

HETEROSIS, COMBINING ABILITY AND STABILITY ANALYSIS IN PEARL MILLET (*Pennisetum glaucum* (L.) R. Br.): A COMPREHENSIVE REVIEW

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ABSTRACT

Pearl millet is an important climate-resilient cereal widely cultivated in semi-arid regions. The present review synthesizes findings on heterosis, combining ability and stability analysis based on multi-environment evaluation of hybrids developed through a Line $\times$ Tester design. Significant genetic variability was observed across environments, with analysis of variance revealing strong genotype and genotype $\times$ environment interaction effects for grain yield and associated traits. Several hybrids exhibited substantial positive heterosis over better parent and standard checks, indicating the predominance of non-additive gene action. Combining ability analysis identified promising CMS and restorer lines with desirable general combining ability effects, while specific cross combinations showed high specific combining ability for yield. Stability parameters and AMMI analysis facilitated identification of broadly adapted and specifically adapted hybrids. The integration of heterosis estimation, combining ability analysis and multi-location stability testing provides a robust framework for accelerating pearl millet hybrid breeding under semi-arid conditions.

KEYWORDS: Pearl millet, Heterosis, Combining ability, Genotype $\times$ environment interaction, Stability analysis

1. INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is one of the oldest domesticated cereal crops, with archaeological evidence confirming its cultivation in West Africa nearly 4,500 years ago [1]. From its center of origin, the crop gradually spread to Asia, particularly India, where it became an integral component of dryland agriculture. Its remarkable adaptation to harsh climatic conditions has enabled it to sustain food production in regions characterized by erratic rainfall and high temperatures.

In India, pearl millet plays a significant role in ensuring food security in arid and semi-arid zones. Continuous genetic improvement programs have led to the development of improved varieties and hybrids suited to diverse agro-ecological conditions [2]. Over the decades, systematic breeding efforts have enhanced yield potential, stress tolerance, and disease resistance, thereby strengthening its position among climate-resilient cereals [3].

With the increasing challenges posed by climate change and land degradation, pearl millet has gained renewed attention as a sustainable crop option. Modern breeding approaches now focus not only on yield enhancement but also on stability, adaptability, and nutritional improvement. Understanding heterosis, combining ability, and genotype $\times$ environment interaction has therefore become essential for sustained genetic progress in this crop.

Importance of Pearl Millet in Global Agriculture

Pearl millet occupies a prominent place in global agriculture, particularly in regions where other cereals struggle to survive. International breeding programs have significantly contributed to expanding its cultivation and improving its productivity across Africa and Asia [4]. The crop's resilience under moisture stress and low-input conditions makes it highly suitable for smallholder farming systems.

In addition to its adaptability, pearl millet has become increasingly important due to biofortification initiatives aimed at enhancing micronutrient content. Breeding programs have successfully developed nutritionally improved cultivars, reinforcing the crop's importance in combating hidden hunger and micronutrient deficiencies [5]. Market development and diversification of pearl millet-based products have further strengthened its economic and agricultural relevance [6].

1.2 Nutritional and Economic Significance

Pearl millet is widely recognized for its superior nutritional composition compared to many staple cereals. It contains substantial levels of protein, dietary fiber, and essential micronutrients, making it a valuable component

of balanced diets [5]. Its inclusion in food security programs supports both caloric and nutritional requirements in vulnerable populations.

Economically, pearl millet contributes to both grain and fodder production systems. The development of heterotic gene pools has accelerated hybrid breeding, resulting in enhanced productivity and farmer profitability [7]. The crop also supports livestock sectors by providing quality fodder, thereby strengthening mixed farming systems and rural livelihoods.

