



Cotton Genetic Resilience: Cytogenetic Tools for Biotic and Abiotic Stress Mitigation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: <https://doi.org/10.9734/ijpss/2025/v37i35346>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://pr.sdiarticle5.com/review-history/132278>

Review Article

Received: 01/01/2025

Accepted: 04/03/2025

Published: 13/03/2025

ABSTRACT

Cotton (*Gossypium* spp.) holds immense significance in the global economy due to its prized fiber, produced from the epidermal cells of the ovule. Among the approximately fifty cotton species, *Gossypium arboreum*, *Gossypium herbaceum*, *Gossypium hirsutum*, and *Gossypium barbadense* are primarily cultivated for fiber production. Of these, *Gossypium hirsutum* (Upland cotton) dominates global production, contributing to over 90% of the cotton used worldwide. Advancements in cotton cytogenetics have greatly enhanced fiber yield and quality, driven by the adoption of modern genomic and genetic engineering techniques. Innovations like CRISPR-Cas9, RNA interference (RNAi), and marker-assisted selection have improved cotton's tolerance to biotic and abiotic stresses, including pests and drought. These technologies have also enabled the refinement

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of fiber properties through epigenetic modifications. The development of male sterility systems has further streamlined breeding programs, accelerating genetic improvements in cotton. This analysis highlights the pivotal role of advanced cytogenetic and biotechnological tools in fostering the sustainable growth of cotton. The integration of these cutting-edge techniques marks a transformative era in cotton breeding, with promising prospects for improving fiber quality, enhancing stress resistance, and boosting overall crop performance. These advancements solidify cotton's vital role in global agriculture and its potential for future innovation.

Keywords: *Cotton cytogenetics; CRISPR-Cas9; genetic engineering; marker-assisted selection; RNA interference; upland cotton.*

1. INTRODUCTION

Cotton (*Gossypium* spp.), a cornerstone of the textile industry and a source of livelihood for millions of farmers globally, stands as one of the most significant fiber crops. However, its productivity is severely challenged by various biotic and abiotic stressors, including pests, diseases, drought, and soil salinity (Ahmad et al., 2023). To address these persistent threats, the development of genetically resilient cotton varieties has become essential. While traditional breeding methods have played a crucial role in cotton improvement, their effectiveness is often limited by the narrow genetic diversity within cultivated species and the complex nature of stress tolerance traits. (Wendel, et al., 2003; Hulse-Kemp et al., 2015).

In response to these limitations, cytogenetic tools have emerged as powerful approaches to enhance the genetic resilience of cotton. These techniques enable the transfer of beneficial traits for stress tolerance, leading to improvements in both fiber quality and crop yield. By integrating cytogenetics into cotton breeding programs, the potential to develop robust and high-performing cultivars is significantly increased, addressing the challenges posed by environmental and biological stresses (Alishah & Ahmadikhah, 2009).

Cotton is a staple crop in both the food and textile industries, making it a staple crop worldwide (Bansal et al., 2014). The textile industry, which makes comfortable, fashionable, and culturally expressive clothing possible, depends on its fibers to improve human existence. Apart from its primary usage in the textile industry, cottonseed is a significant source of vegetable oil that is widely used in mayonnaise, baking fats, and baking a range of snack foods (Bassine et al., 2023). Cotton is so adaptable that its oil extraction by product is utilized in animal feed, fertilizers, and even the

paper industry (Nadeem et al., 2024). Across more than 100 nations, 36 million hectares are used to grow cotton. (Li, et al., 2022).

Cytogenetics, which focuses on the structure, function, and behavior of chromosomes, has played a vital role in understanding the genetic makeup of cotton and in developing varieties that are more resistant to stress (Dong & Liu, 2014). Cotton has a highly intricate genome, including both diploid and tetraploid species with notable structural diversity. Cutting-edge cytogenetic techniques such as FISH, GISH, polyploidization, and chromosome engineering have been pivotal in enhancing cotton's stress tolerance. These methods allow for the precise identification of genetic differences, incorporation of beneficial traits from wild species, and manipulation of chromosomes to improve resilience (Dhunde et al., 2022).

