Plant Genetic Resources: Characterization and Utilization

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Classification of maize inbred lines into heterotic groups based on yield and yield attributing traits

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Research Article

Cite this article: Bhatla A, Angidi S, Thomas N, Madankar K, Shahi JP (2025). Classification of maize inbred lines into heterotic groups based on yield and yield attributing traits. *Plant Genetic Resources: Characterization and Utilization* **23**, 27–39. https://doi.org/10.1017/S1479262124000509

Received: 3 April 2024
Revised: 3 September 2024
Accepted: 4 September 2024
First published online: 4 October 2024

Keywords:
general combining ability; heterosis; heterotic grouping; specific combining ability

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Abstract

This study investigated the combining ability, heterosis and heterotic grouping of maize (Zea mays L.) inbred lines to enhance hybrid performance and productivity. Twenty-four hybrids were developed by crossing eight inbred lines with three testers, and their performance was evaluated for two years at Banaras Hindu University's agricultural research farm. Data on yield and yield-attributing traits were collected from selectively centred competitive plants in each row, avoiding border plants to reduce errors. Biometrical techniques, cluster analysis, and statistical tools were employed to measure general combining ability (GCA), specific combining ability (SCA), and standard heterosis, providing insights into hybrid performance. Analysis of variance revealed significant mean square values for GCA and SCA across most traits studied. Various methods were utilized, including SCA effects, HGCAMT (Heterosis Grouping by Combining Ability of Multiple Traits), and HSGCA (Heterotic Grouping based on Specific and General Combining Ability). The study identified HUZM-242 × CML-286 and HUZM-53 × CML-286 as crosses displaying higher grain yield compared to the check line DKC 7074 and exhibiting positive heterosis. The findings offer valuable guidance for maize breeding programmes by accurately identifying heterotic groups, enabling breeders to select inbred lines more likely to produce high-performing hybrids. This targeted selection reduces the number of necessary cross-breeding trials, saving time and resources. Additionally, hybrids derived from crosses between lines from different heterotic groups exhibit superior performance due to higher heterosis. These conclusions support advancements in maize breeding strategies, ultimately contributing to agricultural sustainability through increased productivity, resource efficiency, and economic benefits for farmers.

Introduction

Maize (*Zea mays* L.) is a vital crop that has played a pivotal role in the sustenance and evolution of human societies for millennia (Goodman and Galinat, 1988; Nankar and Pratt, 2021). Often referred to as the 'Miracle Crop' and the 'Queen of Cereals,' maize holds a significant position in New World civilizations, highlighting its exceptional productivity and genetic diversity (Azam-Ali, 2021). Its importance transcends geographical boundaries, with India ranking it second among cereal crops and witnessing a doubling of production since 2000 B.C. (FAO, 2021). Globally, maize ranks as one of the primary crops, contributing to over half of the total production, with the USA being a leading producer (FAO, 2021). One of the distinctive features of maize is its capacity for both self-pollination and cross-pollination, although the rate of cross-pollination remains low. This characteristic raises concerns about inbreeding depression resulting from the mating of closely related individuals (Howard *et al.*, 2017). To counteract such genetic limitations and enhance productivity, breeders have long exploited the phenomenon of heterosis, also known as hybrid vigour.

Heterosis, or Hybrid vigour, refers to the phenomenon whereby hybrid progeny exhibit superior traits compared to their parental lines (Das *et al.*, 2021). Despite its extensive application in crop production, the precise mechanisms underlying heterosis remain elusive. This phenomenon has been observed across various traits such as growth rate, size, fertility and yield, motivating the widespread adoption of heterosis in crop breeding programmes. Several studies have explored heterosis using different mating designs and performance evaluations. Gurjar *et al.* (2022) utilized a line × tester mating design to evaluate heterosis, per se performance, and combining ability for yield and yield-attributing traits. Basser *et al.* (2022) examined the performance of Quality Protein Maize (QPM) inbred lines and single crosses, analysing the magnitudes of mid-parent and better-parent heterosis for grain yield and its components. Additionally, Ramadan *et al.* (2021) analysed five pure maize lines through diallel and reciprocal crosses, estimating heterosis based on the deviation of the first-generation average from the higher and mean parents in growth and yield attributes. These studies collectively contribute to our understanding of heterosis in maize through various mating designs and performance evaluations, shedding light on its implications for maize breeding and production. The present study employs the line × tester mating design, a widely recognized approach in plant breeding that evaluates the combining ability and genetic potential of maize inbred lines. This design involves crossing multiple inbred lines (female parents) with various testers (male parents) to assess the performance of progeny and identify promising parental combinations. Recent applications of this design highlight its effectiveness in improving maize varieties by systematically evaluating parental lines and optimizing breeding strategies. By utilizing the line × tester approach, breeders can make informed decisions on which parental lines to advance, leading to the development of improved crop varieties with enhanced traits such as higher yields and better stress resilience. This design continues to be a vital tool in maize breeding, ensuring significant advancements in crop improvement and agricultural productivity (Tiwari *et al.*, 2021).

Traditionally, heterotic grouping in maize breeding has relied on assessing the specific combining ability (SCA) effect on grain yield, but this approach is limited by interactions between parental pairs and genotype-environment interactions, potentially leading to inconsistent results across studies (Fan *et al.*, 2001; Wu *et al.*, 2007). To address these limitations, alternative methods such as the HSGCA (Heterotic Grouping based on Specific and General Combining Ability) method and the HGCAMT (Heterosis Grouping by Combining Ability of Multiple Traits). HSGCA integrates SCA and GCA effects providing a more complete understanding of the genetic potential of parental lines. HSGCA also helps in the identification of the best parental combinations, leading to the development of high-yielding and resilient hybrids (Fan *et al.*, 2009). HGCAMT focuses on GCA effects across multiple traits, providing a comprehensive assessment of the parental lines' combining abilities. (Badu-Apraku *et al.*, 2013). By accurately identifying heterotic groups, breeders can produce hybrids with superior performance due to heterosis. Comparative studies by researchers have highlighted the efficiency of these alternative methods in assigning maize germplasm lines into heterotic groups across diverse environments (Badu-Apraku *et al.*, 2015; Amegbor *et al.*, 2017; Ajala *et al.*, 2020). While the HSGCA method emerges as the most efficient approach across diverse environments, the HGCAMT method showed superiority, particularly for delineating heterotic groups among early maturing yellow maize inbreds (Badu-Apraku *et al.*, 2016).