Major nutritional and economic advantages:

High protein and micronutrient content Contribution to biofortification and nutritional security Development of superior hybrids through heterotic grouping Expanding market potential for value-added products Support for dual-purpose grain and fodder systems [6]

1.3 Evolution of Pearl Millet Hybrid Breeding: From Domestication to Genomics-Assisted Hybrid Development

Pearl millet (*Pennisetum glaucum* (L.) R. Br.; syn. *Cenchrus americanus* (L.) Morrone) is among the earliest domesticated cereal crops and represents one of humanity's oldest adaptations to harsh agroecological environments. Archaeological and molecular evidence indicates that domestication occurred approximately 4,500–5,000 years ago [1] in the western Sahel region of Africa, particularly in present-day Mali, Mauritania, Niger and surrounding areas. Following domestication, the crop spread eastward across Africa and subsequently reached the Indian subcontinent through ancient trade and migration routes. Pearl millet is a highly cross-pollinated species with natural outcrossing rates frequently exceeding 80–85%. This reproductive behavior results primarily from protogyny, wherein stigmas emerge before pollen shedding. These biological characteristics of pearl millet made it particularly suitable for heterosis exploitation. High levels of genetic diversity, extensive natural outcrossing and strong hybrid vigor provided the prerequisites necessary for successful hybrid development. The development of cytoplasmic male sterility (CMS) systems represents the most transformative event in pearl millet breeding history. The release of HB-1 in India during 1965 marked the beginning of the hybrid era in pearl millet and represented a landmark achievement in global cereal breeding. One of the most important advances in modern hybrid breeding has been the establishment of heterotic pools and heterotic groups.

Pre-1950	↓
↓	Molecular marker applications
Landraces and farmer selection	↓
↓	Heterotic grouping studies
1950–1960	↓
↓	2015–2020
Discovery of CMS systems	↓
↓	Genome sequencing
1965	↓
↓	GWAS and transcriptomics
Release of HB-1	↓
↓	2020–Present
1970–1985	↓
↓	Genomic selection
Expansion of hybrid breeding	AI-assisted breeding
↓	Climate-smart hybrids
1985–2000	Predictive hybrid breeding
↓	
Disease resistance breeding	
↓	
Diversification of parental lines	
↓	
2000–2015	

Figure 1. Historical Timeline of Pearl Millet Hybrid Breeding

The last decade has witnessed a transition from conventional breeding toward genomics-assisted hybrid development. Advances in sequencing technologies have transformed understanding of pearl millet genetics and hybrid performance.

1.4 Challenges in Pearl Millet Production

Despite its resilience, pearl millet production faces several constraints. Hybrid breeding relies heavily on cytoplasmic male sterility systems, and limited diversification of these systems can restrict genetic progress [8]. Identification and utilization of diverse sterility sources are essential to ensure sustainable hybrid development [9].

Advances in molecular mapping have facilitated the identification of fertility restoration loci, improving the efficiency of hybrid seed production [10]. However, stable expression of combining ability and resistance traits remains a critical requirement for consistent hybrid performance across environments [11].

Diseases such as downy mildew continue to pose major threats to productivity. The development of resistant hybrids has reduced vulnerability, yet historical evidence highlights the risks associated with genetic uniformity in crop populations [12,13].

1.5 Rationale for Heterosis, Combining ability and Stability Studies

Exploitation of heterosis has been a key strategy in pearl millet improvement, leading to significant yield enhancement over traditional varieties. Effective hybrid breeding requires stable male sterility systems and reliable fertility restoration mechanisms to ensure seed production efficiency [14].

Studies on genic male sterility have improved understanding of inheritance patterns and sterility expression, thereby supporting the development of superior parental lines [15]. Combining ability analysis further helps in identifying parents with strong general and specific combining ability for yield and related traits.

In addition, multi-environment testing and stability analysis are essential to select hybrids that maintain consistent performance under variable climatic conditions. Integrating heterosis estimation, combining ability analysis, and stability parameters provides a comprehensive framework for developing high-yielding and widely adaptable pearl millet hybrids suitable for sustainable agriculture.

