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GENETIC EVALUATION FOR SEED YIELD AND ITS COMPONENT TRAITS IN SUNFLOWER (*HELIANTHUS ANNUUS L.*) USING LINE × TESTER APPROACH

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Abstract *Sunflower is a vital oilseed crop, but yield improvement remains a major objective. Exploring germplasm and exploiting non-additive gene action can help overcome yield limitations and identify superior parental lines and crosses for hybrid development through GCA and SCA. Four parental lines (A-1, A-2, A-3, and A-4) were crossed with three testers (B-1, B-2, B-3) in a line × tester fashion to generate F_0 seeds. These seeds were planted using a randomized complete block design (RCBD) with three replicate plots in the field to ensure unbiased results. Data on various growth and yield parameters is collected and analyzed using Analysis of variance and combining ability analysis. Lines A-3 and A-4 and tester B-3 demonstrated consistently positive GCA effects for several traits, signifying their broad genetic value for hybrid development. The hybrid A-4 × B-1 emerged as the top performer, exhibiting exceptional SCA effects in days to flowering, seed yield per head and height of the plant, indicating exceptional compatibility between these parental lines. Lines A-3 and A-4 displayed significant positive GCA effects for key parameters like head diameter and seed yield. Tester B-3 also contributed to enhanced yield traits in hybrid combinations. Lines A-3 and A-4, tester B-3, and hybrid A-4 × B-1 emerged as promising parents for sunflower hybrid development due to their superior GCA and SCA effects. These findings offer valuable resources for future breeding programs that enhance sunflower yield and productivity.*

Introduction

Sunflower (*Helianthus annuus* L.) holds a distinguished position among oilseed crops, playing a crucial role in global food security as the fourth-largest source of edible oil**.** It is a rich source of vitamins, including A, B, C and K, with a fatty acid profile dominated by polyunsaturated fats like oleic and linoleic acid (Kapoor et al., 2021). These polyunsaturated fats regulate cholesterol (Sokoła-Wysoczańska et al., 2018). Additionally, sunflower provides phosphorus, calcium and nicotinic acid (Rauf et al., 2020). It possesses phytoremediation properties, which means it can assist in reducing pollutants in soils and water. Sunflower is one of the temperate zones of cross-pollinated crops and belongs to the "*Asteraceae*" family. Achene consists of 40-50% oil and 17-20% protein content and represents a valuable genetic resource for breeding programs to overcome the challenge of securing qualitative edible sunflower oil and sustainable animal feed (Radanović et al., 2023). However, sunflower yield in Pakistan is lower than in other countries. Sunflower is a non-conventional and early-maturing crop (90-110 days) with multiple yearly growing cycles under favorable conditions. In 2021-22, the total domestic production of edible oil was 0.496 million tons, which was insufficient to meet the demand, so 2.681 million tons edible oil was imported. In 2022-23, 179 thousand acres of Pakistan were under sunflower cultivation (Nungula et al., 2023). The local sunflower seed and oil production were 124 thousand and 47 thousand tons, respectively (Sonawane et al., 2019). This gap can be overcome by developing high-yielding cultivars and hybrids, expanding the planted area and increasing seed and oil quality (Debaeke et al., 2021). The number of seeds per head, head diameter and 100-

seed weight have a positive and substantial relationship with the yield (Sirel and Aytac, 2016).

The primary goal of sunflower breeding is to develop high-yielding hybrids. Superior hybrids are developed by crossing male and female parents with significant combing ability values (Dhillon and Tyagi, 2016). Inbred lines are assessed and classified by their performance in crosses using Combining ability estimates. Identifying parental lines with high GCA is crucial for developing base populations with desirable traits. We need to produce hybrids well-suited to our environment to attain better productivity. Hybrids are preferred due to their strong self-fertility, uniform maturation, great yield potential and stability. SCA helps to identify which specific crosses lead to superior hybrids (Ali et al., 2017; Meena et al., 2022). Line × tester analysis provides valuable information on the genetic potential of different lines and testers (Ahmad et al., 2012; Ali et al., 2015; Bhoite et al., 2018; Memon et al., 2015). The studies help breeders make more efficient and effective choices in developing new hybrids with improved traits. It informs the development of appropriate breeding methodologies by investigating the genetic architecture of target traits (Ali et al., 2013; Ali et al., 2016; Ali et al., 2014; Casadebaig et al., 2022). The main objective of the present investigation was to assess parents and their hybrids for estimating the magnitude of gene action, yield and its component parameters through line × tester analysis (Ali et al., 2014; Memon et al., 2015).

