



Genetic Innovation in Sunflower (*Helianthus annuus* L.) Exploring Combining Ability and Heterosis Revealed by Line \times Tester Analysis.

Tehreem Mariam¹, Saima Bibi², Sehar Nawaz³, Nazar Hussain Khan⁴, Sidra Naheed⁵, Zulaikha Aman⁶, Muhammad Ahsan Iqbal (Corresponding Author)⁷

^{1,6} MSc (HONS.) student, Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan

^{2,5} M.SC.(HONS.) student, Department of Botany, University of Agriculture Faisalabad, Pakistan

³ Lecturer, Center for Agriculture Biochemistry and Biotechnology, University of Agriculture, Faisalabad, Pakistan

⁴ Lecturer, Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan

⁷ Associate professor, Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan, Email: ahsanuaf2003@yahoo.com

Abstract

Sunflower (*Helianthus annuus* L.) is a globally significant oilseed crop valued for its high-quality oil content. This study, conducted at the University of Agriculture, Faisalabad, aimed to assess the genetic potential of sunflower hybrids through combining ability analysis. The experiment spanned two growing seasons, Autumn 2023 and Spring 2024, involving four female lines (A-39, A-42, A-45, and A-47) and three male testers (B-31, B-33, and B-34). Data were collected for eight yield-related traits: plant height, number of leaves per plant, leaf area, internodal length, stem diameter, head diameter, seed yield per plant, and 100-achene weight. The results highlighted the significant genetic contributions of lines and crosses, with A-42 emerging as an excellent general combiner (GCA) for traits like internodal length (-0.93*), stem diameter (-0.34**), seed yield (6.64**), and 100-achene weight (-0.21**). Similarly, A-39 and A-47 exhibited strong combining abilities for key traits, including seed yield and head diameter. Among testers, B-34 demonstrated pronounced additive gene effects, particularly for plant height (2.92**), head diameter (-0.4**), and seed yield (-3.94**). Specific combining ability (SCA) analysis revealed superior cross combinations, such as A-39 \times B-33 for seed yield (7.19**) and A-45 \times B-33 for head diameter (-1.98**). Crosses also showed prominent substantial better parent heterosis across all traits. Overall, the study identifies A-42, A-45, and A-39 as promising candidates for advancing sunflower breeding programs focused on yield improvement. By following superior lines, testers, and cross combinations, can develop high-yielding, stress-tolerant hybrids with improved oil content. The identified genotypes can serve as a foundation for exploring adaptability under diverse environmental conditions, ensuring sustainable sunflower production in the face of climate variability.

Keywords: *Helianthus annuus* L, General Combining Ability, Specific Combining Ability, Better parent heterosis, Testers (male), Lines (female).

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Introduction

After rapeseed, soybean and groundnuts, sunflower is the fourth most important oilseed crop globally (Arshad *et al.*, 2010). Sunflower (*Helianthus annuus* L.) has emerged as a competitive oilseed crop due to its excellent nutritive properties, grown worldwide as a source of premium oil and dietary fibres that significantly contribute to human health (Adesina, 2018). It contains a high amount of polyunsaturated fatty acids, making its oil good quality edible and reducing the risk of cardiac diseases (Rani *et al.*, 2017). Sunflower seeds contain a high number of vitamins like vitamin E, B, and folate and minerals like iron, magnesium, sodium, potassium, calcium, copper, phosphorous and zinc. By and large, the therapeutic potential of sunflower seeds has been proven medically curative for colds and coughs (Islam *et al.*, 2016).

Sunflower, an essential oilseed crop, is widely cultivated globally, with leading producers including the USA, Argentina, Brazil, China, and parts of Europe and Africa (Cheng *et al.*, 2019). Favourable climates, particularly semi-arid regions with temperatures between 20-26°C, support optimal sunflower growth, though extreme heat and frost negatively impact yield (Onemli *et al.*, 2012; Thomaz *et al.*, 2012). Despite global advancements, Pakistan's sunflower productivity remains low at 2200-3000 kg/ha compared to other countries' 3000-5000 kg/ha. Challenges include poor-quality seeds, a lack of markets, and expensive hybrid seeds from multinational corporations (Tahir and Shehzadi, 2017).

In Pakistan, sunflower cultivation spans approximately 0.253 million acres, producing 0.141 million tons of seeds and 0.054 million tons of oil annually. However, this production is insufficient to meet the country's growing edible oil demands,

leading to imports of 2.681 million tons of edible oil worth US\$ 3.562 billion (GOP, 2022-2023). The gap between production and consumption continues to widen due to population growth and changing dietary habits, emphasising the need to improve local sunflower yield and oil production (Iqbal *et al.*, 2018).

