

Prospectus of Heterosis Breeding in Pearl Millet

Kavita, Lalit Kumar and Naresh

Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar

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Introduction

Heterosis, also known as hybrid vigour, is a naturally occurring phenomenon that occurs when the offspring of genetically distinct individuals (parental line) are superior to their parents in terms of physical characteristics such as growth, development, and functional ability (Shull, 1948; Landge *et al.*, 2022). Heterosis is essential for hybrid development because it enables plant breeders to better harness the hybrid vigour of both inbred and non-inbred parental material, thus improving success rates of hybrid breeding. Crop heterosis is an increase in yield, biomass tolerance, stress resistance, and growth rate (Kalloo *et al.*, 2006). It is also imperative to agricultural productivity as hybrid breeding has been demonstrated to be one of the most effective methods for increasing grain yield in a variety of crops (Schnable and Springer, 2013). The degree of heterosis is often higher in naturally cross-pollinating species like pearl millet, maize, rye and other grasses than in self-pollinating crop plants like rice, wheat, oats and barley.

Different hypothesis for Heterosis

- A. **Dominant hypothesis:** The very first and universally accepted explanation for genetic basis is that dominance influences gene activity in superior hybrids that exploit heterosis (Charlesworth and Willis, 2009). This hypothesis proposes that the harmful genes of one parent may be masked by the positive dominant genes of a second parent.
- B. **Overdominance hypothesis:** This theory proposes that heterozygosity at a single locus results in heterosis. In accordance to the over dominance theory (Lippman and Zamir, 2007) the interaction between different alleles produces a large number of allelic pairings that play a role in the superiority of F₁ hybrids.
- C. **Epistatic Theory:** Powers (1944) suggested that heterosis arises by the combined effects of nonalleles in hybrids at discrete loci; heterosis research in maize and rice supported this theory.

- D. **Transcriptome view:** Gene interaction among the nucleus and cytoplasm occurs during hybridization of two inbred parental lines, which leads to cellular and molecular changes and an altered series of gene expression. These changes in gene expression and genome function will result in heterosis in the F₁ hybrid.
- E. **Proteomics view:** States that heterosis may be caused by proteins that express in different ways (DEP). DEP discovered in parental inbred lines and their hybrids is associated with numerous plant metabolic pathways, including photosynthesis, glycolysis, transcriptional regulation, carbon metabolism, disease resistance, amino acid and protein metabolism, illustrating different levels of heterosis.
- F. **Epigenomics View:** While contrasting parental inbred lines are crossed, epigenetic modifications such as Histone acetylation, DNA methylation, and RNAi regulation take place. DNA methylation is the most important modulator of genome-related activity and cellular development in the majority of crop species. The occurrence of heterosis as a result of DNA methylation primarily occurs achieved by inhibiting the procedure of transcription of regulatory genes that is responsible for elevating inbreeding depression or by encouraging the expression of heterosis genes.
- G. **Unifying theory of heterosis:** Evolving theory of heterosis depends on the efficacy of energy utilisation and protein metabolism among hybrids and inbreds. Goff proposed this theory, which he termed energy-use efficiency for expressing multi-genic heterosis. This approach suggests that the vigour of hybrids is a result of a significant decrease in the energy-intensive process of protein metabolism when compared to its inbred counterparts. This change in metabolism in hybrids conserves energy from fundamental pathways and utilises it for increased growth rates and biomass. This form of energy-use efficiency has been discovered in *Brassica napus* hybrids, which resulted in a 5% yield increase relative to the in bred. During the early phases of development, hybrids of *Arabidopsis* exhibited increased activity in metabolism and greater energy-use efficiency than inbreds.

Heterosis in Pearl millet

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a significant cereal crop with nutritious grains that is capable of thriving in some of the world's harshest and most hostile environments. In this highly cross-pollinating crop, heterosis was exploited by a

commercially successful cytoplasmic male-sterility (CMS) system consisting of a three-lines breeding system (A-, B-, and R-lines)(Fig. 1).

