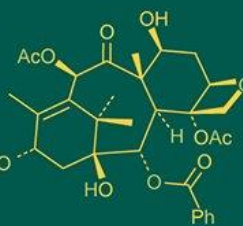
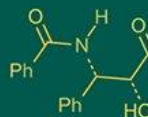


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Assessment of combining ability of double haploid-derived maize (*Zea mays* L.) Inbred lines for grain yield and yield-attributing traits

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Abstract

The present investigation was carried out to assess combining ability and gene action among 88 single-cross maize (*Zea mays* L.) hybrids of inbred line developed through Double Haploid (DH) technology, evaluated along with two commercial check hybrids (90 entries in total) at two locations Dharwad, Karnataka (Site 1) and Banda Milaram, Telangana (Site 2) during Kharif 2025, using a Line $\times$ Tester mating design under an Alpha Lattice Design with two replications. Analysis of variance for combining ability revealed highly significant mean squares due to lines (GCA) for most yield-attributing traits at both individual locations and pooled analysis, confirming the predominance of additive gene action in governing trait expression. At Dharwad, lines GCA for grain yield (6.34***) substantially exceeded the corresponding SCA estimate (3.31***) and a similar trend was observed at Banda Milaram, where lines GCA (2.09***) again surpassed SCA (0.70***), further confirming the additive nature of gene action. Nevertheless, significant SCA effects detected for grain yield, plant height and ear height at both locations indicated a meaningful contribution of non-additive (dominance and epistatic) gene action alongside the predominant additive effects. Among the 44 DH-derived inbred lines evaluated, L25 recorded the highest positive GCA effect for grain yield at Dharwad (+ 3.08*), followed closely by L21 (+ 2.79*), both lines also recording significant positive GCA for cob weight, establishing them as the most outstanding general combiners for yield improvement at this location. At Banda Milaram, L21 again emerged as the best general combiner with a significant positive GCA of + 2.05*. In pooled analysis across locations, L21 secured the top rank with the highest positive GCA effect of + 2.45*, confirming its consistent performance and broad adaptability across environments, followed by L25 with a pooled GCA of + 2.19*. For SCA L21 showed significant positive SCA for grain yield (1.25*), supporting its dual importance as both a good general and specific combiner line making both lines the most stable and valuable parental lines for grain yield improvement.

Keywords: Maize, double haploid, line $\times$ tester, general combining ability, specific combining ability, grain yield

1. Introduction

Maize (*Zea mays* L.) is one of the most versatile and widely cultivated cereal crops in the world, belonging to the family Poaceae with a chromosome number $2n = 20$. It is the third most important cereal crop in India after rice and wheat, and its diverse end-uses as food, feed, fuel, and forage (the “4F” usage) underline its strategic importance for global and national food and industrial security (DA & FW, 2023) ^[1].

Globally, maize was harvested on approximately 208.2 million hectares in 2023, with a production of 1.24 billion tonnes and an average productivity of 5.66 t ha⁻¹. India cultivated maize on about 10.74 million hectares during 2023-24, producing 30.88 million tonnes at a productivity of 3.45 t ha⁻¹ (DA & FW, 2023) ^[1], nearly 40% lower than the global average. This yield gap, largely attributable to the predominance of rainfed cultivation and associated climatic constraints, represents a significant opportunity for productivity enhancement through improved hybrid development.

Being a monoecious, protandrous and predominantly cross-pollinated crop, maize is exceptionally suited to hybrid cultivar development, with single-cross hybrids preferred globally for their superior yield potential, uniformity and heterosis expression. The development of genetically diverse, homozygous inbred lines is fundamental to hybrid breeding; however, conventional inbred development through repeated selfing is time-

consuming. Double Haploid (DH) technology has emerged as a powerful alternative, enabling the rapid fixation of complete homozygosity in a single generation and thereby accelerating the breeding cycle (Khulbe *et al.*, 2023; Kaur *et al.*, 2023) [3, 2]. CIMMYT established DH production facilities in India in 2023, generating the first indigenous genome-wide DH lines for use in national maize improvement programmes.