Edible oil is a critical component of the Pakistani diet, yet low sunflower yields are attributed to the unavailability of quality seeds, inadequate technological adoption, and pest infestations. Furthermore, reliance on expensive hybrid seeds from multinational corporations exacerbates production costs, creating barriers for local farmers. Developing cost-effective, high-yielding local hybrids with improved oil content is essential to address this challenge and reduce the nation's dependence on imports (Tahir and Shehzadi, 2017).

To bridge the edible oil production-consumption gap, enhancing sunflower yield and oil content through heterosis-based breeding programs is critical. Hybrids outperform open-pollinated varieties in yield, disease resistance, and adaptability, with over 90% of sunflower production in Turkey attributed to hybrid seeds (Sujatha and Reddy, 2009; Golabadi *et al.*, 2015). Line × tester analysis, an efficient breeding tool, evaluates combining abilities, identifying promising genotypes and parental combinations for hybridization (Sanghera and Hussain, 2012; Rajane *et al.*, 2022).

Efforts to develop heterotic hybrids with superior oil content and seed yield require leveraging genetic diversity and morphological traits as selection criteria (Rauf, 2019; Dimitrijevic and Horn, 2018). This study focuses on identifying high-performing genotypes and effective cross combinations, facilitating the development

of high-quality hybrids tailored to local environmental conditions.

Materials and Methods

Experimental Design

The research was performed at the sunflower experimental area, Department of Plant Breeding and Genetics, University of Agriculture Faisalabad during the autumn season of 2023. The mean temperature during the autumn season of 2023 was recorded at 23.07°C, accompanied by an average relative humidity of 69.5%, at an altitude of 184.4 meters above sea level. In the spring season of 2024, the trials were conducted at a longitude of 73°06' E, and experienced a slightly higher mean temperature of 23.73°C, providing favourable conditions for the experimental study. Seven genotypes were acquired including four lines (female) and three testers (male) and seeds were planted manually keeping row × row (75cm) and plant × plant distance (25cm). Twelve hybrids were developed in the autumn season in the first season. At the end of the season, seeds of lines, testers and crosses were kept separated for evaluation in the next season. During the spring season, the sowing of seed from hybrids was done following Randomized Complete Block Design (RCBD) using three replications. All agronomic practices were followed for maximum accuracy of results from seedling till harvesting.

Crossing

Sunflower is a highly cross-pollinated protandrous crop normally Entomophily and Anemophily. The season is the main factor and flower opening depends upon it. In the winter season flower opening is completed in 8-10 days but 5-8 days are required for complete blooming. Anthesis is completed in 2-3 days but pollen viability is very short about 12 hours. To develop hybrids and avoid contamination heads were covered with butter paper or a muslin

cloth bag until fertilization. A camel hair brush was used to spread pollens, 8-10 am is considered a good time for crossing.

Germplasm for Experimentation

The germplasm was imported from USDA including four lines (A-39, A-42, A-45 and A-47) and three testers (B-31, B-33 and B-34). By crossing lines × testers twelve cross combinations were developed and further screening was carried out among these crosses for future hybrid development.

Morphological Characters Measured

Data on the following pre-harvest and post-harvest plant traits including No. of leaves/plant, plant height (cm), leaf area (cm²), internodal length (cm), stem diameter (cm²), head diameter (cm²), with the help of measuring tape, seed yield per plant and 100 seed weight(g) with the help of electronic balance was recorded.

Biometrical Approach

After recording the data on morphological traits according to [Steel et al. \(1997\)](#), Analysis of Variance was used to study the significance level among parents and F1 hybrids, which was further subjected to combining ability analysis ([Kempthorne, 1957](#)).

Results

The two-way ANOVA results revealed significant effects of various sources of variation on different traits. Key findings include significant F-values for plant height (F = 3.5318, p < 0.05) and internodal length (F = 6.1522, p < 0.01); head diameter was not significant (F = 1.2645). Genotypic effects were highly significant across all traits, with substantial genetic variability noted for leaf area (F = 10.4252), internodal length (F = 9.29748), and 100-achene weight (F = 53.5606).

Cross variation was significant for most of the recorded traits, particularly stem (26.7957) and head diameter (35.6637), indicating strong hybrid vigour.

