

Cytogenetic Markers and Their Importance in *Gossypium* Breeding Programs

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Abstract The primary goal of this study is to explore the significance of cytogenetic markers in the breeding programs of *Gossypium* species, particularly focusing on their application in enhancing genetic diversity, improving fiber quality, and increasing yield. The study highlights several key findings from recent research. Simple Sequence Repeat (SSR) markers have been effectively used to analyze genetic diversity and DNA fingerprinting in *Gossypium hirsutum*, revealing significant polymorphisms and genetic variations among cultivars. High-density genetic maps constructed using various molecular markers, such as SSRs, SNPs, and others, have facilitated the identification of numerous quantitative trait loci (QTLs) associated with important agronomic traits, including fiber quality and yield. The integration of advanced genomic techniques, such as single-molecule real-time sequencing and high-throughput chromosome conformation capture, has led to the development of reference-grade genome assemblies for *Gossypium* species, providing deeper insights into cotton evolution and aiding in the identification of loci associated with superior fiber quality. Additionally, marker-assisted selection (MAS) and genome-wide association studies (GWAS) have been instrumental in identifying elite alleles and stable QTLs, which are crucial for modern cotton breeding programs. The findings underscore the critical role of cytogenetic markers in *Gossypium* breeding programs. These markers not only enhance our understanding of genetic diversity and genome structure but also enable the precise identification of valuable traits for cotton improvement. The integration of these markers into breeding strategies promises to accelerate the development of high-yielding, superior fiber quality cotton cultivars, thereby meeting the demands of the textile industry and contributing to sustainable agricultural practices.

Keywords *Gossypium*; Cytogenetic markers; SSR markers; Genetic diversity; QTL mapping; Fiber quality; Marker-assisted selection; Genome sequencing

1 Introduction

Gossypium, commonly known as cotton, is a genus of flowering plants that holds significant economic importance due to its fiber, which is a primary raw material for the textile industry. The two most widely cultivated species, *Gossypium hirsutum* and *Gossypium barbadense*, are known for their high yield and superior fiber quality, respectively. Breeding programs in *Gossypium* aim to combine these desirable traits to enhance both yield and fiber quality. Traditional breeding methods, while effective, are time-consuming and labor-intensive. The advent of molecular markers has revolutionized plant breeding by enabling marker-assisted selection (MAS), which accelerates the breeding process and increases precision (He et al., 2007; Han et al., 2006).

Cytogenetic markers, including simple sequence repeats (SSRs), sequence-related amplified polymorphism (SRAP), random amplified polymorphic DNA (RAPD), and retrotransposon-microsatellite amplified polymorphism (REMAP), play a crucial role in the genetic mapping and marker-assisted breeding of *Gossypium*. These markers facilitate the identification and mapping of quantitative trait loci (QTLs) associated with economically important traits such as lint yield, fiber strength, and seed index. For instance, a high-density molecular marker linkage map constructed using SSRs, SRAPs, RAPDs, and REMAPs has been instrumental in detecting 52 distinct QTLs related to various phenotypic traits in cotton (He et al., 2007). Additionally, the development of EST-SSR markers from expressed sequence tags (ESTs) has further enriched the genetic maps, aiding in the identification of QTLs and comparative genomics studies (Han et al., 2006).

This study aims to provide a comprehensive overview of the role and importance of cytogenetic markers in *Gossypium* breeding programs. It will cover the development and application of various molecular markers, their integration into genetic maps, and their utility in identifying QTLs for key agronomic traits. By synthesizing findings from recent studies, this study will highlight the advancements in marker-assisted selection and its impact on improving cotton breeding efficiency and effectiveness. The scope of this study includes an analysis of the current state of cytogenetic marker research in *Gossypium*, the challenges faced, and future directions for enhancing marker-assisted breeding strategies.

2 Overview of Cytogenetic Markers

2.1 Definition and types of cytogenetic markers

Cytogenetic markers are specific sequences of DNA that can be visualized and identified on chromosomes using various cytogenetic techniques. These markers are essential tools in genetic studies as they help in the identification and characterization of chromosomal structures and abnormalities. The primary types of cytogenetic markers include:

Simple Sequence Repeats (SSRs): These are short, repetitive DNA sequences that are highly polymorphic and widely used in genetic mapping and diversity studies. SSRs are user-friendly and cost-effective markers.

Single Nucleotide Polymorphisms (SNPs): These are single base-pair variations in the DNA sequence that occur at specific loci. SNPs are valuable for high-resolution genetic mapping and association studies (Zhang et al., 2019).

