stable under different environments. The CottonSNP80K chip is a typical example. Its polymorphism and detection rate in hundreds of germplasm materials indicate that it can basically meet the diverse breeding demands (Figure 2) (Billings et al., 2022; Chen et al., 2022).

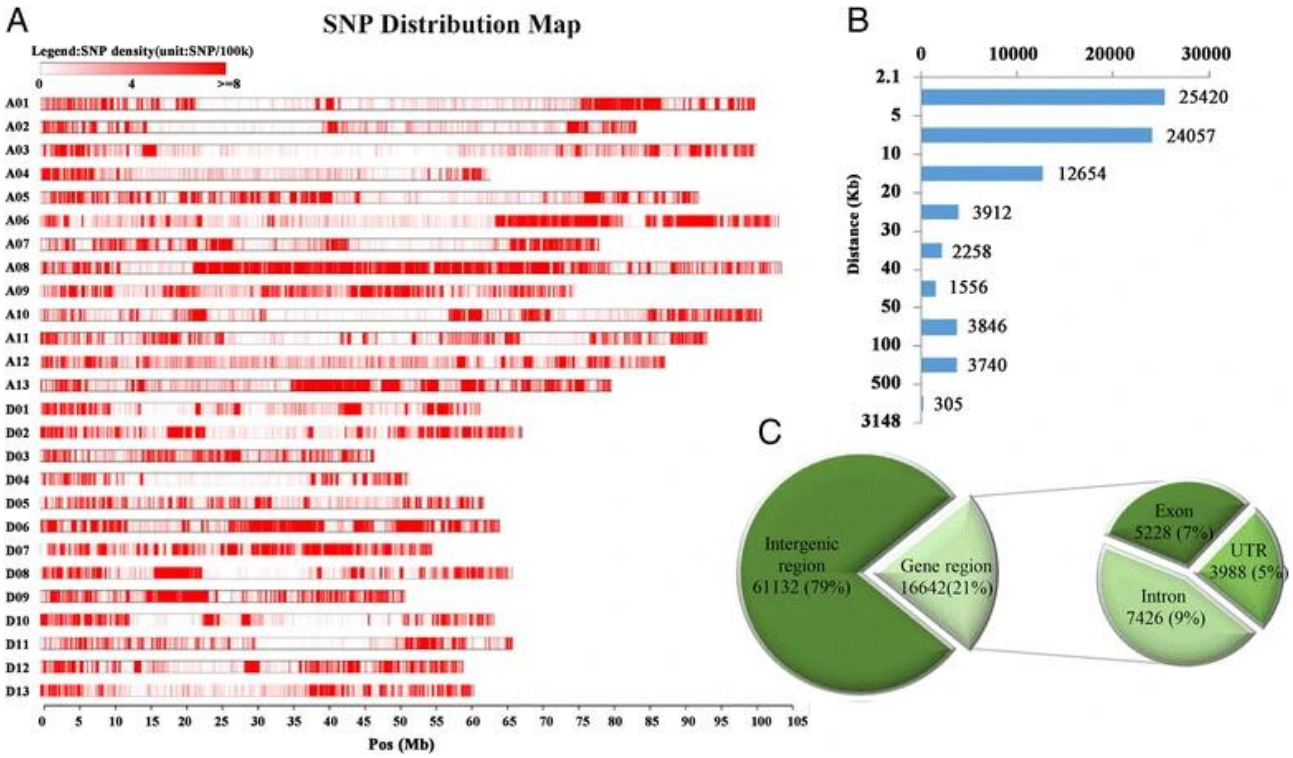

Figure 2 Description of SNPs in CottonSNP80K array. a: SNPs distributions on the 26 chromosomes of upland cotton. A01-A13 and D01-D13 in vertical axis are the serial number of 26 chromosomes; the horizontal axis shows chromosome length (Mb); the red region depicts SNP density (the number of SNPs per window). b: Distances between the SNPs. The vertical axis represents distances range (Kb) of SNPs. c: Distribution of genic and intergenic regions of selected SNPs (Adopted from Cai et al., 2017)