Differences among lines were significant for internodal length (28.254), stem diameter (F = 20.0105), and seed yield per plant (F = 77.4854). Tester effects were significant for plant height (4.5907) and seed yield (65.2206), showing their effectiveness in distinguishing traits. The replication effects for all the measured traits were non-significant with exceptions of plant height and internodal length. The uniformity in blocks and optimal growing conditions are the main reasons because of why no difference was observed in all experimental units. These outcomes indicated that germplasm has maximum homogeneity.

Table 1. Two-way ANOVA for all studied traits plant height, No. Of Leaves/plant, Leaf area, Internodal length, Stem diameter, Head diameter, Seed yield/plant and 100-achene weight. * Indicate significant results ** indicate highly significant results when p<0.05 and p<0.01. ns indicate non-significant. Reply. Stands for replication, Geno. Represents Genotypes, L. stands for lines and T stands for testers.

Source of Variation	D F	Plant height	No. of leaves/plant	Leaf area	Internodal length	Stem diameter	Head diameter	Seed yield/plant	100-achene weight
Repl.	2	3.5318*	0.5567ns	0.5234ns	6.1522**	2.4071ns	2.4558ns	1.2645ns	1.6318ns
Geno	18	3.272**	7.5583**	10.4252**	9.29748**	37.1868**	36.2484**	45.7436**	53.5606**
Cross	11	3.3989**	4.701**	2.0976*	11.6832**	26.7957**	35.6637**	53.0922**	41.4135**
Lines ©	3	1.8252ns	8.6186**	0.569ns	28.254**	20.0105**	11.6484**	77.4854**	10.8614**
Tester ©	2	4.5907*	0.5722ns	1.6694ns	4.407*	14.902**	33.3576**	65.2206**	6.6455**

L × T ©	6	3.7885**	4.1186**	3.0046*	5.8232**	34.153**	48.44**	36.8529**	68.2789**
Parent	6	2.1868ns	2.0236ns	2.89*	3.6152**	22.147**	8.8593**	10.1858**	9.1809**
Lines (p)	3	3.6463**	2.5361ns	3.22**	0.8686ns	26.6919**	13.3943**	11.2561**	15.8296**
Tester (p)	2	0.2879ns	1.732ns	1.087*	7.8003**	25.5156**	4.3988*	7.3441*	2.1663ns
L (p) × T (p)	1	1.6063ns	1.0692ns	2.453*	3.4847**	1.7755ns	4.1752*	12.6585**	3.2639ns
Cross vs Parent	1	8.3869**	72.1972*	164.5809*	17.1466**	241.7269*	207.0149*	178.2553**	453.4563*
Error	36								
Total	56								

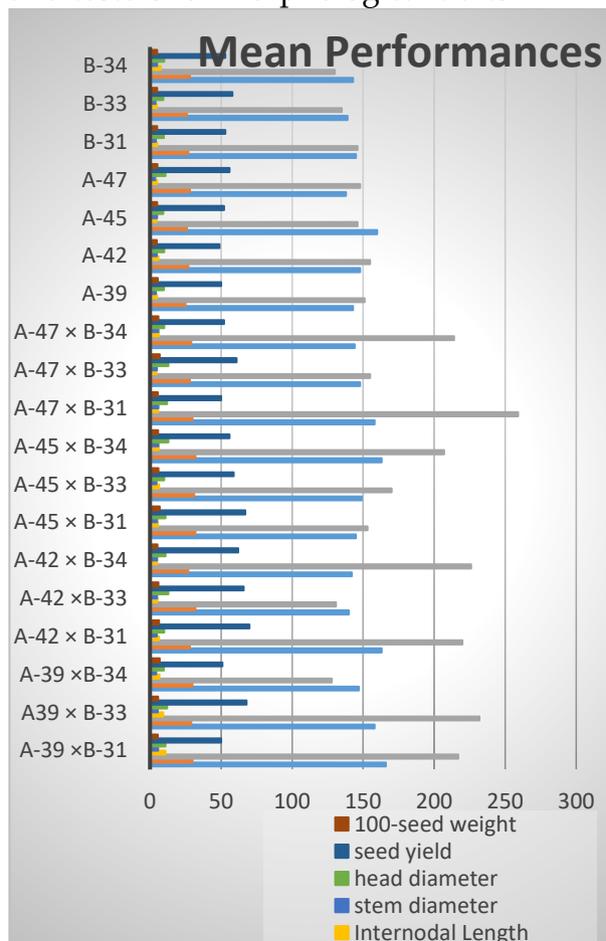
Mean Performances Of Lines, Testers and Crosses:

