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MODERN DEVELOPMENTS IN GENETIC MAPPING OF COTTON FOR YIELD, FIBER EXCELLENCE, AND ABIOTIC STRESS RESISTANCE

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Abstract

Cotton (Gossypium spp.) is among the world's most significant cash crops, supplying natural fiber and supporting both the textile and agricultural sectors. Its productivity, however, is often limited by pests, diseases, and various abiotic stresses, which are inadequately addressed by conventional breeding approaches. Recent advances in genome sequencing and molecular breeding have opened new avenues for improving fiber quality and stress tolerance. This review highlights recent progress in genetic mapping, quantitative trait locus (QTL) analysis, and marker-assisted selection (MAS) for identifying genes linked to key agronomic traits. Innovations in high-throughput sequencing and genome-editing technologies, particularly CRISPR/Cas9, have enabled precise manipulation of target genes to enhance cotton performance. The integration of high-density molecular markers with genomic selection has accelerated breeding cycles by allowing early prediction of desirable traits. Furthermore, combining traditional breeding with genomic tools has helped overcome challenges associated with polyploidy and genetic bottlenecks, leading to more stable yields under stress conditions. Future research should focus on the wider application of genome-assisted breeding, functional genomics, and high-throughput phenotyping to improve cotton's adaptability to climate variability and biotic pressures. Overall, genomics-integrated breeding offers a promising strategy for achieving sustainable gains in cotton yield, fiber quality, and stress resilience.

INTRODUCTION

Cotton, the world's largest fibre and oilseed crop in the world (Shahzad et al., 2022). It grows in over 100 countries, with an area of around 33M hectares. As a major cash crop, it produces around 31% of the world's natural fibre. Furthermore, it makes a significant contribution to global production(Nabi et al., 2015). Cotton is a globally important natural fibre and oilseed crop with significant economic implications(HE et al., 2019).

The Gossypium genus contains approximately fifty species with ploidy levels of diploid (2n=26) and tetraploid (2n=52). Diploid species contain genomes A, B, C, D, E, F, G, or K. These are found in both tropical and subtropical regions and are geographically connected (Mei et al., 2004a). Plant height ranges up to 120 cm, has a boll with separate branches. It is a tap- rooted plant with a zone depth of 90cm that may be grown under warm and irrigated circumstances(Aydoğdu et al., n.d.).

Despite the opportunities of cotton breeding, it is devastated by a high frequency of pests and insect attacks, weeds and herbicide resistance due to climatic insecurities such as drought, floods, and heat waves (Mollaee et al., 2019). The huge and

complex genome of the Gossypium species is an assembly of high-quality genomes is challenging. The major goal of genome research is to utilize genomic tools to develop crops with great genetic improvement(H. Bin Zhang et al., 2008a). Functional genomics has accelerated cotton breeding to genomic breeding (Z. Yang et al., 2020).

QTL mapping is primarily a discovery tool that assists researchers in understanding the genetic architecture of complex traits and identifying possible target genes. It is not directly utilized for selection but rather informs other methods such as MAS(Pan et al., 2024). MAS is especially beneficial for features influenced by a few significant QTLs. It enables more efficient selection than traditional approaches, particularly for features that are difficult or expensive to quantify phenotypically. Genomic selection can forecast individuals' breeding value more accurately and sooner in their life cycle than traditional approaches, particularly for traits with many small-effect QTLs(Lübberstedt et al., n.d.).

Even though new sequencing methods have substantially decreased prices, complex polyploid genomes continue to pose a difficulty in terms of short-read assembly. Polyploidy creates genetic and gene expression novelty, but it also provides repetition, making sequence annotation and assembly more difficult. We developed an integrated technique for sequencing and assembling an allopolyploid cotton genome, which might be used to sequence difficult genomes from other polyploid crops (T. Zhang et al., 2015). One of the key reasons for cotton cultivar diversity is the use of wild genetic resources, which causes linkage dragging of undesirable traits. Another factor is a lack of creative strategies for incorporating genetic differences from exotic cotton plants of the Gossypium genus into breeding cultivars. All these causes contributed to the genetic bottleneck in evolution(Iqbal et al., 2001). This review paper discusses the potential method of genome sequencing that can improve cotton resistance through genetic mapping.