7 Integration of Genotyping with Breeding Decision Platforms

7.1 Integration with genomic selection (GS) models

Today's high-throughput genotyping is not merely about providing a vast amount of SNP data; it has almost become a prerequisite for the operation of GS models. In the past, the prediction efficiency of breeding values was limited by data density and the operational threshold of model tools. Now, with platforms like AutoGP and IP4GS, the situation has changed (Li et al., 2023; Wu et al., 2025). They have user-friendly interfaces. Even breeders who don't know programming can model and predict by themselves. From genotype extraction, model parameter adjustment to the final phenotypic prediction, everything is packaged (Hickey et al., 2017; Yan et al., 2021). How to select the model, whether the prediction is accurate, and which parent is the best can all be quickly tested through these tools. Ultimately, they have transformed GS from merely an "expert-only" tool into a practical and operational productivity tool.

7.2 Joint analysis of high-throughput phenotyping and genotyping data

Just looking at the genotype is not enough. In actual breeding, there are too many phenotypic and environmental interference factors. It is necessary to comprehensively consider the typing data, high-throughput phenotypic information and environmental background together in order to predict complex traits more accurately. Nowadays, some intelligent breeding strategies simply feed genomic, phenome and environmental data into the model together, and run the analysis in combination with machine learning or deep learning algorithms. The effect is actually more stable (Adunola et al., 2024). In terms of platforms, it's not necessary to set up your own server. Tools like Breedbase and IBP can basically help you organize, analyze, and even visualize your data (Varshney et al., 2016; Rasheed et al., 2017; Morales et al., 2020). Once the process is clear and the results are intuitive, the decision-making of the breeding plan will also be more confident.

7.3 The role of cloud computing and smart platforms in decision support

Facing huge amounts of omics data, it is no longer realistic to rely on traditional desktop systems for analysis. At this point, the cloud platform becomes particularly crucial. For instance, the breeding management system (BMS)

in IBP not only enables cross-regional teams to share data and tools, but also allows analysis to be completed on the cloud. The integration of AI technology has further accelerated this process. Nowadays, many analyses no longer require manual operation; systems can run predictions and recommend mating combinations on their own (Xu et al., 2022; Crossa et al., 2025). The breeding process that used to rely on experience to be refined gradually can now be assisted by intelligent analysis for many decisions, which has significantly increased the speed and improved the accuracy.

8 Conclusion

The progress of high-density SNP chip technology has indeed been rapid in recent years. Chips like CottonSNP40K, CottonSNP63K and CottonSNP80K have made the genotyping of cotton both fast and accurate, and not very costly. With the addition of sequencing-based methods, these tools have basically clarified the genetic information of various germplasm materials. Nowadays, there are no technical barriers to constructing saturation maps, locating QTLS, or conducting GWAS. This technological breakthrough has also made it more reliable to screen key trait loci, and the efficiency of marker-assisted selection has significantly improved. Moreover, once these high-throughput typing techniques are combined with functional omics research and mutant libraries, the genetic basis of cotton will be expanded, and the adaptability of germplasm will also extend outward.

However, relying solely on classification is not enough. Truly complex traits, such as yield, quality and resistance, cannot be clearly explained by data at the omics level alone. Thus, multi-omics integration has become the mainstream direction nowadays. Bring in genomics, transcriptomics, phenomics and even environmental data to jointly analyze the mechanisms of trait formation. This kind of analytical method is also less likely to get stuck when it comes to the interaction between genotypes and the environment. More importantly, this combination of measures can push us to design "super cotton"-that is, varieties that not only produce a lot but also have strong resistance and good quality. Of course, achieving these is not merely about "measuring accurately". The accumulation of gene editing tools like CRISPR and pan-genome resources actually gives us the space to further adjust the target traits. As long as these variant information can be accurately identified and well utilized, the goal of what traits to improve next will be very clear.

Ultimately, it still depends on platform-based solutions to catch up with these technological achievements. High-throughput data does bring abundant resources, but it also means the need for powerful data processing capabilities. The intelligent cloud breeding system might be one of the solutions to this problem: it can not only store data but also automatically analyze and assist in decision-making. Especially after the intervention of AI, the breeding process will become much simpler than before. As sequencing costs continue to fall, multi-omics integration methods may become a "routine operation". By then, the gap between genotypes and phenotypes will become smaller and smaller, and cotton breeding will be better able to cope with the dual challenges of climate change and global demand.

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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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