For plant height, among lines, A-45 (160 cm) showed maximum mean performance, followed by A-42 (137.73 cm), and the lowest value in lines was observed in A-47 (134.67 cm). Among testers, B-31 (144.67 cm) had the highest value, while the lowest value was exhibited in B-33 (139.33 cm). For crosses, the highest value was observed in A-39 × B-31 (166.33 cm), while the lowest value was observed in A-42 × B-33 (140.00 cm). For the number of leaves maximum value was exhibited by A-39 (27.667), followed by A-47 (27.333) and A-45 (26.00), while among testers maximum mean value was observed in B-31 (28.00). These significant variations indicated that genetic diversity exists between selected germplasm that will be a helping hand to develop hybrids in future.

For crosses, the highest value was observed in A-45×B-31 (259.3 cm²), while

the lowest value was observed in A-42×B-34 (231.9 cm²) for leaf area. For internodal length, the highest value was observed in A-39×B-31 (11.133 cm), while the lowest value was observed in A-47×B-33 (4.533 cm). Among lines for seed yield per plant, maximum mean performance was exhibited by A-47 (56.333) followed by A-45 (52.333), while among testers, the maximum mean value was observed by B-33 (57.667) followed by B-31 (53.333). These outcomes predicted that following parents have a higher proportion of additive effects. Among crosses, for 100-achene weight, the cross A-45×B-31 (6.9333g) exhibited high mean performance that indicated this to be a potential source for hybrid development, while A-42×B-34 (6.1g) showed minimum values among all crosses.

Fig. 1. Mean comparisons of crosses, lines and testers for morphological traits



Genotypic Compatibility Assessment General Combining Ability effects

The study of general combining ability (GCA) effects revealed varying magnitudes and directions among lines and testers across traits. Line A-39 exhibited a highly negative GCA for seed yield (-3.03**) and head diameter (-0.41**) which indicates that dominance-controlled variation is less significant. But had a significant positive value for the internodal length (2.52) while line A-42 showed highly negative GCA for internodal length (-1.11**) indicating lower distance more lodging-resistant hybrids. Line A-47 demonstrated a significant positive GCA for stem diameter (0.4**) and head diameter (0.53**) but a highly significant negative value for the 100-achene weight (-0.96**). Contrarily, line A-45 displayed a significant positive GCA for 100-achene weight (0.21**) which highlights it to be a good source to enhance yield. All lines exhibited notable GCA values for seed yield, while non-significant GCA values were observed for the number of leaves as shown in Table 2. The highest value of SE was observed for leaf area which indicated this trait is highly influenced by environmental variations. These outcomes for SE indicated heterogeneity in genetic contributions among lines.

Table 2 general combining ability effect for lines, PH (plant height), NL (No. of leaves), LA (leaf area), IL internodal length, SD (stem diameter), HD (head diameter), SY (seed yield), SW (seed weight). S.E (standard error). *Indicates significant and ** shows highly significant. ns show non-significant

Lines	PH	NL	LA	IL	SD	HD	SY	SW
A-39	5.31 ns	- 0.11 ns	- 4.05 ns	2.52 **	- 0.07 ns	- 0.41 **	- 3.03 **	0.7n s
A-42	- 3.58 ns	- 0.89 ns	1.14 ns	- 0.93 *	- 0.34 **	- 0.16 ns	6.64 **	- 0.21 **

A-45	0.47 ns	1.89 ns	- 14.9 5	- 0.48 ns	0.01 ns	0.04 ns	1.19 *	0.21 **
A-47	- 2.19 ns	- 0.89 ns	17.8 6	- 1.11 **	0.4* *	0.53 **	- 4.81 **	0.96 ns
S. E	3.52 7	0.47 2	22.9 7	0.32 8	0.06 7	0.12 2	0.53 3	0.05 4

Tester B-31 exhibited a significant negative GCA for head diameter (-0.27*) and while positive GCA for stem diameter (0.09**). Meanwhile, tester B-34 showed significant negative GCA values for head diameter (-0.40**) and seed weight (-0.13**). Tester B-33 demonstrated a significant GCA for stem diameter (0.25**) and a positive, though non-significant, GCA for plant height and number of leaves. All testers displayed negative GCA values for the number of leaves. These findings align with previous reports by [Dhillon et al. \(2016\)](#) and [Hilli et al. \(2020\)](#) as indicated in Table 3.

