



Heterotic Grouping: A Novel Strategy to Enhance Hybrid Breeding Efficiency in Crop Plants: A Review

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ABSTRACT

Heterosis refers to the increased vigour, yield and overall performance of F_1 hybrids compared to their parental lines. Heterotic grouping is an essential strategy in hybrid breeding that aims to classify germplasm lines into distinct groups to maximize heterosis and hybrid performance. Various methods of heterotic grouping include pedigree analysis, combining ability analysis, phenotypic clustering and marker-assisted heterotic grouping. Pedigree analysis classifies lines based on their genetic background and ancestral information. Combining ability analysis is a widely used approach that classifies lines based on their performance in hybrid combinations. Phenotypic clustering utilizes multivariate analysis to classify germplasm based on agronomic and morphological characters. Marker-assisted heterotic grouping involves various molecular markers like RFLP, AFLP, RAPD, SSR and SNP. Advances in genetic mapping techniques and incorporating genomic selection may improve the understanding and precision of heterotic grouping. Heterotic grouping is a powerful strategy in hybrid breeding that enables breeders to efficiently create superior hybrids by exploiting genetic diversity and heterosis.

Key words: Combining ability analysis, Heterosis, Heterotic grouping, Marker-assisted heterotic grouping.

Plant breeding is a vital agricultural technique aimed at developing new crop varieties with higher yield, improved quality and better adaptability. A key method within this field is hybrid breeding, which involves crossing two genetically distinct genotypes to produce hybrids exhibiting desirable traits. A central concept in hybrid breeding is *heterosis*, or hybrid vigour. The term heterosis was coined by Shull in 1914 and was first detected in maize. Heterosis refers to the superiority of an F_1 hybrid in one or more characters over its parents. Shull (1952) defined heterosis as, the 'Interpretation of increased yield, vigour, fruitfulness, size, resistance to abiotic and biotic stress or speed of development, manifested by cross-bred organisms as compared with corresponding inbreds, as the specific results of unlikeness in the constituents of the uniting parental gametes. Heterosis is a chief cause of non-additive gene action which is the maximum in cross-pollinated crops. It is particularly significant in cross-pollinated crops, where outbreeding increases heterozygosity and improves hybrid performance.

To maximize heterosis, breeders classify germplasm into *heterotic groups*-collections of genotypes that produce superior hybrids when crossed with members of another group. This categorization helps identify optimal parental combinations and utilize genetic diversity effectively. The main objective of heterotic grouping is to develop superior hybrid combinations within a short period of time. By exploiting heterosis through careful parental selection and grouping, plant breeding significantly enhances crop productivity and resilience. The present study is a review about the importance of heterotic grouping, its objectives and different methods employed for the formation of heterotic groups.

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Principles of heterotic grouping

Heterotic grouping is vital in hybrid breeding, based on principles like genetic diversity, heterosis, combining ability and genetic distance. Genetic diversity enables classification by maximizing variability between lines, which enhances hybrid vigor when complementary traits combine. Heterosis is another important principle around which the heterotic grouping is done. The lines were classified into different groups and evaluated for heterosis in the F_1 hybrid. Combining ability analysis helps to classify the germplasm into heterotic groups. Lines within the same group tend to have similar combining ability than between groups. Genetic distance, often measured using molecular markers, further aids in grouping; greater distance between lines from different groups indicates more variability and higher heterosis potential.

Heterotic grouping

Heterotic grouping is a pre-requisite step in hybrid breeding. It is the process of grouping different germplasm lines into various heterotic groups. Melchinger and Gumber (1998) defined a heterotic group 'as a group of related or unrelated genotypes from the same or different populations, which display similar combining ability and heterotic response when crossed with genotypes from other genetically distinct germplasm groups'. When two divergent heterotic groups are crossed, it results in the formation of superior hybrid combinations. These hybrids were found to be more productive than the hybrids developed from the same heterotic group. Heterotic grouping involves identifying diverse germplasm groups that when crossed with one another, produce high-quality hybrids with greater heterosis. A heterotic group is a group of genotypes which exhibits similar combining ability and a heterotic behaviour when crossed with genotypes of other groups.

Steps in heterotic grouping

The steps given by Melchinger and Gumber (1998) for the identification of heterotic groups were,

1. Grouping germplasm based on genetic similarity.
2. Selection of representative genotypes from subgroups for producing diallel-cross.
3. Evaluation of diallel crosses along with parents in replicated trials.
4. Selection of most promising cross combinations as potential heterotic groups.

If heterotic patterns are present already, the superior genotypes in a heterotic group can be taken as testers and used for evaluation and classification of existing germplasm. Test cross is performed and the lines with similar combining ability and heterotic behaviour can be grouped into a new heterotic group. If the lines have similar combining ability and heterotic behaviour as of the existing heterotic group then it can be merged to the existing heterotic groups.

Melchinger and Gumber (1998) recommended the following criteria for the choice of heterotic patterns:

- (1) High mean performance and large genetic variance in the hybrid population.
- (2) High per se performance and good adaption of the parent population to the target region(s).
- (3) Low inbreeding depression, if hybrids are produced from inbred lines.

Heterotic pattern

The heterotic pattern is a key factor for utilizing germplasm to maximize the performance of the population crosses and derived hybrids (Eberhart *et al.*, 1995). The term heterotic pattern refers to a specific pair of two heterotic groups, which express high heterosis and consequently high hybrid performance in their cross (Melchinger and Gumber, 1998). A heterotic pattern is improved by exploiting genetic variation generated within heterotic

groups (Riedelsheimer *et al.*, 2012). The primary criteria to develop a heterotic pattern proposed by Reif *et al.* (2005) was the hybrid form the heterotic pattern should show high heterosis and yield with a reduced SCA and lower SCA/GCA ratio or higher GCA/SCA ratio. Heterotic patterns can determine the type of genotype in hybrid breeding for a long period.

Objectives of heterotic grouping

1. To obtain improved hybrid performance and mean heterosis.
2. To lessen the specific combining ability (SCA) variance and reduce the ratio of SCA to GCA variance.
3. To eliminate unnecessary crosses which were made to find out superior hybrids by classifying lines into heterotic groups.
4. To save the time and resources for hybrid development.
5. To broaden the genetic base of hybrid by utilizing new germplasm lines.

