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Study of Combining Ability and Gene Action for Seed Yield in Sunflower (*Helianthus annuus* L.) Using Line x Tester Analysis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The choice of suitable parental lines is vital in crop improvement programs, as the performance of the resulting hybrids reflects their relative advantages. Therefore, selecting parents with strong combining ability is a key component in the success of any breeding effort. A study was undertaken to assess the extent of heterosis and combining ability in sunflower using six cytoplasmic male sterile (CMS) lines and seven restorer lines, crossed in a line × tester mating design during the

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Kharif 2024 season. The resulting hybrids were evaluated in the *Rabi* 2024–25 season at the Oilseeds Research Station, Latur. Observations were recorded for ten agronomic traits, including days to 50% flowering, days to maturity, plant height, head diameter, seed filling percentage, 100-seed weight, volume weight, hull content, oil content and seed yield per plant. Among the female lines, CMS-82A exhibited significant GCA effects for days to 50% flowering, seed filling percentage, volume weight, hull content and oil content, whereas CMS-852A was found to be a strong general combiner for seed yield per plant, oil content and seed filling percentage. Among the male lines, LT-02 showed favorable GCA for days to 50% flowering, head diameter, seed filling percentage, hull content, oil content and seed yield per plant. LT-07 also emerged as a promising general combiner for days to 50% flowering, seed filling percentage, 100-seed weight, volume weight, hull content, oil content and seed yield. LT-01 was specifically identified for its strong GCA for seed yield. Notable hybrids with significant SCA effects for seed yield and other desirable traits included CMS-112A × LT-02 and CMS-10A × LT-05. These crosses exhibited Specific combining ability (SCA) variance that was greater than general combining ability (GCA) variance for all the studied traits, indicating the predominance of non-additive gene action in trait expression. Thus, the study confirms that utilizing parents with high GCA and selecting crosses with favorable specific combining ability (SCA) effects is an effective approach for evolving superior sunflower hybrids.

Keywords: Sunflower; line x tester; cross; general combining ability; specific combining ability.

1. INTRODUCTION

Sunflower (*Helianthus annuus L.; 2n = 2x = 34*) is a globally important oilseed crop, primarily cultivated for its nutrient-rich edible oil, known for its abundance of essential fatty acids and antioxidants. Its remarkable adaptability to diverse soil types and resilience against abiotic stresses have positioned it as a key crop across various agro-climatic regions (Patel et al., 2023). Originally native to North America, sunflower was formally introduced into Indian agriculture in the late 1960s to diversify oilseed production and address the rising demand for vegetable oils (Deshmukh et al., 2022).

Currently, sunflower ranks as the fourth major oilseed crop in India, trailing groundnut, mustard and soybean. According to projections for 2024–25 from the Indian Institute of Oilseeds Research (IIOR) and the Government of India, the crop covers approximately 2.28 lakh hectares, yielding 2.13 lakh tonnes with an average productivity of 934 kg/ha (IIOR, 2024).

In recent decades, hybrid technology has driven significant progress in sunflower breeding. The utilization of *cytoplasmic male sterility (CMS)* systems along with compatible restorer lines has facilitated the development of high-yielding hybrids characterized by improved uniformity, oil quality and resistance to various diseases (Singh et al., 2021). The commercial release of hybrid sunflower cultivars began in the early 1970s and quickly gained momentum worldwide due to their superior agronomic performance. In India, hybrids now account for over 95% of total sunflower cultivation, with predominant production in states such as Karnataka, Maharashtra, Andhra Pradesh and Tamil Nadu (IIOR, 2024) The *Line × Tester mating design* remains a widely employed method in sunflower genetics to evaluate *general combining ability (GCA)* and *specific combining ability (SCA),* facilitating the identification of superior parental combinations for hybrid development (Rambilash et al., 2023). The present study focuses on assessing these combining abilities in sunflower for key yield-related traits.