Table 3. Additive effect values for tester, PH (plant height), NL (No. of leaves), LA (leaf area), IL (internodal length), SD (stem diameter), HD (head diameter), SY (seed yield), SW (seed weight). *Indicates significant and ** shows highly significant. ns show non-significant.

Tester	PH	NL	LA	IL	SD	HD	SY	SW
B-31	6.21 ns	0.28 ns	20.9 2ns	0.62 ns	0.19 **	- 0.2 7*	- 0.19 ns	0.04 ns
B-33	6.21 n	0.03 ns	- 19.5 5	- 0.09 ns	0.25 **	0.6 7*	4.14 **	0.09 ns
B-34	2.92 **	0.31 ns	-1.37	0.53 ns	0.06 ns	- 0.4 **	- 3.94 **	- 0.13 *

Specific Combining Ability Effects:

The study revealed significant specific combining ability (SCA) effects for various traits as shown in Table 4. Cross A-47×B-34 showed a highly significant negative SCA value for head diameter (-1.16**), indicating that the larger the head size more yield losses, while A-45×B-34 displayed a highly positive SCA for plant

height and the highest positive SCA for head diameter pointing it to be good source for hybrid development. Cross A-39 × B-33 exhibited the highest positive SCA for seed yield per plant which is a major concern for any breeding programme. This cross combination highlights that genetic diversity exists between these two parents and traits are controlled by dominance gene action. No hybrids showed notable SCA values for leaf area. Cross A-39 × B-34 demonstrated a highly significant positive SCA for 100-achene weight, whereas A-45 × B-33 displayed a highly significant negative SCA for head diameter.

Table 4. Specific Combining ability effects for all studied traits. PH (plant height), NL (No. of leaves), LA (leaf area), IL (internodal length), SD (stem diameter), HD (head diameter), SY (seed yield), SW (seed weight). *Indicates significant and ** shows highly significant. ns show non-significant.

Crosses	PH	NL	LA	IL	SD	HD	SY	SW
A-39×B-31	2.9 ns	- 0.0 6ns	8.47 ns	1.5* ns	0.3 9**	0.3 5ns	- 5.8 1**	- 0.5 9**
A-39×B-33	4.4 ns	- 0.8 1ns	64.0 6ns	0.4 2ns	0.5 6**	0.3 1ns	7.1 9**	- 0.3 8**
A-39×B-34	- 7.3 1ns	0.8 6ns	- 72.5 ns	- 1.9 2**	- 0.9 5**	- 0.6 5**	- 1.3 9ns	0.9 8**
A-42×B-31	8.4 6ns	- 0.9 4ns	6.91 ns	0.1 8ns	- 0.3 1*	- 0.7 8**	3.8 6**	0.4 5**
A-42×B-33	- 5.0 4ns	- 0.9 4**	- 42.2 ns	- 0.1 4ns	0.3 9**	0.9 2**	- 3.8 1**	0.0 3**
A-42×B-34	- 3.4 2ns	- 1.6 9ns	35.2 4ns	- 0.0 4ns	- 0.0 8ns	- 0.1 4ns	- 0.0 6ns	- 0.4 8**
A-45×B-31	- 13. 26*	0.2 8ns	- 44.5 ns	- 1.2 3ns	- 0.2 9*	0.0 2ns	6.3 1**	0.7* *
A-45×B-33	- 0.6 ns	- 0.8 1ns	13.1 3ns	0.4 8ns	- 0.4 2**	- 1.9 8**	- 6.0 3**	- 0.2 9**
A-45×B-34	13. 86*	0.5 3ns	31.3 5ns	0.7 5ns	0.7 1**	1.9 6**	- 0.2 8ns	- 0.4* *
A-47×B-31	1.9 ns	0.7 2ns	29.0 9ns	- 0.4 4ns	0.2 2ns	0.4 ns	- 4.3 6**	- 0.5 68*

A-47×B-33	1.9 ns	- 1.0 3ns	- 35.0 ns	- 0.7 6ns	- 0.5 4**	0.7 6**	2.6 4**	0.6 5**
A-47×B-34	- 3.1 4ns	0.3 1ns	5.94 ns	1.2 1ns	0.3 2*	- 1.1 6**	1.7 2ns	- 0.0 9**

Heterosis Manifestation

Heterosis, also known as hybrid vigour, is the better performance of crosses over their parents. The analysis of better parent heterosis across different crosses revealed significant genetic potential for key traits (table 4). Crosses such as A-39×B-31, A-39×B-33, and A-42×B-31 exhibited highly significant positive heterosis for plant height, with respective values of 14.98**, 10.98**, and 10.14*. Similarly, the number of leaves demonstrated robust positive heterosis, with A-45×B-33 achieving the highest value (19.23**). For seed yield/plant, A-42×B-31 (30.36**) and A-45×B-31 (25**) outperformed other crosses, indicating superior performance in yield-related traits. However, heterosis for leaf area showed limited significant variation, with a few crosses such as A-45 × B-34 (5.78**) and A-42 × B-31 (3.04**) showing modest improvements. These overall findings illustrated that the germplasm used for experimentation has genetic diversity and cross combinations in one way or another contribution towards hybrid development in future.