Understanding the genetic relationships among testers and their efficacy in grouping other inbred lines is crucial for the success of hybrid breeding programmes. Plant breeders continuously evaluate inbred testers to ascertain their ability to classify other inbred lines. In India, since the late 1990s, there has been a significant shift from breeding open-pollinated maize varieties to developing hybrids. Various methods, including phenotypic data, combining ability effects, and molecular markers, have been employed to categorize Indian maize inbred lines into heterotic groups, yet a comprehensive classification remains elusive (Krishna *et al.*, 2019; Kumar *et al.*, 2020; Rajkumar *et al.*, 2021; Singh and Guleria, 2020). CIMMYT's maize improvement programme has identified several promising testers over the past two decades, but their specific heterotic groups, particularly in relation to Indian maize germplasm, are not fully established (Singh and Guleria, 2020). While initial attempts have been made to group inbreds based on heterotic patterns using CIMMYT testers like CML 451 and CML 286 (Krishna *et al.*, 2019; Singh and Guleria, 2020), the classification based on Indian testers has yet to begin. The grouping of Indian maize inbred lines with indigenous testers could yield more meaningful results, as these testers are likely more suitable for grouping all available Indian maize inbred lines. This study aimed to address this gap by using three inbred testers, CML 286, LM 13 and LM 14 to classify maize inbred lines.

Heterotic grouping facilitates the development of superior hybrids, enhancing the efficiency of hybrid breeding programmes and maximizing hybrid vigour (Carena and Hallauer, 2001). By categorizing inbred lines into specific heterotic groups, breeders can systematically create and test hybrids. This reduces the need for extensive random crossing and testing, thereby saving time and resources (Labroo *et al.*, 2021). Also, grouping helps in developing hybrids that are better adapted to specific environmental conditions. For example, certain heterotic groups may perform better under drought or low-nitrogen conditions, allowing breeders to develop hybrids tailored to different agroecological zones. By using defined heterotic groups, breeding programmes can more efficiently allocate resources towards the most promising hybrid combinations, thus enhancing the overall productivity of the breeding process (Olutayo, 2021). Therefore, this study aims to determine general combining ability (GCA) effects for the inbred lines and SCA effect for the hybrids for grain yield and yield attributing traits, estimate standard heterosis, and classify parental lines into heterotic groups using SCA effect, HSGCA, and HGCAMT grouping methods.

Materials and methods

Plant materials and experimental locations

A total of eight inbred lines developed from BHU, Varanasi were crosses with three elite inbred testers (CML-286, LM13, and LM14) obtained from CIMMYT and IIMR Ludhiana (Sravya *et al.*, 2023) (Table 1). A small number of inbred lines were selected based on their advanced breeding status within the local HUZM population, which focuses on enhancing maize genetics for key traits such as grain yield, stress tolerance and nutritional quality. Preliminary work was essential to identify and develop these lines, ensuring they exhibit desirable characteristics. Extensive assessments were conducted to evaluate genetic diversity, yield potential and specific traits like zinc concentration in grains, as well as a low anthesis-silking interval, as demonstrated in studies by Sravya *et al.* (2023) and Amin *et al.* (2023). Tester CML-286 (Sravya *et al.*, 2023; Amin *et al.*, 2023) is used as a tester as it has a high combining ability within maize (Table 1). LM-13 and LM-14 are national testers with opposite heterotic groups (Amin *et al.*, 2023) (Table 1). A total of 24 F1 hybrids were developed by crossing 8 inbred lines with 3 elite testers using a line × tester mating design, with DKC-7074, a well-known hybrid, used as a check variety (Kempthorne, 1957) (Table 1). The hybridization in maize was performed by placing a paper bag over the tassel to collect pollen, and the cob was bagged to protect it from foreign pollen. The pollen collected from the tassel was then transferred to the cob. Emasculation (removal of anthers) was done with the help of forceps before anther dehiscence between 4 and 6 PM, one day before the anthers dehisce, ensuring the gynoecium was not injured. Immediately after emasculation, the inflorescence

Table 1. List of genotypes used in the present study

S.No.	Genotypes	Type	Source[a,b,c]	KC[d]	KT[e]	M[f]	Pedigree	Specific character
1	HUZM-147	Inbred	BHU, Varanasi	Normal yellow	Semi dent	Late	PHS 4705 (W)	Charcoal rot resistance, Drought tolerant
2	HUZM-242	Inbred	BHU, Varanasi	Normal yellow	Flint	Medium	BH 3309	Stem borer Resistant, Drought tolerant
3	HUZM-246	Inbred	BHU, Varanasi	Normal yellow	Semi Flint	Early	BH 3447	Turcicum leaf Blight resistance, Heat stress resistance
4	HUZM-343	Inbred	BHU, Varanasi	Normal yellow	Flint	Early	HUZM 45 X Local 41-2	Charcoal rot and Turcicum leaf Blight
5	HUZM-345	Inbred	BHU, Varanasi	Normal yellow	Flint	Early	Local 121-5-7-8-1	Downy Mildew and Stem borer tolerant
6	HUZM-379	Inbred	BHU, Varanasi	Normal yellow	Flint	Early	Local 234-8-3-7-1-5-9	Stay green character
7	HUZM-53	Inbred	BHU, Varanasi	Normal yellow	Flint	Late	ISO2 × 1381 WA	Cold tolerant
8	HUZM-79	Inbred	BHU, Varanasi	Normal yellow	Dent	Late	DMR WN -8X Local	Resistance to Heat and Drought stress
9	CML-286	Tester	CIMMYT	Normal yellow	Dent	Early	P24STE-C1-FS16-1-3-3-1-2-B	*A. flavus* and *F. verticilloides* resistance, High combining ability
10	LM13	Tester	IIMR, Ludhiana	Normal yellow	Flint	Late	LCY3-7-1-2-2-1-1-f	High combining ability
11	LM14	Tester	IIMR, Ludhiana	Normal yellow	Flint	Late	CA 00310-xb-xb-xb-1-1-1-1-1	High combining ability
12	DKC-7074	Check	Monsanto	Normal yellow	Flint	Early	–	Strong vigour and stay-green characteristic

[a] BHU, Banaras Hindu University.
[b] CIMMYT, International Maize and Wheat Improvement Centre.
[c] IIMR, Indian Institute of Maize Research.
[d] KC, Kernal colour.
[e] KT, Kernal texture.
[f] M, Maturity.

was enclosed with suitable bags of appropriate size to prevent random cross-pollination. The pollen grains collected from a desired male parent were transferred to the emasculated flower in the morning hours during anthesis. The flowers were bagged immediately after the artificial crossing and tagged just after bagging. Tags with the date of emasculation, date of pollination, parentage and the number of flowers emasculated were attached to the inflorescence with the help of a thread. The study was conducted at the Agricultural Research Farm of Banaras Hindu University (BHU), Varanasi, India (25.28°N, 83.08°E, 76 m a.s.l.). The experimental site was used for the improvement of different maize genotypes. The study consisted of two trials conducted in 2020 and 2021. The plants were cultivated in a randomized complete block design with two replications for each genotype for two seasons (Rabi 2020-21 and Rabi 2021-22), with a planting density of 8.33 plants per square metre.

