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HETEROSIS, COMBINING ABILITY AND STABILITY ANALYSIS FOR YIELD AND FIBRE QUALITY TRAITS IN UPLAND COTTON (GOSSYPIUM HIRSUTUM L.): A COMPREHENSIVE REVIEW

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ABSTRACT
Upland cotton (*Gossypium hirsutum* L.) is a globally important fibre crop, and improving seed cotton yield and fibre quality remains a primary objective of cotton breeding programs. Over the past decades, extensive research has focused on heterosis, combining ability, and stability analysis to identify superior parental lines and high-performing hybrids. This review critically synthesizes published studies employing classical biometrical approaches such as Line × Tester and diallel mating designs, multi-environment evaluations, and emerging molecular methodologies including transcriptomics, QTL mapping, and multi-omics integration. Previous investigations consistently demonstrate the involvement of both additive and non-additive gene action in controlling yield and fibre traits, while genotype × environment interaction significantly influences hybrid performance and adaptability. Although substantial progress has been achieved through conventional breeding, limitations persist in terms of restricted environmental testing, narrow genetic bases, and limited integration of molecular tools. Recent advances highlight the potential of combining phenotypic selection with genomic-assisted breeding to enhance selection efficiency and hybrid stability. This review further identifies key research gaps and outlines future directions emphasizing integrated breeding strategies, expanded multi-location trials, and utilization of diverse germplasm resources. Collectively, the synthesis provides a comprehensive framework for developing high-yielding, fibre-quality superior, and environmentally stable cotton hybrids to support sustainable cotton production.

KEYWORDS: Upland cotton, heterosis, combining ability, stability analysis, hybrid breeding

1. INTRODUCTION

Cotton (*Gossypium hirsutum L.*) is one of the most important commercial fibre crops in the world, widely cultivated for its lint, seed and by-products. Genetic improvement in upland cotton has primarily focused on enhancing seed cotton yield and fibre quality traits through systematic breeding approaches. Among the available genetic tools, combining ability analysis and heterosis studies using Line × Tester mating designs have provided valuable insights into gene action governing yield and fibre traits. Several studies have demonstrated the effectiveness of this approach in identifying superior parental lines and promising hybrid combinations for commercial exploitation [1]. These investigations highlight the importance of both additive and non-additive gene effects in cotton improvement programs [2].

In recent decades, emphasis has also been placed on evaluating hybrids across diverse environments to understand genotype × environment interactions and performance stability. Since cotton is grown under varied agro-climatic conditions, identifying stable and high-performing hybrids is essential for consistent productivity [3]. Therefore, integrating heterosis estimation, combining ability analysis and multi-environment testing has become a central strategy in modern cotton breeding research.

1.1 Global and National Importance of Cotton

Cotton plays a crucial role in the global agricultural and industrial economy. It serves as the primary raw material for the textile industry and supports millions of livelihoods worldwide. The economic relevance of cotton extends beyond fibre production to oil extraction, animal feed and various industrial applications. Its contribution to employment generation and rural development makes it a strategically important crop in many cotton-producing countries [4].

Global cotton trade patterns have undergone significant shifts over the past decades, with changes in production hubs and import centers. Recent analyses indicate a gradual transition of cotton import dominance toward countries in the Global South, reflecting evolving trade dynamics and consumption patterns [5]. These changes highlight the need for continuous genetic improvement to maintain competitiveness and sustainability in international markets.

1.2 Economic Significance in India

In India, cotton is often referred to as "white gold" due to its substantial contribution to the national economy. It plays a vital role in supporting the textile and garment industries, which form a major component of the country's industrial sector. The crop provides direct and indirect employment to millions of farmers, laborers and workers engaged in processing and marketing activities.

The broader economic structure of India has witnessed significant transformation over the decades, with the tertiary sector expanding rapidly alongside agricultural development [6]. Within this evolving framework, cotton continues to hold strategic importance by linking agriculture with industry and trade. Enhancing cotton productivity and fibre quality is therefore essential for strengthening both farm income and industrial growth.

1.3 Constraints in Cotton Productivity

Despite its importance, cotton productivity remains constrained by several biotic and abiotic factors. Major challenges include irregular rainfall, pest and disease incidence, soil fertility limitations and inadequate input management. In many cotton-growing regions of India, yield levels remain below global averages due to these structural and environmental limitations [7].

