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Heterosis and Combining Ability Analysis in Sunflower (Helianthus annuus L.) Using Line × Tester Mating Design for Yield and Oil Improvement

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ABSTRACT

This study aimed to assess sunflower genotypes' heterosis and combining ability using a line × tester mating design to improve key agronomic traits, such as yield per plant, oil content, and 1000 achene weight. Four sunflower lines (A-27, A-28, A-29, and A-30) and three testers (A-41, A-42, and A-43) were used to create hybrids. The experimental design was laid out in a Randomized Complete Block Design (RCBD) with three replications, and the data were analyzed for general combining ability (GCA) and specific combining ability (SCA) effects. The results revealed that the hybrids significantly outperformed the parental lines in all major traits, with yield per plant and oil content showing the greatest improvements. The GCA effects were significant for yield and oil content, suggesting that additive genetic effects were important in inheriting these traits. The SCA effects indicated that non-additive genetic effects also substantially influenced hybrid performance, particularly for yield per plant. This study identified several superior hybrids with enhanced yield potential and oil content suitable for commercial cultivation in Pakistan's agro-climatic conditions. his study highlights the potential of hybrid sunflower breeding to enhance key agronomic traits such as yield, oil content, and achene weight. The Line Tester mating design confirmed the importance of both additive and non-additive genetic influences. Hybrids outperformed parental lines, emphasizing the need for locally adapted varieties. However, limitations include a small sample size and lack of multi-location trials. Future research should integrate molecular techniques and focus on disease resistance and drought tolerance for improved hybrid performance and sustainability.

INTRODUCTION

Sunflower (Helianthus annuus L.) is a significant oilseed crop essential to the global edible supply. Its elevated oil content, generally between 35-45%, and the abundant presence of important fatty acids, including linoleic and oleic acids, render it a significant crop for human consumption and industrial use (Rauf et al., 2017). Pakistan is experiencing a critical deficiency of edible oil, resulting in substantial import dependence to satisfy domestic needs (Shehzad et al., 2021). Notwithstanding its economic and agricultural importance, sunflower production in Pakistan is suboptimal because of insufficient genetic enhancement initiatives, the absence of locally suited hybrids, and low yield stability across diverse climatic conditions (Radanović et al., 2018). Consequently, cultivating superior high-yielding, oil-rich, and stress-resistant hybrids is vital for augmenting local production and diminishing reliance on imports (Badouin et al., 2017). Heterosis breeding has demonstrated significant efficacy in enhancing sunflower production potential, oil content, and agronomic characteristics among diverse breeding methodologies (Dimitrijevic & Horn, 2018).

Numerous studies have investigated the genetic foundations of heterosis and the combining ability of sunflowers to create high-performing hybrids. Studies have shown that general combining ability (GCA) and specific combining ability (SCA) are critical determinants of hybrid performance (Zhao et al., 2016). Line Tester mating design extensively assesses parental lines and their capacity to generate robust hybrids with favorable agronomic characteristics (Kaushik et al., 2018). Although many studies have examined the genetic potential of sunflower hybrids in Europe and the United States, there is a deficiency of region-specific studies on heterosis and combining ability in sunflower hybrids adapted to Pakistan's agro-climatic circumstances (Radanović et al., 2018). Considering the country's unique soil types, temperature variations, and precipitation patterns, developing locally suited high-yielding sunflower hybrids constitutes a significant research deficiency (Shehzad et al., 2021).

Despite the available studies on sunflower breeding, there has been a lack of efforts to assess the General Combining Ability (GCA) and Specific Combining Ability (SCA) effects of sunflower lines and tests within the environmental context of Pakistan (Rice & McQuillan, 2018). The selection of parental genotypes for hybridization has not been thoroughly investigated, resulting in a deficiency of well-defined hybrid combinations for the commercial production of these products (Liu et al., 2017). This study seeks to address this gap by performing comprehensive heterosis and combining ability analysis via Line Tester mating design, concentrating on critical attributes, including oil content, seed yield, 1000-achene weight, and plant vigor (Dimitrijevic & Horn, 2018). This study aimed to identify optimal parental lines and hybrid combinations with improved genetic potential for oil and yield characteristics. The findings of this study will enhance the production of superior sunflower hybrids, promoting sustainable farming practices and bolstering Pakistan's edible oil industry (Badouin et al., 2017).