Data collection

Observations were recorded on two randomly selected competitive plants from the centre of each row, chosen from a total of five plants, excluding border plants to reduce the error of border effect. The selection of these plants was performed using a random number generator to ensure unbiased selection. These competitive plants were assessed for various characteristics in maize, using specific modes of determination, except for silking and anthesis. This method ensured that the selected plants were representative of the trial plots, providing reliable data for analysis. Firstly, days to 50% silking (DTS) is the number of days from planting to when 50% of the plants had emerged silks, and days to anthesis (DTA) when 50% of the plants had shed pollen. The antithesis-silking intervals (ASI) were determined as the time difference between pollen shed and silk emergence. Plant height (cm) was measured from the base to the first node at maturity, excluding any tassel. Ear height (cm) was measured from the base to the base of the first ear placement at maturity. Ear diameter (mm) was determined by measuring the girth diameter of three randomly selected cobs. Ear length (cm) was measured from the butt end to the tip of the apical bud after removing the husk cover. The meter scale was used to measure the plant height (cm), ear height (cm), ear diameter (mm), and ear length (cm). The number of kernels per row and the number of rows per ear were assessed by counting and averaging. The 100-grain weight (gm) was determined by randomly selecting and weighing 100 grains per replication. Finally, grain yield per plant (q/ha) was calculated by shelling dried cobs separately and determining the yield per hectare, averaged across plants.

Following the harvest, a spring balance with a precision of ±0.001 g was employed to ascertain the field weight of the ears in each row. Subsequently, a superpro portable digital grain moisture meter was utilized to determine the moisture content of the grains after manually shelling the dried harvested ears from each row. The shelling per cent and grain yield were calculated by the following equation:-

$$\textbf{Shelling percent} = \frac{\textbf{Grain Weight}}{\textbf{Ear Weight}} \times 100 \text{ (Uba et al., 2018)}$$

$$\textbf{Grain yield(q/ha)} = \frac{\textbf{Grain Weight x } (100 - \textbf{MC})}{\left(\frac{100 - \textbf{Adjusted MC}}{\textbf{Plot Area}}\right)} \times \frac{10,000}{\textbf{Plot area}} \times \frac{1}{\textbf{Shelling Percentage}} \times 0.01$$

Where, Grain Weight was the weight of the harvested maize grains, in grams; MC (Moisture Content) was the actual moisture content of the grain at harvest; adjusted MC (Adjusted Moisture Content) was the standard moisture content to which one wants to adjust the grain weight (usually 15% for maize); plot area was the area of the plot harvested, in square meters; shelling percentage was the proportion of the grain to the total ear weight, expressed as a decimal (e.g., 0.80 if the shelling percentage is 80%) and 0.01 was the conversion factor from grams to quintals (since 1 quintal = 100 kg, and there are 100,000 g in 100 kg) (ASTM, 2001).

Statistical analysis

After data collection, the Levene test was performed to assess the homogeneity of variance between the two trials, given there were four replications. The Levene test was chosen because it is robust to non-normality and provides a reliable assessment of variance equality across different groups (O'Neill and Mathews, 2002). Once homogeneity of variance was confirmed, the replications were combined for a unified analysis, as the means were found to be similar. A combined analysis of variance (ANOVA) was then conducted for each character to detect significant genotypic differences (Panse and Sukhatme, 1985). This analysis was performed using RStudio software (version 3.6.1) (Knezevic *et al.*, 2007). The ANOVA model used was:

$$Y_{ij} = m + g_i + r_j + e_{ij}$$

Where Y_{ij} = Phenotypic observation of *i*th genotype in the *j*th replication, m = General mean, g_i = Effect of *i*th genotype, r_j = Effect of *j*th replication, e_{ij} = Random error associated with *i*th genotype in the *j*th replication.

The *F*-test was applied to verify significant differences among genotypes and replications. The F-test is widely used in ANOVA to determine if the means between different groups are significantly different, which helps in understanding the variability attributed to genotypic effects as opposed to random errors (Costa *et al.*, 2024). Significant genotypic differences detected using ANOVA were then subjected to further phenotypic analysis to interpret the results in the context of the traits studied. This approach ensured that the statistical analysis was rigorous and provided meaningful insights into the performance of the maize hybrids.

The estimates of general and SCA and their variances were obtained by using the covariance half-sibs and full-sibs (Kempthorne, 1957). Standard heterosis was expressed as a per cent increase or decrease observed in F_1 over the standard check (Virmani *et al.*, 1982).

$$\textbf{Standard heterosis (\%) (H}_3\textbf{)} = \frac{F_1 - SC}{SC} \times 100$$

Where SC = Mean of standard check

Eight inbred lines were classified into heterotic groups using the SCA effect, HSGCA and HGCAMT. The HSGCA values were calculated as HSGCA = Cross mean (X_{ij}) + Tester mean (X_i) = GCA + SCA, where X_{ij} is the mean yield of the cross between *i*th tester and *j*th line, X_j is the mean yield of the *i*th tester (Oyetunde *et al.*, 2020). The statistical model used by the HGCAMT method to assign the inbreds into the heterotic groups is as follows:

$$Y = \sum_{i=1}^{n}\left[\frac{G_i - g_i}{s}\right] + \varepsilon ij$$

where Y is HGCAMT, which is the genetic value measuring relationship among genotypes based on the GCA of multiple traits *i* to *n*; Y_i is the individual GCA effect of genotypes for trait *i*, μ is the mean of GCA effects across genotypes for trait *i*, s_i is the standard deviation of the GCA effects of trait *i*, ε_{ij} is the residual of the model associated with the combination of where inbred *i* and trait *j* (Badu-Apraku *et al.*, 2016). The grouping by HGCAMT was achieved by standardizing the GCA effects with significant mean squares to minimize the effects of different scales of the traits.

In comparing the efficiencies of three heterotic grouping methods, the 24 test crosses were arranged in descending order based on their mean grain yield across various environments. Each method's total number of hybrids generated by each tester was divided into two major groups: inter-group and intra-group crosses. These groups were then further categorized into three distinct yield groups: high-yielding, intermediate-yielding, and low-yielding hybrids. To calculate the breeding efficiency (Fan *et al.*, 2009; Badu-Apraku *et al.*, 2016), the following equation was used.

$$\frac{\frac{\textbf{HYINTERGH}}{\textbf{TN INTERGH}} \times 100 + \frac{\textbf{LY INTRAGH}}{\textbf{TN INTRAGH}} \times 100}{2}$$

Where, HYINTERGH = Numbers of high-yielding inter-heterotic group hybrids; TN INTERGH = Total number of inter-heterotic group hybrids; LY INTRAGH = Number of low-yielding intra-heterotic group hybrids; TN INTRAGH = Total number of intra-heterotic group hybrids.

