



A REVIEW ON HETEROSIS, COMBINING ABILITY AND STABILITY FOR YIELD AND YIELD-CONTRIBUTING TRAITS IN PEARL MILLET

[*Pennisetum glaucum* (L.) R. Br.]

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Abstract

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a fascinating diploid cereal ($2n = 14$) that thrives in the hands of millions of smallholder farmers in the arid and semi-arid regions of India and sub-Saharan Africa. It's cherished not just as a staple food grain but also as a versatile fodder crop. Thanks to its unique flowering behavior and a well-established cytoplasmic-genic male sterility (CMS) system, pearl millet was the first cereal to successfully embrace commercial hybrid technology, starting in India back in the 1960s. The ongoing genetic enhancement of both grain and fodder yields in this crop hinges on three interconnected areas of research: (i) understanding the extent and patterns of heterosis in breeding populations, (ii) evaluating the combining ability of parent lines and the genetic factors influencing yield and its traits, and (iii) ensuring that superior hybrids remain stable across the diverse environments where they are cultivated. This review brings together insights from various studies, including diallel, line $\times$ tester, and multi-environment trials conducted over the past fifty years. It delves into these three key themes, discusses the statistical models frequently employed (like Eberhart–Russell regression, AMMI, and GGE biplot), and wraps up with the breeding implications for creating high-yielding, genetically stable pearl millet hybrids that can thrive in challenging agro-ecological conditions.

Keywords: Pearl millet, *Pennisetum glaucum*, heterosis, combining ability, GCA, SCA, gene action, genotype $\times$ environment interaction, stability analysis, hybrid breeding

1. Introduction

Pearl millet holds the title of the sixth most important cereal crop globally and is the most widely cultivated millet species, thriving on about 27 million hectares across Asia and Africa. It's particularly

well-suited for areas with low and unpredictable rainfall, high temperatures, and poor soil fertility—conditions where major cereals like maize, wheat, and rice struggle to grow (Krishnamurthy et al., 2007; Gemenet et al., 2015). In India, you'll find it mainly in states like Rajasthan, Gujarat, Maharashtra, Uttar Pradesh, and Haryana. Meanwhile, in Africa, it serves as a dietary staple in the Sahelian belt, which stretches across Senegal, Mali, Niger, Burkina Faso, and northern Nigeria (Pucher et al., 2015; van Staveren and Stoop, 1985).

This species is naturally allogamous due to its protogyny, and the breakthrough discovery of the A1 cytoplasmic male sterility system by Burton in 1965 paved the way for large-scale commercial hybrid seed production. India was the first country to release a commercial hybrid of any millet crop (HB-1, 1965), and since then, pearl millet has become a key model for showcasing how to effectively harness heterosis in a cereal system that combines self-pollination and cross-pollination. Given that F1 hybrids are the backbone of the seed industry for this crop, studying heterosis, combining ability, and stability is not just an academic exercise; it's crucial for the entire commercial breeding process.

This review brings together a wealth of research, organizing it into three main categories: heterosis, combining ability and gene action, and stability analysis. It then explores how these elements come together in a real-world hybrid breeding program.

2. Heterosis for Yield and Yield-Contributing Traits

Heterosis, often called hybrid vigor, highlights how the F1 hybrid outshines its parent plants. This can be measured in a few ways: relative heterosis or mid-parent heterosis compares the hybrid to both parents, heterobeltiosis looks at the hybrid's performance against the better parent, and standard heterosis measures it against a commercial benchmark. In the case of pearl millet, researchers have used line × tester and diallel mating designs to estimate heterosis across a diverse range of germplasm.

Chittora and Patel (2017) explored heterosis for grain yield and its components in pearl millet, finding impressive hybrid vigor for grain yield per plant, along with several traits that contribute to yield. Similarly, Barathi et al. (2020) examined an indigenous germplasm collection and reported significant heterosis and combining ability estimates, pinpointing several cross combinations that hold high commercial potential. More recently, in studies involving elite Indian hybrid parents evaluated in Deesa, Gujarat, standard heterosis for grain yield varied from about -48.5% to 96.6%. Notably, hybrids like PMGH-5, PMGH-24, PMGH-26, PMGH-29, PMGH-31, and PMGH-32 exhibited the highest positive

standard heterosis, along with favorable heterosis for related yield components. This suggests a strong connection between heterosis for grain yield and the traits that contribute to it.