2. MATERIALS AND METHODS

The present investigation was carried out at the Oilseeds Research Station, Latur (18.40° N latitude and 76.57° E longitude). The field trial was conducted during the *Rabi* 2024–25 season following hybridization during *Kharif* 2024. The study aimed to evaluate heterosis and combining ability in sunflower using six cytoplasmic male sterile (CMS) lines and seven restorer (R) lines of diverse genetic backgrounds. A total of 42 hybrids were developed using the line × tester mating design as proposed by Kempthorne (1957). A total of 42 hybrids, along with 13 parental lines and three standard checks (LSFH-171, KBSH-90and KBSH-44), were evaluated in a Randomized Complete Block Design (RCBD) with two replications, using a spacing of 60 cm between rows and 30 cm between plants.
Observations were recorded on five randomly selected plants for ten quantitative traits days to

50% flowering, days to maturity, plant height (cm), head diameter (cm), seed filling percentage, 100-seed weight (g), volume weight (g/100 ml), hull content (%), oil content (%) and seed yield per plant (g). The recorded data were subjected to analysis of variance (ANOVA) as described by Gomez and Gomez (1984). Combining ability analysis was carried out using the line × tester method to estimate general combining ability (GCA) and specific combining ability (SCA) effects. The nature of gene action was determined based on the ratio of GCA to SCA variances. All statistical analyses were performed using TNAUSTAT software (Manivannan, 2014).

3. RESULTS AND DISCUSSION

The analysis of variances for combining ability revealed significant differences among treatments, parents, crosses and L x T for all the characters, indicating the presence of adequate variability in the experimental population, while parents vs crosses also showed significant differences for all the characters except hull content (Table 1). Line × tester analysis revealed significant variance for all traits due to line effects and for most traits due to tester effects except head diameter and volume weight. Interactions between lines and testers were significant for all characters except plant height (Table 2). General combining ability (GCA) effects were supported by significant mean squares for both lines and testers (Binodh et al., 2008; Andarkhor et al., 2011; Jondhale et al., 2012; Choudhary 2018; Sharma and Shadakshari, 2021; Dhanalakshami et al., 2022, Varalakshmi 2019).

Examination of general combining ability (GCA) estimates in Table 3, revealed that lines CMS-148A, CMS-112A and testers LT-02 and LT-07 had significant negative GCA effects for days to 50% flowering, indicating their usefulness in breeding for early flowering. This aligns with findings by Dhanalakshmi et al. (2022) and Ramaraju et al. (2021). For days to maturity, CMS-148A, CMS-82A and testers LT-03, LT-04 and LT-05 showed favorable GCA effects. CMS-148A, CMS-112A and LT-07 were superior for reducing plant height, supporting the results by Farrokhi et al. (2008). Positive GCA effects for head diameter were recorded in LT-02, CMS-852A, CMS-10A and CMS-82A. Seed filling percentage showed strong GCA effects in CMS-852A, CMS-10A, CMS-82A, CMS-COSF-6 A and testers LT-01, LT-02 and LT-07. Noteworthy GCA effect for 100-seed weight and volume weight were seen in CMS-852A, CMS-82A, CMS-10A, CMS-148A and LT-07 which is consistent with reports by Patil et al. (2012), Shinde et al. (2016). CMS-10A, CMS-82A, CMS-COSF-6A, LT-01 and LT-02 showed favorable GCA effects for hull and oil content, corroborating Kaya and Atakisi (2004). For seed yield per plant and oil content, the genotypes viz; CMS-852A, CMS-10A, CMS-82A, LT-01, LT-02, LT-05 and LT-07 were identified as excellent combiners, consistent with Shinde et al. (2016).