2. LITERATURE REVIEW

Pearl millet breeding research has evolved significantly in recent years, with strong emphasis on heterosis exploitation, combining ability analysis, gene action studies, and stability evaluation across diverse environments. Modern improvement programs focus on identifying genetically diverse parental lines capable of producing superior hybrids with high yield potential and enhanced nutritional traits. Researchers have increasingly utilized structured mating designs such as Line $\times$ Tester and diallel analysis to understand the inheritance patterns governing important agronomic and quality traits. These approaches have helped clarify the relative importance of additive and non-additive gene effects in hybrid performance. The following table 1: summarizes key research findings relevant to cotton improvement, highlighting methodologies, major outcomes and their implications for breeding strategies.

Table 1:

Author(s)	Research Focus	Methodology Used	Major Findings	Relevance to Present Study
(Punia et al., 2026) [16]	Combining ability and gene action for yield and biofortification traits	Line $\times$ Tester analysis	Identified parents with high GCA and SCA effects for grain yield and micronutrients	Supports combining ability analysis for high-yielding nutrient-dense hybrids
(Verma et al., 2026) [17]	Gene action and heterosis through diallel analysis	Half diallel design	Demonstrated importance of non-additive gene action in hybrid performance	Strengthens concept of heterosis estimation methodology
(Kapoor et al., 2026) [18]	Multi-trait selection and stability in diverse environments	Stability analysis and ideotype selection	Identified stable genotypes across environments	Direct relevance to multi-location stability analysis
(Aher and Palakolanu, 2025) [19]	Genetic enhancement strategies	Review of breeding advancements	Emphasized resilience and stress-tolerant hybrids	Provides broader breeding background

(Yadav et al., 2025) [20]	Inheritance of biochemical traits	Generation mean analysis	Confirmed additive and non-additive gene effects	Supports gene action studies in heterosis breeding
(Rasitha et al., 2025) [21]	Fertility restoration genetics in CGMS lines	Hybrid evaluation studies	Identified effective restorer lines	Important for CMS-based hybrid development
(Khandelwal et al., 2024) [22]	Stability analysis using multi-trait index	Multi-environment trials	Selected superior stable hybrids	Strong support for stability parameters in your study
(Yadav et al., 2024) [23]	Strategies for productivity and resilience	National breeding strategies review	Emphasized hybrid technology for food security	Aligns with broader impact of heterosis breeding
(Papanna and Gupta, 2024) [24]	Identification of efficient testers	Heterotic grouping and tester evaluation	Strengthened heterotic pool classification	Direct link to Line $\times$ Tester mating design
(Gaoh et al., 2023) [25]	Combining ability for Fe and Zn traits	Line $\times$ Tester analysis	High SCA effects for micronutrient traits	Supports combining ability for biofortification
(Pallavi et al., 2023) [26]	Combining ability for rancidity traits	Hybrid analysis	Identified parents with desirable quality traits	Shows broader trait improvement through combining ability
(Rajpoot et al., 2023) [27]	Genetic variability and multivariate analysis	Germplasm evaluation	High variability for yield traits	Provides genetic base for hybrid selection
(Pravinbhai, 2022) [28]	Combining ability and stability using isonuclear lines	Line $\times$ Tester with stability parameters	Identified stable and superior hybrids	Strongly related to combining ability + stability framework
(Naranbhai, 2022) [29]	Genetic component analysis in F1 and F2	Combining ability analysis	Confirmed role of additive and dominance effects	Supports genetic interpretation of heterosis
(Lenka, 2022) [30]	Heterosis and combining ability across environments	Multi-environment Line $\times$ Tester	Reported significant G $\times$ E interaction	Directly supports multi-location evaluation approach
(Suryawanshi et al., 2021) [31]	Heterosis for yield components	Hybrid performance evaluation	High heterosis for grain yield traits	Validates heterosis exploitation in pearl millet
(Balasaheb, 2021) [32]	Heterosis and combining ability studies	Line $\times$ Tester analysis	Identified superior cross combinations	Reinforces parent selection strategy
(Srivastava et al., 2020) [33]	Review on heterosis exploitation	Comprehensive review	Highlighted importance of hybrid vigor	Provides theoretical foundation for heterosis breeding
(Sahadeo, 2019) [34]	Fertility restoration and heterosis	Hybrid and CMS evaluation	Identified stable restorers and heterotic hybrids	Integrates fertility restoration with heterosis studies

3. Research Methodological Approaches in Previous Studies

Research on heterosis, combining ability and stability in upland Pearl millet (*Pennisetum glaucum* (L.) R. Br. 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.