In research focused on fodder-type pearl millet, line $\times$ tester analyses of 54 hybrids derived from nine lines and six testers revealed that the crosses JMSA 20042 $\times$ BIB 19, ICMA 97111 $\times$ BIB 16, ICMA 93333 $\times$ BIB 31, and ICMA 843-22A $\times$ BIB 4 showed the highest standard heterosis and SCA effects. These hybrids are promising candidates for multi-locational testing and potential commercial use.

In a broader look at genetic resources, Dutta et al. (2021) took a deep dive into a seven-parent diallel of genetically diverse Sahelian pearl millet populations hailing from countries like Senegal, Mali, Benin, Burkina Faso, Niger, Sudan, and Nigeria. They tested these populations across six different environments in West Africa. The results were intriguing: the average panmictic mid-parent heterosis for grain yield was around 24%, with a range from -1.5% to 64.7% . This shows that even hybrids formed at the population level, rather than just from inbred lines, can achieve significant heterosis in the tough growing conditions typical of the Sahel.

More recent studies focused on biofortification—looking at grain iron, zinc, and protein content alongside yield—have found that some elite Indian crosses exhibit mid-parent and better-parent heterosis surpassing 100% for grain yield. Interestingly, yield-related traits displayed the most extensive positive heterosis across all categories examined, which included phenological, morphological, yield, and biofortification traits. On the flip side, phenological traits like days to flowering and maturity mostly showed negative heterosis, which is actually a good thing for agronomists as it indicates a selection for earlier maturation.

When we look at these studies as a whole, a clear pattern emerges:

- High heterosis is observed in grain yield per plant, fodder yield per plant, 1000-grain (test) weight, ear-head weight, harvest index, and the number of effective tillers per plant.
- Moderate heterosis can be seen in ear-head (panicle) length and the threshing index.
- Low or even negative (favorable) heterosis is noted for days to 50% flowering and days to maturity.

3. Combining Ability and Gene Action

- The ideas of general combining ability (GCA) and specific combining ability (SCA) were introduced by Sprague and Tatum back in 1942 and have since been put into practice using mating designs like the diallel method (Griffing, 1956) and the Line $\times$ Tester approach (Kempthorne, 1957). GCA represents how a parent performs on average across all its crosses, which is linked to additive gene effects. On the other hand, SCA shows how a particular cross deviates from what you'd expect based on the GCA of its two parents, and it's tied to non-additive gene effects, such as dominance and epistasis.
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- In most studies on combining ability in pearl millet, researchers have consistently found that the ratio of GCA to SCA variance for grain yield and many traits contributing to yield is less than one. This suggests that non-additive gene action is more prevalent. For instance, in a Line $\times$ Tester study involving 96 hybrids from eight lines and twelve testers conducted in Kharif 2022, the analysis of variance for combining ability revealed significant variation among hybrids for all traits examined. The GCA:SCA variance ratio was below one for every trait, reinforcing the idea that non-additive gene action is dominant and highlighting the importance of heterosis (hybrid) breeding. In this study, the parental lines ICMB 02777 and PT 6679 stood out as excellent general combiners, showing positive GCA effects and strong performance in terms of grain yield, test weight, single ear-head weight, and single ear-head threshed weight. Additionally, ICMB 02444, ICMB 93222, PT 6067, and PT 6476 were also identified as valuable combiners.
- In a thorough 5 $\times$ 5 diallel study focusing on grain yield and related traits, the General Combining Ability (GCA) effects were found to be highly significant for days to 50% flowering, panicle length, and plant height. However, they were not significant for grain yield per plant and the number of productive tillers. This highlights the distinct genetic control mechanisms at play, where phenological and morphological traits are largely influenced by additive effects, while yield itself tends to be more non-additive in nature.
- In another study involving six inbred lines crossed in every possible combination, researchers discovered that GCA variance was greater than Specific Combining Ability (SCA) variance for grain yield and its components within that specific parental set. Notably, the lines MS. 7625 and MS. 7373 emerged as standout general combiners, while MS. 7625, MS. 7373, and J. 104 were recognized as superior specific combiners. This illustrates how the balance between additive and non-additive gene action can shift depending on the specific germplasm used, even though the overall trend in literature leans towards non-additive control of yield.