Cotton Genomic Sequencing

Cotton has been domesticated as Gossypium hirsutum and G. barbadense (Tetraploid species), G. arboreum and G. herbaceum (Diploid species)(Wendel & Cronn, 2003). Moreover, 95% of the world's cotton crop is G. hirsutum, which is also known as Upland cotton, with extra-long staple (Z. J. Chen et al., 2007).

G. hirsutum L. is an allotetraploid, as well as a polyploid.Assembly of allopolyploid plant genomes is a difficult process due to the genomes' extremely complex structure(Saski et al., 2017). To exploit this complexity, researchers applied advanced sequencing and genetic mapping techniques on cotton(P. Wang et al., 2024). For this, it is sequenced and assembled withtheallotetraploid genome ofG. hirsutum L. using DNA from the TM-1 pure strain, which isa homozygous species. They compared the G. hirsutum L. assembly to the hypothesized ancestral species, G. raimondii and G. arboreum, to study sub-genome evolution and gene function, including genes involved in fibre biology(F. Li et al., 2015; Z. MA, 2020).

Table no 1

This table explains the specific and common genes located in the species:

Species 1	Specific genes	References	Species 2	Specific genes	Common genes	References
G. arboretum	16,918	(Du et al., 2018)	G. arboretum	19,236	24,042	(Huang et al., 2020)
G. raimondii	25,964	(Paterson et al., 2012)	G. raimondii	29,202	11,541	(Udall et al., 2019)

G. barb aden se	6,504	(Z. J. Chen et al., 2020)	G. barb aden se	3,240	68,057	(M. Wan g et al., 2019)
G. barb aden se	27,304	(Hu et al., 2019)	G. barb aden se	23,534	47,763	(J. Wan g et al., 2019)
G. barb aden se	25,093	(Hu et al., 2019)	G. barb aden se	24,573	49,988	(Z. J. Chen et al., 2020)

This table lists Gossypium species as well as the specific genes found in each. In the context of species 2, common genes were given alongside specific genes.

Genetic Mapping using Molecular Markers

Genetic mapping identifies the gene and markers with specific traits to improve breeding. In cotton breeding, markers are the main source to locate desirable genes(Malik et al., 2014). In breeding, these markers are highly valuable in detecting, characterizing, and identifying genetic variants, as well as removing linkage drag in breeding programs to find desirable features that are difficult to measure by visual observation(Kalia et al., 2011). SNPs are mostly and widely used molecular markers with a high level of polymorphism. SNPs are found in coding and non-coding regions of the genome, which allows detection variation (Agarwal et al., 2008). In the past decade, an extensive collection of QTL- mapping tools has been introduced for incorporating a variety of methodologies to locate genes. These methods are based on concepts of parametric and nonparametric linkage analysis, as well as innovative methodologies utilizing the study of dispersion components association analysis and multipoint mapping(JIA et al., 2014; Liu & Muse, 2005; Y.-M. Zhang & Gai, 2009; Z.-S. Zhang et al., 2009).

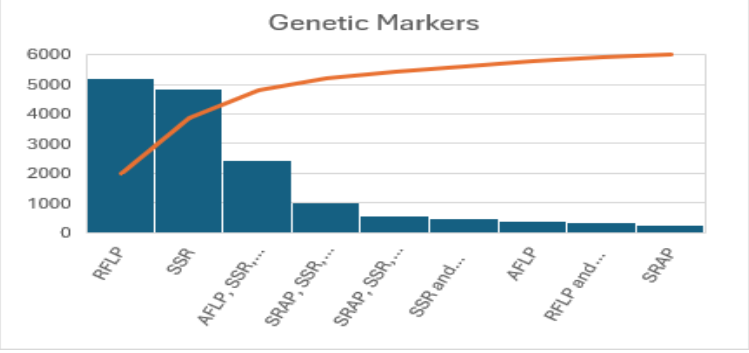


Figure 1

These are the markers widely used in genomics (H. Bin Zhang et al., 2008b)

AFLP: Amplified Fragment Length Polymorphism

SSR: Simple Sequence Repeats

SRAP: Sequence-Related Amplified Polymorphism

RFLP: Restriction Fragment Length Polymorphism

This indicates the uses of different genetic markers along with their usage over different amplifications.