Table 5. Better parent heterosis for crosses compared to their parents. PH (plant height), NL (No. of leaves), LA (leaf area), IL (internodal length), SD (stem diameter), HD (head diameter), SY (seed yield), SW (seed weight). *Indicates significant and ** shows highly significant. ns show non-significant.

Crosses	PH	NL	LA	IL	SD	HD	SY	SW
A-39×B-31	14.9 8**	9.76 **	- 1.9 5*	18.3 **	32.9 8**	11.6 **	- 5.62 *	- 1.21 ns
A-39×B-33	10.9 8**	11.5 4**	0.1 78*	19.1 **	30.4 3**	22.0 7**	17.3 4**	3.64 ns

A-39×B-34	3.04 ns	8.33 *	3.5 8ns	- 13.9 7ns	- 14.0 9**	- 2.66 ns	- 3.16 ns	24.2 4**
A-42×B-31	10.1 4*	3.66 ns	3.0 4**	9.14 ns	- 1.38 ns	- 1.63 ns	30.3 6**	22.0 8**
A-42×B-33	- 5.41 ns	15.8 5**	2.7 8*	- 8.57 ns	4.14 ns	24.1 8**	15.0 3**	21.2 3**
A-42×B-34	- 4.05 ns	- 3.57 ns	1.5 4*	- 34.5 **	- 2.01 ns	3.27 ns	17.7 2**	3.33 ns
A-45×B-31	- 9.17 *	18.2 9**	4.7 3ns	5.88 ns	2.67 ns	12.9 7**	25**	35.0 6**
A-45×B-33	- 7.19 ns	19.2 3**	3.2 ns	32.4 1ns	- 8.67 *	8.33 *	1.73 ns	20.8 1**
A-45×B-34	2.08 ns	14.2 9**	5.7 8**	- 18.3 4ns	20**	27.9 1**	6.96 *	13.3 3**
A-47×B-31	9.1 ns	8.43 *	2.1 3ns	9.15 *	29.5 9**	9.17 **	- 11.2 4**	2.5 ns
A-47×B-33	5.98 ns	1.2 ns	3.8 2**	- 7.48 ns	25**	21.1 **	6.36 *	26.2 5**
A-47×B-34	0.7 ns	3.57 ns	3.4 5*	- 20.5 2**	20.8 1**	- 6.42 *	- 7.1* *	8.12 **

Discussion

The results highlighted in Table 1, that two-way ANOVA interaction significantly influenced all traits, especially head diameter ($F = 68.2789$) and seed yield (36.8529), highlighting the importance of specific combinations. Parental interactions were statistically significant for most traits, particularly stem diameter (26.6919) and seed yield (15.8296), indicating variability among parental lines. Overall, significant differences were noted across all traits, most notably for seed yield (453.4563), suggesting effective enhancement through crossing. In support of these results, [Telangre et al., \(2019\)](#), [Lakshman et al. \(2019\)](#) and [Asif et al., \(2013\)](#) also found similar findings. These results emphasize the potential for selective breeding and hybridization strategies to improve key agricultural traits.

The analysis of agronomic traits, including plant height, leaf area, internodal length, stem diameter, head diameter, 100-seed weight, and seed yield per plant, revealed significant genetic diversity among parents and F1 hybrids ($P < 0.05$ – 0.01). While crosses were significant for most traits, 100-seed weight and the number of leaves were exceptions. Both lines and testers demonstrated substantial genetic variation, with their interactions yielding highly significant ($P < 0.01$) results. These findings align with studies by Rizwan *et al.*, (2020), and others, supporting the potential for genetic improvement in breeding programs. For 100-seed weight, crosses A-45 \times B-31, A-47 \times B-33, A-39 \times B-34, and A-42 \times B-33 showed significant positive SCA values, while A-39 \times B-31, A-39 \times B-33, A-47 \times B-34, and A-47 \times B-31 exhibited significant negative SCA values (Table 4). These findings were consistent with observations by Imran *et al.* (2015).