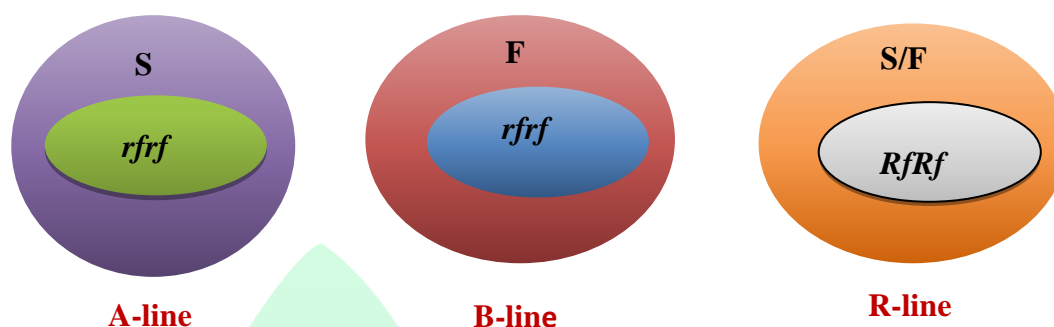


Fig. 1 Three-line breeding system in pearl millet

The interaction of the sterility factor and the recessive fertility restorer (*rf*) allele causes male sterility in the A-line. Tift 23A₁ and Tift18A₁ were the first male-sterile lines utilised to develop hybrids in the early 1960s in Tifton, Georgia, United States. This resulted in a breakthrough in the global production of hybrids. This discovery laid the groundwork for hybrid breeding and the enhancement of hybrid yield potentials by 25–30% and resistance to abiotic and biotic stress over OPVs. In 1965, Punjab Agricultural University (PAU), Ludhiana, India, utilised Tift 23A largely to develop the world's first single-cross pearl millet hybrid, HB 1 (Srivastava *et al.*, 2020). However, the cultivation of a small number of hybrids over a period of years caused an outbreak of downy mildew (DM) in the early 1970s. Such epidemics recurred annually whenever a small number of hybrids inhabited a large area. But no association between A₁ cytoplasm and the DM epidemic has been established (Yadav, 1996). Regular DM epidemics in pearl millet hybrids in India encouraged intensified efforts for diversification of cytoplasm and nuclear genome of parental lines of hybrids, particularly after the 1980s, and can be viewed as a substitute method for the development of hybrids with the objective of enhancing yield and yield stability under different stresses. Presently Six CMS cytoplasm (A₁, A₂, A₃, A₄, A₅, and A_{egp}) are available which are different from one another due to the reorganisation of mitochondrial genes, such as *cox1* and *atp6*, and *cox3*. Due to the greater extent of R-line availability, A₁ CMS source has been widely utilised for the development of hybrids compared to other reported CMS sources. Male sterility is more stable with A₄, A₅, and A_{egp} cytoplasm than with the widely accessible A₁ cytoplasm.

To further enhance heterosis, a number of ideas have been proposed; one of them is to promote heterozygosity by crossing genetically distinct parental materials or materials from distinct heterotic pools (Melchinger *et al.*, 1998). To achieve this, it is required to arrange existing germplasm into heterotic groups so as to improve the efficacy of any hybrid breeding programme. Prediction of F₁ performance and heterosis from the parental generation could considerably increase the efficacy of breeding hybrid or synthetic cultivars by reducing the costs associated with making crosses and evaluating the field for selecting heterotic crosses (Teklewold and Beckwold, 2005). The pearl millet community ^{has} made major advances over the past 50 years in the field of cytoplasmic and nuclear diversification of the hybrid parental lines, resulting in an ongoing improvement in pearl millet productivity from 305 kg ha⁻¹ in the 1950s to the present yield of 1,420 kg ha⁻¹ and adaptability of the developed hybrids, resulting in substantial genetic gains. In order to continue the drive of genetic gains in pearl millet, information on the heterotic pool patterns in the existing germplasm/pool of hybrid parents and on the prediction of heterosis are prerequisite (Reif *et al.*, 2005; Singh *et al.*, 2019).