Cotton's genetic resilience to biotic and abiotic stresses is essential for ensuring sustainable cotton production in the face of global concerns including pest pressure, climate change, and limiting natural resources (Campbell et al., 2010; Khan et al., 2023). Cytogenetic techniques, which involve the investigation and alteration of chromosomal structure, function, and behavior in relation to genetic and environmental factors, have been demonstrated to significantly increase cotton's resilience (Dhunde, 2022). This introduction explains how cotton varieties that are more resistant to various stresses may be produced by cytogenetic approaches, safeguarding fiber quality, yield, and the overall financial viability of cotton farming (Bayramova et al., 2024).

The ability to precisely identify and manipulate cotton genes important for drought, heat, salt, and other adverse environmental conditions (abiotic stressors) as well as resistance to pests and diseases (biotic stressors) has been made possible by advances in cytogenetics (Kulkarni et

al., 2024). Thanks to methods like chromosomal mapping, genomic selection, and gene editing (like CRISPR-Cas9), scientists are now able to identify and alter particular genetic components, promoting the growth of cotton cultivars that can withstand harsh environments (Grover & Wendel, 2010).

CS lines generated via hypoaneuploidy-based backcrossing with "TM-1" as the recurrent parent were used in this study to examine particular reproductive and physiological parameters associated with yield. The study examined how well CS lines with genetic makeup derived from *G. barbadense* and *G. tomentosum* performed when integrated into the TM-1 background. It evaluated how these lines responded to heat and drought stress in terms of leaf gas exchange, pigment content, biophysical characteristics, and reproductive traits in real-world settings (Xu & Chen, 2024). The CS lines were divided into groups based on how well they tolerated these pressures, as determined by a variety of vegetative, physiological, and reproductive parameters. The objective was to identify CS lines that would be beneficial for future cotton breeding initiatives aimed at overcoming the critical obstacles of high temperature and drought in sustainable cotton production by correlating the observed phenotypes with certain exotic chromosomal alterations (Reddy, et al., 2020; Sardar, 2023).

The study explores how abiotic factors, such as heat and drought, affect cotton in complex ways. Previous research has shown that in cotton substitution (CS) lines, chromosomes and chromosomal segments obtained from wild species and an adapted germplasm are associated with unique characteristics. These CS lines haven't, however, been examined for reproductive and physiological characteristics that could enhance field performance. The ultimate goal is to find genetic polymorphisms that might lower yield losses in normal cotton-growing settings by screening novel germplasm and breeding lines for abiotic stress resistance. The goal of genetic enhancements is to supplement stress-reduction techniques used in agriculture, stabilizing cotton production in the face of more erratic weather patterns. Since the CS lines are true-breeding and primarily derived from Upland cotton, those that exhibit stress resilience can be easily incorporated into breeding programs aimed at sustainability and mitigating the effects of climate change, which is

predicted to result in an increase in the frequency of heatwaves and drought conditions.

2. GENETIC CLASSIFICATION AND ANCESTRY OF *Gossypium hirsutum*

The *Gossypium* genus, comprising approximately 50 species, thrives in tropical and subtropical regions and is classified into eight diploid genome groups (A-G, K) based on chromosomal pairing similarities. Species with B and F genomes are native to Africa, while A-genome species are predominantly found in Asia and Africa (Abdurakhmonov et al., 2016, 2008). In contrast, C-, G-, and K-genome species are unique to Australia, E-genome species are located in Arabia, and D-genome species are distributed across Central and South America. This wide geographic distribution highlights the remarkable adaptability of *Gossypium*. Although molecular phylogenetic research has enhanced our understanding of evolutionary relationships within the genus, certain complexities in genome evolution remain unresolved (Grover & Wendel, 2010; Yadav et al., 2023).

Among the genus, five well-known allotetraploid species are recognized: *Gossypium hirsutum*, *G. barbadense*, *G. tomentosum*, *G. darwinii*, and *G. mustelinum*. More recently, *G. ekmanianum* and *G. stephensii* were identified, with the latter discovered at Wake Atoll in the Pacific. These allotetraploid species, collectively referred to as New World cottons, are predominantly found in seasonally dry areas of the Americas and nearby islands, reflecting their ability to disperse over large distances. For example, *G. ekmanianum* is native to the Dominican Republic, while *G. mustelinum* is confined to Brazil. The overlapping distributions of *G. barbadense* and *G. hirsutum* in the Caribbean and northwest South America further exemplify the dynamic habitats of these species.