Negative heterosis was observed in specific traits such as internodal length and head diameter for certain crosses, highlighting variability in trait-specific responses and specifying that lower the internodal length and optimum head size can be more beneficial for high yield as enlisted in Table 5. Similar findings were also observed by (Kanwal *et al.*, 2015). Crosses like A-47 \times B-34 exhibited significant negative heterosis for internodal length (-20.52^{**}) and head diameter (-6.42^*), whereas A-39 \times B-34 showed negative heterosis for plant height (-13.97_{ns}) and seed yield (-3.16_{ns}). Notably, positive heterosis for seed weight was observed in A-45 \times B-31 (35.06^{**}) and A-47 \times B-33 (26.25^{**}), reflecting the genetic potential for enhancing this trait Bhoite *et al.*, (2018), Nehru *et al.*, (2021) and Hussain *et al.*, (2017) also recorded similar findings for these traits. Overall, these results underscore the

importance of hybrid selection to exploit heterosis effectively for targeted breeding programs.

Conclusion:

This study highlights the genetic potential of combining ability and heterosis for yield improvement. Among the genotypes, A-42, A-39, and A-45 demonstrated strong general combining ability (GCA) across key traits indicating prominent additive gene action, for seed yield, 100-achene weight, and morphological parameters. On the tester side, B-34 exhibited superior GCA for yield-related traits, making it a valuable contributor to breeding efforts. Notable specific combining ability (SCA) was observed in crosses like A-45 \times B-34 for stem diameter, plant height, and 100-achene weight, and A-45 \times B-31 for seed yield per plant. These parents and cross combinations are remarkable for future hybrid development. The significant heterosis effects, particularly better-parent heterosis, underscore the genetic potential of these combinations for yield enhancement. Lines A-42, A-39, and A-45 emerge as promising candidates for future breeding programs, offering robust potential for yield improvement and genetic advancement.

Conflicts Of Interest

The authors declare no conflicts of interest.

Authors Contribution

Tehreem Mariam and Saima Bibi conceived the idea, designed the study, conducted the field trial, collected and analyzed the data and drafted the manuscript. Nazar Hussain Khan, Sidra Naheed, Zulaikha Aman, and Zainab Irfan helped in data collection. Sehar Nawaz and Muhammad Ahsan Iqbal helped in providing a critical evaluation of the manuscript and constructive feedback on this project.

