The Role of Heterotic Grouping in Hybrid Breeding: A Comprehensive Review

Mohmed Juned Contractor[1], K. S. Shashidhara*[1], P. Mahadevu[2], H. C. Lohithaswa[3]and G. Jadesha[4]

[1]Department of Genetics and Plant Breeding, College of Agriculture, V. C. Farm, Mandya-571 405, Karnataka.
[2]AICRP on Maize, Zonal Agricultural Research Station, V. C. Farm, Mandya-571 405, Karnataka.
[3] AICRP on Pigeonpea, Zonal Agricultural Research Station, GKVK, UAS, Bangalore-560 065, Karnataka.
[4]AICRP on Nematodes, Department of Plant Pathology, College of Agriculture, GKVK, UAS, Bangalore-560 065, Karnataka.
Corresponding Author: shashi24k@gmail.com

Introduction

Heterosis is due to a high degree of heterozygosity in the genome. Heterozygosity can be increased by crossing genetically distinct inbreds. Adequate characterization of germplasm, assignment of genotypes into heterotic groups, and extensive testing facilitated the utilization of heterosis by breeders (Reif *et al*., 2005). Assigning genotypes into heterotic groups has been the key to the economic success of the crop primarily because; it allows the exploitation of heterosis particularly for grain yield (Moll *et al*., 1965). Therefore, the choice of right type parents that are genetically diverse for hybrid breeding programme is very crucial. Hence, categorizing the germplasm into genetically distinct groups, known as heterotic groups, is a pre-requisite. When large number of inbred lines or genetic material is available, it becomes difficult and costly to examine relative performance and combining abilities of all the introduced germplasm by use of too many testers. This problem can be overcome by developing heterotic groups with divergent testers of known origin with knowledge of their heterotic pattern which can be used for evaluation of large number of inbred lines. Let's now move on to understand the Heterotic group and Heterotic pattern. A heterotic group is a collection of related or unrelated genotypes from the same or different populations that, when crossed with genotypes from other genetically dissimilar germplasm groups, exhibit similar heterotic response and combining ability (Melchinger and Gumber, 1998). A particular pair of two heterotic groups exhibits a high rate of heterosis and hybrid performance in their cross combinations is known as heterotic pattern. Because, there is a significant amount of genetic variation in germplasm, heterotic patterns are crucial for crop breeding. By considering the genetic diversity established among heterotic groups, a heterotic pattern can be improved.

Methods of Heterotic grouping

Earlier method used for grouping was the Pedigree analysis in which they used this method to identify the Reid and Landcaster groups in the corn belt. Quantitative genetic analysis method that can be employed in identifying heterotic groups include making crosses in the Line × Tester mating design that is modification of North Carolina Design II mating design (Robinson *et al*., 1958) and using DNA markers to classify the germplasm (Melchinger, 1999). These are the older methods used but now-e-days due to advancement in the Research, novel methods of grouping are introduced they are mentioned as follows.

Heterotic grouping by using Specific combining ability (*sca*) effects if grain yield

This method popularly known as SCA method which was first used by Vasal *et al.* (1992). Inbred lines exhibiting negative *sca* effect with the tester "A" should be grouped under Heterotic group "A" and inbred lines exhibiting negative *sca* effect with the tester "B" should be grouped under Heterotic group "B". Using this method Vasal *et al.* (1992) classified tropical maize lines developed at CIMMYT. Lines showing negative *sca* effect with tester- 1 "Pop 21" (Tuxpeno-1) were classified under Tropical Heterotic Group (THG-A). Whereas, lines showing negative *sca* effect with tester-2 "Pop 25" (Blanco Cristalino) were classified under Tropical Heterotic Group (THG-B). The hypothesis was that positive *sca* effects between inbred lines generally indicate that lines are in opposite heterotic groups and lines in the same heterotic group tended to exhibit negative *sca* effects when crossed. Following is the hypothetical example representing the procedure for classification of inbred lines.