In Table 4, Analysis of specific combining ability (SCA) effects identified several promising sunflower hybrids exhibiting significant and desirable performance across key traits. Negative SCA effects for days to 50% flowering, maturity, plant height and hull content were found beneficial, with crosses like CMS-10A × LT-02, CMS-COSF-6A × LT-01 and CMS-82A × LT-02 exhibiting favorable earliness (Kulkarni and Supriya, 2017).The hybrids such as, CMS-852A × LT-07 and CMS-82A × LT-02 also showed desirable SCA for early maturity, while CMS-COSF-6A × LT-04 and CMS-852A × LT-01 proved superior for reduced plant height. For head diameter, seed filling and volume weight, hybrids such as CMS-COSF-6A × LT-02, CMS-10A × LT-07 and CMS-148A × LT-04 exhibited positive SCA effects, which is consistent with reports by Vairam et al. (2016), Ghodekar et al. (2021). CMS-10A × LT-05, CMS-82A × LT-07 and CMS-COSF-6A × LT-02 showed strong SCA for 100-seed weight, aligning with Vairam et al. (2016) and Lakshman et al. (2021). Hull content and oil content were significantly improved in CMS-148A × LT-05, CMS-82A × LT-01 and CMS-112A × LT-02, corroborating findings by Nehru et al. (2021), Salem and Ali (2012) and Varalakshami and Neelima (2019). Exceptional yield performance was recorded in CMS-112A × LT-02, CMS-COSF-6A × LT-06 and CMS-82A × LT-07, with parental GCA combinations indicating both additive and non-additive gene action (Sujatha and Reddy, 2009; Sreedhar et al., 2010). Top-performing hybrids showed superiority over commercial checks in seed yield, oil content, 100-seed weight and head diameter, supported by Rukminidevi et al. (2006), Ingle et al. (2017), Salke et al. (2018) and Kale et al. (2018). These findings reinforce the importance of GCA and SCA effects in developing heterotic hybrids with enhanced yield and quality traits (Kaya and Atakisi, 2004; Chandra et al., 2013).

Table 1. Analysis of variance for combing ability for different characters in sunflower

	Source of variation								
Characters	**Replication MSS**	**Treatments MSS**	**Parents MSS**	**Lines MSS**	**Tester MSS**	**Crosses MSS**	**L x T MSS**	**Parents *v/s* Crosses MSS**	**Error MSS**
Days to 50 % flowering	0.736	30.201**	8.153**	3.35*	13.404**	13.946**	7.52**	961.19**	1.032
Days to maturity	1.31	76.411**	7.05 *	0.133	4.904 *	46.196 **	2.679	2147.54 **	1.420
Plant Height (cm)	91.875	1090.411**	47.497 **	4.304	68.913	1382.75**	746.430**	1619.515**	78.349
Head diameter (cm)	0.49	7.004*	3.725*	6.220*	2.245	7.147*	10.078**	40.454**	0.859
Seed Filling (%)	16.83	373.028**	159.971**	192.35**	157.429**	349.231**	54.988 *	3905.406**	4.769
100-seed weight (g)	0.017	0.680**	0.602**	0.854**	0.484**	0.711**	2.851**	0.325*	0.050
Volume weight (g/100ml)	0.327	13.791**	14.782**	5.283*	13.619**	12.897**	32.813**	38.569**	1.031
Hull content (%)	1.961	21.538**	10.687**	9.926**	12.720**	25.176**	0.001	2.608	0.979
Oil content (%)	1.120	10.138**	10.928**	3.765**	10.468**	9.862**	0.004	11.929**	0.333
Seed yield /plant (g)	2.377	156.666**	64.319**	82.597**	57.886**	144.901**	37.667*	1747.163**	4.839

**and ** indicated significance at 5 and 1 percent level, respectively*

Table 2. Analysis of variance for line × tester sets for different characters in Sunflower

Sources of variation	**d.f**	**Days to 50 % flowering**	**Days to maturity**	**Plant height (cm)**	**Head diameter (cm)**	**Seed Filling (%)**	**100-Seed weight (g)**	**Volume weight (g/100ml)**	**Hull content (%)**	**Oil content (%)**	**Seed yield / plant (g)**
Replicates	1	0.42	0.29	149.68	0.61	13.44	0.0072	0.58	3.6	0.85	0.56
Crosses	41	36.43**	96.80**	282.96**	2.19*	178.25**	0.53**	9.19**	10.06**	8.63**	91.30**
Line effect	5	252.96**	618.21**	1316.53**	4.05**	474.55**	0.60**	27.90*	16.91**	28.96**	355.11**
Tester Effect	6	9.88**	59.63**	257.16**	1.27	206.14**	0.31*	2.70	15.42**	12.60**	38.71*
L x T Eff.	30	5.65**	17.33**	115.85	2.06*	123.29**	0.56**	7.36**	7.85**	4.45**	57.84**
Error	41	1.03	1.54	84.86	0.97	5.43	0.06	1.04	0.79	0.32	6.06
Estimates of variance components											
σ^2 GCA		0.15	0.45	21.45	0.01	4.59	0.002	0.04	0.23	0.10	1.71
σ^2 (SCA)		3.22	13.08	203.71	1.01	77.51	0.28	4.97	7.11	2.53	34.71
GCA/SCA		0.04	0.03	0.10	0.009	0.05	0.007	0.008	0.03	0.039	0.04