Methods of heterotic grouping

A heterotic group classification can be done either based on a quantitative or molecular approach. The quantitative methods of classification include HGCAMT, HSGCA and SCA methods which use combining ability estimates of pedigree lines and hybrid field data to classify inbreds. The molecular-based techniques classify lines based on genetic distance (GD) or genetic similarity (GS) from molecular markers which is very useful for describing heterotic groups and studying associations among inbreds at molecular level. Results from both quantitative and molecular approaches vary depending on the environment, the test material or the molecular technique used. The different methods of heterotic grouping along with its advantages were presented in Table 1.

Pedigree analysis

Heterotic grouping with pedigree analysis is a traditional method of heterotic grouping. Classification of germplasm lines into different heterotic groups can be done using pedigree analysis. Pedigree analysis helps to obtain the genetic diversity present in the population with which the germplasm is assigned to different heterotic groups. The Reid and Lancaster heterotic groups of maize were formed using the pedigree method of analysis which is utilized in the Corn Belt (Region of United States). Reif *et al.* (2003) classified sub-tropical maize into two distinct heterotic groups (flint and dent) using SSR markers and pedigree information.

Pedigree analysis was performed by Reid *et al.* (2011) to classify 129 maize inbred lines out of which 119 maize inbred lines were classified into different heterotic groups using pedigree analysis combined with the SSR marker. The main concept of pedigree analysis is to trace the inbred's genetic background to their ancestral varieties using pedigree information. The inbred is grouped based on the percentage of the parent's contribution. The inbred will be assigned to a heterotic group of parents which

contributed more than 50% of the genetic background in that particular inbred. For example, The inbred CM105 from (V3 X B14) consist of genes from B14 about 87.5% and is grouped based on the similarity of genetic background in BSSS germplasm to which B14 belongs. In the case of the inbreds CO343 and CO344 whose parents were Mo17 and CO255 are classified as the group with the genetic background of CO255. The contribution of these parents is equal in the inbreds CO343 and CO344 but it is grouped to CO255 as it is early maturing which is most suitable in Canada. Using the pedigree method 94 inbreds were classified into 8 groups but 35 inbreds lines cannot classified into any of these 8 groups. Table 2 depicts the classification of groups based on the major sources of parents and the number of genotypes in each group.

Simple sequence repeats (SSR) analysis revealed that inbred lines having closer pedigree relationships have more genetic similarity. The average genetic similarity within the BSSS group is found to be greater than the average genetic similarity between BSSS and another group (Fig 1). Discriminant analysis reveals that SSR analysis is in accordance with the pedigree groups.

Combining ability analysis

Combining ability is the ability of the genotype to transmit superior performance to its crosses. It is the ability of the parents to combine well with each other. It is the capacity of a genotype to pass superior traits to its crosses, classified as general combining ability (GCA) and specific combining ability (SCA). GCA reflects average performance across crosses, while SCA shows performance in specific crosses, measured via diallel or line $\times$ tester mating designs. Combining ability analysis is crucial for classifying germplasm into heterotic groups, enabling the development of superior hybrids. Line $\times$ tester mating, using proven testers, is commonly used for this purpose; if testers are unavailable, diallel mating is an alternative. Overall, combining ability analysis is essential in hybrid breeding and heterotic grouping. Heterotic grouping in pigeon pea can also be done using combining ability along with next generation sequencing (Patel *et al.*, 2022). The heterotic grouping of mungbean lines were done using combining ability studies through diallel analysis by Singh *et al.* (2016) based on seed size and yield.

Combining ability can be used in three ways to formulate a heterotic group.

1. Using SCA effects of grain yield.
2. Using both GCA and SCA effects of grain yield.
3. Using GCA effects for multiple component traits.

Heterotic grouping using SCA

In this method, inbred lines are grouped based on their SCA effects for traits like grain yield. The inbred lines are crossed to proven testers and their SCA effects for grain yield are determined. Lines with similar SCA effects are placed in the same heterotic group. For this, the identification of a proven tester is the preliminary step. The inbred lines are crossed with some testers and a pair of testers which shows contrasting positive and negative SCA effects are chosen as proven testers. Based on these estimates of GCA and SCA the inbreds are classified into different heterotic groups. The inbred lines which showed positive GCA effects are selected. Out of these lines, the inbreds which show a positive SCA effect with one tester and with higher grain yield than the grand mean can be grouped into one heterotic group and the inbreds which show a positive SCA effect with other testers with higher grain yield than grand mean can be grouped into another heterotic group. Hence when crossing inbreds present in different heterotic groups maximum heterosis can be obtained.

This method was used in a study by Singh *et al.* (2021) to classify 61 maize inbred lines into heterotic groups using the SCA estimates for grain yield. First, the 61 inbred lines were crossed by two inbred testers in line $\times$ tester mating design and 122 test-crosses were generated. BML 6 and BML 7 were used as testers and these inbred testers are the parents of DHM 117, a well-adapted maize variety. Three hybrid checks were maintained along with the test crosses in a randomized block design and morphological observations are recorded to estimate GCA and SCA effects. Along with this, the economic heterosis of F_1 is calculated by comparing the standard checks. Out of 61 inbred lines, 29 lines showed positive GCA and SCA effects with higher mean grain yield. The SCA effects of these 29 inbred lines in the test cross are then compared with the mean yield of testers. The lines which show positive SCA effects with the tester BML 7 and test cross mean grain yield greater than or equal to the mean yield of test cross of inbreds with both

Table 1: Methods of heterotic grouping.

Methods	Advantages
Pedigree method	Helps to identify heterotic groups with greater genetic diversity using pedigree information.
Heterotic grouping using SCA	Identifies heterotic group using SCA effects.
Heterotic grouping using SCA and GCA	Identifies heterotic group using SCA and GCA effects.
Heterotic grouping using GCA for multiple traits	Identifies heterotic group using GCA effects for multiple traits under different environment conditions.
Phenotypic clustering	Formation of heterotic groups based on multivariate analysis.
Marker assisted heterotic grouping	Most reliable and accurate heterotic groups can be created using SSR and SNP markers.

the testers grouped to heterotic group A (12 lines) and the lines which show positive SCA effects with the tester BML 6 and with test cross mean grain yield greater than or equal to the mean yield of test cross of inbreds with both the testers was grouped to B (17 lines).

Heterotic grouping using SCA and GCA

Fan *et al.* (2008) proposed a heterotic group's specific and general combining ability (HSGCA) model to classify the inbred lines based on both GCA and SCA effects.