Results

ANOVA and mean performance analysis

The ANOVA conducted for line × tester combinations revealed a significant mean sum of squares for all characters except the Anthesis Silking Interval (ASI) (Table 2). Crosses also exhibited a significant mean sum of squares for all characters, while checks were significant for all characters except ASI, ear diameter, ear height, ear per row and grain yield. Testers displayed significance for ASI and ear length. These significant mean sums of squares indicate the presence of appreciable genetic variability within the experimental material. The range of mean performance for days to 50% anthesis varied from 108 days for HUZM-242 × CML-286 to 124 days for HUZM-147 × LM13, respectively (Table 3). Similarly, days to 50% silking ranged from 110 to 125 days across hybrids, with HUZM-242 × CML-286 and HUZM-147 × LM13 representing the extremes (Table 3). Anthesis-silking interval ranged from 1 to 4 days among test crosses, including HUZM 246 × LM13, HUZM-53 × LM13, and HUZM-343 × CML-286 (Table 3). Plant height varied among test crosses, ranging from 107.5 for HUZM-53 × LM14 to 141.75 cm for HUZM-53 × CML-286. Ear height ranged from 47.5 cm for HUZM-53 × LM14 to 78.34 cm for HUZM-147 × LM13, respectively (Table 3). Ear diameter varied from 32.08 mm for HUZM-53 × LM14 to 41.30 mm for HUZM-147 × LM13. Ear length ranged from 10.73 cm for HUZM-246 × LM14 to 17.30 cm for HUZM-147 × LM14 (Table 3). The number of kernels per row ranged from 19.84 for HUZM-147 × CML-286 to 32.09 for HUZM-246 × CML-286 (Table 3). The number of rows per ear ranged from 10.34 for HUZM-345 × LM13 to 14.67 for HUZM-379 × LM13 (Table 3). The 100-grain weight varied from 16.02 g for HUZM-147 × CML-286 to 30.48 g for HUZM-246 × CML-286 (Table 3). According to average data, the yield ranged between hybrids from 27.15 to 80.04 qt/ha, with HUZM-246 × LM14 and HUZM-53 × CML-286. (Table 3).

GCA effects of lines and testers

In terms of grain yield, certain inbred lines such as HUZM 242, HUZM 246, HUZM 343 and HUZM 53 exhibited positive GCA effects, with HUZM 53 demonstrating notably positive effects (Fig. 1). Conversely, inbred lines like HUZM 379, HUZM 246, HUZM 147 and HUZM 242 displayed negative GCA effects concerning flowering traits such as days to 50 per cent anthesis and days to 50 per cent silking (Fig. 1). Thus, overall, inbred line HUZM 246 emerges as a promising general combiner and could be recommended for utilization in developing synthetic varieties. Among the various testers, CML-286 showcased the highest positive GCA effect for grain yield (3.78) and the most significant negative GCA effect for days to 50% silking and days to 50% anthesis (Fig. 1). Consequently, CML-286 demonstrates good GCA, high yield potential, and early maturity. Similar observations regarding GCA effects for grain yield per plant (Fig. 1).

SCA effects of test crosses

The analysis of SCA effects indicated that hybrids such as HUZM-242 × CML-286, HUZM-242 × LM-13, HUZM-246 × LM13, HUZM-246 × LM14, and HUZM-345 × LM14 displayed early maturity concerning days to 50% anthesis and silking, along with lower plant height and ear height (Fig. 2). Additionally, hybrids including HUZM-147 × LM14, HUZM-

Table 2. Analysis of variance for randomized block design for grain yield and its component characters in maize

Source	df	Days to 50% anthesis	Days to 50% silking	ASI	Plant Height	Ear diameter	Ear height	Ear length	100 seed weight	Kernels per row	Ear per row	Grain yield
Replication	3	0.852	0.935	0.002	4.497	0.04	8.595	1.578	29.748***	0.006	0.348	1.02
Treatments	26	43.784***	39.946***	0.935	197.783***	8.869***	123.371***	6.467***	28.432***	28.874***	2.89***	426.102***
Crosses	23	40.407***	36.453***	1.042**	158.976***	9***	133.679***	6.855***	24.953***	25.542***	3.014***	420.636***
Checks	2	66.646***	64.896***	0.115	152.963***	0.741	56.712	3.914***	61.977***	80.241***	2.889	1.816
Testers	2	89.269	64.199	3.897***	75.558	7.36	198.608	22.604***	40.364	30.731	1.294	172.261
Lines × Testers	14	45.853***	43.765***	0.617	109.884***	11.847***	103.526***	2.923***	11.569***	13.738***	3.711***	347.692***
Error	26	1.308	1.283	0.523	7.927	1.068	34.395	0.629	3.563	3.094	0.993	56.815

Note: Significance levels are indicated as follows: *** = $P < 0.01$; ** = $0.01 \leq P < 0.05$; * = $P < 0.05$.

Table 3. Mean performance analysis of test crosses for yield and yield contributing traits

Test crosses	Days to 50% anthesis	Days to 50% silking	ASI[a]	Plant height (cm)	Ear height (cm)	Ear diameter (mm)	Ear length (cm)	Kernel per rows	Ear per row	100 Seed wt.(gm)	Grain yield(q/ha)
HUZM-242 × CML-286	108	110	2	116.00	70.75	40.24	16.00	30.17	12.00	19.70	78.72
HUZM-246 × CML-286	113	116	3	113.34	63.34	34.87	17.04	32.09	11.84	30.48	41.78
HUZM-379 × CML-286	121	123	2	119.91	69.17	35.64	14.45	31.67	14.33	23.81	67.17
HUZM-53 × CML-286	108	111	3	141.75	77.68	40.05	13.67	30.34	11.67	19.13	80.04
HUZM-79 × CML-286	112	115	3	132.50	60.83	36.16	11.75	25.84	13.83	16.52	62.98
HUZM-345 × CML-286	121	124	3	123.45	67.50	33.98	11.34	23.67	11.67	19.74	61.72
HUZM-147 × CML-286	116	118	2	128.34	61.67	33.85	12.77	19.84	13.34	16.02	35.09
HUZM-343 × CML-286	113	117	4	124.89	72.50	36.00	12.60	25.67	13.67	19.31	64.50
HUZM-242 × LM13	109	111	2	116.73	59.17	37.59	12.60	21.50	13.33	17.97	59.15
HUZM-246 × LM13	113	115	1	114.50	72.74	37.04	12.30	25.67	11.67	19.56	72.01
HUZM-379 × LM13	112	115	3	121.28	68.96	37.89	16.03	31.33	14.67	20.84	70.30
HUZM-53 × LM13	122	123	1	135.84	74.28	36.64	13.42	26.50	10.67	19.22	65.80
HUZM-79 × LM13	117	119	3	117.50	59.94	35.00	15.27	29.34	12.00	20.00	61.49
HUZM-345 × LM13	110	113	3	124.17	52.50	35.29	16.97	27.67	10.34	25.57	41.72
HUZM-147 × LM13	124	125	2	129.02	78.34	41.30	14.40	29.50	14.00	24.65	50.30
HUZM-343 × LM13	115	118	3	131.17	68.33	35.60	14.60	28.34	13.50	25.58	66.62
HUZM-242 × LM14	117	120	3	111.50	54.31	38.70	13.60	24.67	12.67	23.30	53.54
HUZM-246 × LM14	109	111	2	119.17	61.67	38.13	10.73	20.67	12.67	24.73	27.15
HUZM-379 × LM14	116	118	2	110.17	65.96	36.00	13.34	28.17	13.67	17.42	36.15
HUZM-53 × LM14	112	115	3	107.50	47.50	32.08	15.20	29.33	13.00	20.40	42.15
HUZM-79 × LM14	112	115	3	108.27	54.17	37.46	12.50	22.13	14.67	18.23	53.13
HUZM-345 × LM14	114	118	3	120.34	69.77	36.47	14.07	23.00	12.67	18.59	66.96
HUZM-147 × LM14	116	119	3	114.17	73.29	35.66	17.30	25.61	11.00	24.98	72.87
HUZM-343 × LM14	121	123	2	126.77	70.84	36.42	12.07	24.77	13.34	19.41	69.23
DKC 7074	108	110	3	97.51	64.92	38.68	11.63	21.00	12.33	24.69	73.66
Minimum	108	110	1	97.51	47.50	32.08	10.73	19.84	10.34	14.94	27.15
Maximum	124	125	4	141.75	78.34	41.30	17.30	33.67	14.67	30.48	80.04
C.D[b].	1.37	1.34	1.29	1.82	5.98	1.14	3.64	3.64	2.06	3.90	12.89
C.V.[c]	3.25	2.58	2.98	2.36	8.97	2.81	5.73	6.60	7.82	9.10	12.53

