

Heterotic grouping facilitates continuous heterosis exploitation for efficient hybrid breeding in maize

Bijendra Pal

Abstract The terms “heterotic groups” and “heterotic patterns” are often used interchangeably, leading to confusion regarding the concept of heterotic grouping. This concept, developed centuries ago, has been refined and extensively utilized for identifying and developing superior corn hybrids, particularly in the USA. Heterotic groups are identified based on the genetic diversity between parental lines or populations. By increasing this genetic distance through inter-population mating, distinct and diverse genetic expressions emerge over time, forming heterotic patterns. These patterns are crucial for developing hybrids with heterosis. It is often questioned whether heterotic groupings can be established at any time. The answer is yes; however, developing heterotic patterns requires multiple generations of intermating. Inbred lines derived from populations based on heterotic patterns will exhibit consistent reactions across different hybrids, climatic conditions, and stress environments, showing no variation in their expression over seasons or years. Misunderstandings arise when heterotic grouping is perceived as a static concept, leading to questions about the varying heterotic reactions of different lines under different climatic conditions. The explanation lies in the genetic diversity that produces heterosis and the interaction between hybrids and their environment, which influences yield performance and trait expression, independent of heterotic grouping. To initiate a breeding program without pre-established heterotic patterns, one should start by grouping materials into different heterotic

groups and then establish heterotic patterns within the program. This approach simplifies the development of heterotic hybrids, allowing for continuous enhancement of genetic diversity between groups. Consequently, this enables the development of heterotic hybrids suited for various environments, maturities, climates, and geographies. In this research review article, I will use the information generated by several researchers and try to put it in simpler form for better use with four clear objectives 1) Combining ability and heterotic grouping. 2) Evaluate the pros and cons of heterotic groups and patterns. 3) Historical understanding of heterotic patterns across regions. 4) A strategy for Heterotic Grouping for Heterotic Patterns.

Keywords: Breeding · Facilitates · Grouping · Heterotic · Hybrid · Maize

Introduction

Maize serves as a model organism for plant breeding and genetic studies, demonstrating the highest heterotic value among crop plants. The exploitation of heterosis for hybrid development has opened opportunities for its application across various crops. Heterosis is determined by the genetic diversity created between parental populations, allowing for the accommodation of favourable alleles in both parental groups. These heterotic values can be sustained over time through intermating within the population, ultimately developing stable heterotic patterns. In the USA and Europe, the importance of maintaining these patterns through intermating within diverse groups was recognized early. Reif *et al.* (2005) highlighted that the status of heterotic patterns was described for the U.S. Corn Belt, Central Europe, Japan, China, and tropical

✉ Bijendra Pal: Bijendra.Pal@pmsrseeds.com

Research and Development Director, Premier Seeds Co., Ltd., Wangchao District, Tak Thailand-63180

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maize-growing regions. While in most regions heterotic patterns have been established, grouping failed in the tropical germplasm due to the complex population structure.

Conversely, in the tropical regions where the hybrid concept was adopted later, heterotic patterns were often mixed or mashed up to develop cultivars with specific traits, ease of multiplication, and adaptability to a wide range of climatic conditions. However, these cultivars exhibited limited yield potential, prompting the initiation of hybrid breeding around 1957. Efforts to develop inbred lines from mixed populations, which had already lost their heterotic patterns, posed significant challenges. To have heterotic hybrids some groups employed the concept of “tropical by temperate” crosses, where the likelihood of background mixing was minimal. High-yielding combinations were achieved using these materials for double-cross and three-way hybrids. However, as the system transitioned to single-cross hybrids, the direct use of temperate inbred lines became impractical due to limitations in large-scale seed production. Consequently, developing inbred lines from mixed populations became necessary. Trait-based classification, initiated by the Directorate of Maize Research (now the Indian Institute of Maize Research, IIMR), categorized flint lines as CM101, CM102, CM103, etc., and dent lines as CM201, CM202, CM203, etc. Crosses between flint and dent lines (eg., CM1 with CM2) often resulted in successful hybrids. However, this classification, based solely on grain type, did not progress as heterotic patterns. Occasionally, favourable combinations were found between flint lines, but these were chance occurrences involving diverse parents from different backgrounds.

The debate on developing heterotic patterns in the country began with a limited number of testers, leading to confusion regarding heterosis reactions and interactions with the environment. Here, we will cover two main elements: the basis for grouping materials and the progression from heterotic grouping to heterotic patterns.

Heterotic grouping and combining ability

In this note, I aim to provide a theoretical understanding of two key concepts in plant breeding: combining ability and heterotic grouping. Combining ability refers to the capacity of a material to combine with other counterparts and produce maximum heterosis over the mid-parent value.