3.1 Mating Designs for Combining Ability and Stability Used in Previous Studies

Accurate estimation of combining ability and stability depends on the experimental design used to generate hybrid populations. Several mating designs have been employed in pearl millet breeding, each possessing distinct advantages and limitations.

Line × Tester Design

The Line × Tester design, introduced by Kempthorne (1957), has become the most widely used mating design in pearl millet breeding.

In this approach:

Female parents (lines) are crossed with a selected group of male parents (testers).

Every line is crossed with each tester.

GCA effects of lines and testers are estimated simultaneously.

SCA effects are estimated for each hybrid combination.

Eberhart and Russell Stability Model:

regression model of Eberhart and Russell (1966) represents the most widely applied parametric approach in pearl millet research. This model extends Finlay and Wilkinson's regression analysis by incorporating both the regression coefficient (b_i) and the variance of deviations from regression (S^2_{di}).

According to this model, an ideal genotype should exhibit:

High mean performance.

Regression coefficient close to unity ($b_i \approx 1$).

Non-significant deviation from regression ($S^2_{di} \approx 0$).

Following table 2: for comparison of Major Mating Designs Used in Pearl Millet Combining Ability and Stability Analysis.

Table 2: Comparison of Major Mating Designs

Mating design	Genetic parameters estimated	Advantages	Limitations	Typical application in pearl millet
Diallel (Griffing, 1956) [35]	GCA, SCA, reciprocal effects	Detailed genetic analysis; estimates both additive and non-additive effects	Number of crosses increases rapidly with parental number; labour-intensive	Genetic studies, small breeding populations
Line × Tester (Kempthorne, 1957) [36]	GCA of lines and testers; SCA of crosses	Efficient, economical, suitable for large breeding programmes and CMS-based hybrid development	Reciprocal effects usually not estimated; depends on tester selection	Standard method for commercial pearl millet hybrid breeding
North Carolina Design I	Additive and dominance variance	Useful for population improvement	Limited application in hybrid evaluation	Quantitative genetic research
North Carolina Design II	Additive and dominance variance	Balanced design; suitable for quantitative genetics	Requires structured mating populations	Population improvement programmes
North Carolina Design III	Additive, dominance and epistatic effects	Useful for validating genetic models	Complex implementation	Advanced genetic investigations
Finlay–Wilkinson (1963) [37]	Regression coefficient (b_i)	Simple; evaluates environmental responsiveness	Ignores deviation from regression	Preliminary adaptation studies
Eberhart–Russell (1966) [38]	Mean yield, b_i , S^2_{di}	Simultaneous evaluation of adaptability and stability; easy interpretation	Assumes linear responses	Most widely used regression model
Perkins–Jinks (1968) [39]	Modified regression coefficients	Reduced environmental bias	Limited practical application	Moderate
Tai (1971) [40]	α and λ stability parameters	Statistical rigor	Computationally demanding	Limited

Huehn/Nassar Non-parametric	Rank-based stability statistics	Robust to non- normality	Ignores yield magnitude	Complementary analysis
AMMI	ANOVA + PCA	Captures complex G×E interaction; high predictive ability	Requires balanced data	Excellent for MET analysis
GGE Biplot	Genotype + G×E effects	Visual interpretation; identifies mega- environments and ideal genotypes	Sensitive to scaling and interpretation	Excellent for hybrid evaluation
Mixed Models (BLUP)	Random genetic effects	Handles unbalanced data; high prediction accuracy	Requires advanced statistical expertise	Increasingly important
Genomic Prediction	Genome-wide marker effects	Early prediction of hybrid performance	Requires large training populations	Future breeding approach

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).