Before development in commercial hybrid development, inbred lines must be evaluated for combining ability and the stability of resulting hybrids across diverse environments (Negi *et al.*, 2018) [5]. Combining ability analysis, particularly through the Line $\times$ Tester mating design (Subba Vaskar *et al.*, 2022) [9], elucidates the relative importance of additive (General Combining Ability, GCA) and non-additive (Specific Combining Ability, SCA) gene action. GCA effects identify parents with superior average performance across crosses, whereas SCA effects identify specific cross combinations exhibiting exceptional performance due to dominance or epistatic interactions. Understanding the genetic architecture of yield and yield-attributing traits including plant height, ear height, number of cobs, days to 50% flowering and silking, cob weight, grain moisture, shelling percentage and grain yield is therefore essential for designing efficient hybrid breeding strategies (Negi *et al.*, 2018) [5].

2. Material and Methods

2.1 Experimental Locations

The experiment was conducted at two locations during Kharif 2025. Location 1, Nath Bio-Genesis R&D Farm, Garag, Dharwad, Karnataka (15°34'31.6" N, 74°56'59.5" E; altitude 700-800 m. The climate is tropical sub-humid to semi-arid, receiving 700-800 mm annual rainfall and medium to deep black soils. Location 2, Nath Bio-Genesis R&D Farm, Banda Milaram, Telangana (17°41'33.8" N, 78°30'03.6" E; altitude 590-600 m above mean sea level), and black soil.

2.2 Experimental material and Design

The experimental material comprised 88 single-cross hybrids generated through a Line $\times$ Tester mating design, using 44 Double Haploid (DH)-derived inbred lines as female parents crossed with two testers as male parents. These were compared against two commercial check hybrids (Entry 89 and Entry 90) giving a total of 90 entries evaluated at each location. The use of fully homozygous DH lines as parents ensured that the genetic variation observed among test-cross hybrids reflected true genetic differences rather than residual heterozygosity, enabling a more precise evaluation of combining ability and heterosis.

At both locations, the trial was arranged out in an Alpha Lattice Design with two replications. This design was preferred over a Randomized Complete Block Design (RCBD) because it provides more efficient control of field heterogeneity when evaluating a large number of genotypes, by grouping plots into incomplete blocks within each replication, thereby reducing error variance and improving the precision of genotype comparisons. Each hybrid was grown in a single-row plot of 4 m length, with 60 cm inter-row and 20 cm intra-row spacing, consistent with recommended commercial maize plant density. Uniform agronomic practices, including fertilization, irrigation and plant protection, were applied across all plots at both

locations to ensure that observed differences among genotypes were attributable to genetic rather than management factors.

2.3 Observations Recorded

Observations on the following yield and yield-attributing traits were recorded on tagged, randomly selected plants per plot, with border plants excluded to avoid edge effects: initial plant count, plant vigour, days to 50% flowering (anthesis), days to 50% silking, plant height (cm), ear height (cm), number of ears per plant, cob weight (g/plot), final plant stand, grain moisture (%), shelling (%), and grain yield (kg/ha). Plant height and ear height were measured on five randomly selected plants per plot using a measuring scale.

2.4 Statistical Analysis

Subjected to combining ability analysis using the Line $\times$ Tester method of Subba Vaskar *et al.* (2022) [9], which partitions genetic variation among test-cross hybrids into General Combining Ability (GCA) effects of lines and testers, and Specific Combining Ability (SCA) effects of specific line $\times$ tester combinations. All computations were performed using SAS/GENSTAT software appropriate for Alpha Lattice designs.