- On top of that, the effects of cytoplasm are important when it comes to combining ability. Research comparing isonuclear line pairs that only varied in their cytoplasmic sources showed that the A1 cytoplasm—often used in commercial CMS-based hybrid breeding—tended to have a positive effect on general combining ability (GCA) estimates for plant height and grain yield. However, it negatively affected the days to flowering. This indicates that the cytoplasmic background should be a crucial factor to consider when selecting or developing A-line (male-sterile) seed parents.
- In addition, Balami et al. (2022) investigated combining ability for *Striga hermonthica* tolerance among pearl millet inbred lines using a Line $\times$ Tester cross. This study broadened the scope of combining ability analysis beyond just yield, incorporating important biotic-stress resistance traits that are vital for West African production systems, where *Striga* parasitism poses a significant threat to yield. A recent diallel study (Griffing's Method 2, Model 1) involving ten genetically diverse inbred lines evaluated across two environments for grain yield and biofortification traits (like iron, zinc, and protein content) reinforced the trend of strong non-additive gene action for yield while also identifying several parents with beneficial additive effects for micronutrient density. This suggests that analyzing combining ability can effectively guide breeding efforts aimed at both yield and nutritional quality.

The consistent observation that SCA (specific combining ability) is greater than GCA for grain yield has two key implications for breeding strategies: first, it supports the idea that hybrid (F1) breeding should be the main approach for improving yield in pearl millet, as non-additive genetic variance can't be fixed in an inbred line and can only be harnessed through specific hybrid combinations; and second, it highlights that the yield performance of a particular cross can't be accurately predicted just from the GCA of its parents—extensive testcross evaluations across various parental combinations are still essential.

4. Stability Analysis for Yield across Environments

Pearl millet is cultivated in a variety of challenging and diverse agro-ecological zones, which makes the genotype $\times$ environment (G $\times$ E) interaction a significant hurdle when it comes to pinpointing hybrids that perform reliably across different locations and seasons. As a result, conducting multi-environment trial (MET) analyses and formal stability assessments is essential before any hybrid can be recommended for varietal release.

In the realm of pearl millet research, three main types of stability models have taken center stage:

(a) Regression-based models. Eberhart and Russell (1966) suggested that each genotype should be characterized by its regression coefficient (b_i), which reflects its response to an environmental index, and its deviation from regression (S^2_{di}), which captures the unpredictable aspects of $G \times E$ interaction. A genotype with b_i close to 1 and S^2_{di} near 0, along with a high mean, is deemed widely adapted and stable. This model, along with similar methods proposed by Perkins and Jinks (1968) and Freeman and Perkins (1971), has been extensively utilized in pearl millet research. For example, in a study involving 24 hybrids tested across eight environments in Gujarat, the genotypes GHB-788, GHB-832, and GHB-840 consistently emerged as the most stable and widely adapted according to all three models, with the Eberhart–Russell model being favored for its simplicity and ease of computation (Pabale and Pandya, 2010). Likewise, Yahaya, Echekwu, and Mohammed (2006) performed yield stability analyses of pearl millet hybrids in Nigeria, further applying these regression-based methods to breeding programs in West Africa.

(b) *Multiplicative/biplot models.* AMMI (Additive Main effects and Multiplicative The interaction between genotypes and environments ($G \times E$) has been explored through various methods, including the Gauch (1988) and Zobel et al. (1988) approaches, as well as the GGE (Genotype and Genotype $\times$ Environment effects) biplot techniques introduced by Yan et al. (2000) and Yan and Kang (2002). These methods utilize principal component analysis to break down the $G \times E$ interaction, making it easier to visualize how different genotypes adapt and to pinpoint "mega-environments." When researchers compared these methods with the Eberhart–Russell model, they discovered that the combination of AMMI and GGE could explain a significantly larger portion of the $G \times E$ sum of squares—around 50% in one study—compared to the Eberhart–Russell model, which only accounted for about 9–16% over two years. Despite its popularity for being straightforward to compute, the Eberhart–Russell model falls short in this regard. A notable example is the release of the pearl millet variety "Gora" (EK 01024-1-2), which boasted a 17% seed-size advantage over standard checks. This case highlighted the importance of using multiple stability parameters instead of relying on a single statistic, as different parameters can reveal distinct aspects of genotype performance across various environments, even when yield outcomes are similar.