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

3.3 Molecular and Genomic Methodologies

Advance in sequencing technologies have transformed understanding of pearl millet genetics and hybrid performance. Availability of reference genome assemblies, high-density SNP markers and next-generation sequencing platforms has accelerated genetic analysis at unprecedented scales.

Modern genomic resources now support:

Molecular Diversity Analysis

Genome-wide markers facilitate characterization of parental diversity and heterotic relationships.

QTL Mapping

Quantitative trait loci associated with grain yield, flowering time, drought tolerance, nutrient content and disease resistance can be identified more accurately.

Genome-Wide Association Studies (GWAS)

GWAS enables dissection of complex traits using natural populations and diverse germplasm collections.

Transcriptomics

Gene expression studies provide insights into molecular mechanisms underlying heterosis and stress adaptation.

Genomic Selection

Prediction models estimate breeding values using genome-wide markers, thereby reducing breeding cycle duration.

Phenomics Integration

High-throughput phenotyping platforms facilitate precise characterization of hybrid performance across environments.

These technologies are gradually transforming hybrid breeding from an empirical process into a predictive science.

Importantly, genomic approaches offer opportunities to integrate:

1. Genetic diversity.
2. Combining ability.
3. Heterosis prediction.
4. Stability analysis.
5. Environmental adaptation.

3.4 Molecular concept of Heterosis: Future Hybrid Breeding Strategies

The remarkable success of hybrid breeding in pearl millet has historically been driven by phenotypic selection, combining ability analysis and empirical exploitation of heterosis. However, despite decades of successful hybrid development, the molecular mechanisms underlying hybrid vigour remain only partially understood. Traditional

breeding approaches have effectively identified superior hybrids but have provided limited insight into the biological processes responsible for their enhanced performance.

These modern models remain relevant, contemporary molecular evidence indicates that hybrid vigor cannot be fully explained by any single mechanism. Current understanding suggests that heterosis results from the combined effects of:

1. Favourable allele complementation.
2. Enhanced regulatory efficiency.
3. Optimized metabolic activity.
4. Improved photosynthetic performance.
5. Epigenetic reprogramming.
6. Superior stress adaptation.

Several recurring patterns have been identified:

Additive Expression

Hybrid gene expression approximates the average of both parents.

Non-Additive Expression

Hybrid expression deviates significantly from parental averages.

Common forms include:

1. Over expression.
2. Under expression.
3. Parent-biased expression.
4. Novel expression patterns.
5. Genes associated with heterosis frequently participate in:
6. Photosynthesis.
7. Carbon metabolism.
8. Nitrogen assimilation.
9. Hormonal signaling.
10. Stress response pathways.
11. Reproductive development.

Recent advances in genomics, transcriptomics, epigenomics and systems biology are transforming our understanding of heterosis from a largely statistical phenomenon into a complex molecular process involving coordinated interactions among genes, regulatory networks, metabolites and environmental signals.

3.5 Molecular Perspectives on Combining Ability

Combining ability analysis has traditionally relied on phenotypic evaluation of hybrid performance through Line $\times$ Tester, diallel and North Carolina mating designs. Recent advances in genomics have shifted the focus from estimating combining ability solely through field experiments to understanding its genetic and molecular basis. This transition represents a significant step toward predictive hybrid breeding, where parental performance can be anticipated using genomic information before extensive field evaluation.

Genome-wide molecular markers, including simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs), diversity arrays technology (DArT) and genotyping-by-sequencing (GBS), have greatly improved the characterization of genetic diversity among pearl millet parental lines. These tools enable breeders to estimate genomic relationships, identify heterotic pools and assess allelic diversity associated with important agronomic traits. Following table 3 is describing comparison between conventional and different molecular techniques used for assessment of combining ability.