Ribosomal DNA (rDNA) Markers: These include 18S and 5S rDNA sequences, which are used to study the organization and evolution of ribosomal genes and their chromosomal locations (Goes et al., 2020).

2.2 Historical development of cytogenetic techniques

The field of cytogenetics has evolved significantly over the years, with several key milestones:

Early Chromosome Staining: Initial cytogenetic studies involved simple staining techniques to visualize chromosomes and identify their number and structure.

Banding Techniques: The development of banding techniques, such as G-banding and C-banding, allowed for the detailed analysis of chromosomal regions and the identification of specific chromosomal abnormalities.

Fluorescence In Situ Hybridization (FISH): This technique enabled the precise localization of DNA sequences on chromosomes using fluorescent probes, revolutionizing the field of cytogenetics.

Molecular Cytogenetics: The integration of molecular biology techniques, such as PCR and sequencing, with traditional cytogenetic methods has led to the development of advanced markers like SSRs and SNPs, enhancing the resolution and accuracy of genetic studies (Kushanov et al., 2022).

2.3 Advances in cytogenetic marker technologies

Recent advancements in cytogenetic marker technologies have significantly improved the efficiency and effectiveness of genetic studies in *Gossypium* breeding programs:

Genotyping-by-Sequencing (GBS): This high-throughput technique allows for the simultaneous discovery and genotyping of thousands of SNPs across the genome. GBS has been used to uncover novel genetic relationships and domestication footprints in *Gossypium hirsutum*, providing valuable insights for breeding programs (Zhang et al., 2019).

In Silico Analysis: The use of computational tools to design and evaluate markers, such as SSRs, has streamlined the process of marker development. For instance, the MlCroSatellite identification tool was used to develop genome-wide SSR markers in *Gossypium barbadense*, facilitating genetic linkage mapping and diversity analyses.

Marker-Assisted Selection (MAS): The identification of quantitative trait loci (QTLs) and candidate genes associated with important traits, such as photoperiod insensitivity, has enabled the use of DNA markers in MAS. This approach accelerates the breeding process by allowing the selection of desirable traits at the molecular level (Kushanov et al., 2022).

These advancements highlight the critical role of cytogenetic markers in enhancing the efficiency and precision of *Gossypium* breeding programs, ultimately contributing to the development of superior cotton varieties.

3 Cytogenetic Markers in *Gossypium*

3.1 Chromosomal structure and karyotyping in *Gossypium*

Chromosomal structure and karyotyping are fundamental in understanding the genetic makeup and diversity within *Gossypium* species. Karyotyping involves the examination of chromosome number and structure, which is crucial for identifying genetic variations and abnormalities. For instance, the study of early-maturing upland cotton (*Gossypium hirsutum* L.) using Simple Sequence Repeat (SSR) markers revealed significant polymorphisms and genetic diversity among different cultivars, which is essential for breeding programs aimed at improving cotton varieties (Kuang et al., 2022). Additionally, the use of cytogenetic markers such as 18S and 5S rDNA genes has been instrumental in characterizing hybrids and understanding karyotypic diversity in other species, which can be applied to *Gossypium* as well (Goes et al., 2020).

3.2 Fluorescent in situ hybridization (FISH) in cotton

Fluorescent In Situ Hybridization (FISH) is a powerful technique used to identify specific chromosomes and their rearrangements. This method has been successfully applied in various species to study karyotype evolution and chromosomal organization. For example, FISH has been used to identify and map the distribution of histone H3 genes in Lepidoptera, providing insights into the stable organization of genomes (Provazníková et al., 2021). In *Gossypium*, FISH can be utilized to map important genetic markers and understand chromosomal behavior during hybridization and breeding processes. The application of FISH in cotton breeding programs can enhance the identification of desirable traits and accelerate the development of improved cotton varieties.

3.3 Use of molecular cytogenetics in cotton genome mapping

Molecular cytogenetics involves the use of molecular markers to map the genome and identify quantitative trait loci (QTL) associated with important agronomic traits. In cotton, various molecular markers such as RFLP, AFLP, RAPD, SSR, EST-SSR, and SNP have been employed to map genes controlling traits like fiber quality, drought tolerance, and yield (Shukla et al., 2021). The identification of tightly linked molecular markers with high predictive trait values is crucial for successful QTL analysis and breeding programs. For instance, SSR markers have been used to explore genetic diversity and perform DNA fingerprinting in *Gossypium hirsutum*, providing valuable information for future breeding efforts (Kuang et al., 2022). The integration of molecular cytogenetics in cotton genome mapping facilitates the precise identification of genetic loci and accelerates the breeding of superior cotton varieties.