[a] ASI, Anthesis Silking Interval.
[b] C.D, Critical Difference.
[c] C.V, Coefficient of Variation.

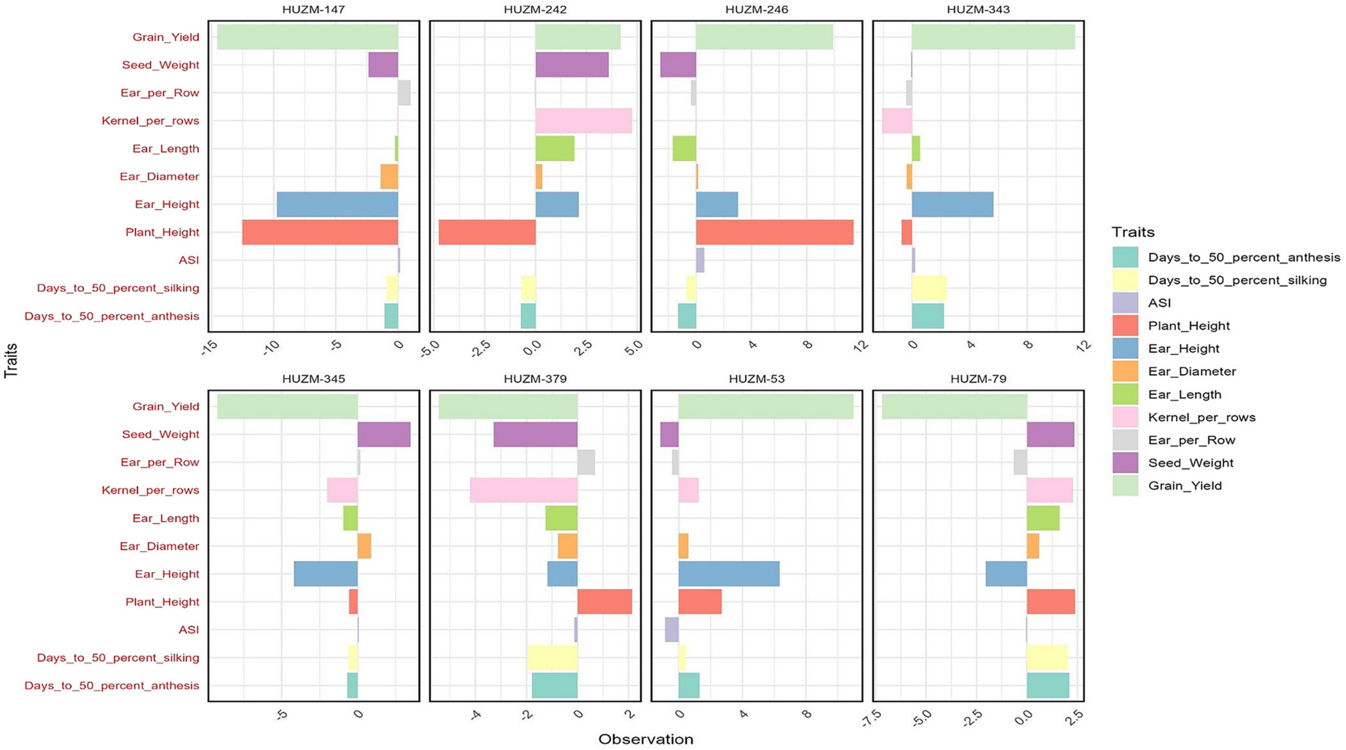

Figure 1. General Combining Ability of Inbred Lines.