Materials and methods

The research was conducted at the Rajawala farm area, University of Agriculture, Faisalabad, during two growing seasons i,e. autumn and spring, in 2020-2021. The imported USA material in this research comprises seven parental genotypes (4 lines and 3 testers). These were crossed to develop 12 F_1 hybrids during 2020. At maturity, the crossed heads were collected, dried and threshed separately from these crosses. To account for potential field variability, a randomized complete block design with three replications was implemented to test the performance of 12 newly developed F1 sunflower hybrids alongside their parents. The seeds were sown by keeping the standard distance of 25cm and 75cm between plants and rows, respectively. All agronomical practices and plant safety measures were adopted per recommendations during the growing season. Total no of 4-5 irrigations was applied to crop according to their requirements. For data collection, seven plants were selected from each replication to obtain various morphological traits such as Days to flower completion (DFC), inter-nodal length (cm), Seed yield per head (SYH), leaf area (LA). No of leaves/plant (NLP), Days to flower initiation (DFI), Head diameter (HD), Number of whorls/plant (NWP) and 100-seed weight (SW). The data was statistically analyzed following ANOVA (Steel *et al.*, 1997) using a randomized block design for combining abilities. According to Kempthorne (1957), the GCA and SCA effects were observed at the 0.01 and 0.05 levels by applying t-test.

Results and discussion

Analysis of Variance (ANOVA)

Table 1. presented highly significant results for all the studied traits of genotypes, replications and crosses. The parental variations were highly significant for all traits except inter-nodal length (1.11) and 100-seed weight (1.22). The variations between parents and crosses revealed pronounced differences for all studied parameters except days to flower initiation (57.281). This indicates the potential for exploiting non-additive gene action in sunflower breeding for yield improvement. Significant variations among the lines were observed for all the characters except the head diameter (0.74) and plant height (37.47). On the other hand, testers exhibited highly significant differences for all studied parameters. It shows inherent genetic diversity within both female lines and male testers, providing a valuable resource for breeding programs. Significant line × testers interaction was observed for all morphological traits. This indicates specific combinations of lines and testers influencing trait expression, highlighting the importance of evaluating specific hybrids rather than individual lines and testers. This aligns with previous research by Hilli and Immadi (2021), Bohra et al. (2022), Memon et al. (2015), Mehmood (2021), Ashraf et al. (2015), and Shyam Sundar et al. (2021),.

Table 1. F-values from analysis of variance for different morphological traits.

SOV	DF	DFI	DFC	NLP	LA	IL	NWP	HD	PH	100-SW	SYH
Replications	2	2.9960**	1.33*	14.36**	444.54*	11.72**	8.91**	5.26*	15.31*	8.29*	20.35*
Genotypes	18	16.0739**	20.45*	35.43*	1265.90**	4.84*	17.55*	15.03**	804.98*	1.97**	62.20*
Crosses	11	17.3754**	7.40**	31.09*	690.55*	6.68*	9.23**	4.02*	308.43*	2.21**	62.43*
Lines (C)	3	51.8514**	23.87*	24.66*	823.34*	0.39*	26.47*	12.84**	1066.16	1.71	79.92*
Testers (C)	2	15.2569**	4.33*	47.25*	857.35*	1.48*	7.52**	1.80*	71.70*	0.81**	74.07*