**and ** indicated significance at 5 and 1 percent level, respectively*

Table 3. Estimates of general combining ability (GCA) effects of lines and testers for ten different characters in sunflower

Sr. No.	Characters	Days to 50 % Flowering	Days to Maturity	Plant height	Head diameter	Seed filling	100 seed weight	Volume weight	Hull content	Oil content	Seed yield/ plant
CMS Lines											
1	CMS-148A	-0.74*	-2.27**	-34.54**	-2.63**	-16.81**	-0.50**	-1.88**	2.92**	-1.75**	-9.85**
2	CMS-112A	-0.74*	-0.13	-23.06**	-1.39**	-8.56**	-0.34**	-0.74*	3.61**	-1.92**	-5.54**
3	CMS-COSF-6A	0.55	2.65**	5.22*	0.49	1.68**	0.11	-1.02**	-1.46**	-0.67**	-1.60**
4	CMS-852A	1.55**	2.73**	26.52**	1.24**	9.39**	0.36**	0.90**	-0.08	1.40**	7.27**
5	CMS-10A	2.26**	2.94**	9.22**	1.57**	5.45**	0.04	1.55**	-1.96**	1.36**	4.98**
6	CMS-82A	-2.88**	-5.92**	16.65**	0.72**	8.85**	0.32**	1.19**	-3.03**	1.58**	4.74**
	SE ±	0.39	0.43	3.45	0.36	0.67	0.08	0.39	0.39	0.21	0.78
	CD at 5 %	0.78	0.87	6.93	0.73	1.36	0.16	0.78	0.79	0.44	1.57
Testers											
7	LT-01	1.95**	2.95**	1.74	-0.03	6.19**	0.02	0.21	-1.13**	1.40**	3.17**
8	LT-02	-0.80*	0.87*	9.64**	0.64*	5.55**	0.11	0.30	-0.61*	0.26	1.35*
9	LT-03	0.29	-1.30**	-3.25	0.33	-3.49**	0.04	0.71*	0.14	0.02	-0.75
10	LT-04	0.79*	-1.55**	-1.84	-0.13	-0.51	-0.05	-0.45	0.57	-1.31**	-0.14
11	LT-05	-0.05	-0.96**	-4.61	-0.61*	-4.15**	-0.05	-1.79**	-0.02	0.51**	-2.68**
12	LT-06	-0.30	-0.63	-7.08**	-0.41	-6.55**	-0.30**	-0.04	1.60**	-0.43*	-3.78**
13	LT-07	-1.88**	0.62	5.40*	0.21	2.94**	0.23**	1.05**	-0.54	-0.46**	2.83**
	SE ±	0.42	0.46	3.72	0.39	0.73	0.08	0.42	0.42	0.23	0.84
	CD at 5 %	0.84	0.94	7.49	0.79	1.47	0.17	0.84	0.86	0.47	1.70

**and ** indicated significance at 5 and 1 percent level, respectively*

Table 4. Estimation of specific combining ability for ten characters in sunflower (*Helianthus annuus* L.)