Table 3: Conventional vs Molecular Approaches for Combining Ability Analysis

Approach	Major Features	Strengths	Limitations	Future Potential
Phenotypic GCA and SCA analysis	Field evaluation using Line $\times$ Tester or diallel designs	Direct assessment of breeding value; well established	Time-consuming; strongly influenced by G $\times$ E interactions	Integration with genomic prediction
Marker-assisted analysis	SSR, SNP, DArT, GBS markers	Characterization of genetic diversity and heterotic groups	Molecular distance alone is a weak predictor of hybrid performance	Marker-assisted parent selection
QTL mapping and GWAS	Identification of loci associated with quantitative traits	Detects genomic regions controlling target traits	Limited functional validation for combining ability	Discovery of candidate genes for hybrid breeding

Multi-omics integration	Genomics, transcriptomics, proteomics, metabolomics and epigenomics	Mechanistic understanding of hybrid performance	High cost and analytical complexity	Predictive systems biology and precision breeding
Genomic selection	Genome-wide prediction models	Early prediction of breeding value; accelerates breeding cycles	Requires large training populations and robust phenotypic datasets	Next-generation hybrid breeding

Integrating Multi-Omics with Combining Ability

The emerging concept of systems biology has fundamentally reshaped the interpretation of combining ability. Rather than viewing GCA and SCA as purely statistical estimates, current research suggests that they represent phenotypic manifestations of complex interactions among genomic, transcriptomic, proteomic, metabolomic and epigenomic processes.

Transcriptomic studies in cereal crops demonstrate that superior parental combinations frequently exhibit coordinated regulation of genes involved in photosynthesis, carbohydrate metabolism, hormone signaling, stress responses and reproductive development.

Epigenetic mechanisms, including DNA methylation, histone modifications, chromatin remodeling and small RNA-mediated regulation, provide an additional layer of complexity.

Integrating epigenomic profiles with genomic marker information may improve the prediction of parental performance beyond what is achievable through DNA polymorphisms alone.

3.6 Predicting Hybrid Performance

One of the most promising applications of genomic selection is hybrid prediction.

Potential targets include:

Grain yield.

Combining ability.

Heterosis.

Stress tolerance.

Nutritional quality.

Genomic prediction offers several advantages:

Reduced field-testing requirements.

Shorter breeding cycles.

Improved selection intensity.

Greater breeding efficiency.

However, prediction accuracy depends on:

Training population size.

Marker density.

Trait heritability.

Environmental representation.

3.7 Artificial Intelligence and Data-Driven Breeding

The rapid expansion of genomic and phenotypic datasets has stimulated interest in artificial intelligence (AI) and machine learning applications.

Potential applications include:

Hybrid prediction.

Genomic selection.

Phenotype prediction.

Stress diagnosis.

Environmental characterization.

Breeding decision support.

Machine learning algorithms can analyze complex nonlinear relationships that are difficult to capture using conventional statistical approaches.

Future breeding systems are likely to integrate:

Genomics.

Phenomics.

Envirotyping.

Artificial intelligence.

Table 4: Summary of Emerging Genomic Technologies and Their Applications in Pearl Millet Hybrid Breeding:

Technology	Primary Application	Major Advantage	Current Limitation
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SSR Markers	Diversity assessment	High polymorphism	Limited genome coverage
SNP Genotyping	Genome-wide analysis	High resolution	Infrastructure requirements
QTL Mapping	Trait dissection	Identification of genomic regions	Environment-specific effects
GWAS	Marker-trait associations	High mapping resolution	Requires large populations
Genomic Selection	Breeding value prediction	Accelerates breeding cycles	Requires extensive training data
Transcriptomics	Gene expression analysis	Functional insights	High analytical complexity
Epigenomics	Regulatory mechanisms	Explains environmental responses	Limited studies in pearl millet
CRISPR/Cas	Functional genomics	Precise gene modification	Regulatory constraints
AI and Machine Learning	Predictive breeding	Handles complex datasets	Requires large datasets

3.8 Critical Evaluation of Current Breeding Paradigms

Despite substantial progress in pearl millet improvement, several conceptual limitations remain.