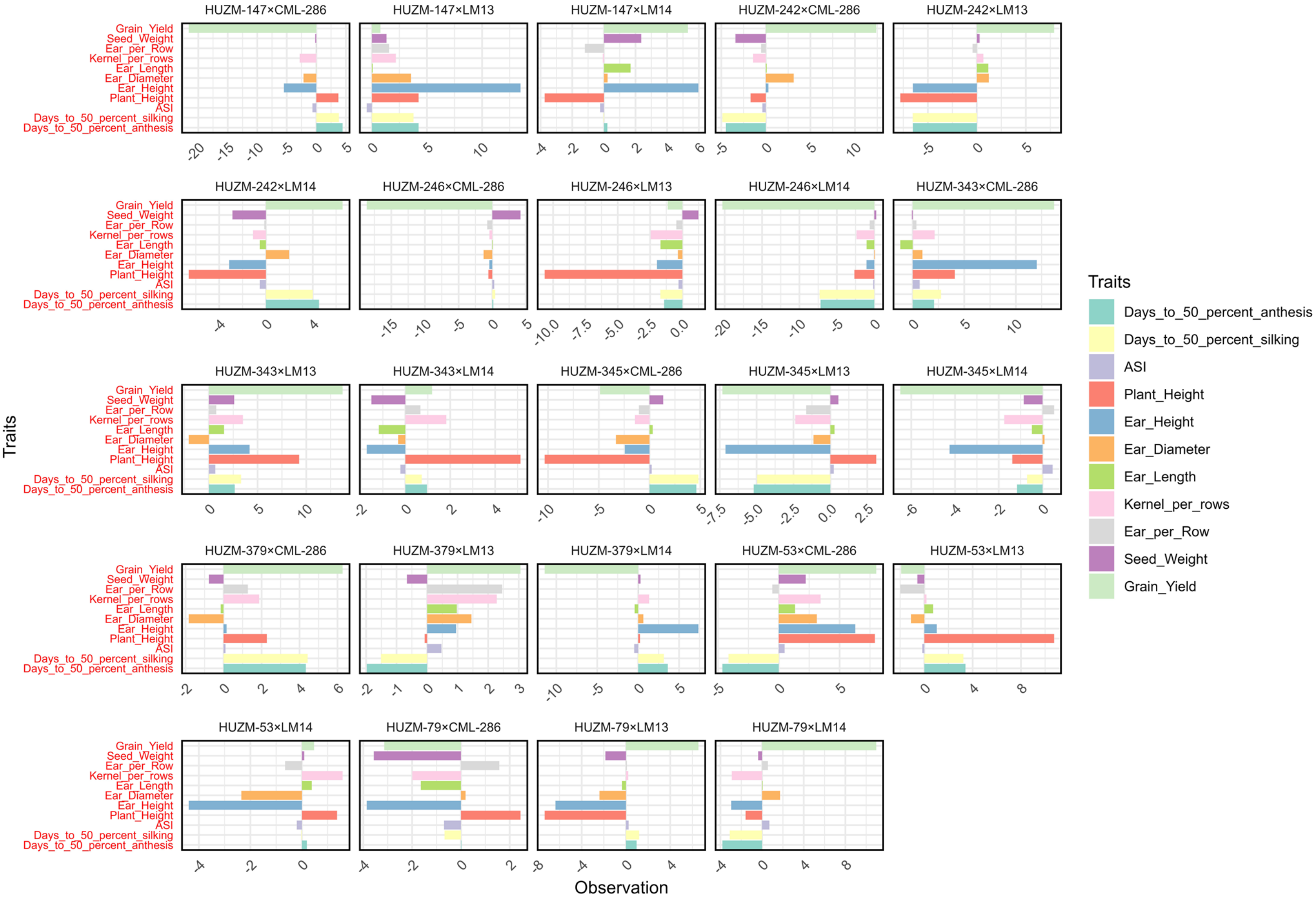

Figure 2. Specific Combining Ability of Test Crosses.

242 × CML-286, HUZM-242 × LM13, HUZM-242 × LM14, HUZM-343 × CML-286, HUZM-343 × LM13, HUZM-379 × CML-286, HUZM-53 × CML 286, HUZM-79 × LM13, and HUZM-79 × LM14 exhibited higher grain yield per plant (Fig. 2).

Standard heterosis for yield and yield contributing traits

Standard heterosis was assessed by comparing three hybrid checks (DKC-7074) with regard to the economic significance of traits in a favourable direction. Inbred lines crossed with CML 286 exhibited the highest positive standard heterosis for test weight, including HUZM 242, HUZM 246, HUZM 345 and HUZM 379 (Table 4). For plant height, inbred lines HUZM 79 and HUZM 147 showed the greatest positive impact of standard heterosis when hybridized with CML 286. Notably, the hybrid HUZM 53 × CML 286 and HUZM 343 × CML 286 displayed the highest positive standard heterosis for ASI and ear height, respectively (Table 4). Among inbred lines crossed with tester LM 13, all exhibited the highest positive standard heterosis over check DKC 7074 for the test weight trait, except HUZM 53, which showed the same effect for ear height (Table 4). Similarly, all inbred lines hybridized with tester LM 14 demonstrated significant standard heterosis for test weight, except for HUZM 79 and HUZM 345, which exhibited a similar pattern for ASI (Table 4).

Heterotic grouping

The results based on the SCA, HSGCA and HGCAMT methods revealed three heterotic groups for each method, except for the SCA effect. Classification by the three methods showed similar but not identical trends. Some inbred lines, such as HUZM 147, HUZM 345 and HUZM 79, were placed in the same group by two of the three methods (Table 5). In terms of the placement of inbred lines into the same group, the SCA and HGCAMT methods appeared to be more similar compared to the other comparisons. For heterotic grouping based on the SCA effect, inbred lines with positive SCA for grain yield were predominantly placed into cluster 4, while those with negative values were distributed into the other three clusters. Specifically, the most negative SCA values were found in cluster 1, and the least negative values were in cluster 3. The HSGCA method, which relies on a quantitative relationship, divided inbred lines into three distinct clusters.

The breeding efficiency of the testers LM-13, CML-286 and LM-14 across diverse yield groups was evaluated using the SCA, HSGCA and HGCAMT methods, resulting in varying numbers of inter- and intra-group crosses (online Supplementary Table S3). Notably, HGCAMT displayed the highest efficiency for LM-13 (50%) and LM-14 (53.34%), whereas HSGCA showed the highest efficiency for CML-286 (25%). HSGCA proved particularly successful in classifying inbred lines into heterotic groups for LM-14 and CML-286, suggesting its potential for enhancing hybrid productivity through crossbreeding. Conversely, HGCAMT demonstrated superior accuracy in categorizing inbred lines into heterotic groups for LM-13, indicating its efficacy in specific scenarios.

Discussion

The realization and stabilization of heterosis are pivotal for maximizing selection gains in crop plants, significantly influenced by the genetic diversity within the germplasm base (Duvick, 1999; Melchinger *et al.*, 2018). Heterosis, or hybrid vigour, plays a critical role in enhancing yield and other desirable traits in maize and other crops (Jiang *et al.*, 2017). The success of exploiting heterosis hinges on the diversity present within the germplasm pool, influencing the extent to which beneficial traits can be combined and stabilized in breeding programmes. This genetic diversity forms the basis for developing hybrids that exhibit superior performance over their parental lines (Jiang *et al.*, 2017). Thus, understanding and leveraging the genetic diversity of the germplasm base is essential for harnessing the full potential of heterosis in maize breeding strategies (Duvick, 1999; Melchinger *et al.*, 2018).

The significant mean sum of squares observed for various traits indicates a rich genetic diversity within the experimental material. This diversity is foundational for any successful breeding programme, offering a wide array of genetic resources to select from. The existence of such variability not only ensures adaptability to diverse environmental conditions but also presents opportunities for developing maize varieties with enhanced agronomic traits. Badu-Apraku *et al.*, 2021 studied genetic diversity and population structure of early and extra-early maturing 439 maize germplasm adapted to sub-Saharan Africa and they found sufficient variability. On similar grounds in India, Patel *et al.*, 2024, Baruah *et al.*, 2024 and Kumar *et al.*, 2024a, 2024b reported significant genetic diversity in normal yellow maize, pigmented maize and sweet corn, respectively. Previous findings by, Darshan and Marker, 2019, and Seyoum *et al.*, 2016, highlight the importance of genetic variability in breeding programmes.

The additive type of gene effects is accountable for GCA, meanwhile, the non-additive type of gene effects is regarded to be responsible for SCA. In general, the SCA variances were greater than GCA variances for all the characters studied suggesting the predominance of non-additive gene action in the expression of various characters. This showed the possibility of exploiting these traits through heterosis breeding (online Supplementary Table S1). These findings appear consistent with the results of Zare *et al.* (2011) who reported that non-additive gene effects were more important than additive gene effects for all the traits except ear length in seven inbred lines and their respective 42 test crosses. The ratio of GCA to SCA variance is less than unity in all the characters. It showed the contribution of line × tester was more than testers and lines for all the characters except plant height, ear length, 100 seed weight, number of kernels per row, and number of ears per row (Kanagarasu *et al.*, 2010; Andayani *et al.*, 2018) (online Supplementary Table S2).