SOV	df	DFI	DFC	NLP	LA	IL	NWP	HD	PH	100-SW	SYH
Lines (C) × Testers (C)	6	0.8436*	0.18	28.91**	568.56**	11.56**	1.19	0.35	8.47**	2.92**	49.80**
Parent	6	6.8198**	6.52**	8.71**	648.08**	1.11	5.30**	9.53**	262.74**	1.22	38.13**
Lines (p)	3	2.9431**	2.97*	14.00**	48.53**	1.23*	2.33*	0.74	37.47	0.85	46.72**
Testers (p)	2	7.3233**	1.44*	4.00**	84.92**	1.05*	4.77**	8.54**	20.12**	2.15**	8.68**
Lines (P) vs Testers (p)	1	17.4432**	27.33**	2.28*	3573.05**	0.86*	15.25**	37.87*	1423.81**	0.48**	71.25*
Crosses Vs Parents	1	57.2811	247.68**	243.60**	11301.63**	7.04*	182.58**	169.17*	9520.48**	3.80*	204.12**
Error	36	5.21	0.14	0.25	324.43	0.10	0.11	0.07	0.05	0.08	0.07

*= 0.05% Probability level;   **= 0.01% Probability level

DFI= Days to flower initiation, **DFC**= Days to flower completion, **NLP**= Number of leaves per plant, **LA**= Leaf area, **IL**= Inter-nodal length, **NWP**= Number of whorls/plant **HD=** Head diameter, **PH**= Plant height, **100-SW=**100 seed weight, **SYH=** Seed yield per head.

General combining ability effects

Different parental genotypes are selected based on GCA effects to develop hybrids with desirable characters. GCA is primarily influenced by additive gene action, where different alleles at a single gene add their effects (either positive or negative) to determine the trait expression. Seven sunflower plants were chosen for this experiment to identify parents with superior genetic potential. Table 2 shows the GCA effects of four female lines (A-1 to A-4) and three male testers (B-1 to B-3) for various morphological parameters in sunflower. Among the parents, lines A-3, A-4 and tester B-3 displayed a remarkably positive GCA effect for days to flower initiation, number of whorls per plant, head diameter, plant height and days to flower completion and seed yield per head. It might be due to favourable alleles, complementary or dominant gene action. Due to high positively significant GCA effects for diameter of sunflower head and seed yield per head, these parents can contribute efficiently to develop hybrids with enhanced yield. Line A-1 displayed highly positive significant values for the number of leaves per plant (2.00**) and 100-seed weight (0.37**), negatively significant values for days to flower initiation (-10.08**), days to flower completion (-2.03**), head diameter (-1.58**), plant height (-14.03**) and seed yield per head (-3.32**) while non-significant result for leaf area (-6.48) and inter-nodal length (0.20). A-2 depicted negatively significant results for all the understudied traits except leaf area (0.23) and 100 seed weight (0.07), which are non-significant. Tester B-1 demonstrated negatively significant results for most of the traits. Hence, these parents (A-1, A-2, and B-1) with strong negative GCA for days to flower initiation and days to flower completion are identified as good general combiners for breeding programs aiming to develop hybrid varieties with shorter durations. Moreover, these parents showed considerable negative GCA effects for height, So they can also be used to develop short stature and lodging-resistant hybrids. Tester B-2 showed high positively significant results for the number of leaves per plant (2.25**), plant height (0.97**) and seed yield per head (1.73**) while non-significant results for the rest of the characters Lamperty (2020), Roberts et al. (2015), EL-Gharbawy (2015), and Mitiku Abdeta (2021) also find out similar results of GCA effects.