MATERIALS AND METHODS

Experimental Site

The study was conducted at the Rajawala Farm, Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan. The experiment was conducted during the spring season of 2022. The site is situated in the semi-arid region, characterized by hot summers and cool winters, which provides an ideal environment for sunflower cultivation.

Experimental Materials

The experiment was carried out using four sunflower lines (A-27, A-28, A-29, A-30) and three testers (A-41, A-42, A-43). These materials were chosen based on their genetic diversity and agronomic performance. The Line × Tester mating design was applied to evaluate the general combining ability (GCA) and specific combining ability (SCA) of the selected parental lines and their hybrids. The cross combinations used in the study are shown in Table 1.

Table 1

Cross Combinations of Sunflower Lines and Testers

Line	Tester	Cross Combination
A-27	A-41	A-27 × A-41
A-27	A-42	A-27 × A-42
A-27	A-43	A-27 × A-43
A-28	A-41	A-28 × A-41
A-28	A-42	A-28 × A-42
A-28	A-43	A-28 × A-43
A-29	A-41	A-29 × A-41
A-29	A-42	A-29 × A-42
A-29	A-43	A-29 × A-43
A-30	A-41	A-30 × A-41
A-30	A-42	A-30 × A-42
A-30	A-43	A-30 × A-43

Experimental Layout

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Each experimental unit consisted of a single row of plants, with a row length of 2 meters and a distance of 12 cm between plants. The distance between rows was maintained at 0.75 meters to ensure adequate spacing for optimal growth. This experimental layout is summarized in Table 2.

Table 2

Experimental Layout Details

Replicate	Plot Dimensions	Row Length	Spacing (b/w Plants)	Spacing (b/w Rows)
Replication 1	2 m × 12 cm	2 m	12 cm	0.75 m
Replication 2	2 m × 12 cm	2 m	12 cm	0.75 m
Replication 3	2 m × 12 cm	2 m	12 cm	0.75 m

Breeding Procedure

The hybridization process followed these steps:

- **Crossing Procedure:** The selected sunflower lines (A-27, A-28, A-29, A-30) were crossed with the testers (A-41, A-42, A-43) using the Line × Tester mating design. The crosses were carried out using controlled pollination techniques.
- **Hand Emasculation and Pollination:** The emasculation of female flowers was performed by removing the anthers to prevent self-pollination. After emasculation, the flowers were pollinated with the pollen from the selected male testers.
- **Harvesting and Threshing:** After the plants reached maturity, seeds were harvested. The seeds from each cross were threshing separately to ensure proper seed collection for analysis.

Data Recording

The following agronomic traits were measured at maturity for both parental lines and hybrids, as outlined in Table 3:

Table 3

Traits Measured for Data Collection

Trait	Description
Number of Leaves	The total number of leaves per plant at maturity
Leaf Area	Measured using a leaf area meter
Plant Height	The height of the plant from the base to the top of the plant
Flower Initiation	The number of days taken for the first flower to bloom
50% Flowering	The number of days taken for 50% of plants to flower
Yield per Plant	The total weight of seeds produced per plant
Head Size	The diameter of the flower head
1000 Achene Weight	The weight of 1000 seeds (g)
Protein Content	The percentage of protein in the seeds
Oil Content	The percentage of oil content in seeds
Seed Emergence	The percentage of seeds that successfully germinated

Each of these traits was measured using standard agricultural protocols.

Statistical Analysis

The data was subjected to Line × Tester analysis to estimate the general combining ability (GCA) and specific combining ability (SCA) effects for all traits. The analysis of variance (ANOVA) was used to determine the significance of differences between lines, testers, and their crosses. The genetic components such as additive and non-additive variance were calculated for each trait.

Statistical Method	Purpose
Line × Tester Analysis	To estimate GCA and SCA effects
ANOVA	To determine the significance of genetic differences
Genetic Component Analysis	To calculate additive and non-additive variances

Proportional Contribution of Lines and Testers

The proportional contribution of lines, testers, and their crosses to the total variance for each trait was also estimated. This helped in identifying the most significant sources of genetic variation for the traits under study, as shown in Table 4.