Genetic factors also play a critical role in productivity gaps. Limited exploitation of heterosis, inadequate parental selection and insufficient multi-environment evaluation often restrict the development of widely adaptable cultivars. Addressing these constraints requires systematic breeding strategies that combine genetic improvement with improved agronomic practices.

1.4 Need for Hybrid Development and Stability Studies

Hybrid development has emerged as a powerful approach to enhance cotton yield and adaptability. The concept of hybrid systems emphasizes the importance of stability and consistent performance under dynamic environmental conditions. Stability analysis frameworks originally developed in system sciences underline the significance of maintaining equilibrium performance despite external disturbances [8].

Recent theoretical advancements in stability analysis further stress the importance of evaluating system behavior across varying conditions [9]. In crop breeding, similar principles apply when assessing genotype × environment interactions and adaptability. Therefore, hybrid development combined with stability studies ensures the identification of cotton genotypes capable of sustaining high productivity under diverse and unpredictable agro-climatic conditions.

2. LITERATURE REVIEW

A substantial body of research has been conducted on heterosis, combining ability and stability analysis in upland cotton (*Gossypium hirsutum L.*) to enhance yield and fibre quality traits. Various genetic designs, including Line × Tester and diallel mating systems, have been widely employed to understand gene action and identify superior parental combinations. Recent advances have also incorporated molecular and multi-omics approaches to decode the biological basis of heterosis and adaptability. Multi-environment testing has further strengthened breeding programs by enabling the identification of stable and widely adaptable genotypes. The following table summarizes key research findings relevant to cotton improvement, highlighting methodologies, major outcomes and their implications for breeding strategies.

Table 2.1. Summary of studies on heterosis, combining ability, stability and genetic improvement in upland cotton.

Author	Study Focus	Genetic Design / Approach	Key Findings	Relevance to Present Review
(Yelimineti et al., 2026) [10]	Combining ability and gene action in cotton hybrids	Line × Tester (Inter & Intra specific hybrids)	Significant GCA and SCA effects observed for yield and fibre traits	Confirms importance of additive and non-additive gene action in cotton breeding
(Dong et al., 2026) [11]	Epigenetic basis of heterosis	Multi-omics integration	Identified epigenetic regulation influencing heterosis expression	Provides molecular insight into heterosis mechanisms
(Greveniotis et al., 2026) [12]	Stability of seed quality traits	Multi-environment trials	Stable genotypes identified across environments	Supports role of stability analysis in cotton improvement

		using stability index		
(Verma et al., 2026) [13]	Combining ability and heterosis in half diallel	Half diallel design	Non-additive gene action predominant for yield traits	Highlights importance of hybrid breeding strategies
(Bhanuchand and Raju, 2025) [14]	Genetic diversity and heterosis in cotton	Genetic divergence analysis	Diverse parents showed high heterotic performance	Emphasizes parent selection for hybrid development
(Ma et al., 2025) [15]	Combining ability and stability of yield	Multi-year evaluation	Stable hybrids with high yield performance identified	Demonstrates importance of multi-environment testing
(Zeng, 2025) [16]	Cotton breeding strategies	Review of genetic enhancement	Focused on enhancing genetic variability for yield and fibre quality	Provides theoretical framework for breeding programs
(Hassan et al., 2024) [17]	Heterosis and combining ability	Line × Tester design	Significant heterosis for yield and fibre quality	Reinforces effectiveness of Line × Tester approach
(Hugar et al., 2024) [18]	Genetic divergence and hybrid potential	Diversity analysis	High genetic divergence associated with superior hybrids	Supports selection of genetically diverse parents
(Gören, 2024) [19]	Drought tolerance in cotton lines	Half diallel crosses	Identified drought tolerant and stable genotypes	Relevant for stress resilience breeding
(Baghyalakshmi et al., 2024) [20]	Fibre quality improvement	QTL and gene analysis	Identified major QTLs controlling fibre traits	Supports integration of molecular tools in breeding
(Suresh, 2023) [21]	Heterosis and stability in intra & inter specific crosses	Line × Tester	Significant heterosis and stable hybrids identified	Aligns directly with stability and heterosis evaluation
(Chorshanbiev et al., 2023) [22]	Combining ability in G. barbadense	Intraspecific hybrid analysis	Additive gene action predominant for fibre traits	Useful for fibre quality improvement strategies
(Abdel-Aty et al., 2023) [23]	Genetic parameters in Egyptian cotton	Diallel analysis	Significant GCA and SCA effects for yield traits	Confirms importance of gene action analysis
(Anwar et al., 2022) [24]	Interspecific hybridization	Hybridization between species	Improved yield and adaptability through interspecific crosses	Supports use of wide hybridization
(Samak et al., 2022) [25]	Multi-environment combining ability	Line × Tester under multiple environments	Identified stable Bt introgressed hybrids	Emphasizes GE interaction studies
(Richika et al., 2021) [26]	Heterosis in interspecific hybrids	Line × Tester	High heterosis for fibre and yield traits	Supports hybrid exploitation
(Rameshbhai, 2021) [27]	Gene action and heterosis	Line × Tester analysis	Both additive and dominance effects significant	Confirms importance of hybrid breeding
(Shahzad et al., 2020) [28]	Molecular basis of heterosis	Transcriptome analysis	Differential gene expression linked to yield heterosis	Connects molecular biology with hybrid vigor