3. Results and Discussion

3.1 Combining Ability Analysis (Line $\times$ Tester Design)

3.1.1 Combining Ability Analysis for Grain Yield

At Dharwad, line GCA for grain yield (6.34***) substantially exceeded SCA (3.31***) with tester GCA also significant (4.22*), revealing that additive gene effects played a major role in governing grain yield expression. Among 44 lines, L21 and L25 emerging as the best general combiners recording significant positive GCA effects of + 2.79* and + 3.08* respectively, indicating their strong potential as superior parents for high-yielding hybrid development, in similar results with Subba Vaskar *et al.* (2022) [9]. At Banda Mailaram, line GCA for grain yield (2.09***) again exceeded SCA (0.70***) with highly significant tester GCA (4.53***), further indicating that additive gene effects were the principal component controlling grain yield at this location, with Line L21 retaining its superiority as the best general combiner (+ 2.05*) across this environment as well, similar finding with Negi *et al.* (2018) [5]. In the pooled analysis across locations, line GCA for grain yield (6.42***) was considerably higher than SCA (1.88***) with significant Site $\times$ Genotype interaction (2.26***) and Site $\times$ Line GCA (2.24***), demonstrating that additive gene effects governed grain yield expression with differential genotypic response across environments; L21 secured the top rank with pooled GCA of + 2.45* and L25 ranked second with + 2.19*, establishing both as the most stable and valuable general combiners for grain yield across diverse environments, similarly reported by Vardhini *et al.* (2023) [10].

3.1.2 Combining Ability Analysis for Cob weight

For cob weight, line GCA (41.87***), tester GCA (74.43**) and SCA (15.92**) were all significant at Dharwad, with Lines L21 and L25 also recording significant positive GCA for this trait alongside grain yield, confirming their value as dual-purpose superior combiners, results with Subba Vaskar *et al.* (2022) [9]. At Banda Milaram, line GCA (11.18***), tester GCA (99.20***) and SCA (3.51***) were all highly

significant, indicating contribution of both additive and non-additive gene actions at this location, similar results reported by Negi *et al.* (2018) [5]. In the pooled analysis, line GCA (37.53***), and SCA (10.44***), were both highly significant with significant Site $\times$ Line GCA interaction (15.36***), indicating location-specific expression of gene effects for cob weight across environments.

3.1.3 Combining Ability Analysis for Plant Height

For plant height at Dharwad, line GCA (636.30**) and genotype mean squares (525.67*) were significant with non-significant tester GCA and SCA, revealing that additive gene effects were the major genetic component with negligible non-additive contribution, suggesting that selection based on GCA effects would be effective for improving plant height. At Banda Milaram, line GCA (674.81***), and tester GCA (7829.29***), were highly significant with non-significant SCA, indicating that both lines and testers contributed through additive effects while non-additive gene action played a negligible role similar results reported by Negi *et al.* (2018) [5]. In the pooled analysis, line GCA (987.40***), tester GCA (6349.84***), and SCA (325.38*) were all significant with highly significant Site $\times$ Tester GCA interaction (2222.37**), indicating contribution of both additive and non-additive effects with strong location-specific tester response; Line L26 (MGE000015939495) recorded desirable negative GCA for plant height and is recommended as a valuable parent for developing compact and lodging-resistant hybrids, similar findings reported by Kireeti Varma and Muniratnam (2026) [4].

3.1.4 Combining Ability Analysis for Ear Height

For ear height at Dharwad, line GCA (419.38***), and genotype mean squares (263.25***), were highly significant with non-significant tester GCA and SCA, indicating strong additive genetic control, suggesting that lines with desirable

GCA effects could be reliably selected as parents for developing hybrids with optimal ear placement. At Banda Milaram, line GCA (183.55***), tester GCA (934.94***), and SCA (180.09***), were all highly significant, indicating the need to identify specific line $\times$ tester combinations for exploiting non-additive effects under high-yield environments, results with Subba Vaskar *et al.* (2022) [9]. In the pooled analysis, line GCA (423.70***), tester GCA (2208.50***), and SCA (142.40***), were all highly significant with significant Site $\times$ Genotype (141.80***), and Site $\times$ Line GCA (171.80***), interactions, indicating strong genotype $\times$ environment interaction for ear height and suggesting that both additive and non-additive effects must be considered for stable ear height performance across environments.