Fragmented Evaluation Systems

Many breeding programmes continue to evaluate:

Heterosis,
Combining ability,
Stability,
as independent criteria.

Such fragmentation limits understanding of how these processes interact.

Limited Molecular Integration

Although genomic resources have expanded rapidly, molecular information is rarely incorporated into routine combining ability or stability analyses.

Inadequate Environmental Characterization

Many multi-environment trials record location effects but provide limited quantitative environmental information, restricting biological interpretation of G×E interactions.

Overemphasis on Yield

Historically, grain yield has dominated hybrid evaluation. Future breeding objectives must simultaneously address:

Nutritional quality.
Climate resilience.
Resource-use efficiency.
Farmer preferences.

3.9 Lessons from Six Decades of Hybrid Breeding

The evolution of pearl millet hybrid breeding illustrates one of the most successful examples of heterosis exploitation among cereal crops. The introduction of CMS systems enabled the hybrid revolution, while sustained improvements in parental lines and disease resistance contributed to long-term productivity gains.

However, several challenges remain unresolved.

Commercial hybrid production continues to depend heavily on A1 cytoplasm, creating potential vulnerability.

Heterotic group development remains less advanced as compared with Maize.

Relationships among genetic diversity, combining ability and heterosis remain inconsistent across environments.

Integration of molecular information into routine hybrid prediction remains limited.

Finally, climate change introduces new breeding challenges requiring simultaneous improvement of productivity, nutritional quality and resilience.

4. Research Gaps Identified from Previous Studies and Emerging Directions:

4.1 Incomplete Understanding of the Molecular Basis of Heterosis

Despite decades of research, heterosis remains only partially understood.

Current evidence suggests contributions from:

Dominance complementation.

Overdominance.
 Epistasis.
 Transcriptomic regulation.
 Epigenetic modifications.
 Metabolic optimization.
 However, the relative importance of these mechanisms remains uncertain.
 Most molecular studies in pearl millet are limited by:
 Small population sizes.
 Restricted environmental sampling.
 Incomplete functional validation.
 Limited integration of multi-omics datasets.

4.2 Weak Integration of Heterosis, Combining Ability and Stability Studies

Historically, these research areas have developed independently.

Many studies report:

Heterosis estimates.

Combining ability effects.

Stability parameters.

without examining relationships among them.

As a result, breeders often lack comprehensive information regarding:

How combining ability influences heterosis.

How heterosis changes across environments.

Which parental combinations produce stable hybrids.

Integrated analytical frameworks remain limited.

4.3 Underdeveloped Heterotic Group Classification

Reliable heterotic groups are fundamental for sustainable hybrid breeding.

Although several studies have attempted heterotic classification using:

Pedigree information.

Genetic distance.

Combining ability.

Consensus heterotic pools remain poorly defined.

Other Gaps are also need to give equal emphasis which are listed in below table:5

Table 5: Major Knowledge Gaps in Pearl Millet Hybrid Breeding

Research Area	Current Limitation	Breeding Consequence
Molecular heterosis	Incomplete mechanistic understanding	Reduced hybrid prediction accuracy
Combining ability genomics	Limited genomic characterization	Inefficient parent selection
Multi-environment testing	Short-term datasets	Uncertain stability estimates
G×E analysis	Poor environmental characterization	Weak biological interpretation
Multi-omics integration	Fragmented datasets	Limited systems-level understanding
Heterotic groups	Poor classification	Reduced breeding efficiency
AI-assisted breeding	Limited implementation	Slower genetic gain
Climate resilience	Inadequate trait integration	Vulnerability to future climate change

4.4 Future Research Priorities

Priority 1: Molecular Dissection of Heterosis

Future studies should integrate:

Transcriptomics.

Proteomics.

Metabolomics.

Epigenomics.

to identify causal mechanisms underlying hybrid vigor.