Allotetraploid cotton species have 13 pairs of homologous chromosomes ($2n = 4x = 52$) and exhibit disomic pairing. Around 1-2 million years ago, an A-genome ancestor from the Old World hybridized with a D-genome ancestor from the New World, resulting in a polyploidization event that gave rise to the allotetraploids (Wendel et al., 2003). This event produced the At and Dt sub genomes, which are derived from diploid ancestors similar to *G. arboreum* and *G. raimondii*, respectively. Comparative genomic studies suggest that these progenitors shared at least nine chromosomal rearrangements before

diverging from a common ancestor 5-7 million years ago (Abdurakhmonov, & Yu, 2016).

Recent advancements in genome sequencing and cytogenetic mapping have illuminated the structural dynamics of the At and Dt sub genomes (Abdurakhmonov & Yu, 2016). Studies have identified two reciprocal translocations and multiple inversions, revealing strong gene collinearity with minimal structural changes following polyploidization. These findings underscore the evolutionary complexity of *Gossypium* species, tracing their development from diploid ancestors to the modern allotetraploids that are critical to contemporary cotton production (Chen, et al., 2010).

2.1 Cytogenetic Tools for Cotton Improvement

In cotton breeding, cytogenetic technologies facilitate gene transfer, chromosomal engineering, and genome analysis. Genetic diversity, stress tolerance, and fiber quality are improved by methods like as chromosomal

substitution, polyploidization, and Fluorescence In Situ Hybridization (FISH). By speeding up breeding initiatives, these instruments guarantee cotton's tolerance to biotic and abiotic challenges and sustainable improvement.

2.2 In Situ Hybridization in Cotton Improvement

Finding particular DNA or RNA sequences on chromosomes can be done cytogenetically using in situ hybridization (ISH). In cotton breeding, methods such as GISH and FISH are essential because they allow for the transfer of desired traits to increase stress tolerance and the discovery of genetic variants. (Wendel & Cronn, 2003).

An effective cytogenetic method for identifying and locating particular DNA or RNA sequences on chromosomes or inside tissues is called in situ hybridization ISH. ISH is essential for gene mapping, genome analysis, and the study of chromosome organization and evolution in cotton (*Gossypium spp.*).

Table 1. Classification and geographic distribution of *Gossypium* species based on genomic diversity supported by cytogenetic evidence

Genome /group	Species	Geographical Distribution	Genomic Characteristics
Old World Diploid (A Genome)	<i>Gossypium herbaceum</i>	Africa (primarily in India, Pakistan)	A genome: smaller chromosome size; distinct from new world diploids
	<i>Gossypium arboreum</i>	Southern Asia (India, Pakistan)	A genome; domesticated for cotton production
New world Diploid (D genome)	<i>Gossypium raimondii</i>	Peru, Ecuador	D genome; related to the diploid ancestor of tetraploid species
	<i>Gossypium thurberi</i>	Southern west united states (Arizona, Mexico)	D genome; wild species, smaller stature
	<i>Gossypium trilobum</i>	Central America	D genome; genetically close to <i>G. raimondii</i>
Tetraploid (AD Genome)	<i>Gossypium hirsutum</i>	Global (native to Central America)	AD genome; most widely cultivated cotton species
	<i>Gossypium barbadense</i>	Peru, West Indies, Egypt, USA	AD genome; long fiber, known for high-quality cotton
Other Diploid (B, E, F, G Genomes)	<i>Gossypisum anomalum</i>	Africa, Arabia	B genome; xerophytic, adapted to dry environments
	<i>Gyossypisum sturtianum</i>	Australia	C genome; adapted to arid regions, smaller in stature
	<i>Gyossypisum longicalyx</i>	East Africa	E genome; important for wild cotton germplasm
Polyploid Species	<i>Gossypium tomentosum</i>	Hawali	AD genome; wild species, likely derived from <i>G. hirsutum</i>
	<i>Gossypium mustelinum</i>	Brazil	AD genome; genetically unique among tetraploids