The estimates of GCA effects identified certain inbred lines, such as HUZM-343, HUZM-53 and HUZM-246, as good general combiners for traits like grain yield per plant. These lines exhibit the capacity to positively influence important traits such as grain yield and early maturity. Conversely, the negative GCA effects observed for few traits underscore the need for careful selection of parental lines to achieve desired trait combinations in hybrids. When addressing inbred lines with negative GCA effects in maize breeding, it is crucial to employ strategies that mitigate these drawbacks and leverage the positive attributes of other lines (Melchinger, 1999; John *et al.*, 2024). The GCA of lines can be improved by Hybridization with compensatory traits, Recurrent selection, Backcross breeding and Population improvement programmes (Melchinger, 1999; Bernardo, 2010; Mebratu *et al.*, 2024 and Abdel-Moneam *et al.*, 2024). The significant positive GCA effects exhibited by testers like CML-286 for grain yield and early maturity further emphasize their suitability for hybridization, aligning with similar findings reported by Belay (2022), Darshan and Marker (2019), and Kamara *et al.* (2020).

The SCA effects analysis identified hybrids like HUZM-343 × LM13, HUZM-343 × CML-286, HUZM-242 × CML-286, HUZM-

Table 4. Standard heterosis of test crosses over DKC 7074 for eleven yield and yield contributing traits

Test crosses	Days to 50% anthesis	Days to 50% silking	ASI[a]	Plant height	Ear height	Ear diameter	Ear length	Kernel per rows	Rows per ear	100 Seed wt	Grain yield
HUZM-242 × CML-286	0.00	0.00	0.00	9.02	22.21	5.92	16.79**	11.04	−14.29	29.95	11.11**
HUZM-246 × CML-286	4.63	5.45	50.00**	6.52	9.41	−8.21*	24.38**	18.11*	−15.43*	68.07**	−41.03**
HUZM-379 × CML-286	12.04**	11.82**	0.00	12.70	19.49	−6.19	5.47	16.56*	2.36	57.06*	−5.19
HUZM-53 × CML-286	0.00	0.91	50.00**	33.22**	34.19**	5.42	−0.22	11.67	−16.64*	26.19	12.97**
HUZM-79 × CML-286	3.70	4.55	50.00**	24.53*	5.08	−4.82	−14.23*	−4.90	−1.21	8.97	−11.11**
HUZM-345 × CML-286	12.04**	12.73**	50.00**	16.02	16.60	−10.56**	−17.23**	−12.88	−16.64*	30.21	−12.89**
HUZM-147 × CML-286	7.41	7.27	0.00	20.62*	6.53	−10.9**	−6.79	−26.98**	−4.71	5.67	−50.47**
HUZM-343 × CML-286	4.63	6.36	100.00**	17.38	25.24*	−5.24	−8.03	−5.52	−2.36	27.37	−8.96**
HUZM-242 × LM13	0.93	0.91	0.00	9.71	2.21	−1.05	−8.03	−20.87**	−4.79	18.54	−16.51**
HUZM-246 × LM13	4.63	4.55	−50**	7.61	25.65*	−2.50	−10.22	−5.52	−16.64*	29.02	9.88**
HUZM-379 × LM13	3.70	4.55	50.00**	13.98	19.12	−0.26	17.01**	15.31*	4.79	37.47	6.82*
HUZM-53 × LM13	12.96**	11.82**	−50**	27.67**	28.31*	−3.55	−2.04	−2.47	−23.79**	26.78	−7.13*
HUZM-79 × LM13	8.33*	8.18*	50.00**	10.43	3.54	−7.87*	11.46	7.99	−14.29	31.93	−13.21**
HUZM-345 × LM13	1.85	2.73	50.00**	16.70	−9.31	−7.11*	23.87**	1.84	−26.14**	68.67**	−41.12**
HUZM-147 × LM13	14.81**	13.64**	0.00	21.26*	35.33**	8.71*	5.11	8.58	0.00	62.6*	−29**
HUZM-343 × LM13	6.48	7.27	50.00**	23.28*	18.03	−6.29	6.57	4.31	−3.57	68.73**	−5.97*
HUZM-242 × LM14	8.33*	9.09*	50.00**	4.79	−6.18	1.87	−0.73	−9.20	−9.50	53.69*	−24.43**
HUZM-246 × LM14	0.93	0.91	0.00	12.00	6.53	0.37	−21.68**	−23.92**	−9.50	63.13*	−61.68**
HUZM-379 × LM14	7.41	7.27	0.00	3.54	13.94	−5.24	−2.63	3.68	−2.36	14.91	−48.98**
HUZM-53 × LM14	3.70	4.55	50.00**	1.03	−17.95	−15.56**	10.95	7.95	−7.14	34.56	−40.51**
HUZM-79 × LM14	3.70	4.55	50.00**	1.76	−6.43	−1.40	−8.76	−18.55*	4.79	20.25	−25.01**
HUZM-345 × LM14	5.56	7.27	50.00**	13.10	20.52	−4.00	2.70	−15.35*	−9.50	22.63	−5.49
HUZM-147 × LM14	7.41	8.18*	50.00**	7.30	26.6*	−6.13	26.28**	−5.74	−21.43**	64.78**	7.24*
HUZM-343 × LM14	12.04**	11.82**	0.00	19.14	22.37	−4.13	−11.90	−8.83	−4.71	28.03	6.27*

[a] ASI, Anthesis Silking Interval, ** = $0.01 \leq P < 0.05$; * = $P < 0.05$.

Table 5. Heterotic grouping of inbred lines

Inbred line	SCA effect	HSGCA	HGCAMT
HUZM-147	Cluster 1	Cluster 3	Cluster 1
HUZM-242	Cluster 4	Cluster 1	Cluster 2
HUZM-246	Cluster 3	Cluster 2	Cluster 3
HUZM-343	Cluster 4	Cluster 1	Cluster 3
HUZM-345	Cluster 2	Cluster 3	Cluster 2
HUZM-379	Cluster 2	Cluster 2	Cluster 1
HUZM-53	Cluster 2	Cluster 1	Cluster 3
HUZM-79	Cluster 1	Cluster 2	Cluster 2

79 × LM14 and HUZM-53 × CML-286 as exhibiting higher grain yield per plant. These hybrids, along with others like HUZM-147 × LM14, HUZM-246 × LM13 and HUZM-379 × LM13, emerged as top performers based on mean grain yield, SCA, and standard heterosis, showcasing their potential for commercial exploitation after thorough evaluation in multilocation trials. Recent research has highlighted the importance of SCA effects in identifying promising maize hybrids for enhanced grain yield. Singh *et al*., 2021 explored combining ability in maize and identified hybrids such as HUZM-55 × CML-176 and HUZM-88 × LM-13 as top performers. Their study emphasized the role of genetic diversity and combining abilities in achieving superior yield potential. Similarly, Sharma *et al*., 2022 investigated SCA effects and found hybrids like HUZM-79 × CML-228 and HUZM-91 × LM-14 to exhibit significant grain yield advantages. These findings underscore the value of strategic hybridization in maize breeding programmes to meet agricultural sustainability goals. Further studies by Rajkumar *et al*., 2023 and Yadav *et al*., 2024 have also contributed insights into the genetic mechanisms influencing grain yield through SCA analysis, highlighting hybrids such as HUZM-63 × CML-176 and HUZM-82 × LM-14 as effective in harnessing heterosis and combining ability for maize improvement.