References

- Arshad M, Khan MA, Jadoon SA, Mohmand AS. Factors analysis in sunflower (*Helianthus annuus* L.) to investigate desirable hybrids. *Pakistan Journal of Botany*, 2010; 42:245-267.
- Adesina S. Effect of processing on the proximate composition of sunflower (*Helianthus annuus*) seeds. *Agro-Science*, 2018; 17(3), 27-33.
- Rani R, Sheoran RK, Sharma B. Studies on variability, heritability and genetic advance for quantitative traits in sunflower (*Helianthus annuus* L.) genotypes. *Research Environment Life Sciences*, 2017; 10:491-493.
- Islam, Rubab Tarannum, Mir Monir Hossain, Kishor Majumder, Amjad Hossain Tipu. "In Vitro Phytochemical Investigation of *Helianthus annuus* Seeds." *Bangladesh Pharmaceutical Journal*, 2016; 19(1): 100-105.
- Cheng H, Zhang H, Li D, Duan H, Liang L. Impact of oil type on the location, partition and chemical stability of resveratrol in oil-in-water emulsions stabilized by whey protein isolate plus gum Arabic. *Food Hydrocolloids*, 2019; 109:106-119.
- Onemli F. Impact of climate changes and correlations on oil fatty acids in sunflower. *Pakistan Journal of Agricultural Science*, 2012; 49: 455-458.
- Thomaz GL, Zagonel J, Colasante LO, Nogueira RR. Yield of sunflower and oil seed content as a function of air temperature, rainfall and solar radiation. *Cienc Rural*, 2012; 42:1380-1385.
- Tahir M, Shehzadi M. Response of spring and autumn sunflower to chemical and bio-fertilizers for yield and quality traits. *Pakistan Journal of Agricultural Research*, 2017; 30(2): 122-128.
- Sujatha M, Reddy AV. Heterosis and combining ability for seed yield and other yield contributing characters in sunflower (*Helianthus annuus* L.). *Journal of Oilseeds Research*, 2009; 26:21-31.
- Iqbal A, Ismail A, Nisar A, Perveen R, Khan SA, Irfan Z. A Study on Heterosis and Combining Ability Through Line × Tester in Some Genotypes of Sunflower (*Helianthus Annuus* L.). (*IJAICR*) *International Journal of Agricultural Innovations and Cutting-Edge Research*, 2023; 1:32-40.
- Government of Pakistan (GOP). (2022-2023). Economic Survey of Pakistan 2022-2023. Ministry of Finance, Islamabad. Retrieved from <https://www.finance.gov.pk>.
- Golabadi M, Golkar P, Shahsavari MR. Genetic analysis of agro-morphological traits in promising hybrids of sunflower (*Helianthus annuus* L.). *Acta Agriculture Slovenica*, 2015; 105:249-260.
- Sanghera GS, Hussain W. Heterosis and combining ability estimates using line × tester analysis to develop rice hybrids for temperate condition. *Notulae Scientiae Biologicae*, 2012; 4:131-142.
- Rajane AR, Nichal SS, Fhatak SU, Vaidya ER, Sasane PR, Karvar SH. Combining Ability Assessment in Sunflower through Line × Tester Analysis. *Biological forum of international journal*, 2022; 14:255-260.
- Rauf S. Breeding strategies for sunflower (*Helianthus annuus* L.) genetic improvement. *Advances in Plant Breeding Strategies: Industrial and Food Crops*, 2019; 6:637-673.
- Dimitrijevic A, Horn R. Sunflower hybrid breeding from markers to genomic selection. *Frontiers in Plant Science*, 2018; 8:320-375.
- Steel, RGD, Torrie GH, Dickey DA. *Principles and Procedures of Statistics: A Biometrical Approach*, 3rd ed.; McGraw-Hill Book International Co.: Columbus, OH, USA, 1997.
- Rizwan M, Sadaqat HA, Iqbal MA, Awan FS. Genetic assessment and combining ability analysis of achene yield and oil quality traits in sunflower (*Helianthus annuus* L.) hybrids. *Pakistan Journal of Agricultural Sciences*, 2020; 57:101-108.
- Dhillon SK, Tyagi V. Combining ability studies for development of new sunflower hybrids based on diverse cytoplasmic sources. *Helia*, 2016; 39:71-79.
- Hilli HJ, Shobhalmmadi CS, Hilli J, Bankapur NS. Combining ability studies and the gene action involved in sunflower lines. *International Journal of Current Microbiology and Applied Sciences*, 2020; 9:2206-2215.
- Imran M, Qasrani SA, Nawaz MA, Shahbaz MK, Asif M, Ali Q. Combining ability analysis for yield-related traits in sunflower (*Helianthus annuus* L.). *American-Eurasian Journal of Agricultural and Environmental Sciences*, 2015; 15:424-436.
- Kanwal N, Sadaqat HA, Ali Q, Ali F, Bibi I, Niazi NK. Breeding progress for morphology and genetic pattern in sunflower (*Helianthus annuus* L.). *Journal of Life Sciences*, 2015; 12:49-56.
- Telangre SS, Kamble KR, Pole SP, Solanki MM. Studies on combining ability of new restorer lines in sunflower (*Helianthus annuus* L.). *Electron Journal of Plant Breeding*, 2019; 10:1339-1344.
- Lakshman SS, Chakrabarty NR, Kole PC. Study on the combining ability and gene action in sunflower through line × tester matting design. *Electron Journal of Plant Breeding*, 2019; 10:816-826.
- Asif M, Shadakshari YG, Naik SJ, Venkatesha S, Vijayakumar KT, Kumar KV. Heterosis and combining ability thorough the line × tester analysis in sunflower. *Field crops research*, 2013; 23:56-67.
- Bhoite KD, Dubey RB, Vyas M, Mundra SL, Amet KD. Evaluation of combining ability and heterosis for seed yield in breeding lines of sunflower (*Helianthus annuus* L.) using line × tester analysis. *Journal of pharmacognosy and phytochemistry*, 2018; 7:1457-1464.
- Nehru S, Timmanna BA, Ramesh S, Bhat D, Author C, Umar Farooq M, Shadakshari Y, Uma M. Identification of inbred lines with good combining ability and hybrids surpassing the best checks in sunflower (*Helianthus annuus* L.). *Journal of Pharmaceutical Innovation*, 2021; 10:259-265.
- Hussain T, Pooni HS, Philimon BMH. The nature of seed oil contents variation in a large set of sunflower test crosses. *Journal of Genetics and Breeding*, 2017; 54:207-211.