The heterotic group's specific and general combining ability (HSGCA) method proposed by Fan *et al.* (2009) was computed as follows:

$$\text{SCA} = \text{Cross mean (X}_{i.}) \times \text{Line mean (X}_{.j}) \times \text{Tester mean (X}_{i.}) + \text{Overall mean (X}_{..})$$

$$\text{GCA} = \text{Line mean (X}_{.j}) \times \text{Overall mean (X}_{..})$$

$$\text{HSGCA} = \text{Cross mean (X}_{i.}) \times \text{Tester mean (X}_{i.}) = \text{GCA} + \text{SCA}$$

Where,

X_{ij} = Mean yield of the cross between *i*th tester and *j*th line.

X_{i.} = Mean yield of the *i*th tester.

X_{.j} = Mean yield of *j*th line.

This method also involves the identification of testers and the grouping of lines into different heterotic groups.

1. The inbred lines with negative HSGCA effects were grouped into the same heterotic group in which their corresponding tester was present. So the inbreds will be classified into two known tester groups. Here a line can be grouped into more than one heterotic group.
2. In the second step, the line which is grouped into more than one heterotic group will be grouped into the heterotic group with the smallest value or largest negative value and can be removed from other heterotic group.
3. The inbred lines with positive HSGCA effects were grouped into different unknown heterotic groups different from the two testers.

Identification of representative tester lines is key for the HSGCA method (Fan *et al.*, 2009). This method combines both GCA and SCA effects and hence it is more efficient than the grouping method where only the SCA effect is used. This is because in SCA the interaction of hybrid and the environment may lead to mismatching of lines into different heterotic groups.

For example, a study was conducted for the classification of 21 quality protein maize (QPM) inbred lines into heterotic groups and was done using the HSGCA method by Mekasha *et al.* (2021). The 21 lines were crossed with two elite QPM lines as testers CML 159 and CML 144 in a line × tester mating design. The estimates of SCA effects, GCA effects and grain main yield were obtained for 21 maize inbred lines. Then HSGCA was calculated based on the formula given by Fan *et al.* (2009) for the inbreds. The procedure mentioned by Fan *et al.* (2009) was also followed for the classification of heterotic groups.

These maize inbred lines were classified into 4 different groups as in Table 3. The lines which show a

negative HSGCA effect with tester CML 159 were grouped along with that tester in group Heterotic group A. The lines which showed a negative HSGCA effect with tester CML 144 were grouped in Heterotic group B along with the tester. The lines which show negative HSGCA with both the testers were grouped into Heterotic group AB and the lines with positive HSGCA were grouped into unknown heterotic group.

HSGCA method was found to be most effective in classifying the maize inbred lines into different heterotic groups. To obtain heterosis for grain yield a breeder should choose both the lines and testers with positive GCA effects or at least the lines with positive GCA effects which can be used as female parents. Similarly, in wheat heterotic groups have been made using GCA and SCA effects (Deviren *et al.*, 2024).

Heterotic grouping using GCA with multiple traits

Badu-Apraku *et al.* (2013) proposed heterotic grouping based on the GCA of multiple traits (HGCAMT) method.

Badu-Apraku *et al.* (2015) explained that grouping by the HGCAMT method was achieved by standardizing the GCA effects (mean of zero and standard deviation of 1) of the traits that had significant mean squares for genotypes under each study condition using the following statistical model:

$$Y = \sum_{i=1}^n (Y_i - \bar{Y}_i | S) + \varepsilon_{ij}$$

Where,

Y = HGCAMT, which is the genetic value measuring the relationship among genotypes based on the GCA of multiple traits *i* to *n*.

Y_i = Individual GCA effect of genotypes for trait *i*.

$\bar{Y}_i$ = Mean of GCA effects across genotypes for trait *i*.

S = Standard deviation of the GCA effects of trait *i*.

ε_{ij} = Residual of the model associated with the combination of inbred *i* and trait *j*.

Grouping of Inbred lines based on a single trait *i.e.*, grain yield can mislead the classification as grain yield is a quantitative character and controlled by polygenes. In case of grain yield, if the yield gets reduced subsequently the heritability of grain yield will also be decreased. The

Table 2: Heterotic grouping in maize by pedigree method (Reid *et al.*, 2011).

Groups	Major sources	No. of genotypes
1	BSSS, B73, B14, B37 and BSTE	18
2	INRA 258, Pfister 44 and lethbridge gene pool	19
3	Mo17, Oh43, LH83, W153, H99	15
4	Minnesota 13 or commercial hybrid pride 5	7
5	Early butler	7
6	Iodent	4
7	Pioneer 3990	11
8	Pioneer 3994	13

HGCAMT method uses multiple component traits of inbreds with significant GCA effects which have a positive strong correlation with grain yield under different environmental conditions.

A study was conducted at IITA, Nigeria by Okunlola *et al.* (2023) to find the combining ability of early-maturing quality

Table 3: Heterotic grouping in maize by HSGCA (Mekasha *et al.*, 2021).

Groups	Inbred lines
HGA	L12, L14, L16, L18, L19 and L 20
HGB	L3, L10, L11
HGAB	L1, L2, L3 and L13
UNKNOWN	L5, L6, L7, L8, L9, L15, L17 and L21

Table 4: Heterotic grouping in maize by HGCAMT (Okunlola *et al.*, 2023).

Groups	Number of inbred lines
1	22
2	1
3	14
4	10

protein maize inbred lines under *Striga*-infested conditions and low nitrogen conditions. After finding the GCA and SCA estimates they classified the inbreds into different heterotic groups using the HGCAMT method. A total of 47 inbred lines and 4 testers along with 2 hybrid checks that have combined resistance for both *Striga*-infested and low N conditions were classified (Fig 2). 188 test crosses were done in striga-infested conditions, low N conditions and optimum conditions. Observations are recorded for morphological and yield-related traits under each experiment. Heterotic grouping using HGCAMT proposed by Badu-Apraku *et al.* (2015) was followed in this study.

Forty seven inbreds were successfully placed into 4 heterotic groups along with the tester are mentioned in Table 4.

The QPM hybrids obtained from crossing lines in the different heterotic groups will be resistant to *Striga*-infested conditions, tolerant to low N content in soil and will be early-maturing hybrids.