Genetic Mapping using QTL Mapping

The cotton QTL study was conducted on 392 genetic loci, comprising 333 AFLP, 47 SSR, and 12 RFLP(X.-Y. Wang et al., 2012). A genetic map of cotton was constructed for QTL using composite interval mapping and permutation tests. This study discovered that seven QTLs for six fibre-related traits, of which five are found on the A-subgenome chromosomes, are responsible for fibre traits(Syed & Gao, 2010). Prior study also found that QTLs in both the A and D sub-genomes are responsible for fibre-related phenotypes, which are influenced by homoeologous genes(Razzaq et al., 2022). QTL clusters on certain chromosomes are likely to contribute to the high phenotypic diversity in fibre-related characteristics(Mei et al., 2004b; Tan et al., 2018).

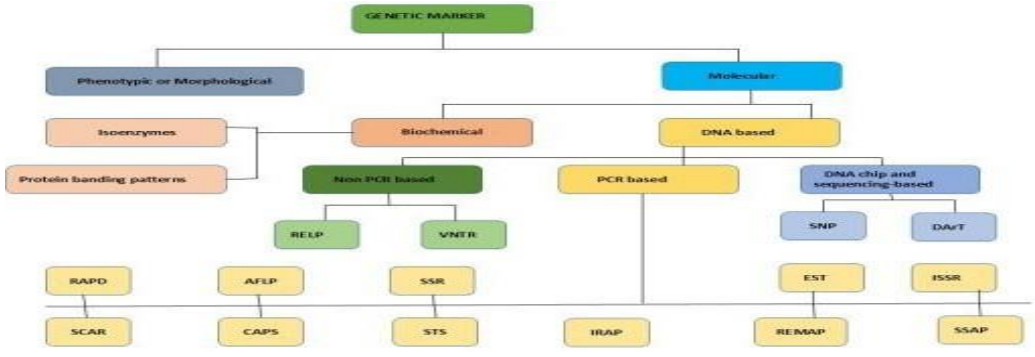
Another study uses the high-density cotton molecular marker linkage map based on PCR (Kushanov et al., 2021). An F2 population of a hybrid between "Handan208" and "Pima90" examined the use of SSR, sequence-related amplified polymorphism, RAPD , and retrotransposon microsatellite amplified polymorphism(D. He et al., 2007). Fifty-two distinct QTLs were identified for lint index, seed index, lint yield, seed cotton yield, number of seeds per boll, fibre strength, fibre length, and micronaire. The current map and QTL analysis are a useful tool for breeders to transfer advantageous traits from G. barbadense to the most farmed species, G. hirsutum(D.-H. He et al., 2007). Furthermore, gene expression analysis using RNAseq data identified 40 possible genes, including 23 stable and 17 novel genes. These genes are transcriptionally active at different stages of fibre, ovule, and seed development. These studies have revealed a rich tapestry of genetic elements, including SNPs, QTLs,and candidate genes, and have a high potential for increasing fibre yield in future breeding initiatives for cotton(Joshi et al., 2023).

Genetic Mapping using Marker-Assisted Breeding

Molecular markers provided a significant opportunity in increasing the precision of crop development operations through MAS breeding technology(Collard & Mackill, 2008a). MAS technology enables selection at any stage of plant growth and development. In short, the development of MAS technology has enabled advanced selection resulting in genomics, which has since become an essential component of agricultural science(Kushanov et al., 2021b).

Figure 2

(Kushanov et al., 2021c)This figure implies the types of markers, including morphological and molecular markers.



This figure represents the phylogenetic tree of molecular markers at the morphological and molecular levels.

Genetic Mapping for improved fibre quality

Cotton is the leading natural fibre crop because of its increasing demand in the agricultural market. Fibre length, consistency, strength, elongation, and micronaire value are some of the most common fibre quality characteristics (Wendel et al., 2010). Fibre

length and strength both have an impact on yarn quality. In the textile industry, fibre strength is an important aspect. The micronaire value has a direct effect on fibre processing(Ijaz et al., 2019; Rodgers et al., 2017; X. Yang et al., 2016).

Yield is a complex feature; the lint fraction is the percentage of lint extracted from seed cotton that is positively connected with yield. It has given an indirect and cost-effective method of breeding for increased yield potential, resulting in around 10% increases over the last 60 years. However, lint fraction can be inversely associated with seed size, thereby lowering seedling vigour (Constable et al., 2015).