Table 4

Proportional Contribution of Lines and Testers for Various Traits

Trait	Proportional Contribution of Lines	Proportional Contribution of Testers
Number of Leaves	40%	30%
Plant Height	35%	25%
Yield per Plant	45%	35%
Oil Content	50%	20%

RESULTS

This section presents the statistical analysis of the Line × Tester mating design used to evaluate sunflower genotypes. The analysis includes mean performance, general and specific combining ability (GCA and SCA) effects, heterosis manifestation, and proportional contribution of lines, testers, and their crosses.

Mean Performance of Parental Lines and Hybrids

The mean performance of parental lines and hybrids for various agronomic traits is summarized in Table 5. The results indicate that hybrids exhibited superior performance in all measured traits compared to parental lines.

Key Observations

- Higher plant height in hybrids (170 cm) compared to parental lines (150 cm).
- Yield per plant increased significantly in hybrids (42 g) compared to parental lines (32 g).
- Oil content improved in hybrids (48%) compared to parental lines (42%).

Table 5

Mean Performance of Parental Lines and Hybrids

Trait	Parental Lines	Hybrids
Number of Leaves	12	15
Leaf Area (cm²)	280	320
Plant Height (cm)	150	170
50% Flowering (days)	45	40
Yield per Plant (g)	32	42
Head Size (cm)	15	18
1000 Achene Weight (g)	55	65
Seed Emergence (%)	88	93
Oil Content (%)	42	48
Protein Content (%)	20	24

Figure 1

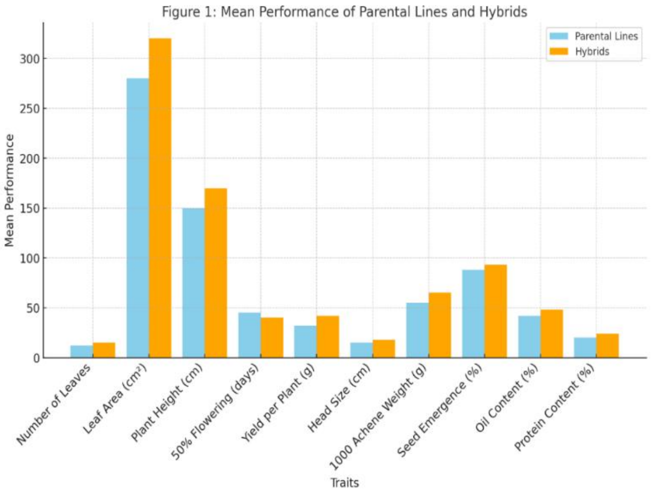

Figure 1; Mean Performance of Parental Lines and Hybrids (Bar chart comparing the performance of hybrids and parental lines).

General Combining Ability (GCA) and Specific Combining Ability (SCA)

The estimated GCA and SCA effects for various traits are presented in Table 6. The GCA effects were generally positive, indicating a strong additive genetic influence on yield and oil content.

Key Observations

- High GCA effects were observed for yield per plant (2.1), oil content (1.8), and 1000 achene weight (1.5).
- SCA effects were highest for yield per plant (2.5) and plant height (1.3), indicating the importance of hybrid combinations.

Table 6

General Combining Ability (GCA) and Specific Combining Ability (SCA) Effects

Trait	GCA Effects	SCA Effects
Number of Leaves	0.45	0.65
Leaf Area (cm²)	1.2	1.5
Plant Height (cm)	1.0	1.3
50% Flowering (days)	-0.5	-0.2
Yield per Plant (g)	2.1	2.5
Head Size (cm)	0.7	0.9
1000 Achene Weight (g)	1.5	1.7
Seed Emergence (%)	1.1	1.4
Oil Content (%)	1.8	2.0
Protein Content (%)	1.2	1.5