Phenotypic clustering

It is a conventional heterotic group classification method. Multivariate analysis is used to classify the germplasm into various heterotic groups based on the differences in

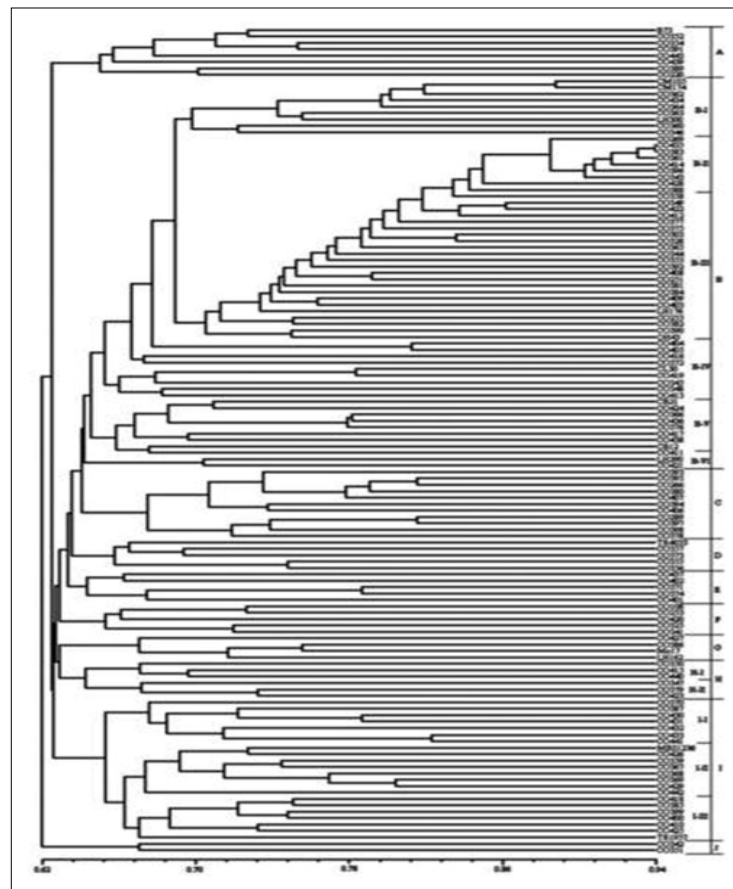


Fig 1: Dendrogram constructed with an unweighted paired group method using the arithmetic average (UPGMA) clustering algorithm from the genetic similarities (GS) among 129 maize inbred lines (Reid *et al.*, 2011).

agronomic and morphological characters. Due to environmental influences and unknown genetic mechanisms, the reliability of classification is uncertain. Hence phenotypic clustering along with genetic characterization of the population using molecular markers proved to be an effective way to classify germplasm into different heterotic groups.

The clustering method was effectively used for the development of heterotic groups in sunflower by Ibrar *et al.* (2024). 109 Sunflower genotypes were classified into different heterotic groups and the efficiency of identification of these groups was evaluated using the performance of F_1 in line $\times$ tester method. Phenotypic, genotypic and biochemical data were combined and using 3 different algorithms (hierarchical, k-means, hybrid), the lines were assigned to the heterotic groups.

Table 5: Heterotic grouping in sunflower by phenotyping clustering (Ibrar *et al.*, 2024).

Groups	No. of genotypes
Subgroup 1A	6
Subgroup 1B	3
Subgroup 1C	8
Subgroup 1D	6
Subgroup 1E	2
Subgroup 1F	6
Subgroup 2A	8
Subgroup 2B	11
Subgroup 2C	7
Subgroup 2D	20
Subgroup 2E	20
Subgroup 2F	12

The hierarchical algorithm clustering (Fig 3) was found to be the best for the classification of sunflower genotypes into heterotic groups. The D^2 method was used for the combined data of morphological, biochemical and genotypic characterization. The result of this clustering revealed two distinct groups of genotypes. Out of 109, 31 genotypes were grouped under Cluster 1 and 78 genotypes were grouped under Cluster 2 respectively. Cluster 1 consists of restorer lines and Cluster 2 consists of CMS lines, B lines and self-pollinated lines. Based on an estimate of genetic distance the clusters are further divided into subgroups *i.e.*, heterotic groups. Cluster 1 was divided into 6 subgroups and Cluster 2 was divided into another 6 subgroups as in Table 5.

Twelve genotypes from each heterotic group (sub-group) were crossed in line $\times$ tester mating design and 36 F_1 hybrids were evaluated for their performance. In each cross, one genotype from a heterotic group of CMS lines is crossed with other genotype from a heterotic group of restorer lines. The final results revealed that the hybrids produced by crossing lines from two different heterotic groups showed remarkable heterosis for the traits studied in a desirable direction and can be used in the future hybrid breeding of Sunflower. Multivariate analysis of fifty pea genotypes conducted by Singh *et al.* (2021) to identify genetic divergence based on phenotypical traits and grouped them into six clusters. Maximum value of inter-cluster distance ($D^2 = 6.471$) was recorded between cluster I and cluster IV genotypes these clusters can be used as distant parents in any breeding programme for pea.

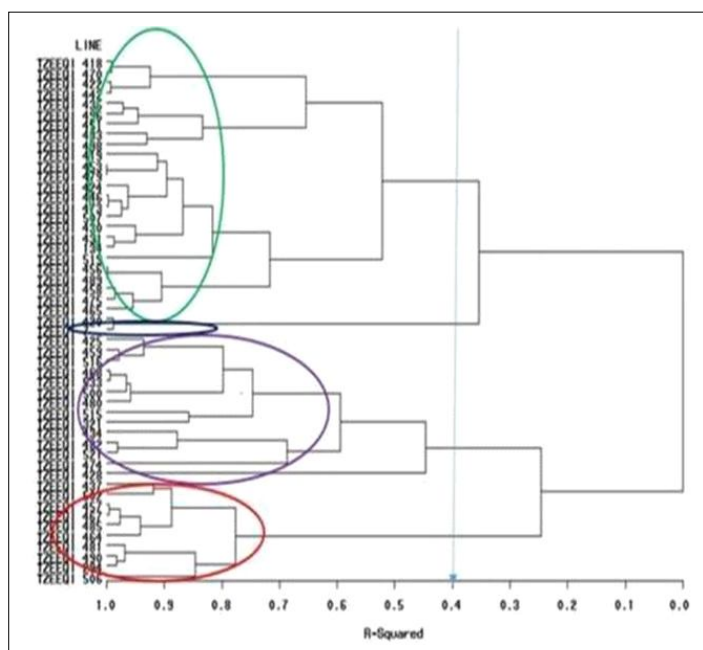


Fig 2: Heterotic grouping based on the general combining ability of 47 extra-early quality protein maize across research environments (Okunlola *et al.*, 2023).