For improved fibre development, Marker-assisted breeding technology has gained importance because it can locate specific genes with greater efficiency(Tan et al., 2015). Recent advances in molecular breeding have enabled the combination of traditional breeding and biotechnology procedures with the desired output. However, integration of cotton traditional breeding with marker-assisted breeding helped in accelerating breeding with potential results(Morales-Aranibar et al., 2024). Recent investigations revealed that the paternal BC population investigates the genetic mechanism of fibre quality, detecting 19 and 8 QTLs in the BC/P and
populations shared three QTLs for fibre strength and elongation: qFS-Chr21-2, qFE- Chr2-3, and qFE-Chr3-1. This demonstrates isolating novel elite alleles of the male father for fibre quality(L. MA et al., 2020).

Another study identified ninety-one QTL loci associated with fibre quality indicators in a BC5S5 upland population of 107 lines. Their PEV ranged from 4.53% to
20.92. Among them, the favourable alleles of QTLs qFS-A02-1 and qSCI-A02-1
discovered in stable detection were all from
G. tomentosum, with PVE ranging from 9.8-16.71% and 14.78-20.92%, respectively. This shows that the G. tomentosum significantly improved the fibre quality in upland cotton. Fourteen genes were discovered in the candidate interval, includingGhir_A02G012730, Ghir_A02G012790, and Ghir_A02G012830, which are involved in cellulose and cell wall production and have a reasonably high expression during fibre formation (Chang et al., 2023). Furthermore, Crisper/CAS can also be used to validate the results and alleles. Cotton fibre quality has been successfully improved through the introduction of foreign genes pertinent to fibre manufacturing. However, more research is needed in addition to the advanced methodologies previously stated(Ahmed et al., 2020). Discovering and integrating more distant fibre-related genes into cotton can increase fibre characteristics even further. Understanding the molecular foundation of diverse fibre creation mechanisms necessitates additional research to improve
fibre features(Baghyalakshmi et al., 2024).

Genetic Mapping for stress resistance

Extensive research has been conducted in cotton production and quality over the last few years using traditional breeding. However, this has increased susceptibility
BC/M populations, respectively. Both BC against stresses(Maqbool et al., 2010). Stress
tolerance is influenced by genetic as well as environmental factors, which are lacking in conventional breeding due to genetic diversity(H. Sun et al., 2019). Abiotic stress typically includes drought, heat, salinity and cold stress. In addition to enhancing yield, quality, and resistance to diseases and insects, it also pays more attention to tolerance to stresses and efficient use of soil resources(Abdelraheem et al., 2021; Diouf et al., 2017). Besides abiotic stress, cotton is highly susceptible to Cotton leaf curl virus, which is a biotic stress.

To address these issues, the meta- analysis program Biomercator was used by researchers to examine 661 stress resistance QTLs. This included QTL for drought tolerance in a greenhouse and field conditions, salt tolerance in a greenhouse, resistance to Verticillium wilt, resistance to Fusarium wilt, and resistance to uniform nematodes and root-
knot nematodes(Abdelraheem et al., 2017). Recent breakthroughs in functional genomics, genetic and analytical methods, particularly complete gene expression profiling of cotton fibre

cells, along with the availability of a sequenced genome, have opened new avenues for improving cotton fibre properties through genetic manipulation. Several fibre-specific genes involved in fibre cell initiation, elongation, or cell wall biosynthesis have been identified as possibilities for genetic manipulation to improve fibre yield and/or quality(Walford et al., 2011).

Another study on the investigation on drought stress uses a total of 1,116 SNPs and 782 SSRs. In which nineteen QTLs were found in one chromosome 3, 4, 5, 7, 8, 12,

13, 15, and 26 for plant morphological features. This mapping approach identified one QTL hotspot on chromosome 8 using public domain mapping data. These findings suggest candidate alleles for

drought tolerance in upland cotton, which can be used to produce cotton varieties with stress resilience through marker- assisted selection (MAS) breeding programs(Shukla et al., 2021).