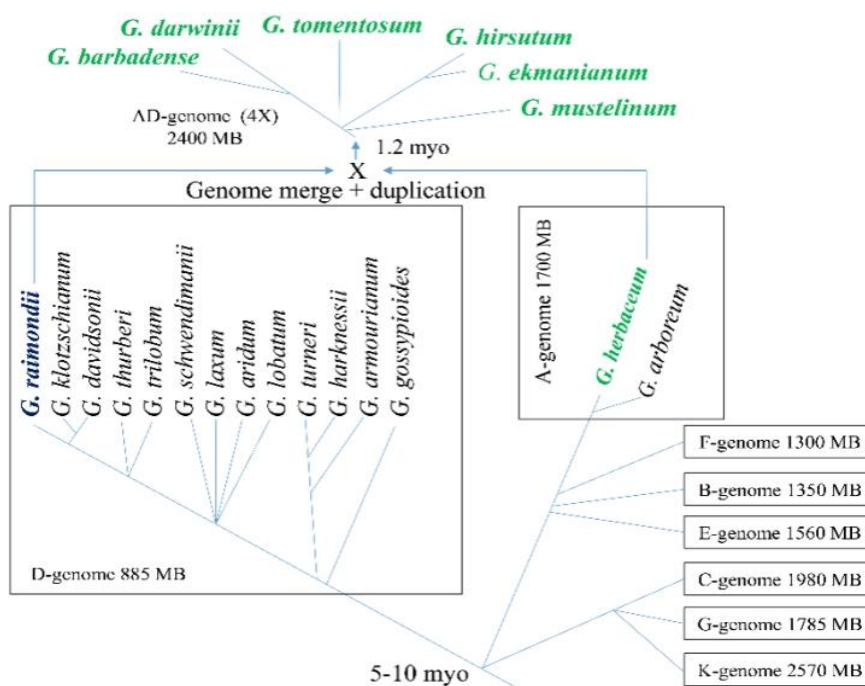


Fig. 1. The development of tetraploid species and the evolution of the *Gossypium* genus (Islam, et al., 2022)

Two main types of ISH are commonly used in cotton research are

1. FISH (Fluorescence in situ hybridization)

In the field of cotton genetics, the FISH has become an essential technology since it increases the crop's resistance to a variety of stresses. This method makes it possible to analyse the cotton genome in great depth and provides highly precise positions for genes and DNA sequences of interest on chromosomes. Fluorescently labelled probes that precisely attach to complementary DNA regions within the cotton genome are used in FISH operations. A fluorescent microscope is then used to view this binding, revealing the spatial distribution of these sequences within the structure of the chromosome (Wang, et al., 2013).

Many advances in cotton genetics have been made possible by the use of FISH, especially in the fields of disease resistance, improved fiber quality, and tolerance to environmental challenges like salt and drought. Breeders can expedite the production of improved cotton varieties by more effectively selecting for desired features in breeding programs by determining the chromosomal regions linked to these qualities. For example, the development of cotton strains

with markedly enhanced fiber characteristics has resulted from the discovery of genes important for fiber strength and length by FISH.

Furthermore, the investigation of polyploidy and chromosomal rearrangements in the cotton genome has benefited greatly from the use of FISH. Effective breeding techniques need an awareness of the genetic and genomic linkages between various cotton species as well as within cultivars, as cotton species display a range of ploidy levels. The imaging of chromosomal duplications, deletions, and translocations all of which are frequent in polyploid genomes are made possible by FISH aids in this comprehension (Andres, et al., 2013).

2. GISH (Genomic in situ hybridization)

GISH has emerged as a vital tool in cotton genetics, significantly advancing the study of genomic relationships and introgressions among *Gossypium* species. This technique utilizes whole genomic DNA as a probe against chromosomal DNA within cytological preparations, enabling the detection of foreign chromatin in a host genome and distinguishing genomes in polyploid organisms. Given the complexity of cotton's genetic structure, which includes both diploid and polyploid species,

GISH is particularly beneficial for unraveling these intricate genomic interactions.