3. RESEARCH METHODOLOGICAL APPROACHES IN PREVIOUS STUDIES

Research on heterosis, combining ability and stability in upland cotton (*Gossypium hirsutum* L.) has employed diverse experimental designs and analytical frameworks. Over the years, methodological approaches have evolved from classical biometrical techniques to integrated multi-environment and molecular strategies. A systematic review of previous research methodologies provides insight into experimental designs, genetic models, statistical analyses, and emerging genomic tools applied in cotton improvement programs.

3.1 Mating Designs Used in Previous Studies

The majority of heterosis and combining ability studies in cotton have utilized structured mating designs, primarily Line × Tester and diallel systems.

Line × Tester Design

The Line × Tester mating design has been widely adopted for estimating general combining ability (GCA) and specific combining ability (SCA). Preetha and Raveendran (2008), Monicashree et al. (2017), and Thiyagu et al. (2019) employed Line × Tester analysis to evaluate hybrid performance for yield and fibre traits. Similarly, Hassan et al. (2024) and Samak et al. (2022) applied this design under multi-environment conditions.

This design allows:

- Partitioning of additive and non-additive gene effects
- Identification of superior parents (GCA)
- Identification of promising crosses (SCA)
- Efficient evaluation of large breeding material

Diallel Analysis

Diallel mating systems, including full and half diallel crosses, have also been extensively used. Abdel-Aty et al. (2023) utilized diallel analysis to estimate genetic parameters in Egyptian cotton. Verma et al. (2026) employed half diallel analysis to reveal gene action patterns. Chorshanbiev et al. (2023) also adopted intraspecific hybrid analysis using diallel crosses.

Diallel analysis provides:

- Estimation of additive and dominance variance
- Detailed genetic parameter estimation
- Insight into inheritance patterns

Interspecific Hybridization

Anwar et al. (2022) and Richika et al. (2021) adopted interspecific hybridization between *G. hirsutum* and *G. barbadense* to broaden genetic variability.

Table 3.1. Major Mating Designs Used in Previous Cotton Studies

Author	**Year**	**Design Used**	**Purpose**
Preetha and Raveendran	2008	Line × Tester	GCA & SCA estimation
Monicashree et al.	2017	Line × Tester	Heterosis and gene action
Thiyagu et al.	2019	Line × Tester	Yield and fibre evaluation
Abdel-Aty et al.	2023	Diallel	Genetic parameter estimation
Verma et al.	2026	Half Diallel	Gene action analysis
Samak et al.	2022	Line × Tester (Multi-environment)	Stability and combining ability
Richika et al.	2021	Interspecific crosses	Hybrid vigor assessment

3.2 Statistical and Biometrical Analysis Techniques

Most studies applied analysis of variance (ANOVA) to test significance of genotype differences. Line × Tester analysis followed the methodology proposed by Kempthorne (1957 framework), as adopted by Preetha and Raveendran (2008) and Hassan et al. (2024).

Common statistical tools included:

- ANOVA for partitioning variance
- Estimation of GCA and SCA effects
- Heterosis estimation (mid-parent, better-parent, standard heterosis)
- Genetic variance components
- Heritability estimates

Rameshbhai (2021) estimated additive and dominance components to understand gene action. Abdel-Aty et al. (2023) computed genetic parameters including heritability and degree of dominance.