Table 2. General combing ability (GCA) effects of lines and testers for various morphological traits

SOV	DFI	DFC	NLP	LA	IL	NWP	HD	PH	100-SW	SYH
A-1	-10.08 **	-2.03 **	2.00 **	-6.48	0.20	-2.31 **	-1.58 **	-14.03 **	0.37 **	-3.32 **
A-2	-3.42 **	-0.47 **	-2.00 **	0.23	-0.26 *	-0.31 *	-0.26 *	-2.77 **	0.07	-0.94 **
A-3	5.25 **	0.75 **	0.33	-7.19	-0.08	1.36 **	0.77 **	6.20 **	0.18	0.44 **
A-4	8.25 **	1.75 **	-0.33	13.44	0.14	1.25 **	1.07 **	10.60 **	-0.63 **	3.82 **
Standard Error	0.14	0.14	0.12	0.24	0.08	0.09	0.11	7.55	0.11	0.10

	DFI	DFC	NLP	LA	IL	NWP	HD	PH	100-SW	SYH
B-1	-1.00 **	-0.67 **	-1.50 **	-9.74	0.40 **	-0.78 **	-0.38 **	-2.78 **	0.15	-2.85 **
B-2	0.00	0.17	2.25 **	5.43	-0.16	-0.03	-0.01	0.97 **	0.15	1.73 **
B-3	1.00 **	0.50 **	-0.75 **	4.31	-0.25 *	0.81 **	0.39 **	1.81 **	-0.30 **	1.12 **
Standard Error	0.12	0.12	0.10	0.20	0.07	0.08	0.09	6.54	0.09	0.08

* = 0.05 probability level; ** = 0.01 probability level

DFI= Days to flower initiation, **DFC**= Days to flower completion, **NLP**= Number of leaves per plant, **LA**= Leaf area, **IL**= Inter-nodal length, **NWP**= Number of whorls/plant **HD**= Head diameter, **PH**= Plant height, **100-SW=**100 seed weight, **SYH=** Seed yield per head.

Specific combining ability effects

Table 3 shows all Specific Combining Ability (SCA) effects observed in the experiment. These values indicate the performance of specific hybrid crosses, beyond what would be expected based on the GCA of individual parents. This represents the unique interaction between specific parents when crossed, resulting in performance deviation from the expected GCA combination. Such as in table 3, hybrid A-2 × B-1 showed positive and highly significant results for seed yield per head (2.38**) that deviated from its parents A-2 (-0.94**) and B- (-2.58**). All the crosses displayed non-significant SCA effects for days to flower completion and leaf area, suggesting average performance based on parental combinations. A-1×B-1 and A-4×B-2 showed negatively significant

and non-significant SCA effects for all traits, depicting inferior performance compared to other crosses. Hybrid combinations A-1×B-2, A-1×B-3, Hybrids A-1×B-2, A-1×B-3, A-2×B-1, A-2×B-2 and A-4×B-3 displayed high positively significant results of SCA for head diameter and seed yield per head. Hybrid progeny A-1×B-2, A-2×B-1, A-2×B-3, A-3×B-3, and A-4×B-2 demonstrated high negatively significant SCA for plant height that is considered as lodging resistance traits. The hybrid A-1×B-3 had a significant positive SCA effect for half of the given traits, indicating potentially superior performance compared to other crosses. These results resonate with the with the outcomes of Singh et al. (2016), Fichera (2023), ABIY (2017), and Sheunda (2019).

Table 3. Specific combining ability (SCA) effects for various morphological traits.