In cotton breeding and genetic research, GISH has played a pivotal role in identifying and tracing the introgression of genomic segments from wild species into domesticated cultivars. These introgressions have been crucial for broadening cotton's genetic base and improving traits such as biotic and abiotic stress resistance, and fiber quality. By pinpointing the genetic origin of specific chromosomal segments, GISH enhances the precision of breeding programs, facilitating the development of superior cotton varieties with desirable agronomic traits.

Moreover, GISH has proven to be invaluable in identifying structural chromosomal changes, including translocations and inversions, which are often associated with the development of novel traits or improvements in existing ones. By providing a genome-wide perspective on chromosomal organization and structural variation, GISH deepens our understanding of cotton's genome architecture and informs strategies for targeted crop improvement.

2.3 Karyotyping in Cotton Improvement

Karyotyping is a cytogenetic technique used to analyze chromosome number, structure, and

organization in cotton (*Gossypium* spp.). In breeding programs, it is essential for determining chromosomal variations, genome evolution, and genetic stability.

Cotton species display a range of ploidy levels, including diploid species (*Gossypium arboreum* and *G. herbaceum*, $2n = 2x = 26$) and tetraploid species (*Gossypium hirsutum* and *G. barbadense*, $2n = 4x = 52$). Karyotyping serves as a valuable tool for differentiating these species and identifying structural variations such as duplications, deletions, and translocations. This technique is particularly significant in wide hybridization and chromosome substitution breeding, facilitating the incorporation of beneficial traits from wild species into cultivated cotton varieties.

Advanced karyotyping techniques, such as FISH and GISH, have improved chromosomal identification, allowing for precise genome mapping and alien gene introgression (Li et al., 2020). These tools help cotton breeders develop high-yielding, stress-resistant varieties with superior fiber quality.

Karyotyping remains a fundamental tool for understanding the genetic architecture of cotton, supporting evolutionary studies, and enhancing breeding efficiency through chromosome-level selection.

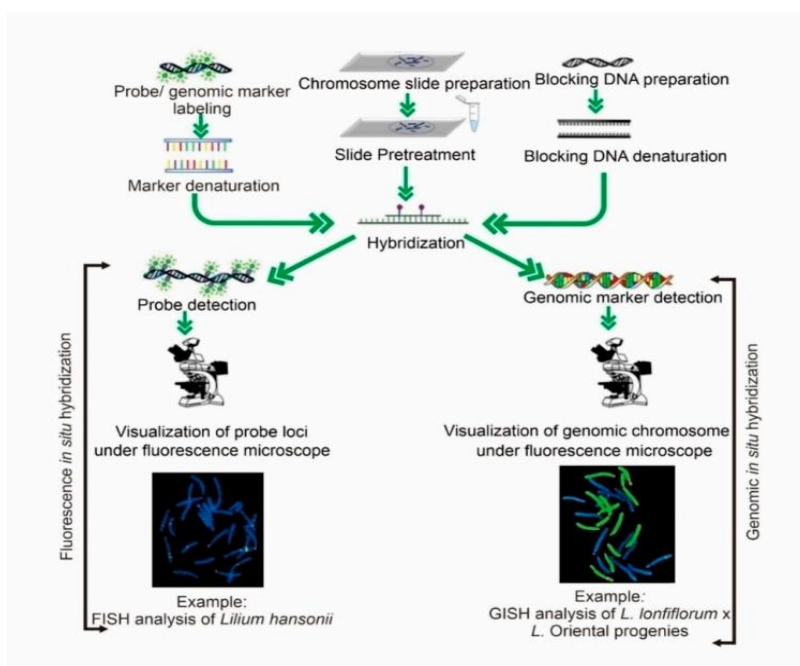


Fig. 2. Representation of FISH and GISH Analysis (Islam et al., 2022)

2.4 Alien Gene Introgression in cotton Improvement

In order to enhance characteristics like yield, fiber quality, insect resistance, and stress tolerance, beneficial genes from wild or related species are introduced into cultivated cotton by alien gene introgression. This method is crucial for adding unique features not found in cultivated germplasm and broadening the genetic foundation of cotton.