Sr. No.	Characters	Days to 50% Flowering	Days to Maturity	Plant height	Head diameter	Seed Filling (%)	100 seed weight	Volume weight	Hull content	Oil content	Seed yield/ plant
1	CMS 148 x LT01	-0.88	-0.35	-5.48	-0.38	-0.93	-0.11	0.43	2.38 **	-0.4	1.13
2	CMS 148 x LT02	-1.05	0.74	6.79	-0.68	-7.04**	-0.39*	-2.32 **	-0.08	-0.58	-4.23 *
3	CMS 148 x LT03	0.79	-2.35*	-1.99	-0.1	-1.77	0.05	0.01	-1.78 **	1.84 **	-4.68 **
4	CMS 148 x LT04	1.04	-1.68	-4.06	0.35	8.81**	0.44*	0.26	-0.56	1.11 **	4.99 **
5	CMS 148 x LT05	-0.55	-2.18*	-8.87	-0.03	-3.44*	0.01	-0.99	-3.81 **	-0.87 *	0.88
6	CMS 148 x LT06	1.2	3.32**	1.63	0.76	3.86*	0.26	2.10 **	0.96	-1.72 **	2.97
7	CMS 148 x LT07	-0.55	2.49**	11.99	0.08	0.51	-0.26	0.51	2.89 **	0.63	-1.06
8	CMS 112 x LT01	0.4	1.44	4.94	-0.31	-2.26	0.11	-2.00 **	-2.82 **	-1.27 **	-1.31
9	CMS 112 x LT02	-0.26	0.02	-3.41	0.58	6.65**	0.65**	2.25 **	-1.82 **	2.18 **	9.04 **
10	CMS 112 x LT03	-0.93	-0.56	-5.74	-0.51	-7.05**	-0.71**	-0.92	3.29 **	-0.08	-7.89 **
11	CMS 112 x LT04	2.23**	2.11*	12.36	-0.25	-4.95**	-0.24	-1.17	1.87 **	-2.31 **	-2.65
12	CMS 112 x LT05	-0.76	-0.89	-2.9	0.05	5.36**	0.38*	0.08	0.54	-0.93 *	4.80 **
13	CMS 112 x LT06	-1.51*	-3.39**	-4.25	0.02	-5.21**	-0.40*	0.17	-0.16	0.96 *	-3.50 *
14	CMS 112 x LT07	0.74	1.27	-1	0.41	7.45**	0.21	1.58 *	-0.91	1.44 **	1.52
15	CMS COSF-6 x LT01	-2.45**	-0.7	9.78	-1.32	-7.78**	-0.46*	1.79 *	2.15 **	0.73	-7.28 **
16	CMS COSF-6 x LT02	1.88*	0.88	10.67	2.29**	8.03**	0.80**	0.04	-0.68	-0.03	5.77 **
17	CMS COSF-6 x LT03	-0.29	0.8	5.11	-0.68	-1.96	-0.09	1.37	-1.26	-1.40 **	1.06
18	CMS COSF-6 x LT04	0.96	-2.54**	-12.8	-0.6	2.54	0.23	1.12	-0.86	0.59	0.59
19	CMS COSF-6 x LT05	0.88	-1.04	-3.49	-0.17	-1.95	-0.34	-0.13	-1.43 *	-0.56	-4.08 **
20	CMS COSF-6 x LT06	-0.87	-0.04	-1.59	0.89	17.91**	0.55**	-0.05	0.69	0.74	8.87 **
21	CMS COSF-6 x LT07	-0.12	2.63**	-7.71	-0.42	-16.79**	-0.68**	-4.13 **	1.39 *	-0.08	-4.94 **
22	CMS 852 x LT01	-0.45	4.58**	-11	0.22	2.47	-0.24	0.21	1.24	0.35	0.81
23	CMS 852 x LT02	2.88**	5.17**	2.4	0.05	-2.59	-0.57**	-1.54 *	1.33*	-0.76	-2.84