Marker assisted heterotic grouping

Molecular technology has become a vital tool in modern plant breeding, offering advantages over conventional methods through higher efficiency, reduced costs and errors and independence from environmental influences. Molecular markers allow for whole-genome assessment and have proven effective in classifying inbred lines into heterotic groups. Barata and Carena (2006) emphasized using molecular approaches as preliminary tools in field evaluations to create distinct heterotic groups with strong within-group genetic similarities.

In hybrid breeding, particularly in maize, molecular markers are widely used to assess genetic distances,

which underpin heterotic grouping. Genetically similar lines within a group have smaller distances, while genetically dissimilar lines between groups exhibit larger distances. For accurate correlations between genetic distance and hybrid performance, essential requirements include appropriate phenotypic evaluation, the right marker choice and a sufficient number of inbred lines.

Restriction fragment length polymorphism

RFLP was among the first markers used for heterotic grouping, particularly in maize. It proved useful in assessing genetic diversity and grouping lines by geographic origin. However, limitations were noted-RFLP alone could not reliably differentiate between testers from opposite

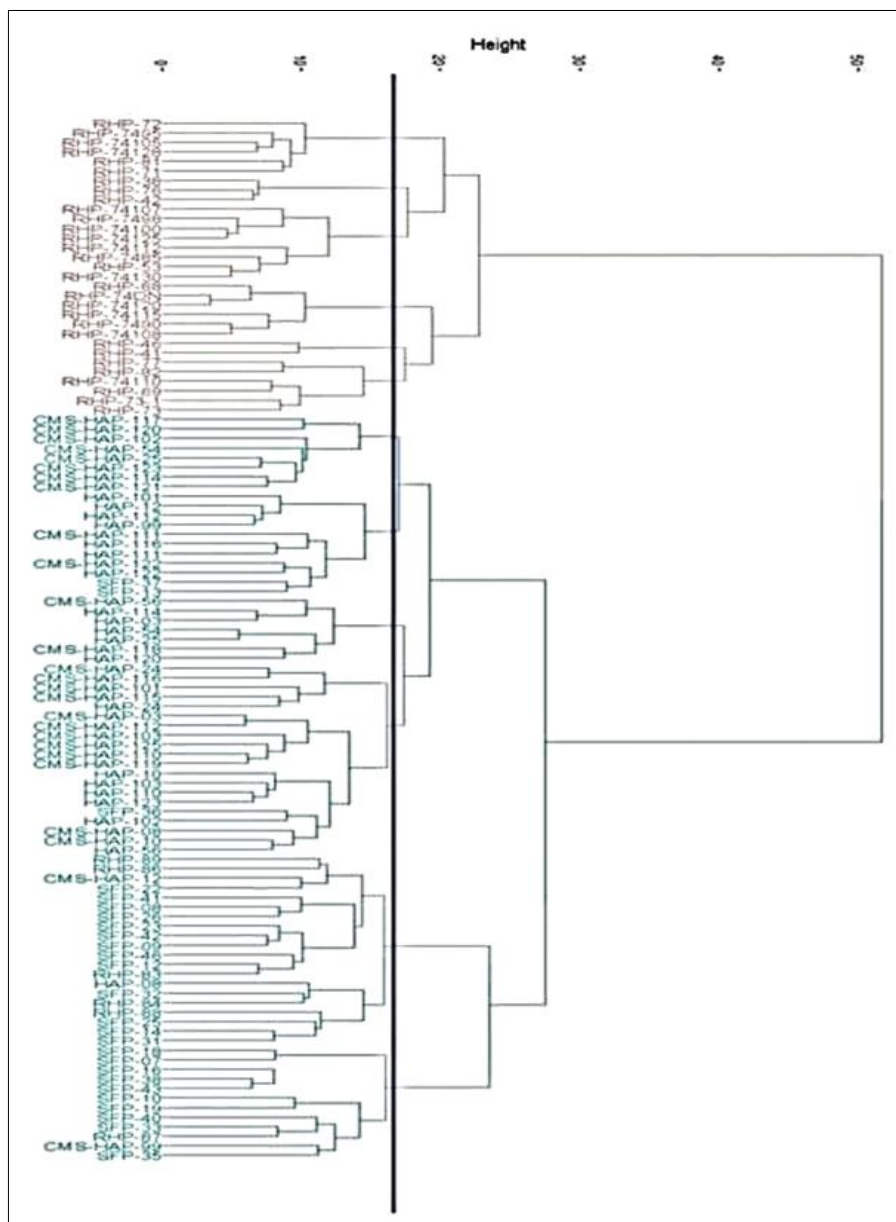


Fig 3: Hierarchical clustering of 109 sunflower genotypes through the Ward D^2 method (Ibrar *et al.*, 2024).

heterotic groups (Warburton *et al.*, 2005) and was better at predicting intra-group cross performance than inter-group performance (Melchinger *et al.*, 1998).

Amplified fragment length polymorphism

AFLP emerged as an improvement over RFLP, offering more polymorphism and efficiency by evaluating many loci in a single PCR. Studies by Ajmone Marsan *et al.* (1998) and Pejic *et al.* (1998) showed its usefulness in identifying genetic diversity. AFLP-based genetic distance measurements were moderately correlated with hybrid performance, making AFLP a reliable but somewhat limited predictor of F_1 yield. Li *et al.* (2004) successfully classified Italian and Chinese inbred maize lines using AFLP, demonstrating its utility in global germplasm classification.

Random amplified polymorphic DNA

RAPD markers have also been employed to assess genetic diversity in maize, wheat, rice and barley. Though easy to use and capable of revealing high polymorphism, RAPD markers tend to show low correlation between genetic distance and hybrid performance. For instance, Parentoni *et al.* (2001) used RAPD to group 28 maize varieties, while Bruel *et al.* (2007) classified 16 inbred lines into five heterotic groups. Bootstrap methods confirmed sufficient RAPD markers for reliable results, though predictive accuracy for hybrid yield remained low.