Hybrids	DFI	DFC	NLP	LA	IL	NLP	HD	PH	100-SW	SYH
A-1 x B-1	-0.67 **	-0.22	-0.17	4.07	-2.08 **	-0.11	0.13	0.16	-1.27 **	-4.41 **
A-1 x B-2	0.33 **	-0.06	-0.25	-1.99	1.51 **	-0.19	0.22	-1.16 **	0.76 **	3.92 **
A-1 x B-3	0.33 **	0.28	-3.25 **	-2.08	0.57 **	0.31	-0.35 *	1.00 **	0.51 **	0.49 **
A-2 x B-1	-1.33 **	0.22	-1.50 **	9.23	2.17 **	-0.44 *	-0.23	-0.77 **	1.29 **	2.38 **
A-2 x B-2	-0.33 **	-0.28	0.75	-4.74	0.23	-0.19	-0.14	1.01 **	1.29 **	0.67 **
A-2 x B-3	1.67 **	0.06	0.75	-4.49	-2.41 **	0.64 **	0.36 *	-0.23	-0.39 *	-3.05 **
A-3 x B-1	-2.00 **	0.00	-3.83 **	-23.32	0.23	0.56 *	-0.15	0.42 *	0.02	-2.16 **
A-3 x B-2	0.45	0.17	0.42	9.55	-0.28	0.47 *	-0.16	1.78 **	0.48 *	0.16
A-3 x B-3	2.00 **	-0.17	3.42 **	13.77	0.05	-1.03 **	0.31	-2.20 **	-0.50 *	2.00 **
A-4 x B-1	4.00 **	0.65	1.83 **	10.02	-0.33	0.33	0.25	0.19**	-0.04	4.19 **
A-4 x B-2	0.00	0.17	-0.92 *	-2.82	-1.47 **	-0.08	0.08	-1.62 **	-0.34	-4.75 **
A-4 x B-3	-4.00 **	-0.17	-0.92 *	-7.20	1.79 **	0.08	-0.33	1.43 **	0.38 *	0.56 **

Standard Error	0.24	0.24	0.20	0.41	0.15	0.16	0.19	13.08	0.19	0.17

* = 0.05 probability level; ** = 0.01 probability level

DFI= Days to flower initiation, **DFC**= Days to flower completion, **NLP**= Number of leaves per plant, **LA**= Leaf area, **IL**= Inter-nodal length, **NWP**= Number of whorls/plant **HD=** Head diameter, **PH**= Plant height, **100-SW=**100-seed weight, **SYH=** Seed yield per head.

Proportional contribution of lines, tester and their interaction

In Figure 1, Lines played a prominent role in most traits, such as days to flower initiation and plant height. The number of whorls per plant suggests their genetic makeup significantly influenced the observed variation. This is following the result of Cortés-Fernández et al. (2022). Testers in the present study showed their minimum parental contribution towards all characters included as depicted by Hughes et al. (2018) earlier. However, their interaction was maximum for characters like inter-nodal length, 100-seed weight, number of leaves per plant, seed yield per head and leaf area. The given study's findings correlate with the outcomes of (Ali et al., 2014); GB (2019); (Iqra et al., 2020); Shabaz et al. (.

Table 4. Proportional contribution of lines, tester and their interaction to the total variance for indicated plant traits of sunflower genotypes

Traits	Contribution (%)		
	Lines	Tester	Line x Tester
DFI	92.16	1.18	6.66
DFC	87.99	10.64	1.36
NLP	21.64	27.63	50.73
LA	32.52	22.57	44.91
IL	1.62	4.04	94.34
NWP	78.14	14.81	7.05
HD	87.08	8.15	4.78
PH	94.27	4.23	1.50
100-SW	21.16	6.66	72.18
SYH	34.91	21.57	43.52

DFI= Days to flower initiation, **DFC**= Days to flower completion, **NLP**= Number of leaves per plant, **LA**= Leaf area, **IL**= Inter-nodal length, **NWP**= Number of whorls/plant **HD=** Head diameter, **PH**= Plant height, **100-SW=**100 seed weight, **SYH=** Seed yield per head.
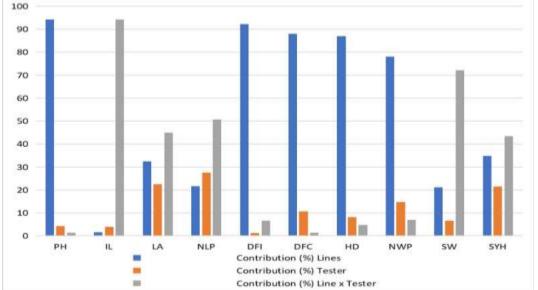
Fig 1. Proportional contribution of lines, testers and their interactions