Table 3.2. Statistical Approaches Applied in Previous Studies

Author	Year	Statistical Tools	Traits Evaluated
Monicashree et al.	2017	ANOVA, GCA, SCA	Yield & fibre traits
Rameshbhai	2021	Variance components	Gene action
Hassan et al.	2024	Line × Tester ANOVA	Yield & fibre
Abdel-Aty et al.	2023	Diallel analysis	Growth, yield, fibre
Yelimineti et al.	2026	Combining ability analysis	Seed cotton yield

3.3 Multi-Environment Evaluation and Stability Models

To assess genotype × environment interaction (G × E), several researchers adopted multi-location or multi-year testing.

Samak et al. (2022) evaluated Bt-introgressed hybrids across multiple environments. Ma et al. (2025) conducted two-year evaluations to determine stability of yield traits. Greveniotis et al. (2026) applied stability index methods to evaluate seed quality traits.

Common stability approaches included:

- Environmental index model
- Stability index calculation
- Regression-based stability parameters
- G × E interaction ANOVA

Gören (2024) evaluated drought tolerance using stress-based stability assessment.

Table 3.3. Stability and Multi-Environment Methodologies

Author	Year	Environment Type	Stability Method
Samak et al.	2022	Multi-location	G × E interaction
Ma et al.	2025	Multi-year	Yield stability analysis
Greveniotis et al.	2026	Multi-environment	Stability Index
Gören	2024	Drought conditions	Stress stability analysis

3.4 Molecular and Genomic Methodologies

Recent studies have incorporated molecular tools into heterosis research.

Shahzad et al. (2020) performed comparative transcriptome analysis between inbred lines and hybrids to identify gene expression differences associated with heterosis.

Dong et al. (2026) applied multi-omics integration including epigenetic analysis to decode heterosis mechanisms.

Baghyalakshmi et al. (2024) reviewed QTL mapping and gene editing approaches for fibre quality improvement.

These approaches represent a methodological shift from classical quantitative genetics toward integrative genomics.

Table 3.4. Molecular Approaches in Cotton Improvement

Author	Year	Molecular Tool	Objective
Shahzad et al.	2020	Transcriptome analysis	Molecular basis of heterosis
Dong et al.	2026	Multi-omics integration	Epigenetic regulation
Baghyalakshmi et al.	2024	QTL analysis	Fibre quality improvement

3.5 Evolution of Research Methodologies

The progression of research methodologies in cotton breeding can be summarized in three phases:

1. Classical Phase
   - Line × Tester and diallel designs
   - Focus on yield traits
2. Multi-Environment Phase
   - Incorporation of G × E interaction
   - Stability analysis
3. Molecular Phase
   - Transcriptomics
   - Epigenetics
   - QTL mapping

While classical biometrical approaches remain foundational, modern studies increasingly integrate molecular tools to complement phenotypic evaluation.

4. Research Gaps Identified from Previous Studies and Emerging Directions

Despite extensive research on heterosis, combining ability, and stability analysis in upland cotton (*Gossypium hirsutum* L.), several methodological and conceptual gaps remain evident. While classical breeding approaches have generated valuable insights, limitations in experimental scope, environmental representation, and molecular integration restrict the development of universally adaptable and high-performing hybrids. A critical examination of previous studies highlights key areas requiring further refinement.

4.1 Limited Integration of Multi-Trait Evaluation

Most earlier investigations primarily emphasized seed cotton yield and a few fibre quality parameters. Studies by Preetha and Raveendran (2008), Monicashree et al. (2017), and Thiyagu et al. (2019) focused largely on yield-contributing traits, while fibre characteristics were treated as secondary objectives.

Although some researchers incorporated fibre quality (Hassan et al., 2024; Abdel-Aty et al., 2023), very few studies simultaneously integrated yield, fibre quality, stress tolerance, and stability parameters within a unified analytical framework. This fragmented approach limits comprehensive genotype evaluation and restricts selection of truly superior hybrids suitable for commercial deployment.

Future research must prioritize holistic trait assessment by combining productivity, quality, and adaptability traits within single experimental designs.

Table 4.1. Major Research Gaps Identified from Previous Studies

Area	Observed Limitation	Suggested Direction
Trait evaluation	Focus mainly on yield	Integrate yield, fibre, and stress traits
Environmental testing	Limited locations/years	Long-term multi-environment trials
Methodology	Dominance of classical statistics	Combine biometrics with molecular tools
Molecular integration	Minimal application	Expand omics and QTL-based breeding
Genetic diversity	Narrow parental base	Utilize diverse and interspecific germplasm

4.2 Inadequate Multi-Environment Representation

Although multi-environment testing has been recognized as essential, many studies relied on limited locations or short-term evaluations. Samak et al. (2022) and Ma et al. (2025) conducted multi-environment assessments; however, most experiments were confined to two or three environments or single growing seasons.