Simple sequence repeats

SSR markers are among the most widely used for heterotic grouping due to their high polymorphism and reproducibility. They enable morphological and molecular profiling, making them ideal for identifying desirable heterotic patterns. SSR-based groupings often align with pedigree and combining ability analyses. Reif *et al.* (2003) and Aguiar *et al.* (2008) found SSR markers useful for systematic introgression of exotic germplasm. Sruthi *et al.* (2020) used 50 SSR markers to classify 96 rice parental lines into two distinct heterotic

groups-Maintainer (B lines) and Restorer (R lines) with results matching pedigree data.

Single nucleotide polymorphism

Recently SNP are in great demand for molecular studies especially in the categorization of heterotic groups. SNP markers based on genetic distance show a high correlation with the heterosis of F_1 so it can be used to predict the performance of F_1 along with the classification of heterotic groups. It is a more advantageous and effective method of heterotic grouping when compared to other methods of grouping like HSGCA and HGCAMT heterotic grouping (Badu-Apraku *et al.*, 2015). A study was performed by Silva *et al.* (2021) in sorghum to evaluate the genetic diversity and identify heterotic groups. Genotyping of 160 sorghum lines was done using 86,342 SNP markers. Population structure analysis was analysed using a Bayesian model-based clustering algorithm in a STRUCTURE software program. Principal component analysis was done on a marker-based similarity depicted in Fig 4. The clustering method was conducted using the Neighbor-Joining model as in Fig 5. Then four heterotic groups were identified using both PCA and clustering methods. The cluster G1 and G2 consists mainly of R lines, G3 has both B lines and R lines and G4 has B lines. The PCA and clustering analysis was found to be correlated with pedigree analysis.

SNPs were also used in sorghum for classifying 96 inbred lines into different heterotic groups (Zhang *et al.*, 2025). The inbreds were critically analyzed by whole-genome sequencing and categorized into group 1 which contains restorer lines and group 2 with male sterile lines respectively. Hybrids exhibiting strong heterosis were obtained by crossing group 1 and group 2.

Efficiency of methods of heterotic grouping

The above mentioned heterotic grouping methods by SCA for grain yield, HSGCA, HGCAMT and SNP-GD were assessed for their efficiencies in the classification of

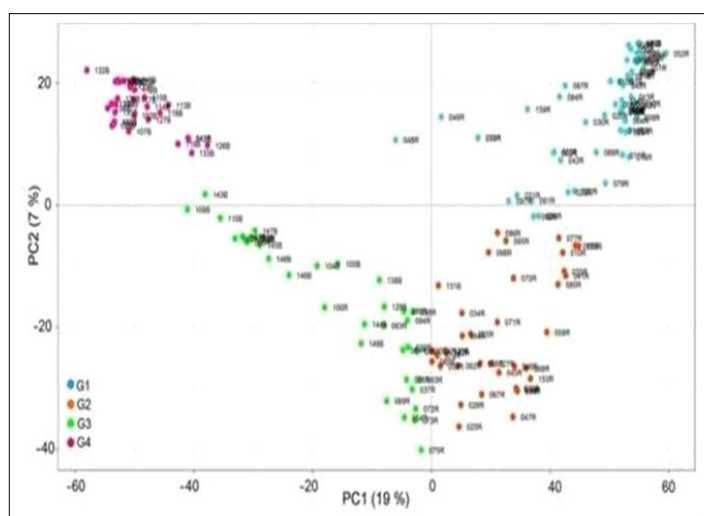


Fig 4: PCA analysis based on 29,649 SNP markers for 160 sorghum lines (Silva *et al.*, 2021).

heterotic grouping. Fan *et al.* (2009) and Badu-Apraku *et al.* (2016) proposed a formula for estimating the efficiency of heterotic grouping methods.

Breeding efficiency =

$$\left(\frac{HY_{intergroup}}{TN_{intergroup}} \times 100 \right) + \left(\frac{LY_{intergroup}}{TN_{intergroup}} \times 100 \right)$$

Where,

$HY_{intergroup}$ = Number of high yielding inter-heterotic group hybrids.

$TN_{intergroup}$ = Total number of inter-heterotic group hybrids.

$LY_{intergroup}$ = Number of low yielding inter-heterotic group hybrids.

$TN_{intergroup}$ = Total number of inter-heterotic group hybrids.

Fan *et al.* (2009) state that the strength of the inter-heterotic group crosses in relation to the within-group crosses determines the effectiveness of a heterotic grouping strategy. But it also depends upon other factors like the percentage of available inbred lines, the genetic diversity among genotypes and heterotic patterns obtained from the group. Several researchers have used these techniques to look into the grouping of maize germplasm lines. Furthermore, Akinwale *et al.* (2014) compared the efficiency of SCA, HSGCA and SSPr-based GD techniques for classifying early ripening yellow maize inbreds assessed under *Striga*-infested and *Striga*-free growth conditions using orthogonal comparisons. The HSGCA approach was the most effective method under the conditions for research.

Factors affecting heterotic grouping

Heterotic grouping is influenced by genetic structure, linkage disequilibrium (LD), molecular markers, genetic

divergence measures, combining ability and phenotypic data.

Genetic structure

The genetic makeup of a population, including diversity and subpopulation, plays a vital role. High genetic variability allows broader heterotic group formation, improving breeding outcomes. Subpopulations, often shaped by geography or breeding programs, frequently form distinct groups.

Molecular markers

Markers like SSR, RFLP and RAPD are essential for estimating genetic distances between lines. Greater genetic distances typically correlate with stronger heterosis. Advancement in molecular markers with high throughput technology played a vital role in estimation of genetic diversity and analysis of heterosis with marker assisted selection (Nair and Pandey, 2024).

Linkage disequilibrium

LD between genes and markers influences grouping precision. High LD enhances accuracy and impacts epistatic variance and breeding strategies. LD can also highlight overdominant loci, aiding hybrid performance predictions.

Genetic divergence measure

Methods like cluster analysis using SNPs offer precise heterotic grouping by directly assessing genetic diversity.

Combining ability

GCA and SCA help classify lines based on hybrid performance. HSGCA and HGCAMT methods are effective under stress conditions like drought or pests.