**and ** indicated significance at 5 and 1 percent level, respectively*

Table 4. Continued…

Sr. No.	Characters	Days to 50% Flowering	Days to Maturity	Plant height	Head diameter	Seed Filling (%)	100 seed weight	Volume weight	Hull content	Oil content	Seed yield/ plant
24	CMS 852 x LT03	1.21	2.08*	7.51	1.17	7.36**	0.56**	-1.70 *	0.01	-0.47	4.99 **
25	CMS 852 x LT04	-1.54*	-1.25	-3.89	-1.01	-2.36	0.07	1.55 *	-1.77 **	-0.3	-1.47
26	CMS 852 x LT05	0.38	0.75	-2.98	-0.83	-0.77	-0.60**	0.8	1.69 **	0.24	-1.56
27	CMS 852 x LT06	-0.87	-3.25**	7.52	0.36	-2.32	0.58**	-0.62	-1.67 *	-0.09	-0.68
28	CMS 852 x LT07	-1.62*	-8.08**	0.42	0.04	-1.8	0.19	1.3	-0.83	1.02 *	0.75
29	CMS 10 x LT01	0.33	-2.99**	-2.95	0.6	1.73	0.40*	0.36	0.06	-1.75 **	4.37 *
30	CMS 10 x LT02	-2.33*	-3.40**	-7.54	-2.20**	-9.14**	-0.44*	-3.39 **	-0.53	0.49	-11.01 **
31	CMS 10 x LT03	0.5	0.01	-1.8	0.13	-0.5	-0.01	2.44 **	1.71 **	-1.77 **	3.56 *
32	CMS 10 x LT04	-0.75	3.18**	6.24	0.37	5.52**	-0.35	-0.81	1.16	0.28	3.17
33	CMS 10 x LT05	1.17	2.18*	6.32	2.22**	6.83**	1.16**	-0.06	0.61	2.41 **	5.81 **
34	CMS 10 x LT06	-1.08	0.68	-1	-0.93	-13.40**	-0.55**	0.52	-0.98	-0.16	-3.27
35	CMS 10 x LT07	2.17**	0.35	0.72	-0.2	8.97**	-0.2	0.94	-2.03 **	0.5	-2.62
36	CMS 82 x LT01	3.05**	-1.99*	4.69	1.18	6.76**	0.3	-0.79	-3.02 **	2.34 **	2.28
37	CMS 82 x LT02	-1.12	-3.40**	-8.91	-0.04	4.09*	-0.04	4.96 **	1.78 **	-1.31 **	3.27
38	CMS 82 x LT03	-1.29	0.01	-3.09	-0.02	3.92*	0.19	-1.2	-1.96 **	1.88 **	2.96
39	CMS 82 x LT04	-2.04**	0.18	2.12	1.14	-9.55**	-0.14	-0.95	0.15	0.62	-4.62 *
40	CMS 82 x LT05	-1.12	1.18	11.92	-1.25	-6.04**	-0.61**	0.3	2.39 **	-0.29	-5.85 **
41	CMS 82 x LT06	3.13**	2.68**	-2.31	-1.11	-0.85	-0.44*	-2.12**	1.16	0.27	-4.39*
42	CMS 82 x LT07	-0.62	1.35	-4.42	0.09	1.66	0.74**	-0.2	-0.5	-3.51**	6.35**
	SE ±	0.46	0.2	11.67	0.33	10.17	0.08	1.37	0.21	0.85	10.92
	CD at 5 %	1.57	1.51	8.09	1.508	4.97	0.226	2.674	1.096	1.059	4.189
	CD at 1 %	2.1	0.97	17.77	2.013	6.52	0.301	3.56	1.462	1.358	5.59

**and ** indicated significance at 5 and 1 percent level, respectively.*

4. CONCLUSION

The study established significant GCA and SCA variances across key sunflower traits, confirming robust genetic diversity and hybrid potential. CMS-82A, CMS-852A, LT-02 and LT-07 are the good combiner. Hybrids like CMS-112A × LT-02 and CMS-82A × LT-07 consistently outperformed checks in yield and quality traits. The findings affirm that selecting parents with high × high or low × high GCA enables successful heterosis breeding through additive and non-additive gene action. Furthermore, the predominance of non-additive genetic effects reinforces the utility of selective intermating and recurrent selection approaches in capturing diverse gene actions and accelerating genetic gain in sunflower breeding programs.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below:

1. Google Scholar
2. Google

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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