4 Applications of Cytogenetic Markers in *Gossypium* Breeding

4.1 Genetic diversity and germplasm characterization

Cytogenetic markers play a crucial role in assessing genetic diversity and characterizing germplasm in *Gossypium* species. By utilizing these markers, researchers can identify and catalog the genetic variations present within and between different cotton species. This information is vital for the conservation of genetic resources and for the selection of parent lines in breeding programs. For instance, the study by (Kushanov et al., 2021) highlights the use of molecular markers to explore the genetic diversity within the *Gossypium* genus, which aids in the creation of high-yielding cultivars with superior fiber quality and stress adaptation (Figure 1).

Kushanov et al. (2021) compares conventional breeding with marker-assisted selection (MAS). Conventional breeding relies on phenotypic selection, which is challenging to automate, cannot distinguish between heterozygotes and dominant homozygotes, and is influenced by environmental factors. MAS, on the other hand, utilizes genotypic selection through DNA markers, enabling almost complete automation, accurate identification

4.4 Enhancing fiber quality traits

Improving fiber quality traits such as fiber length, strength, and uniformity is a primary goal in cotton breeding. Cytogenetic markers enable the precise mapping of QTLs associated with these traits, allowing breeders to select for superior fiber quality more effectively. Studies like Chen et al. (2018) and Fan et al. (2018) have identified specific QTLs and candidate genes that contribute to enhanced fiber quality in *Gossypium* species. These findings are crucial for developing cotton varieties that meet the high standards of the textile industry. Furthermore, Ijaz et al. (2019) and Li et al. (2018) discuss the integration of multi-omics approaches with QTL mapping to dissect the genetic control of fiber quality traits, providing a comprehensive strategy for fiber quality improvement.

By leveraging cytogenetic markers, *Gossypium* breeding programs can achieve significant advancements in genetic diversity assessment, marker-assisted selection, disease resistance, and fiber quality enhancement, ultimately leading to the development of superior cotton varieties.

5 Case Studies and Success Stories

5.1 Successful integration of cytogenetic markers in breeding programs

The integration of cytogenetic markers has significantly advanced cotton breeding programs, particularly in improving fiber quality and disease resistance. For instance, the identification and utilization of quantitative trait loci (QTLs) for fiber quality traits such as fiber length, strength, and elongation have been pivotal. In one study, introgressed alleles from *Gossypium barbadense* were mapped in *G. hirsutum*, leading to the detection of multiple QTLs associated with superior fiber quality without negatively impacting lint yield (Chen et al., 2018). Similarly, the development of functional markers through kompetitive allele-specific PCR (KASP) assays has enabled the high-throughput selection of superior cotton varieties with enhanced fiber length and strength (Li et al., 2022).

5.2 Case study: cytogenetic mapping of fiber length genes

A notable case study involves the cytogenetic mapping of fiber length genes in *Gossypium hirsutum*. Researchers developed functional markers (FMs) for key genes underpinning fiber length and strength using KASP assays. These markers were validated across 360 cotton accessions, demonstrating their efficacy in differentiating genotypes with superior fiber traits. The study identified two FMs, D11_24030087 and A07_72204443, which were highly consistent with phenotypic variations in fiber length and strength. This successful mapping and marker development have provided valuable tools for marker-assisted selection (MAS) in cotton breeding programs, facilitating the production of varieties with longer and stronger fibers (Li et al., 2022).

5.3 Case study: development of disease-resistant cotton varieties

The development of disease-resistant cotton varieties has also benefited from the use of cytogenetic markers. For example, a study on *Verticillium wilt* resistance in *Gossypium hirsutum* utilized association mapping to identify genetic markers linked to disease resistance. The research identified several single nucleotide polymorphism (SNP) markers associated with resistance to different pathotypes of *Verticillium dahliae*. These markers were located on genes known to be involved in biotic and abiotic stress responses, providing a genetic basis for developing *Verticillium wilt*-resistant cotton varieties through MAS (Bardak et al., 2021).

5.4 Case study: introgression of wild cotton traits

Another success story is the introgression of desirable traits from wild cotton species into cultivated varieties. A study involving *Gossypium thurberi* and *Gossypium trilobum* developed a genetic map using simple sequence repeat (SSR) markers. This map facilitated the identification of genes within segregating distortion regions (SDRs) that are crucial for fiber development. The introgression of these genes into cultivated cotton has the potential to enhance fiber quality and stress resistance, demonstrating the value of wild species in cotton breeding programs (Figure 2) (Li et al., 2018).