3.1.5 Combining Ability Analysis for Shelling%

For shelling percentage at Dharwad, line GCA (14.38***), tester GCA (14.65**) and SCA (3.30*) were all significant, indicating contribution of both additive and non-additive gene action, suggesting that both GCA-based line selection and specific hybrid combinations would be effective for improving shelling efficiency. At Banda Milaram, line GCA (11.45***), and tester GCA (78.17***), were highly significant with non-significant SCA, confirming additive gene effects as the principal genetic component, indicating that high GCA lines could be directly utilized as reliable parents for superior shelling hybrids, in accordance with Kireeti Varma and Muniratnam (2026) [4]. In the pooled analysis, line GCA (21.07***), and SCA (5.26**) were both significant with non-significant Site $\times$ Genotype interaction (3.55), confirming consistent additive genetic control across both environments and indicating that superior GCA lines for shelling percentage would perform reliably across diverse locations, similar results reported by Vardhini *et al.* (2023) [10].

Table 1: ANOVA (mean sum of squares) for combining ability using the Line $\times$ Tester design at Dharwad (Site 1)

Source of Variation	d.f	Anthesis (days)	Silking (days)	Plant Height (cm)	Ear Height (cm)	No. of cobs per plant	Cob wt (t/ha)	Grain moisture (%)	Shelling (%)	Grain yield (t/ha)
Replication	1	1.51*	3.47**	188.51	34.08	7.19**	139.60***	0.08	0.38	28.09***
Genotype (testcross)	87	3.46***	4.44***	525.67*	263.25***	8.81*	29.42***	8.89***	8.90***	4.82***
Line (GCA)	43	5.53***	7.16***	636.30**	419.38***	8.75	41.87***	10.18***	14.38***	6.34***
Tester (GCA)	1	2.09	6.65*	541.61	1283.74***	22.97*	74.43**	45.69***	14.65**	4.22*
Line $\times$ Tester (SCA)	43	1.43**	1.67*	414.67	83.38	8.55	15.92**	6.74***	3.30*	3.31***
Residuals	53	0.67	0.99	311.55	90.08	5.70	6.64	1.72	1.96	1.00

*Significant at 5%; **Significant at 1%; ***Significant at 0.1% probability.

Table 2: ANOVA (mean sum of squares) for combining ability using the Line $\times$ Tester design at Banda Milaram (Site 2)

Source of Variation	d.f	Anthesis (days)	Silking (days)	Plant Height (cm)	Ear Height (cm)	No. of cobs per plant	Cob wt (t/ha)	Grain moisture (%)	Shelling (%)	Grain yield (t/ha)
Replication	1	15.27	38.60*	948.76***	164.12***	3.11	45.69***	0.91	36.12***	6.91***
Genotype (testcross)	87	2.85**	5.69**	494.30***	190.48***	6.52**	8.40***	4.20***	8.52***	1.43***
Line (GCA)	43	3.84***	8.12***	674.81***	183.55***	7.97**	11.18***	2.49*	11.45***	2.09***
Tester (GCA)	1	2.23	3.82	7829.29***	934.94***	0.94	99.20***	177.45***	78.17***	4.53***
Line $\times$ Tester (SCA)	43	1.87	3.31	143.22	180.09***	5.21	3.51***	1.88	3.97	0.70***
Residuals	53	1.33	2.57	105.37	39.63***	3.41	1.11	1.33	3.08	0.27

*Significant at 5%; **Significant at 1%; ***Significant at 0.1% probability.

Table 3: ANOVA (mean sum of squares) for combining ability using the Line × Tester design across locations