Example 1:

Inbred line	*sca* effects with		Heterotic group
	Tester "A"	Tester "B"	
L1	-2.03	2.03	A
L2	7.69	-7.69	B
L3	11.45	-11.45	B
L4	8.64	-8.64	B
L5	-1.26	1.26	A
L6	-3.10	3.10	A

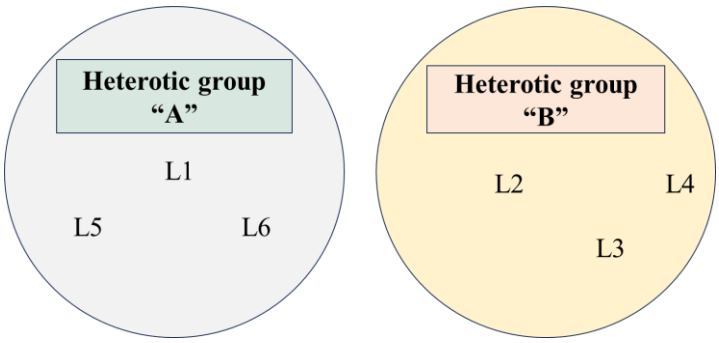

Fig 2. Distribution of inbred lines into different heterotic groups using SCA method

From the Example 1, Among 6 lines L1, L5 and L6 exhibited negative *sca* effect with tester A. Hence, these are assigned to the Heterotic group "A". L2, L3 and L4 revealed *sca* effect with tester "B" and were allocated to Heterotic group "B". This method was also used by Elmyhun *et al.* (2020) for classifying the inbred lines.

Heterotic group's Specific and General Combining Ability (HSGCA) method

This method was given by Fan *et al.* (2009) for classification of inbreds. This model takes in account both *gca* and *sca* effects for grain yield.

$$SCA = \text{Cross mean } (X_{ij}) - \text{Line mean } (X_{i.}) - \text{Tester mean } (X_{.j}) + \text{Overall mean } (X_{..})$$

$$GCA = \text{Line means } (X_{i.}) - \text{Overall mean } (X_{..})$$

$$HSGCA = \text{Cross mean } (X_{ij}) - \text{Tester mean } (X_{.j}) = GCA + SCA$$

Where, Xij = Mean yield of the cross between i^{th} line and j^{th} tester

$X_{i.}$ = Mean yield of i^{th} line

$X_{.j}$ = Mean yield of j^{th} tester

Inbred lines showing negative HSGCA with tester "A" were assigned to Heterotic group "A". Inbred lines showing negative HSGCA with tester "B" were assigned to Heterotic group "B". If the inbred lines exhibit negative HSGCA with both the testers "A" and " Inbred line is assigned to the that group which reveal highest negative value and inbred lines exhibiting positive HSGCA with both the tester were grouped as "AB" group, they neither belong to Heterotic group "A" nor belong to Heterotic group "B". Following is the hypothetical example depicting the procedure of classification.

Example 2:

Line	HSGCA with		Heterotic Group
	Tester "A"	Tester "B"	
L1	-2.88	2.75	A
L2	3.25	-8.02	B
L3	-7.49	-31.05	B
L4	6.16	25.98	AB
L5	4.78	17.01	AB
L6	-19.16	3.97	A

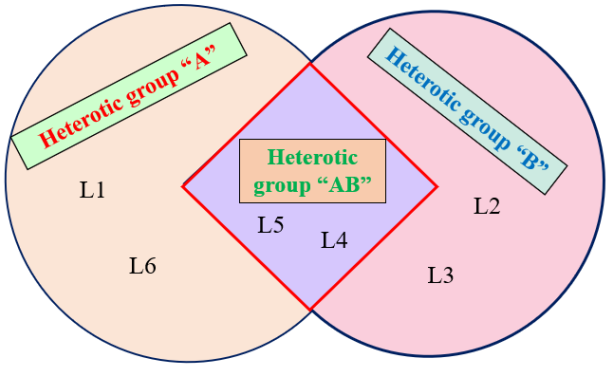

Fig 2. Distribution of inbred lines into different heterotic groups using HSGCA method

From the Example 2, Among 6 lines L1 and L6 exhibited negative HSGCA with tester A. Hence, these are assigned to the Heterotic group "A". L2 and L3 revealed negative HSGCA with tester "B" and were allocated to Heterotic group "B". Remaining, L5 and L4 exhibited positive HSGCA with both the testers hence, they are grouped under "AB" (Fig. 2). They neither belong to "A" nor "B", but they belong to some

other group. Several researchers like Fan *et al.,* 2009, Badu-Apraku *et al.,* 2015, Annor *et al.,* 2020 and Bhatla *et al.,* 2024 used HSGCA method for identifying the heterotic groups.