Li et al. (2018) presents genetic maps of the 13 chromosomes in the F2 progeny from *G. thurberi* and *G. trilobum* crosses. Markers in blue indicate distorted markers, while those in red highlight regions with significant segregation distortion (SDRs). The study used 849 polymorphic markers to construct a linkage map spanning 1,012.458 cM with an average marker distance of 1.193 cM. Chr09 had the highest marker density (93 markers),

while Chr02 had the fewest (21 markers). The longest chromosome was Chr12 (103.563 cM), and the shortest was Chr02 (28.665 cM). Notably, 15.783% of the markers deviated from the Mendelian ratio, with Chr07 and Chr11 having the most distorted loci. Eight SDRs were identified, with large clusters of segregation distortions on Chr02, Chr06, Chr07, and Chr11, skewed towards heterozygous alleles. This comprehensive mapping provides valuable insights for understanding genetic inheritance and potential regions of interest for further breeding programs.

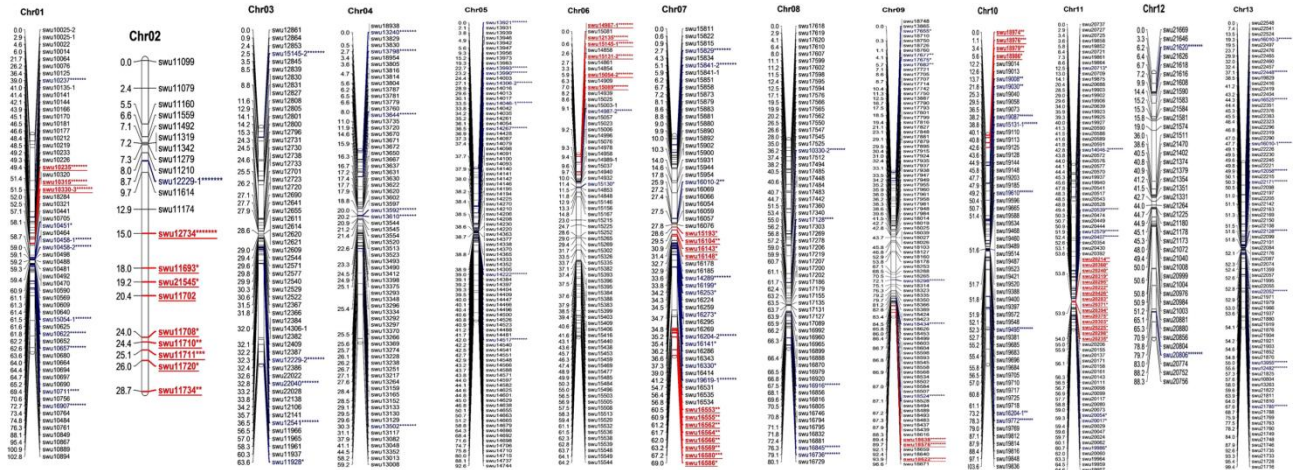


Figure 2 Genetic maps for the 13 chromosomes of the F₂ interspecific individuals derived between *G. thurberi* and *G. trilobum* (Adopted from Li et al., 2018)

Image caption: The markers in blue are distorted while markers in red and underlined indicates the distorted regions per chromosomes (Adopted from Li et al., 2018)

5.5 Case study: QTL mapping for fiber quality and yield

QTL mapping has been instrumental in improving both fiber quality and yield in cotton. A study on introgression lines derived from *Gossypium hirsutum* and *G. tomentosum* identified 74 QTLs associated with fiber quality and yield traits. These QTLs were distributed across multiple chromosomes, with significant positive additive effects observed for both fiber and yield traits. The identified QTLs and developed introgression lines provide a valuable resource for molecular breeding aimed at enhancing fiber quality and yield in Upland cotton (Keerio et al., 2018).

6 Challenges and Future Directions

6.1 Technical challenges in cytogenetic marker application

The application of cytogenetic markers in *Gossypium* breeding programs faces several technical challenges. One significant issue is the complexity of the cotton genome, which includes a high level of repetitive DNA sequences such as tandem repeats (TRs) and satellite DNA (satDNA). These repetitive elements can complicate the identification and mapping of specific markers (Kroupin et al., 2019). Additionally, the presence of cryptic species and species complexes within the *Gossypium* genus can lead to difficulties in establishing common genetic pools, further complicating the use of cytogenetic markers for hybrid identification and breeding. The variability in heterochromatin distribution among different strains and hybrids also poses a challenge, as it can affect the consistency and reliability of marker-based identification (Goes et al., 2020).