Wide hybridization usually occurs between wild or diploid cousins (*G. arboreum* and *G. herbaceum*) and cultivated tetraploid cotton species (*G. hirsutum* and *G. barbadense*). However, there are major obstacles because to ploidy variances and genetic incompatibilities. To get over these obstacles, methods including embryo rescue, bridge species, and chromosomal doubling are frequently employed (Smith, et.al., 1999)

Chromosome substitution lines, alien chromosome additions, and backcrossing strategies have been widely applied to transfer specific traits, such as pest resistance to *Helicoverpa armigera* or abiotic stress tolerance, from wild species. Marker-assisted selection (MAS) and genomic tools, including high-throughput sequencing, have significantly improved the precision and efficiency of alien gene transfer (Chen, et al., 2010).

The introduction of alien genes has resulted in the development of superior cotton cultivars with improved adaptability, higher productivity, and better fiber quality, supporting the sustainability of cotton production across various agro-climatic regions (Abdurakhmonov, 2016).

2.5 Chromosome Painting in Cotton Improvement

Chromosome painting is a molecular cytogenetic technique used to visualize and distinguish individual chromosomes or chromosomal regions using fluorescently labeled DNA probes. This method, primarily based on FISH, is widely applied in cotton research to study genome structure, chromosome evolution, and introgression of alien genes.

Chromosome painting in cotton (*Gossypium spp.*) aids in tracing genomic rearrangements that have taken place during evolution and identifying homologous and homeologous

chromosomes in polyploid species such as *G. hirsutum* (tetraploid, $2n = 4x = 52$) (Wang, et al., 2015). In extensive hybridization research, it is especially helpful since it enables breeders to track the introgression of desired genes from wild relatives.

This technique also facilitates chromosome substitution breeding, where specific chromosomes from wild cotton species are introduced into cultivated varieties. By precisely mapping alien chromosome segments, chromosome painting enables efficient gene transfer and enhances breeding strategies for improving fiber quality, yield, and stress resistance with advancements in molecular cytogenetics, chromosome painting is becoming an essential tool for genome characterization, aiding in the precise manipulation of genetic material to enhance cotton breeding programs. (Grover & Wendel, 2010).

2.6 Polyploidization in Cotton Improvement

Polyploidization is the process of increasing the number of chromosome sets in a plant, either naturally or through artificial methods, to enhance its genetic potential. Many cultivated cotton species, including *Gossypium hirsutum* (upland cotton) and *G. barbadense* (Egyptian cotton), are naturally tetraploid ($2n = 4x = 52$), a characteristic that has contributed to their adaptability, high productivity, and superior fiber quality.

Artificial polyploidization is induced using chemical agents like colchicine, which disrupts spindle fiber formation during mitosis, resulting in chromosome doubling. This technique is particularly useful for converting diploid species such as *G. arboreum* ($2n = 2x = 26$) into synthetic tetraploids. These synthetic polyploids can then be hybridized with naturally occurring tetraploid cotton to incorporate desirable traits like drought resistance, pest tolerance, and enhanced fiber properties (Chen et al., 2010).

Polyploidization also boosts genetic variability and heterozygosity, enabling plants to become more resilient and adaptable to environmental stressors. Additionally, it helps breeders overcome reproductive barriers in wide hybridization, facilitating the transfer of beneficial traits from distant species into cultivated cotton varieties. This process has played a crucial role in improving cotton and developing new

germplasm for breeding programs (Stewart et al., 2015).

2.7 Induced Polyploidy in Cotton Improvement

The deliberate doubling of chromosomes in plants to enhance their genetic and agronomic characteristics is known as induced polyploidy. It contributes significantly to the enhancement of cotton by improving the output, quality, and flexibility of the fiber. Some wild or cultivated diploid species, such as *G. arboreum* ($2n = 2x = 26$), are underutilized in breeding, but the majority of farmed cotton species, like *G. hirsutum* and *G. barbadense*, are tetraploids ($2n = 4x = 52$) (Chen, Z. J., & Ha, M. et al., 2010).

Colchicine, a mitotic inhibitor, is commonly used to disrupt spindle fiber formation during cell division, leading to chromosome doubling. This technique enables the conversion of diploid species into synthetic polyploids with improved traits, making them compatible for hybridization with naturally tetraploid species. Induced polyploidy has also facilitated the introgression of traits like pest resistance and stress tolerance from diploid species into tetraploids. Moreover, polyploids exhibit enhanced heterozygosity and greater genetic variation, benefiting cotton breeding programs

The application of induced polyploidy in cotton contributes not only to trait improvement but also to studying genome evolution, gene expression, and genetic diversification, providing valuable germplasm for future breeding efforts.