It is crucial to specify the trait for which heterosis is being measured, such as grain yield, total biomass, stress tolerance, or disease resistance. Evaluating these traits separately is essential, as their responses can vary significantly. Consequently, trials conducted under different environmental conditions may offer insights but not definitive solutions. Recent studies have redefined combining ability in various ways. As a commercial plant breeder, I simplify this concept: when breeding for higher yield and other desirable traits, combining ability should be computed based on these specific traits. The focus should be on the final economic yield, which reflects the plant's ability to optimize photosynthesis and store photosynthates.

For effective heterotic grouping based on combining ability, it is crucial to consider specific crosses that yield the best results, indicating that the lines belong to different heterotic groups. Relying solely on general combining ability (GCA) can be misleading, as it identifies materials with the potential to combine with others and express complementary traits, ultimately leading to higher yields. Yadav *et al.* (2002) suggested that inbreds can be divided into different heterotic groups based on specific combining ability (SCA). However, they highlighted the limitation of separating environmental effects, as the total heterotic value is influenced by inbred $\times$ inbred and hybrid $\times$ environment interactions. Similar observations and limitations were reported by Oloyede and Oyekale (2015) and Annor *et al.* (2019).

Fan *et al.* (2008) introduced the concept of Heterotic Groups Specific and General Combining Ability (HSGCA), which considers both the GCA of lines and the SCA of combinations. The SCA-PY and HSGCA methods use SCA and/or GCA of grain yield as the criterion. Grain yield is a complex trait governed by polygenes with low heritability and is highly influenced by the environment. Therefore, any method for heterotic grouping will have limitations. Heterotic grouping can divide materials into two or three groups, but this problem remains unsolved until we move towards heterotic patterns. Continuous efforts are being made to find the best method for developing heterotic groups. Badu-Apraku *et al.* (2013) proposed a concept based on component traits with high heritability and strong correlation with grain yield, enhancing grouping efficiency. This third method, Heterotic Grouping by GCA of Multiple Traits (HGCAMT), measures additive gene effects for each trait.

Subsequent reports revealed inconsistencies in the efficiencies of these heterotic grouping methods. SCA-PY was reported to be more efficient in later studies. Fan *et al.* (2008) found that the HSGCA method increased maize hybrid breeding efficiency over SSR marker and SCA-PY methods. Badu-Apraku *et al.* (2013) reported higher efficiency of the HSGCA method. Laouali (2014) found higher efficiency of the SCA method over the HSGCA method and concluded that the HSGCA method offered no additional advantage. Oyetunde *et al.* (2020) reported marginally higher efficiency of SCA-PY over the HSGCA method and concluded that both HSGCA and SCA-PY methods were the most efficient for heterotic grouping.

In summary, no single method provides complete clarity. Methods can help split materials into different groups based on heterotic values created by combinations. Emphasizing the background and pedigree of materials can help understand the real distance between them.

Pros and cons of heterotic groups and patterns

Moll *et al.* (1962) proposed a simpler method for utilizing materials from different taxa, suggesting that geographic origin could serve as a basic indicator of genetic distance. This approach has since been extensively applied in U.S. maize breeding programs in 1999, Lamkey and Edwards introduced the term panmictic mid parent heterosis (PMPH) within the framework of Hardy-Weinberg equilibrium. PMPH describes the difference between a hybrid population and the average of its two parent populations. Falconer and Mackay (1996) emphasized that assuming two alleles per locus and no epistasis, PMPH depends on the dominance effect at each locus and the square of the difference in allele frequency between the populations. This difference corresponds to the square of the modified Roger's distance (MRD). Consequently, under these assumptions, PMPH is expected to increase with greater genetic distance between the parents. This genetic distance can be achieved by intermating superior progeny from the same population, which will enhance genetic variability and improve the population mean simultaneously. The concept of heterotic patterns greatly assists breeders in selecting parent crosses for line development and in identifying testers to evaluate the combining ability of newly developed inbreds. This approach significantly streamlines germplasm management

and organization, making the breeding process more efficient and effective.

In contrast, Cress (1967) disagreed with the approach of optimizing heterotic value through heterotic groups. He evaluated inter-population improvement methods in a simulation study and pointed out that maximum genetic potential could not be achieved in a breeding system with two strictly separated groups if the best alleles were present in only one of the populations, assuming a degree of dominance less than one. However, it is debatable whether maximum yield potential is an appropriate criterion for evaluating selection strategies. It is crucial to recognize that the heterotic effect often involves more than just combining favourable alleles. There are complementary effects of certain alleles that may be challenging to achieve while maintaining strict heterotic groups. Additionally, it is important to explore whether simple intermating can develop the products and traits needed to address climate change and multiple stresses.