Genetic variances

Table 5 demonstrates genotypic variations for all the understudied characters. Traits with high GCA and low SCA, such as days to flower initiation, days to flower completion, leaf area, number of whorls per plant, head diameter and height of the plant showed that additive gene effects (σ2A) are prevalent, and they can be improved through direct selection of high-performing lines. On the other hand, parameters with low GCA and high SCA, such as number of leaves per plant, inter-nodal length, seed yield per head and 100-seed weight, are governed by non-additive gene effects (σ2D). Hence, Specific crossing strategies and hybrid testing are needed to identify superior combinations. Leaf area, 100-seed weight and seed yield per head with (σ2D/σ2A)1/2 > 1 showed

dominance plays a substantial role in these traits, suggesting potential for transgressive segregation and heterosis exploitation in hybrids. Additive gene effects dominate the parameters with $(\sigma2D/\sigma2A)1/2 <$ 1, including plant height, days to flower initiation, number of leaves per plant, head diameter, days to flower completion, and number of whorls per plant. It implies straightforward inheritance patterns and efficient selection during breeding. The results of the given study correlate with the results of Ene et al. (2019), Naheed et al. (2017), Begna (2021), Zhao et al. (2015) for plant height, days to flowering and hundred seed weight. Bhoite et al. (2018), and Habib et al. (2021) noticed higher SCA than GCA for components related to yield, which SCA controlled.

Table 5. Estimation of variance

SOV	Genetic Components					
Traits	$\sigma^2_{GCA} = \left\{\dfrac{(1 + F)}{4}\right\}$ σ^2_A	With F = 0, σ^2_A	$\sigma^2_{SCA} = \left\{\dfrac{(1 + F)}{2}\right\}$ σ^2_D	with F = 0, σ^2_D	$\sigma^2_{SCA} / \sigma^2_{GCA}$	$(\sigma^2_D /\sigma^2_A)^{1/2}$
DFI	7.0252	7.0252	7.5556	30.2222	1.07	1.034
DFC	0.3113	1.2452	0.0036	0.0146	0.0115	0.107
NL/P	0.0938	0.3752	9.5051	38.0202	102.15	10.10
LA	5.2624	21.0496	18.3522	73.4088	3.48	1.86
INL	-0.2104	-0.8414	3.8144	15.2574	-18.16	-4.26
NW/P	0.3471	1.3882	0.3586	1.4343	1.033	1.016
HD	0.1583	0.6332	0.0912	0.3650	0.5761	0.7590
PH	12.9395	51.7579	2.7997	11.1987	0.215	0.464
100-AW	-0.0308	-0.1233	0.9432	3.7729	30.61	5.53
SYH	0.5446	2.1783	16.5747	66.2986	30.43	5.51

DFI= Days to flower initiation, **DFC**= Days to flower completion, **NLP**= Number of leaves per plant, **LA**= Leaf area**, IL**= Inter-nodal length, **NWP**= Number of whorls/plant **HD=** Head diameter, **PH**= Plant height, **100-SW=**100 seed weight, **SYH=** Seed yield per head

Conclusion

Both GCA and SCA were significant, but SCA dominated for all traits, indicating the prevalence of non-additive gene action. This means specific interactions between parental lines were more important than average genetic effects for determining these traits. A-3, A-4 and B-3 showed high GCA for most parameters across lines and testers. This makes them reliable choices for hybridization programs, as they likely transmit desirable traits to their offspring even when crossed with various partners. The cross A1×B3 exhibited the highest SCA for days to flower initiation, inter-nodal length, plant height, 100 seed weight and seed yield per head. This specific combination shows exceptional potential for hybrid development due to its superior performance in these desirable traits. Breeders should focus on exploiting both GCA and SCA for efficient selection. Choosing parents with good GCA ensures overall good performance while identifying crosses with high SCA can lead to exceptional hybrids for specific traits. A-1×B-3 should be further evaluated and potentially used in commercial hybrid development programs targeting improved yield. Investigating the performance of this hybrid across different environments and planting seasons would provide valuable insights into its stability and adaptability.

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Declarations

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AA wrote the initial draft of manuscript. AA, MAI, and SF edit the manuscript for final submission. All authors have read and approved the final manuscript.

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The authors declare that they have no competing interests.