Heterotic gene pool:

To exploit heterosis in hybrid breeding, an approach of heterotic groups and patterns was proposed, which assists breeders in developing inbred lines and maximizing hybrid breeding results through more effective utilizations of available germplasm. A heterotic group is a collection of related or unrelated genotypes from the same or distinct populations that exhibit the same combining ability and heterotic response when crossed with genotypes from other germplasm groups. Heterotic pattern, on the contrary, is a specific pair of heterotic groups, which may be populations or lines, that exhibit high heterosis and, as a result, high hybrid performance in their crosses. Heterotic groups identified through diversity analysis are validated by evaluating intra-pool and inter-pool crosses at multiple locations. This allows for the determination of the optimum genetic distance between parental materials in order to achieve maximum hybrid vigour. Moreover, superior hybrids can be directly utilized as experimental cultivars and then evaluated on farms. In pearl millet, ICRISAT and other public and private sectors are making attempts to establish genetic pools through the introduction of a variety of germplasm material from India and Africa with diversified

phenotypic characteristics, such as earliness, tillering, grain size, panicle size and grain colour etc. (Fig.2)

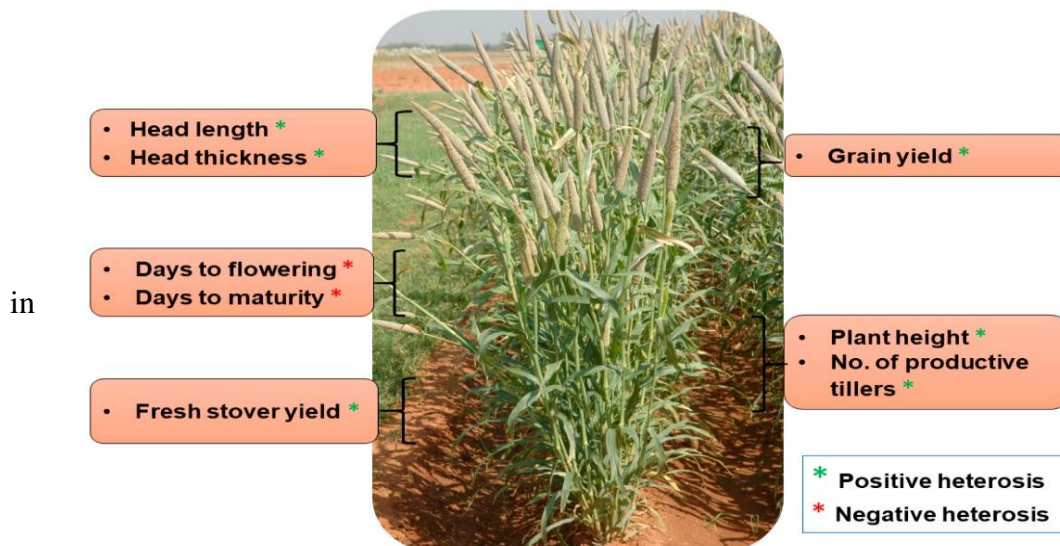


Fig. 2 Heterosis for various traits in HHB 67 Improved hybrid of pearl millet

In order to broaden the genetic base of both seed and restorer parents. There is little knowledge on the categorization of a large number of hybrid parental lines utilizing molecular marker data, and there is insufficient data on the identification of heterotic pools in pearl millet using genomic tools. Precision phenotyping protocols, intensified speed breeding and high-throughput genomic tool development and implementation are required to fully exploit the vast genetic diversity of pearl millet. This will help to accelerate genetic gains and take heterosis breeding to a next level in pearl millet (Yadav *et al.*, 2021).

Conclusion

Pearl millet breeding in India has over time transitioned from the development of open-pollinated varieties to hybrid breeding. The commercial successes of pearl millet breeding are frequently cited as one of the greatest agricultural success stories in India. The presence of a stable cytoplasmic male sterility system with sufficient restorers and the effective utilization of genetic resources from India and SSA established a strong foundation for hybrid breeding. In the past 50 years, the pearl millet community has witnessed an astonishing rise in the cytoplasmic and nuclear diversification of the hybrid parental lines, which has resulted to a gradual increase in the yield and adaptability of the hybrids developed, resulting in substantial genetic gains. Recent applications of genomics and

molecular technologies have improved our understanding of the genetic architecture and distribution patterns of heterotic gene pools. In pearl millet, the introduction of whole-genome prediction models that incorporate heterotic gene pool models, mapped traits, and markers has the potential to contribute to the next quantum leap in yield.

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