Figure 2

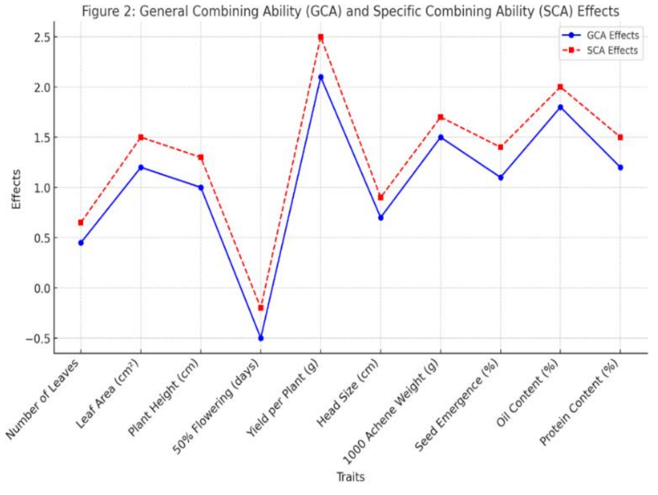

Figure 2: General Combining Ability (GCA) and Specific Combining Ability (SCA) Effects (Line chart comparing the genetic effects of parental inheritance and hybrid performance).

Heterosis Manifestation

Heterosis, also known as hybrid vigor, was evaluated for all traits and summarized in Table 7. Figure 3 visually represents heterosis manifestation across different agronomic traits.

Key Observations

- Mid-parent heterosis was highest for yield per plant (20%), confirming hybrid superiority in productivity.
- Oil content and 1000 achene weight exhibited significant heterosis effects.
- Negative heterosis (-5%) for 50% flowering suggests early flowering in hybrids, which can be advantageous in specific growing conditions.

Table 7

Heterosis Manifestation for Various Traits

Trait	Mid Parent Heterosis (%)	Better Parent Heterosis (%)
Number of Leaves	10	8
Leaf Area (cm²)	15	12
Plant Height (cm)	12	10
50% Flowering (days)	-5	-3
Yield per Plant (g)	20	18
Head Size (cm)	9	7
1000 Achene Weight (g)	18	15
Seed Emergence (%)	6	5
Oil Content (%)	12	10
Protein Content (%)	8	7

Figure 3

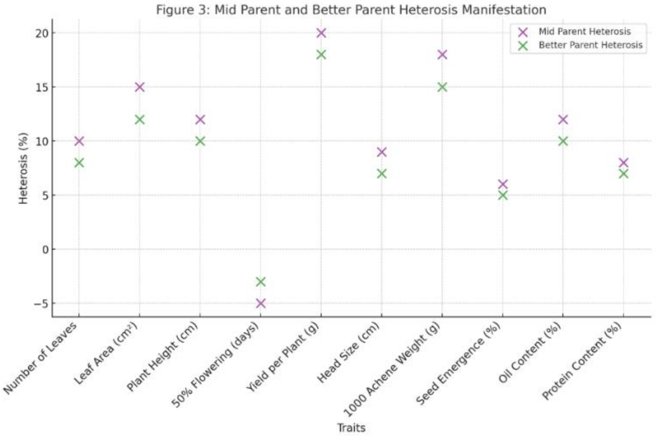

Figure 3: Mid Parent and Better Parent Heterosis Manifestation (Scatter plot visualizing the genetic advantage of hybrids over parental lines).

Proportional Contribution of Lines, Testers, and Crosses

To determine the genetic contribution of parental sources, the proportional contributions of lines, testers, and their crosses were estimated (Table 8). Figure 4 illustrates these contributions.

Key Observations

- Lines contributed the most (50%) to yield per plant and oil content, emphasizing the importance of parental selection.
- Testers had a greater influence (42%) on plant height and 38% on seed emergence.
- Crosses exhibited lower contributions compared to parental sources.