In agriculture, biotic factors are a major cause of large output losses up to 84% for insects and up to 30% for pathogens(Jans et al., 2021). At the same time, attempts to reduce infestations include increasing the internal defensive mechanisms of plants or introducing pathogen-targeted constructs into the genome(Kamburova & Abdurakhmonov, 2018). Classical breeding methods increase the plant's internal defence mechanisms and use cotton germplasm reserves to produce new resistant varieties over time, whereas MAS and QTL mapping have been widely used in the development of cotton varieties resistant to Verticillium and Fusarium wilt. More than 400 QTL indicating resistance to both types of wilt have been found(Kamburova & Abdurakhmonov, 2018; C. Wang et al., 2018a; J. Zhang et al., 2014). These results were achieved by mapping chromosome-substituted and RIL populations using different markers and GWAS. The same meta-analysis showed 74 QTLs for nematode resistance(Kushanov et al., 2021d). Thus, 71 QTLs relate to resistance to root-knot nematode, while three remain associated with resistance to reniform nematodes. Furthermore, this study found two QTLs for resistance to Xanthomonascampestrispv. Malvacearum(C. Wang et al., 2018b). CLcV is one of the most devastating biotic stresses, but we used quantitative trait loci (QTL) mapping in four crosses with different sources of resistance to identify single-nucleotide polymorphism (SNP) markers associated with the resistance trait, allowing for the development of varieties without the need for field screening every

generation. To aid in the analysis of many populations, a new publicly available R/Shiny App was built to simplify genetic mapping utilizing SNP arrays as well as give an easy method to convert and deposit genetic data into the CottonGen database(Schoonmaker et al., 2023).

In comparison to the advancement in cotton resistance is largely sluggish, and there is still a gap in resistance breeding. Future study will be undertaken based on the investigation and evaluation of good resources, the major effect of resistance QTL, and the cloning of excellent key resistance genes, employing marker- assisted selection (MAS) and transgenic technology to polymerize resistance genes(Saud & Wang, 2022).

Future Direction

Genome Editing through CRISPR/Cas

Genome editing plays a crucial role in functional gene studies and crop improvement. The CRISPR/Cas9 uses single guide RNA molecules to control double-strand breaks in the genome sequence, has the potential to revolutionize agriculture (Gao et al., 2017a). The CRISPR/Cas9 technique uses a guide RNA (gRNA) to lead the Cas9 nuclease to a specific genomic sequence, causing a double-strand break (DSB). This break can then be repaired using the cell's natural repair mechanisms, non-homologous end joining (NHEJ) or homology-directed repair (HDR), resulting in specific gene changes. The capacity to construct gRNAs to target almost any sequence in the genome has made CRISPR/Cas9 a very versatile tool for diverse genetic changes in cotton (Thangaraj et al., 2025).

The successful use of the CRISPR/Cas9 system for crop improvement or functional investigation can generate transformed mutants and undertake

phenotypic characterization of homozygous stable

contained in the sgRNA is a critical component influencing the overall mutagenesis efficacy of the CRISPR/Cas9 system(Ma et al., 2015). In cotton, CRISPR/Cas9 has been used to improve a variety of agronomically significant properties. For example, researchers employed this technology to improve fibre quality by targeting genes involved in fibre growth, resulting in longer and stronger fibres (C. Li et al., 2017). CRISPR/Cas9 has also been used to improve resistance to biotic stressors like pests and diseases by removing susceptibility genes or increasing defence-related genes(Gao et al., 2017b). Furthermore, CRISPR/Cas9 has been used to improve abiotic stress tolerance, such as drought and salinity, by editing genes that regulate stress responses, increasing cotton's endurance to harsh environmental conditions(X. Chen et al., 2017).

The successful implementation of the CRISPR/Cas9 system for crop improvement or functional analysis is dependent on the creation of stably transformed mutants in order to characterization of homozygous stable mutants. The sequence of the target site contained in the sgRNA is an important factor affecting the overall mutagenic efficiency of the CRISPR/Cas9 system, as different sgRNAs can result in very different efficiencies when targeting the same gene (Ma et al., 2016).