Priority 2: Genomic Prediction of Hybrid Performance

Develop robust genomic prediction models capable of accurately predicting:

Heterosis.

GCA.

SCA.

Yield stability.

before field testing.

Priority 3: Multi-Environment Genomic Breeding

Integrate:

Genomics.

Envirotyping.

Phenomics.

to improve prediction of genotype performance across diverse environments.

Priority 4: Climate-Smart Hybrid Development

Develop hybrids combining:

High yield.

Heat tolerance.

Drought resilience.

Nutritional quality.

Resource-use efficiency.

to address future climate challenges.

Priority 5: Establishment of Global Heterotic Pools

Develop internationally coordinated heterotic group classification systems using:

Genomics.

Combining ability.

Hybrid performance data.

Priority 6: AI-Assisted Breeding Systems

Implement machine learning frameworks capable of integrating:

Multi-omics datasets.

Environmental variables.

Breeding records.

for predictive hybrid development.

5. CONCLUSION AND FUTURE SCOPE

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) occupies a unique position among cereal crops because of its exceptional adaptation to arid and semi-arid environments, resilience to climatic stresses and growing importance for food, feed, fodder and nutritional security. This review critically synthesized evidence regarding heterosis, combining ability, stability analysis, genotype $\times$ environment interaction and emerging genomic approaches in pearl millet breeding.

The review further demonstrated that heterosis remains one of the most important contributors to genetic gain in pearl millet. Although dominance, overdominance and epistasis continue to provide valuable theoretical explanations, contemporary evidence indicates that heterosis cannot be adequately explained by any single classical model.

Combining ability analysis continues to serve as a cornerstone of hybrid breeding by facilitating identification of superior parental lines and hybrid combinations. Evidence reviewed in this manuscript indicates that both additive and non-additive genetic effects contribute to economically important traits.

The review also highlighted the central role of genotype $\times$ environment (G $\times$ E) interaction in determining hybrid performance. Environmental factors including rainfall variability, temperature extremes, soil fertility, disease pressure and management.

A major conclusion emerging from this review is that heterosis, combining ability, G $\times$ E interaction and stability should not be viewed as independent breeding concepts. Rather, they constitute interconnected components of a unified biological and breeding continuum.

Despite significant advances, several important knowledge gaps remain. The molecular mechanisms underlying heterosis are not fully resolved, genomic dissection of combining ability remains limited, long-term multi-environment datasets are scarce and integration of multi-omics information into breeding programmes remains insufficient.

The future success of pearl millet hybrid breeding will depend on the successful integration of classical quantitative genetics with modern molecular and computational technologies. By combining heterosis exploitation, combining ability analysis, stability assessment, genomic prediction and environmental characterization within unified breeding frameworks, it will be possible to develop next-generation hybrids capable of contributing substantially to global food, nutritional and climate security. Pearl millet possesses enormous untapped genetic potential and continued investment in multidisciplinary research will be essential for realizing this potential in the decades ahead.

5.2 Key Take-Home Messages

CMS-based hybrid breeding remains the foundation of heterosis exploitation in pearl millet.

Heterosis results from complex interactions among genetic, molecular, physiological and environmental factors. Both additive and non-additive gene effects contribute to hybrid performance, with non-additive effects often predominating for grain yield.

Genotype $\times$ environment interaction is a major determinant of heterosis expression, combining ability and adaptation.

Stability analysis is indispensable for identifying hybrids with consistent performance across environments.

Genomics, transcriptomics, epigenomics and phenomics are reshaping understanding of hybrid vigor.

Integration of heterosis, combining ability, G $\times$ E interaction and stability provide a more realistic framework for hybrid breeding.

Genomic prediction, artificial intelligence and multi-omics technologies represent the future of pearl millet improvement.

Climate-resilient, nutrient-dense and stable hybrids should become central objectives of future breeding programmes.

A systems-based breeding paradigm is required to accelerate genetic gain and enhance global food and nutritional security.

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