Heterotic grouping based on General Combining Ability (GCA) of multiple traits (HGCAMT)

This grouping method offers a more accurate technique of heterotic group classification of inbreeds as it considers the *gca* effect of several traits, which quantify additive gene effects for each trait. When the breeders aim for the development of inbreds or hybrids which is tolerance or resistance to several stresses this approach is preferable. Oyetunde *et al.*(2020) used this method for classifying the inbred lines resistance to *Striga* infestation and drought.The following statistical model was used to standardize the *gca* effects (mean of zero and standard deviation of 1) of the traits that exhibited significant mean squares for genotypes under each research condition in order to group by using HGCAMT approach (Badu Apraku *et al*., 2015).

$$Y = \sum_{t=1}^{n} \left(\frac{(Gi - gi)}{si} \right) + \in ij$$

Where,Y is HGCAMT, which is the genetic value measuring relationship among genotypes based on the GCA of multiple traits i to n; G is the individual *gca* effects of genotypes for trait i; g is the mean of *gca* effects across genotypes for trait i; si is the standard deviation of the *gca* effects of trait i; ∈ ijis the residual of the model associated with the combination of inbred i and trait j.

Molecular markers

It is challenging to assess a larger set of parental lines because; it is impossible to generate comprehensive information on combining ability and does not accurately depict genetic relationships. In previous methods, the heterotic groups are established by testing all possible hybrid combinations of the inbred lines at the field level based on morphological data. To get around these problems, it was discovered that using molecular markers was a trustworthy method that assesses genetic differences across inbred lines directly using molecular marker-based genetic distance. Earlier Restriction Fragment Length Polymorphism (RFLP) markers are generally utilized for the assessment of genetic diversity (Pinto *et al.,* 2003). Microsatellite or simple sequence repeats (SSRs) are also the favoured methods in classifying maize germplasm into discrete heterotic groupings(Akinwale *et al.,* 2014). In modern days, Single nucleotide Polymorphism (SNP) markers are used for identifying the heterotic groups (Annor *et al.,* 2020).

Breeding Efficiency

Breeding efficiency is the proportion of superior, high-yielding intergroup-heteroticcrosses produced out of all the crosses (Fan *et al.,*2009). Let's consider there are 150 hybrids. The efficiencies of the different heterotic grouping methods need to be compared by arranging 150 hybrids from the highest to the lowest based on the means of grain yield.The total number of 150 hybrids for each method should be divided into two major groups: inter-heterotic group and intra-group heterotic crosses. These two groups must be subsequently divided into high-yielding hybrids (Yield Group 1 with a mean grain yield ranking among the top 50 hybrids), intermediate-yielding hybrids (Yield Group 2 with a grain yield between the 51th and 100 th hybrid) and low-yielding hybrids (Yield Group 3 with a mean grain yield between 101th and 150 th hybrid).The best classification method can be identified based on the breeding efficiency proposed by Fan et al. (2009) and modified by Badu-Apraku *et al*. (2016).

$$BE = \frac{\left[\frac{HYINTERGROUPH}{TNINTERGROUPH} \times 100\right] + \left[\frac{LYINTRAGROUPH}{TNINTRAGROUPH} \times 100\right]}{2}$$

Where, BE = Breeding efficiency, HYINTERGROUPH = Number of high-yielding inter-heterotic group hybrids, TNINTERGROUPH = Total number of inter-heterotic group hybrids, LYINTRAGROUPH = Number of low-yielding intra-heterotic group hybrids, TNINTRAGROUPH = Total number of intra-heterotic group hybrids

Conclusion

Heterotic grouping is a crucial concept in plant breeding that focuses on the genetic diversity between two distinct groups to produce offsprings with superior traits. Through the combination of two genetically diverse parents from opposite heterotic groups, high heterotic hybrids can be obtained that often exhibit increased vigor, yield, and overall performance compared to their parents. This canbe extensively studied and utilized in various crop species to enhance productivity and adaptability.

Additionally, the identification and selection of optimal parental lines play a significant role in maximizing heterotic effects in hybrid breeding programs. Within a heterotic group inbred improvement programme may be planned through recurrent selection procedures. By identifying the heterotic groups and harnessing its potential, plant breeders can develop improved hybrids that meet the demands of modern agriculture by ensuring sustainability and resilience in the face of environmental challenges.

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