The primary aim of this study is to identify the best-performing maize hybrids by comparing test crosses with the high-yielding DKC 7074 check variety. In India, where maize growers predominantly favour hybrids over composite or synthetic varieties, evaluating hybrids based on standard heterosis is particularly critical. Unlike mid-parent heterosis or better-parent heterosis, which measure hybrid performance relative to the parental genotypes, standard heterosis assesses hybrid vigour against a commercial cultivar, providing a more robust and agriculturally relevant metric. This method is advantageous because it facilitates the identification of hybrids that exhibit superior agronomic traits relative to established industry standards, thereby enhancing the likelihood of their commercial success and adoption by farmers. In this study, hybrids such as HUZM-242 × CML-286 and HUZM-53 × CML-286 demonstrated positive and significant estimates of standard heterosis for grain yield per plant over the check variety DKC-7074, signifying their potential for improved performance and yield compared to current cultivars. The incorporation of diverse parental lines, like HUZM-53 from BHU, Varanasi, known for its late-flowering trait, and CML-286 from CIMMYT, with resistance to *A. flavus* and *F. verticilloides*, highlights the efficacy of heterosis breeding. The positive significant estimates of standard heterosis in these hybrids further validate the use of this metric, as it effectively identifies hybrids capable of surpassing commercial cultivars while offering resilience to both biotic and abiotic stresses (Eberhart and Sprague, 1973). Hybrids HUZM-242 × CML-286 and HUZM-53 × CML-286, derived from parental lines with distinct origins and traits, demonstrated positive significant estimates of standard heterosis for grain yield per plant over the standard check DKC-7074, underlining their potential for enhanced performance and yield compared to established varieties (Table 4). This emphasizes the efficacy of incorporating diverse parental lines like HUZM-53 from BHU, Varanasi, known for its late-flowering trait, and CML-286 from CIMMYT, possessing resistance to *A. flavus* and *F. verticilloides*, into hybrid combinations. The positive significant estimates of standard heterosis further affirm the benefits of heterosis breeding, where hybrid offspring exhibit improved performance beyond that of their parents or standard checks. Additionally, the successful manifestation of positive heterosis and resistance in this cross underscore the importance of strategic hybridization in maize breeding programmes, aiming to develop varieties with increased productivity and resilience to biotic and abiotic stresses.

The demonstration of significant positive standard heterosis for various traits underscores the potential for exploiting hybrid vigour in maize breeding. Recent studies have shown that inbred lines crossed with specific testers exhibit enhanced performance for traits such as test weight and plant height (Dubey *et al*., 2009; Abuali *et al*., 2012; Sandesh *et al*., 2018; Guleria *et al*., 2023; Kumar *et al*., 2024a, 2024b). This highlights the superiority of hybrids over their parental lines, reaffirming the critical role of hybrid breeding strategies in achieving higher yields and improving overall crop performance. The utilization of combining ability and heterosis in these studies provides valuable insights into enhancing maize productivity through strategic hybridization.

The results of this study demonstrate the effectiveness and distinct advantages of three methods – SCA, HSGCA and HGCAMT – for heterotic grouping in maize breeding programmes. SCA effectively identifies specific cross combinations that exhibit superior performance, particularly in traits like grain yield, by recognizing hybrid vigour and specific line interactions. However, SCA is highly specific to the traits evaluated and the environmental conditions of the trials, limiting its applicability for GCA. In contrast, HSGCA offers a more comprehensive assessment by combining both specific and general combining abilities. This method is particularly useful for identifying lines that consistently perform well across various crosses and environments, although its complexity and data requirements can be a drawback. HGCAMT provides a holistic view by incorporating multiple traits into the heterotic grouping, enhancing the accuracy of grouping through a broader range of performance indicators. However, HGCAMT can be resource-intensive and may require sophisticated statistical tools and expertise, posing challenges in scenarios with limited data availability. By using these methods complementarily, maize breeding programmes can leverage the strengths of each approach. SCA can be utilized for initial hybrid selection based on specific traits, HSGCA can offer a broader evaluation of combining abilities, and HGCAMT can refine the grouping by considering multiple traits, ensuring that the selected inbred lines are versatile and high-performing across different conditions. Integrating SCA, HSGCA and HGCAMT methods can optimize the identification and utilization of heterotic groups, thereby enhancing hybrid productivity and breeding efficiency in maize. The differences observed in breeding efficiency across methods emphasize the importance of selecting appropriate grouping techniques tailored to specific breeding objectives and genetic contexts. The breeding efficiency results highlight the importance of selecting appropriate methods and parental combinations to achieve specific breeding goals. This aligns with previous studies by Akinwale *et al.* (2014), Amegbor *et al.* (2017), and Fan *et al.* (2009).

This study specifically focuses on grouping Indian maize inbred lines with Indian-origin testers, which can provide more meaningful and applicable results for local breeding programmes. The identification of two testers, LM13 and LM14, with excellent combining ability (Amin *et al.*, 2023), is a key outcome of this research. These testers are now suitable for grouping all available Indian maize inbred lines. The methods to classify inbred lines are valuable for yield prediction of inbred lines, enabling breeders to avoid unnecessary test crosses, thus saving time and resources. The findings of the heterotic grouping of maize inbred lines significantly contribute to sustainable agriculture by enabling the development of high-yielding, resilient hybrids, which offer substantial environmental and economic benefits. Environmentally, these hybrids improve resource use efficiency, with studies showing up to 30% higher water use efficiency (Blum, 2011) and 20–40% increased nitrogen use efficiency reducing the need for chemical inputs and conserving resources. They also offer cost savings through a 20–30% reduction in pest and irrigation management costs (Tollenaar and Lee, 2002). This yield stability ensures reliable food supply and market stability (Pingali and Pandey, 2001). It is expected that the current collection of inbred lines could introduce advantageous new alleles for improving populations, developing hybrids and creating lines that can bolster future breeding efforts. Future research should focus on the environmental adaptability of the identified high-performing hybrids. Investigating the molecular basis of combining ability could provide deeper insights into the genetic mechanisms underlying heterosis and hybrid performance. Additionally, studies on the long-term stability and performance of these hybrids across diverse environmental conditions would be valuable. This research will help refine breeding strategies further, ensuring the development of maize varieties that contribute to sustainable agriculture and global food security.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1479262124000509

Acknowledgements. We acknowledge CIMMYT, IIMR and BHU for providing the germplasm. We also extend our appreciation to Agricultural Research Farm, BHU, Varanasi, India for providing a research area to conduct research.

Authors' contributions. AB, NT and KM designed, collected data and implemented the experiment. KM and SA analysed the data and wrote a manuscript. All authors read and approved the manuscript.

Funding statement. Not applicable.

Competing interests. None.

Ethical standards. Not applicable.

Consent for publication. Not applicable.

Availability of data and materials. All data produced during this study were included this article.

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