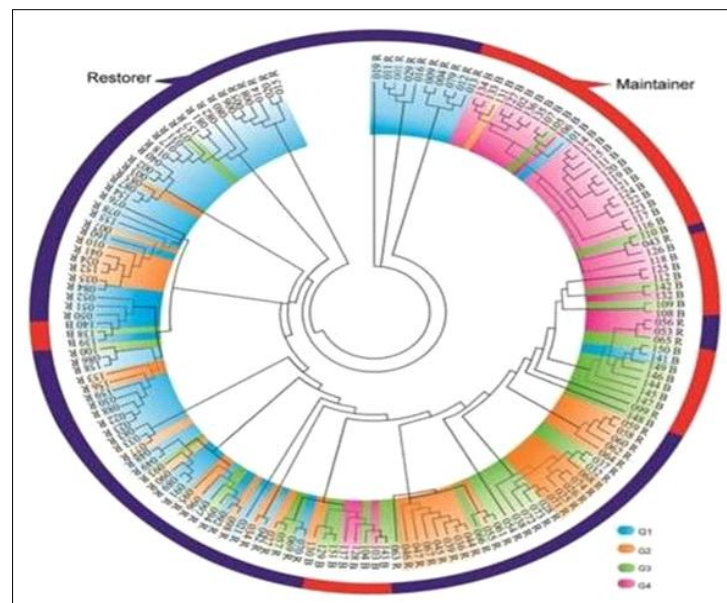


Fig 5: Cluster of 160 lines according to Neighbor- Joining model (Silva *et al.*, 2021).

Phenotypic data

Phenotypic traits such as yield, resistance and stress tolerance validate genetic groupings. High phenotypic diversity suggests greater heterotic potential, guiding the selection of optimal parent lines for hybrid vigor.

Advantages and disadvantages

Heterotic grouping offers several advantages in plant breeding. The advantages and disadvantages of heterotic grouping are listed below.

Advantages	Disadvantages
Helps to maximizes heterosis.	Time and resource intensive process.
Helps to assesses genetic diversity.	Complexity.
Efficient usage of germplasm.	Risk of inbreeding depression.
Reduces unnecessary hybrids and improves selection accuracy.	Sensitive to environmental conditions.

CONCLUSION

It is possible to create a large number of hybrid combinations quickly within a short period if heterotic groups and their patterns are discovered. Exploitation of heterosis successfully creates heterotic groups and places the germplasm lines in appropriate heterotic groupings. Collaborating with global research institutes promotes germplasm exchange and group effectiveness. Though applied in many crops like rice, maize and sorghum, heterotic grouping should expand to all important and minor crops. Advances in genetic mapping, genomic selection and AI in future studies will improve precision in identifying parental lines and evaluating group efficiency under varied environments. By identifying heterotic groupings, the ideal parent combination can be chosen to produce superior hybrids. These heterotic groups are helpful for a breeder to produce superior high-yielding hybrids. Hence it is a novel strategy which must be employed for hybrid breeding.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

No animal procedures were involved.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

REFERENCES

- Aguiar, C.G., Schuster, I., Amaral Junior, A.D., Scapim, C.A., Vieira, E.S.N. (2008). Heterotic groups in tropical maize germplasm by test crosses and simple sequence repeat markers. *Genetics and Molecular Research*. **7(4)**: 1233-1244.
- Ajmone Marsan, P., Castiglioni, P., Fusari, F., Kuiper, M., Motto, M. (1998). Genetic diversity and its relationship to hybrid performance in maize as revealed by RFLP and AFLP markers. *Theoretical and Applied Genetics*. **96(2)**: 219-227.
- Akinwale, R.O., Badu-Apraku, B., Fakorede, M.A.B., Vroh-bi, I. (2014). Heterotic grouping of tropical early maturing maize inbred lines based on combining ability in Striga-infested and Striga-free environments and the use of SSR markers for genotyping. *Field Crop Research*. **156**: 48-62.
- Badu-Apraku, B., Fakorede, M.A.B., Gedil, M., Talabi, A.O., Annor, B., Oyekunle, M., Akinwale, R.O., Fasanmade, T.Y., Akaogu, I.C., Aderounmu, M. (2015). Heterotic responses among crosses of IITA and CIMMYT early white maize inbred lines under multiple stress environments. *Euphytica*. **206(1)**: 245-262.
- Badu-Apraku, B., Fakorede, M.A.B., Talabi, A.O., Oyekunle, M., Akaogu, I.C., Akinwale, R.O., Aderounmu, M. (2016). Gene action and heterotic groups of early white quality protein maize inbreds under multiple stress environments. *Crop Science*. **56**: 183-199.
- Badu-Apraku, B., Oyekunle, M., Fakorede, M.A.B., Vroh, I., Akinwale, R.O., Aderounmu, M. (2013). Combining ability, heterotic patterns and genetic diversity of extra-early yellow inbreds under contrasting environments. *Euphytica*. **192**: 413-433.
- Barata, C. and Carena, M.J. (2006). Classification of North Dakota maize inbred lines into heterotic groups based on molecular and testcross data. *Euphytica*. **151(3)**: 339-349.
- Bruel, D.C., Carpentieri-Pipolo, V., de Fatima Ruas, C., Gerage A.C., de Souza, S.G.H. (2007). Assessment of genetic diversity in maize inbred lines using RAPD markers. *Crop Breeding Applied Biotechnology*. **7(2)**: 173-178.
- Deviren, B., Bilgin, O., Kutlu, I. (2024). Heterotic grouping of wheat hybrids based on general and specific combining ability from line $\times$ tester analysis. *Peer Journal*. **12**: e18136. doi: 10.7717/peerj.18136.
- Eberhart, S.A., Salhuana, W., Sevilla, R., Taba, S. (1995). Principles for tropical maize breeding. *Maydica*. **40**: 339-355.
- Fan, X.M., Chen, H.M., Tan, J., Xu, C.X., Zhang, Y.M., Huang, Y.X., Kang, M.S. (2008). A new maize heterotic pattern between temperate and tropical germplasm. *Agronomy Journal*. **100(4)**: 917-923.
- Fan, X.M., Zhang, Y.M., Yao, W.H., Chen, H.M., Tan, J., Xu, C.X., Han, X.L., Luo, L.M., Kang, M.S. (2009). Classifying maize inbred lines into heterotic groups using a factorial mating design. *Agronomy Journal*. **101(1)**: 106-112.