Environmental variability strongly influences cotton performance, and restricted testing reduces confidence in genotype stability. Greveniotis et al. (2026) and Gören (2024) emphasized the importance of stability indices and stress-based evaluations, yet such approaches remain underutilized across large breeding populations.

There is a clear need for long-term, multi-location trials representing diverse agro-climatic zones to strengthen conclusions regarding hybrid adaptability.

4.3 Overreliance on Classical Biometrical Techniques

Traditional statistical approaches such as ANOVA, GCA, and SCA estimation continue to dominate cotton breeding research (Hassan et al., 2024; Yelimineti et al., 2026). While these methods effectively identify superior parents and crosses, they provide limited insight into the underlying molecular mechanisms governing heterosis.

Only a few studies have attempted to bridge this gap. Shahzad et al. (2020) employed transcriptomic analysis, and Dong et al. (2026) utilized multi-omics integration to decode epigenetic regulation of heterosis. However, these advanced methodologies remain largely isolated from conventional breeding programs.

Future studies must integrate molecular data with phenotypic performance to enhance precision in parental selection and hybrid development.

4.4 Limited Application of Molecular Breeding Tools

Although QTL mapping and gene identification have shown promise for fibre quality improvement (Baghyalakshmi et al., 2024), molecular-assisted selection is still not widely incorporated into routine breeding programs. Most reviewed studies relied exclusively on morphological and agronomic traits.

The lack of marker-assisted breeding, genomic selection, and gene editing limits genetic gain and delays the development of elite cultivars. Integration of molecular tools with Line × Tester and stability analysis could substantially accelerate cotton improvement.

4.5 Narrow Genetic Base in Hybrid Development

Several studies highlighted the importance of genetic diversity in achieving superior heterosis (Bhanuchand and Raju, 2025; Hugar et al., 2024). However, many breeding programs continue to utilize limited parental pools, resulting in narrow genetic bases.

Anwar et al. (2022) demonstrated the value of interspecific hybridization for broadening variability, yet such approaches remain underexploited. Expanding germplasm resources and incorporating wild or exotic lines could enhance resilience and productivity.

4.6 Emerging Directions for Future Cotton Breeding

Based on the reviewed literature, future research should emphasize:

- Integration of phenotypic, molecular, and environmental data
- Expanded multi-location and multi-year testing
- Adoption of genomic selection and marker-assisted breeding
- Inclusion of climate-resilience traits such as drought and heat tolerance
- Development of decision-support systems combining GCA, SCA, and stability indices

Such integrated strategies would improve selection efficiency and support development of stable, high-yielding cotton hybrids.

5. CONCLUSION AND FUTURE SCOPE

The present review critically synthesizes existing research on heterosis, combining ability, and stability analysis in upland cotton (*Gossypium hirsutum* L.), highlighting the substantial progress achieved through classical breeding approaches such as Line × Tester and diallel designs. Previous studies consistently demonstrated the involvement of both additive and non-additive gene action in governing seed cotton yield and fibre quality traits, confirming the effectiveness of hybrid breeding strategies. Multi-environment evaluations further emphasized the importance of genotype × environment interaction in determining hybrid adaptability and performance stability. Recent molecular investigations, including transcriptomic and multi-omics analyses, have provided valuable insights into the genetic and epigenetic mechanisms underlying heterosis. However, most breeding programs continue to rely predominantly on phenotypic selection and limited environmental testing, indicating the need for more integrated and comprehensive methodologies.

Future cotton improvement efforts should focus on combining conventional biometrical techniques with advanced molecular tools such as QTL mapping, genomic selection, and multi-omics integration to enhance selection efficiency and genetic gain. Expanded multi-location and multi-year trials are essential for identifying climate-resilient and widely adaptable hybrids. Greater utilization of diverse germplasm, including interspecific lines, will help broaden the genetic base and improve stress tolerance. Additionally, simultaneous evaluation of yield, fibre quality, and environmental stability within unified breeding frameworks is required to develop commercially viable hybrids. Such integrated strategies will contribute toward sustainable cotton production and support the development of high-yielding, fibre-quality superior, and environmentally stable cultivars suited to diverse agro-climatic regions.

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