6.2 Integration with genomic and bioinformatic tools

To overcome these technical challenges, the integration of cytogenetic markers with advanced genomic and bioinformatic tools is essential. Recent advancements in whole-genome sequencing and bioinformatics have enabled the rapid and cost-effective identification of TRs and satDNA, which can be converted into molecular cytogenetic markers. The development of pipelines for high-throughput screening and the use of quantitative PCR (qPCR) for preliminary estimation of TR abundance facilitate the selection of the most abundant and prospective TRs for marker development. Furthermore, the use of fluorescence in situ hybridization (FISH) with probes prepared through PCR amplification of TR units can enhance the accuracy and efficiency of chromosome karyotyping in *Gossypium* species (Kroupin et al., 2019).

6.3 Future prospects and emerging technologies

Looking ahead, the future of cytogenetic marker application in *Gossypium* breeding programs appears promising with the advent of emerging technologies. The continuous improvement of whole-genome sequencing techniques and bioinformatics tools will likely lead to the discovery of new and more efficient cytogenetic markers. Additionally, the development of more sophisticated pipelines for marker screening and probe preparation will further streamline the process, making it more accessible and practical for breeding programs (Kroupin et al., 2019). The integration of cytogenetic markers with other genomic tools, such as CRISPR/Cas9 for genome editing, could also open new avenues for precise and targeted breeding strategies. As these technologies evolve, they will undoubtedly enhance our ability to utilize cytogenetic markers for the improvement of *Gossypium* species, ultimately contributing to more robust and productive cotton breeding programs.

7 Concluding Remarks

Cytogenetic markers have proven to be invaluable tools in the study and improvement of *Gossypium* species. High-density genetic maps constructed using single nucleotide polymorphism (SNP) markers have facilitated the identification of quantitative trait loci (QTLs) associated with fiber quality and lint yield traits in *Gossypium barbadense* (Fan et al., 2018). Similarly, the use of SSR markers has enabled the mapping of genes related to flowering traits in *Gossypium hirsutum*, providing insights into the genetic basis of photoperiod insensitivity (Kushanov et al., 2022). The integration of advanced sequencing technologies has led to the development of reference-grade genome assemblies for *Gossypium hirsutum* and *Gossypium barbadense*, which have identified extensive structural variations and QTLs associated with superior fiber quality (Wang et al., 2019). Additionally, introgression lines derived from interspecific crosses have been instrumental in identifying QTLs for fiber quality and yield traits, highlighting the potential of wild *Gossypium* species in breeding programs (Keerio et al., 2018; Feng et al., 2021).

Cytogenetic markers are crucial for the future of *Gossypium* breeding programs. They enable the precise identification and mapping of genes associated with desirable traits, such as fiber quality and yield, which are essential for the development of superior cotton varieties. The use of high-density genetic maps and advanced sequencing technologies allows for the fine mapping of QTLs and the prediction of candidate genes, facilitating marker-assisted selection (MAS) (Fan et al., 2018; Wang et al., 2019). Moreover, the identification of structural variations and introgression of favorable chromosome segments from wild species can enhance the genetic diversity of cultivated cotton, leading to improved agronomic traits (Wang et al., 2019; Feng et al., 2021). The application of cytogenetic markers in breeding programs can accelerate the development of cotton varieties with enhanced fiber quality, yield, and other agronomic traits, ultimately benefiting the cotton industry.

Researchers and breeders should continue to leverage cytogenetic markers in their efforts to improve *Gossypium* species. It is recommended to:

- (1) Expand the Use of Advanced Sequencing Technologies: Utilize technologies such as single-molecule real-time sequencing and high-throughput chromosome conformation capture to develop more comprehensive and accurate genetic maps (Wang et al., 2019).
- (2) Focus on Marker-Assisted Selection (MAS): Implement MAS in breeding programs to efficiently select for desirable traits based on identified QTLs and candidate genes (Fan et al., 2018; Kushanov et al., 2022).
- (3) Explore Wild *Gossypium* Species: Investigate the genetic potential of wild species and develop introgression lines to incorporate beneficial traits into cultivated varieties (Keerio et al., 2018; Feng et al., 2021).
- (4) Conduct Functional Analyses: Perform functional analyses of candidate genes associated with important traits to validate their roles and enhance the precision of breeding efforts (Kushanov et al., 2022).
- (5) Collaborate Across Disciplines: Foster collaborations between geneticists, breeders, and agronomists to integrate cytogenetic findings into practical breeding strategies effectively.

By following these recommendations, researchers and breeders can harness the full potential of cytogenetic markers to drive the development of superior cotton varieties, ensuring the sustainability and competitiveness of the cotton industry.

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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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