Source of Variation	d.f	Anthesis (days)	Silking (days)	Plant Height (cm)	Ear Height (m)	No. of cobs per plant	Cob wt (t/ha)	Grain moisture (%)	Shelling (%)	Grain yield (t/ha)
Site	1	88.60***	70.90***	158538.00***	103553.10***	608.00***	2017.16***	133.21***	59.49***	237.02***
Replication (site)	2	10.60***	24.92***	744.60*	83.70	5.07	91.71***	0.56	14.58**	19.37***
Genotype (testcross)	87	4.75***	7.98***	721.81***	305.20***	9.96***	23.72***	5.80***	13.95***	4.10***
Line (GCA)	43	7.39***	12.70***	987.40***	423.70***	10.02***	37.53***	6.35***	21.07***	6.42***
Tester (GCA)	1	3.94*	9.00*	6349.84***	2208.50***	16.87	0.75	22.36***	81.36***	0.00
Line × Tester (SCA)	43	2.13***	3.23**	325.38*	142.40***	9.73***	10.44***	4.87***	5.26**	1.88***
Site × Genotype	87	1.48*	2.03	310.98*	141.80***	5.22	14.01***	7.29***	3.55	2.26***
Site × Line (GCA)	43	1.80**	2.38	348.27*	171.80***	6.52	15.36***	6.30***	4.97*	2.24***
Site × Tester (GCA)	1	0.05	0.01	2222.37**	15.30	5.79	171.31***	205.38***	12.44**	8.02***
Site × Line × Tester (SCA)	43	1.20	1.74	229.25	114.80*	3.91	9.00***	3.67***	1.92	2.14***
Residuals	106	0.99	1.76	208.10	65.20	4.56	3.88	1.53	2.56	0.65

*Significant at 5%; **Significant at 1%; ***Significant at 0.1% probability.

3.2 Identified prominent GCA lines

Among the 44 DH-derived inbred lines evaluated, L25 recorded the highest positive GCA effect for grain yield at Dharwad (+ 3.08*), followed by L21 (+ 2.79*), with both lines also recording significant positive GCA for cob weight; at Banda Milaram, L21 retained its superiority with the highest positive GCA of + 2.05*, while in the pooled analysis, L21 again topped with a GCA of + 2.45*, followed

by L25 (+ 2.19*), confirming both lines as the most stable and outstanding general combiners for grain yield across diverse environments. On the contrary, L44 was consistently identified as the poorest general combiner for grain yield across both Dharwad (−2.75*) and the pooled analysis (−2.03*), while L8 performed poorly at Banda Milaram (−1.70*), and both lines are recommended for elimination from future crossing programmes.

Table 4: Lines with the most prominent positive and negative GCA effects for grain yield

Location	Line	GCA effect (grain yield)	Remarks
Dharwad	L25 (MGE000015938895)	+ 3.08*	Best general combiner; also positive for cob weight
Dharwad	L21 (MGE000015938653)	+ 2.79*	Strong combiner for yield and cob weight
Dharwad	L44 (MGE000015945600)	−2.75*	Poorest combiner for grain yield
Banda Milaram	L21 (MGE000015938653)	+ 2.05*	Consistent positive combiner across locations
Banda Milaram	L8	−1.70*	Poor combiner for yield at this site
Pooled	L21 (MGE000015938653)	+ 2.45*	Most stable and important line for yield improvement
Pooled	L25 (MGE000015938895)	+ 2.19*	Second-best general combiner across locations
Pooled	L44 (MGE000015945600)	−2.03*	Consistently poor combiner across locations

3.3 Identified prominent SCA lines

SCA effects for grain yield were generally larger in magnitude at Dharwad than at Banda Milaram, reflecting the greater overall genetic variance for yield at this location. Across locations, Line L21 again showed significant positive SCA for grain yield (1.25*), reinforcing its dual importance as both a good general and specific combiner, while L6 showed significant negative SCA (−1.10*). Significant SCA effects for plant height, ear height, cob weight and grain moisture were also recorded for several line × tester combinations, confirming a meaningful contribution of non-additive gene action alongside the predominant additive effects.

4. Conclusion

Combining ability analysis of 88 Double Haploid-derived maize hybrids at Dharwad and Banda Milaram confirmed the predominance of additive gene action for most yield traits, with line GCA for grain yield exceeding SCA at both locations. Significant SCA effects for grain yield, plant height and ear height indicated a meaningful contribution of non-additive gene action alongside the predominant additive effects. L21 and L25 were identified as the best general combiners for grain yield with significant positive pooled GCA effects of + 2.45* and + 2.19* respectively. For Specific combining ability (SCA) L21 showed significant positive SCA for grain yield (1.25*), L26 was the best combiner for earliness and compact plant type. Double

Haploid technology proved effective in generating homozygous, uniform parental lines for precise combining ability estimation, and L21 and L25 are strongly recommended for future high-yielding maize hybrid development programmes.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, Copilot, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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