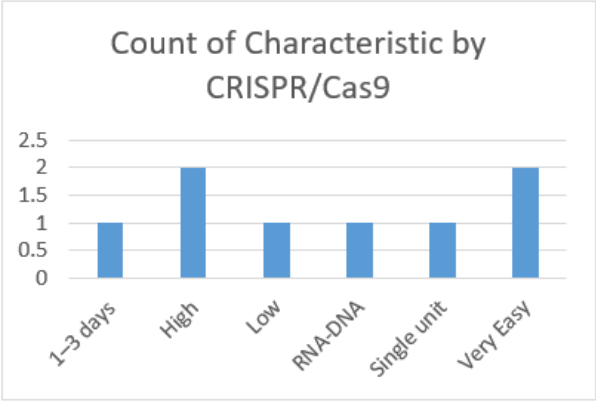


Figure 3

mutants. The sequence of the target site (Khan et al., 2023) This diagram depicts the three gene- editing technologies. CRISPR/Cas9 is the simplest andquickest to design and construct, whereas ZFNs are the most complicated and expensive. CRISPR/Cas9, on the other hand, works as a single unit.

High-Throughput Markers

The high-quality phenotypic data are crucial for enhanced cotton yield. This study reveals that HTP offers a lot of potential for data collection and analysis while assessing phenotypic traits in cotton in the form of cheaper input costs and resources(Bolouri et al., 2024). Imaging and sensor technologies based on spectral, thermal, fluorescence, and 3D sensors are the most useful and powerful tools for evaluating crop characteristics, tracking crop growth and development, and assessing cotton health. With the emergence of HTP technologies, several ground and aerial-based platform systems for phenotypic and agronomic research in cotton have been built(Pabuayon et al., 2019).

The high-throughput phenotyping technology developed in the field reconstructed precise 3D surface models. Multiple morphological parameters at the plot level, such as plant height, projected canopy area, and plant volume, were retrieved concurrently. Because of its relatively large data collection and processing capacity, the device aids in the repetitive scanning of the field. The measured morphological features had the highest correlation with eventual yield between 67 and 109 DAP. Further research will focus on using additional sensor data to derive more phenotypic features from the 3D point cloud. Although this method was only tested on cotton plants, it has proven to be a successful application(S. Sun et al., 2018).

Genomic Breeding

Cotton genomic sequencing has made significant advancements in recent years, with multiple high-quality reference genomes for G. hirsutum and G. barbadense. This research has enabled the researchers to identify crucial genes involved in fibre growth, insect resistance, and stress tolerance in cotton(Collard & Mackill, 2008b). Furthermore, genomic approaches such as molecular markers and gene editing technologies are becoming more common for accurate cotton breeding. However, in the face of several challenges, including ongoing global growth, complex environmental conditions, and decreasing genetic gain effects of breeding new cotton varieties, the cotton research community must urgently rethink and design the future of cotton breeding(Kun et al., 2025). Also, genomic selection, another tool for estimating GEBV, has proven application in testing individuals because it is not dependent on the late measurement phenotype, which significantly reduces the generation interval. It estimates GEBV using marker information from the entire genome, which considerably enhances its accuracy. Furthermore, genomic selection can estimate some features that are difficult to quantify phenotypically (Zhai, 2023).

Digital Technologies

Cotton must continue its fibre market leadership as the most abundant and natural fibre of choice for a wide range of industrial and commercial applications. To do this, cotton production, which has traditionally been input-intensive and has an indelible negative environmental impact, must be continuously refined to use the fewest inputs while optimizing yield, fibre quality, and profit using existing and developing technology. To fully benefit from new advanced techniques and technologies as they are developed globally, these enabling tools

(such as variety breeding, improved irrigation systems/biodegradable mulching, autonomous aerial systems, computer vision/agricultural remote sensing techniques, robotic harvesters and multipurpose platforms, HDPT, and chemical topping) for cotton agronomy optimization must be continuously improved upon. Some of these tools have been thoroughly studied and commercialized in the global cotton industry (Adeleke, 2024).

Conclusion

Cotton is an important cash crop which plays a pivotal role in textile production, and agriculture is subject to many external factors. Changing climatic conditions worsen the situation of cotton development. Both biotic and abiotic stress negatively affected the increasing demand for high-quality cotton. Traditional breeding methods have less genetic diversity, thus leading to a decline in cotton innovation. Integration of marker-assisted breeding and genetic mapping techniques can revolutionize cotton breeding by identifying QTLs which can improve desired traits. Recent studies have also found genes and markers which can directly locate the desired trait. However, integrating conventional breeding through genome editing, high-throughput markers, and molecular breeding designs, many of the constraints of traditional breeding may be overcome for the new era of cotton assisted with cotton.

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