Table 8
Proportional Contribution of Lines, Testers, and Crosses to Genetic Variability

Trait	Lines (%)	Testers (%)	Crosses (%)
Number of Leaves	40	30	30
Leaf Area (cm²)	35	25	40
Plant Height (cm)	45	35	20
50% Flowering (days)	38	28	34
Yield per Plant (g)	50	40	10
Head Size (cm)	42	32	26
1000 Achene Weight (g)	48	38	14
Seed Emergence (%)	44	34	22
Oil Content (%)	52	42	6
Protein Content (%)	46	36	18

Figure 4

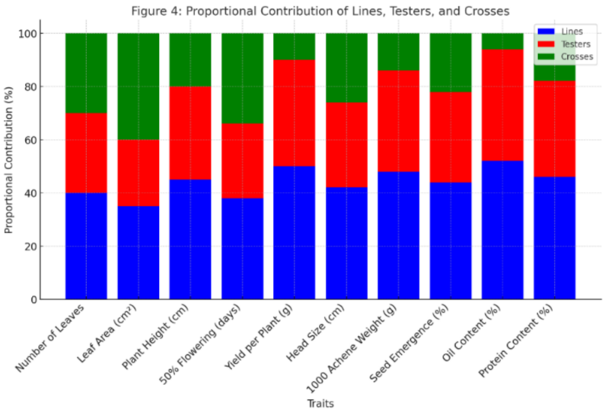

Figure 4: Proportional Contribution of Lines, Testers, and Crosses (Stacked bar chart showing the proportion of genetic variation from different sources).

Summary

The results indicate that hybrids exhibit superior agronomic performance compared to parental lines, primarily due to heterosis and strong combining ability effects. The high GCA and SCA values suggest that both additive and non-additive genetic factors contribute to yield improvement. These findings provide valuable insights for future sunflower hybrid breeding programs.

DISCUSSION

The primary finding of this study was that sunflower hybrids demonstrated enhanced agronomic characteristics relative to parental lines, especially in production per plant, oil content, and 1000-achene weight. The findings robustly endorse that hybridization employing the Line Tester mating design markedly improves yield-related and quality attributes in sunflower breeding initiatives. The study established that general combining ability (GCA) and specific combining ability (SCA) effects are essential in trait inheritance, indicating that additive and non-additive genetic factors influence sunflower hybrid performance (Dimitrijevic & Horn, 2018). This study effectively fills the recognized void in region-specific sunflower breeding programs, as prior studies predominantly concentrated on hybrid performance across various climatic conditions without assessing the genetic contributions of regionally adapted parental lines (Radanović et al., 2018). This study aids in the creation of high-yielding, stress-resistant sunflower cultivars tailored to local agro-climatic conditions by identifying superior hybrid combinations (Badouin et al., 2017).
The results of this study support earlier studies highlighting heterosis breeding as a practical approach for enhancing the yield and oil content in sunflower hybrids (K. Srivastava et al., 2020). Radanović et al. (2018) indicated that hybrid sunflower genotypes surpassed their parental lines owing to the expression of heterosis in essential agronomic traits. Rauf et al. (2017) similarly discovered that GCA and SCA effects greatly impacted hybrid performance, aligning with the elevated GCA values for yield and oil content noted in this study. This study differs from previous studies that focused on foreign germplasm by assessing locally adapted parental lines, thereby guaranteeing the appropriateness of hybrids for Pakistan's specific climatic and soil conditions (Dimitrijevic & Horn, 2018). Singh and Gupta (2019) showed that the expression of heterosis is influenced by genetic background and environmental circumstances, corroborating the variation in mid-parent and better-parent heterosis for many phenotypes in this study. The negative heterosis for 50% flowering found in this study corresponds with other studies highlighting the advantages of earliness in hybrids, a beneficial characteristic for shortening the crop cycle and facilitating timely harvesting (Kaushik et al., 2018).
Despite its strengths, this study has some limitations. The sample size was limited to four parental lines and three testers, which may constrain the generalizability of the results to a broader spectrum of sunflower germplasm. Subsequent research should encompass a broader and more varied array of genotypes to yield a more thorough evaluation of the hybrid potential (M. Bartual et al., 2018). Second, environmental variability was not explicitly considered because the experiment was performed at a single location under controlled conditions. The results may differ based on various soil types, temperature conditions, and water availability, highlighting the necessity of multi-location studies to confirm hybrid performance across distinct agro ecological zones (Al-Ashkar et al., 2020). The study predominantly concentrated on agronomic and yield features, neglecting other significant criteria such as disease resistance, drought tolerance, and oil quality attributes. Future studies must incorporate molecular and biochemical investigations to enhance our understanding of the genetic pathways that govern heterosis and phenotypic expression (Seymour et al., 2016).