(c) *Integrated multi-parameter approaches.* Recent studies have taken a closer look at regression, AMMI, and GGE biplot methods, comparing them side by side using the same dataset. In one interesting study involving 23 pearl millet genotypes tested over four seasons, researchers applied the Eberhart–Russell model, AMMI, and GGE biplot together. They found that the genotype ICMB 07999 stood out as stable, consistently delivering a solid mean yield. Meanwhile, genotypes PT 6679, PT 7058, and PT 7054

demonstrated above-average stability, and these findings were consistent across all three analytical methods. The GGE biplot also uncovered two distinct mega-environments, showing that the Kharif (rainy) season environments were better at distinguishing genotype stability compared to those in the summer season. This suggests that when it comes to pearl millet, recommendation domains might need to be tailored to specific seasons rather than applying a one-size-fits-all approach. Changizi et al. (2014), referenced in this stability discussion, warned that relying solely on classical regression methods to select "stable" genotypes can sometimes lead to favoring those that are stable but only moderately high-yielding. This underscores the importance of combining stability statistics with a yield-stability selection index (Farshadfar, 2008) that takes both mean performance and stability into account.

In addition to grain-type hybrids, researchers have also looked into the stability of dual-purpose and fodder pearl millet. A recent study conducted across four locations and two different agro-climatic zones examined 26 elite genotypes, including some brown midrib (bmr) lines. The findings revealed that genotypic variance was responsible for over 75% of the total variation in various fodder yield and quality traits. Interestingly, environmental factors played a bigger role in traits like the number of days to reach 50% flowering and the acid detergent fibre content. This suggests that while fodder yield is relatively stable, certain phenological and quality traits are more influenced by environmental conditions. Notably, genotypes such as ICMbmr 2401, IGPM 100, and ICMbmr 2404 stood out for their impressive stability and strong agronomic performance.

5. Integrating Heterosis, Combining Ability, and Stability in Hybrid Breeding

These three lines of investigation work together as sequential and complementary stages in a single applied breeding pipeline:

1. Heterosis screening kicks things off by measuring the potential hybrid vigor within a selected group of parental lines or populations. It also helps pinpoint which trait combinations—like yield, component traits, or phenology—are likely to show positive responses in F1 hybrids.
2. Next up, combining ability and gene-action analysis digs deeper to find out which specific parental pairings can consistently turn that heterotic potential into high-quality, reproducible hybrids. This step also sheds light on whether the genetic makeup leans more towards hybrid breeding (where SCA/non-additive effects take the lead, often seen in grain yield) or traditional pedigree/recurrent selection (where GCA/additive effects are more prominent, typically in traits like flowering time, plant height, and panicle length).

3. Finally, stability analysis checks if the top-performing experimental hybrids from step 2 maintain their yield advantage when tested across a broader range of target production environments. This step is crucial and often a regulatory requirement before any varietal release and commercial seed production can take place.

6. Conclusion

After over fifty years of research into pearl millet breeding, the evidence is clear: there's a significant potential for heterosis when it comes to both grain and fodder yields. This is largely driven by non-additive gene action, particularly in terms of yield itself. On the other hand, several phenological and morphological traits that are easier to select for show additive genetic control. When it comes to assessing yield stability, it's important not to rely on just one statistical model. Instead, using a mix of regression-based methods (like Eberhart–Russell), multiplicative approaches (such as AMMI), and biplot-based techniques (like GGE) is the way to go. Ideally, this should be backed by a combined stability-and-yield selection index to provide the best recommendations for pearl millet hybrids, whether for specific areas or broader cultivation. By integrating analyses of heterosis, combining ability, and stability into a single, coordinated breeding strategy, we can effectively develop high-yielding and genetically stable pearl millet hybrids that thrive in the drought-prone and climatically diverse regions where this crop is mainly grown.

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