2.8 Wide Hybridization in Cotton Improvement

The technique of introducing advantageous features from wild relatives into farmed crops by crossing genetically distant species or genera is known as wide hybridization. It is an essential breeding method for cotton that transfers qualities including drought tolerance, pest resistance, and higher fiber quality. (Li et al., 2013).

Interspecific hybridization enables the combination of complementing traits, as demonstrated by crosses between *Gossypium hirsutum* (high-yielding upland cotton) and *G. barbadense* (fine-fiber Egyptian cotton). Similarly, in order to get past sterility and ploidy barriers, crosses between diploid (*G. arboreum*)

and tetraploid species frequently call for chromosome doubling (Li et al., 2018). Methods like bridge species and embryo rescue have been used to improve the success rate of wide crosses and address hybrid incompatibility.

In addition to encouraging genetic variety, extensive hybridization facilitates the introduction of alien genes from wild relatives. Developing cultivars with improved adaptability, disease resistance, and fiber quality has been a key component of this strategy to support sustainable cotton production (Abdurakhmonov, 2016).

3. ROLE OF CYTOGENETIC TOOLS IN BIOTIC AND ABIOTIC STRESS RESISTANCE IN COTTON

Cytogenetic tools are essential for cotton improvement as they aid in transferring stress-resistance genes, detecting chromosomal variations, and promoting genetic diversity. These tools are essential in developing resilient cotton cultivars capable of withstanding biotic and abiotic stresses.

3.1 Cytogenetic Tools for Biotic Stress Resistance

Biotic stressors, including bacteria, viruses, fungi, and pests, pose significant challenges to cotton production. The use of cytogenetic techniques, such as chromosome substitution, alien gene introgression, and Fluorescence In Situ Hybridization (FISH), has enabled the introduction of resistance genes from wild *Gossypium* species into cultivated varieties. For instance, resistance to *Verticillium* and *Fusarium* wilt from *G. barbadense* and *G. tomentosum* has been incorporated into *G. hirsutum* through chromosome substitution lines. Similarly, combining broad hybridization with genomic in situ hybridization (GISH) has allowed the transfer of insect resistance genes against *Helicoverpa armigera*, promoting crop sustainability while reducing dependency on chemical pesticides (Li et al., 2022).

3.2 Cytogenetic Tools for Abiotic Stress Resistance

Abiotic stresses, including drought, salinity, and extreme temperatures, significantly hinder cotton growth and productivity. Polyploidization has been extensively utilized to enhance tolerance to these stresses by promoting beneficial physiological traits. For example, artificially

induced polyploidy in diploid cotton (*Gossypium arboreum*) has increased drought resilience through deeper root systems and improved water retention. Similarly, alien gene introgression has facilitated the transfer of salt tolerance genes from *G. davidsonii*, enabling cultivated cotton to perform well in saline soils. Additionally, chromosome engineering techniques aid in identifying and transferring stress-tolerant genes, ensuring sustained advancements in cotton resilience (Wang, et al., 2013).

4. CONCLUSION

The sustainability and production of this significant crop for the world's population depend heavily on the genetic resilience of cotton. Breeders are able to create resilient cotton cultivars because cytogenetic technologies are at the forefront of recognizing and reducing biotic and abiotic stressors. Cotton genomes and stress tolerance are intricately related, as demonstrated by the detailed genetic research and modification made possible by techniques like karyotyping, FISH, and GISH. Through the use of polyploidization and sophisticated genetic mapping, researchers are finding new resistance genes that can be deliberately bred into commercial lines. Cotton cultivars that are more resilient to environmental changes and insect stressors are becoming possible thanks to modern breeding methods and this genetic insight. Ultimately, by increasing cotton's genetic resistance, the careful application of cytogenetic procedures is assisting the crop in surviving climate change.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of this manuscript. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below:

1. ChatGPT-4
2. AI was used for language enhancement, grammar correction, and rephrasing complex sentences to improve clarity. No content generation or data analysis was performed using AI.

3. summarize the main prompts used when interacting with the AI.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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