Historical understanding of heterotic patterns across regions

Hallauer *et al.* (1988) noted that heterotic patterns were not initially present in the early stages of hybrid breeding in the USA. These patterns gained recognition with the advent of commercial single crosses in the mid-1960s, driven by the need for high-yielding female parents to economically support commercial seed production. Inbreds such as B14, B37, and B73, developed at Iowa State University from the Iowa Stiff Stalk Synthetic (BSSS), became prominent. BSSS originated from the Reid Yellow Dent population, which has been under continuous improvement since 1840. Tracy and Chandler (2004) highlighted that the seed yield of these lines and their combinations made them the preferred female parents. Inbreds that combined well with BSSS were placed in the male pool, leading to the designation of the female group as Stiff Stalk (SS) and the male group as Non-Stiff Stalk (NSS) (Duvick *et al.*, 2004). They also noted that pre-heterotic pattern germplasm was not structured, but with ongoing inter-population improvement, SS and NSS diverged. In the public sector, several germplasm sources were used to establish the male pool, including Lancaster-derived lines such as C103, C123, MO17, and OH43. In Central and Western Europe, high-yielding U.S. dent lines were crossed with adapted

European flint lines (Schnell, 1992), eventually forming the heterotic pattern of Europe. Japan followed a similar approach, using U.S. dent and BSSS derivatives as female parents and Northern flint or European flint as male parents (Enoki *et al.*, 2002). In China, hybrid breeding began in the 1930s, with heterotic patterns based on domestic and foreign crosses. The domestic group consists of lines derived from Tangsipingtou and Ludahonggu germplasm (Li *et al.*, 2002), while the foreign group includes Lancaster Sure Crop (LSC), Reid Yellow Dent (RYD), and germplasm derived from Pioneer hybrids (PN) (Li *et al.*, 2002, 2004).

Wellhausen (1978); Goodman (1985); Vasal *et al.* (1999) have identified several promising heterotic patterns. For example:

- A) Tuxpeno is heterotic with Cuban Flint, Coastal Tropical Flint (Caribbean Flint), Tuson, and ETO.
- B) Cuban Flint is heterotic with Tuxpeno, Tuson, Coastal Tropical Flint, and Perla.
- C) Coastal Tropical Flint is heterotic with Tuxpeno, Cuban Flint, and Chandelle.

While these heterotic patterns are well-documented, their recognition and utilization by breeding groups in tropical regions may not be as clear-cut as in other regions. CIMMYT germplasm is extensively used in tropical and subtropical areas, with strong pre-breeding efforts for population improvement tailored to regional requirements. However, the complex population structure of the germplasm has sometimes hindered the success of complementary heterotic patterns (Xia *et al.*, 2004).

Strategy for heterotic grouping for heterotic pattern

Grouping materials in a breeding program is essential to streamline the development of inbred lines. Relying on a single approach is insufficient; instead, the best strategy begins with grouping materials based on their origin and their interactions with other materials in the program. In the initial phase of developing heterotic groups, it is important to consider heterosis based on General Combining Ability (GCA), Specific Combining Ability (SCA), and the origin of the materials. This approach helps clarify the initial steps, starting with the use of two testers that can serve as parents for commercial hybrids. Since this grouping serves as a starting point for categorizing materials based on heterotic value, it is

advisable to avoid complicating it with environmental interactions or materials that do not show clear reactions. These issues can be managed later. In the early stages, the main objective should be to focus on heterosis for yield. Other traits and segmentation can be addressed when the population improvement program begins.

Once the program has indicative groups, it is advisable to categorize the material for further use as follows: 1) Lines with a clear reaction to testers. 2) Lines showing heterotic reactions with both testers. 3) Lines not exhibiting heterotic reactions with either tester. From this point, we can begin developing heterotic patterns by creating new recombinants within the heterotic groups and developing new parental lines. This can be initiated by developing biparental populations or using multiple parent populations. Lines that did not show clarity in the first phase can be crossed again with new recombinants. Over time, all material will fall into clear heterotic patterns.

Melchinger and Gumber (1998) recommended considering the following important patterns when identifying new heterotic patterns: 1) High mean performance and large genetic variance in the hybrid population. 2) High per se performance and good adaptation of the parent populations to the target environment. 3) Low inbreeding depression if hybrids are produced from inbreds.

In summary, developing heterotic patterns is a process that requires time and cannot be achieved in just one or two seasons. The starting point involves grouping materials based on their reactions with testers. This approach simplifies the breeding program, increases efficiency, and helps establish a well-organized program for long-term sustainability. However, it is important to note that this method has limitations when multiple traits from different heterotic groups need to be combined. Such cases should be managed as special projects rather than disrupting the existing heterotic patterns within the program.

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