- Ibrar, D., Khan, S., Raza, M., Nawaz, M., Hasnain, Z., Kashif, M., Rais, A., Gul, S., Ahmad, R., Gaafar, A.R.Z. (2024). Application of machine learning for identification of heterotic groups in sunflower through combined approach phenotyping, genotyping and protein profiling. *Scientific Reports*. **14**(1): 7333.
- Li, Y., Shi, Y.S., Song, Y.C., Du, J.Y., Tuberosa, R., Wang, T.Y. (2004). Analysis of genetic diversity in maize inbred lines based on AFLP markers. *Maydica*. **49**(2): 89-95.
- Mekasha, G.M., Chere, A.T., Ali, H.M., Seyoum, S.A. (2021). Classifying highland quality protein maize inbred lines into heterotic groups. *African Journal of Range and Forage Sci.* **15**(8): 225-236. <https://doi.org/10.5897/AJPS2021.2160>.
- Melchinger, A.E. and Gumber, R.K. (1998). Overview of heterosis and heterotic groups in agronomic crops. Concepts and Breeding of Heterosis in Crop Plants. CSSA, Madison, WI, 1998, **25**: 29-44
- Nair, R.J. and Pandey, M.K. (2024). Role of molecular markers in crop breeding: A review. *Agricultural Reviews*. **45**(1): 52-59. doi: 10.18805/ag.R-2322.
- Okunlola, G., Badu-Apraku, B., Ariyo, O., Ayo-Vaughan, M. (2023). The combining ability of extra-early maturing quality protein maize (*Zea mays*) inbred lines and the performance of their hybrids in *Striga*-infested and low-nitrogen environments. *Frontiers in Sustainable Food Systems*. **7**: 1238874. <https://doi.org/10.3389/fsufs.2023.1238874>.
- Parentoni, S.N., Magalhaes, J.V., Pacheco, C.A.P., Santos, M.X., Abadie, T., Gama, E.E.G., Guimaraes, P.E.O., Meirelles, W.F., Lopes, M.A., Vasconcelos, M.J.V., Paiva, E. (2001). Heterotic groups based on yield-specific combining ability data and phylogenetic relationship determined by RAPD markers for 28 tropical maize open-pollinated varieties. *Euphytica*. **121**(2): 197-208.
- Patel, K., Varshney, R., Saxena, R., Singh, P.K. (2022). Genomic basis of defining heterotic pools in pigeonpea (*Cajanus cajan* L.): A review. *Agricultural Reviews*. **45**(4): 609-617. doi: 10.18805/ag.R-2450.
- Pejic, I., Ajmone-Marsan, P., Morgante, M., Kozumplick, V., Castiglioni, P., Taramino, G. and Motto, M. (1998). Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs and AFLPs. *Theoretical and Applied Genetics*. **97**: 1248-1255.
- Reid, L.M., Xiang, K., Zhu, X., Baum, B.R., Molnar, S.J. (2011). Genetic diversity analysis of 119 Canadian maize inbred lines based on pedigree and simple sequence repeat markers. *Canadian Journal of Plant Science*. **91**(4): 651-661.
- Reif, J.C., Melchinger, A.E., Xia, X.C., Warburton, M.I., Hoisington, D.A., Vasal, S.K., Beck, D., Bohn, M., Frisch, M. (2003). Use of SSR establishing heterotic groups in subtropical maize. *Theoretical and Applied Genetics*. **107**(5): 947-957.
- Reif, J.C., Hallauer, A.R. and Melchinger, A.E. (2005). Heterosis and heterotic patterns in maize. *Maydica*. **50**: 215-223.
- Riedelsheimer, C., Czedik-Eysenberg, A., Grieder, C., Lisec, J., Technow, F., Sulpice, R., Altmann, T., Stitt, M., Willmitzer, L., Melchinger, A.E. (2012). Genomic and metabolic prediction of complex heterotic traits in hybrid maize. *Nature Genetics*. **44**(2): 217-220.
- Shull, G.H. (1952). Beginnings of the heterosis concept. *Heterosis*. **23**: 31-33.
- Silva, K.J.D., Pastina, M.M., Guimaraes, C.T., Magalhaes, J.V., Pimentel, L.D., Schaffert R.E., Pinto, M.D.O., Souza V.F.D., Bernardino, K.D.C., Silva, M.J.D., Borem, A., Menezes de, C.B. (2021). Genetic diversity and heterotic grouping of sorghum lines using SNP markers. *Scientia Agricola*. **78**(6): e20200039. doi: 10.1590/1678-992x-2020-0039.
- Singh, C.M., Arya, M., Mishra, S.B., Pandey, A. (2016). Combining ability, heterotic grouping and patterning of mungbean [*Vigna radiata* (L.) Wilczek] lines for high seed yield. *Legume Research*. **39**(5): 682-689. doi: 10.18805/lr.v0i OF.9380.
- Singh, S.B.P., Kumar, P., Kasana, R.K., Choudhary, M., Kumar, S., Kumar, R., Karjagi, C.G., Kumar, B., Rakshit, S. (2021). Unveiling ability and heterotic grouping of newly developed winter maize (*Zea mays*) inbreds. *Indian Journal of Agricultural Sciences*. **91**(11): 1586-1591. doi: 10.56093/ijas.v9i11.118535.
- Singh, S., Sharma, V.R., Nannuru, V.K.R., Singh, B., Kumar, M. (2021). Phenotypic diversity of pea genotypes (*Pisum sativum* L.) based on multivariate analysis. *Legume Research*. **44**(8): 875-881. doi: 10.18805/LR-4165.
- Sruthi, K., Divya, B., Senguttuvel, P., Revathi, P., Kemparaju, K.B., Koteswararao, P., Sundaram, R.M., Singh, V.J., Ranjith Kumar, E., Bhowmick, P.K., Vinod, K.K., Krishnan Gopala, S., Singh, A.K., Prasad Hari, A.S. (2020). Evaluation of genetic diversity of parental lines for development of heterotic groups in hybrid rice (*Oryza sativa* L.). *Journal of Plant Biochemistry and Biotechnology*. **29**(2): 237-252.
- Warburton, M.L., Ribaut, J.M., Franco, J., Crossa, J., Dubreuil, P. and Betran, F.J. (2005). Genetic characterization of 218 elite CIMMYT maize inbred lines using RFLP markers. *Euphytica*. **142**(1): 97-106.
- Zhang, H., Lyu, D., Zhang, Y., Wang, W., Zhao, R., Lu, P., Zhao, W., Zhao, Z., Lu, S. (2025). Classification of heterotic groups and prediction of heterosis in sorghum based on whole-genome resequencing. *International Journal of Molecular Sciences*. **26**(16): 7950. <https://doi.org/10.3390/ijms26167950>.