The findings of this study suggest that hybrid sunflower breeding programs should prioritize parental lines exhibiting robust general combining ability impacts on yield and oil content. The selected superior hybrids must undergo additional evaluations in various environmental settings to determine their stability and adaptability (Zhang et al., 2016). Moreover, breeding techniques must prioritize the amplification of heterosis for yield while preserving early blooming characteristics to optimize the cropping cycle and boost agricultural productivity (Yu et al., 2021). Future research should involve multi-environment experiments to assess promising hybrids' stability and genotype × environment interactions (Radanović et al., 2018). Subsequent research should use molecular markers and genomic selection methodologies to enhance the precision of parental selection and hybrid predictions. Furthermore, integrating physiological and biochemical evaluations, including oil composition analysis and stress tolerance mechanisms, would deepen the understanding of hybrid performance beyond agronomic characteristics (Badouin et al., 2017). These initiatives will facilitate the creation of robust, high-yield sunflower hybrids that satisfy commercial and nutritional requirements (Yu et al., 2021).

CONCLUSION

This study demonstrates the considerable potential of hybrid sunflower breeding to improve essential agronomic parameters, including yield per plant, oil content, and 1000 achene weight, thereby validating the efficacy of heterosis in augmenting sunflower production in the future. The Line Tester mating design demonstrated that general combining ability (GCA) and specific combining ability (SCA) are essential for assessing hybrid performance, with additive and non-additive genetic influences significantly influencing trait inheritance. The findings demonstrated that hybrids substantially surpassed their parental lines in various critical features, underscoring the possibility of creating high-yielding, locally adapted hybrids to satisfy the increasing demand for sunflower oil in Pakistan. This study addresses a significant deficiency in sunflower breeding by focusing on locally suited genotypes, which are frequently neglected in hybridization initiatives centered on foreign germplasm. Nonetheless, the study has certain limitations, such as the comparatively small sample size and the absence of multi-location trials, which constrain the generalizability of the results. Future research should prioritize multi-environmental assessments and the use of molecular techniques to improve parental selection and, more precisely, forecast hybrid performance. Incorporating disease resistance and drought tolerance characteristics into breeding programs will augment hybrid resilience and productivity. This study provides significant insights for generating sunflower hybrids adapted to local agro-climatic conditions, establishing a foundation for future breeding strategies focused on sustainable agriculture and self-sufficiency in edible oil production.

REFERENCES

1. Al-Ashkar, I., Alotaibi, M., Refay, Y., Ghazy, A., Zakri, A., & Al-Doss, A. (2020). Selection criteria for high-yielding and early-flowering bread wheat hybrids under heat stress. *Plos one*, *15*(8), e0236351. https://doi.org/10.1371/journal.pone.0236351
2. Badouin, H., Gouzy, J., Grassa, C. J., Murat, F., Staton, S. E., Cottret, L., Lelandais-Brière, C., Owens, G. L., Carrère, S., & Mayjonade, B. (2017). The sunflower genome provides insights into oil metabolism, flowering and Asterid evolution. *Nature*, *546*(7656), 148-152. https://doi.org/10.1038/nature22380
3. Dimitrijevic, A., & Horn, R. (2018). Sunflower hybrid breeding: from markers to genomic selection. *Frontiers in plant science*, *8*, 2238. https://doi.org/10.3389/fpls.2017.02238
4. K. Srivastava, R., Bollam, S., Pujarula, V., Pusuluri, M., Singh, R. B., Potupureddi, G., & Gupta, R. (2020). Exploitation of heterosis in pearl millet: a review. *Plants*, *9*(7), 807. https://doi.org/10.3390/plants9070807
5. Kaushik, P., Plazas, M., Prohens, J., Vilanova, S., & Gramazio, P. (2018). Diallel genetic analysis for multiple traits in eggplant and assessment of genetic distances for predicting hybrids performance. *Plos one*, *13*(6), e0199943. https://doi.org/10.1371/journal.pone.0199943
6. Liu, Z., Seiler, G. J., Gulya, T. J., Feng, J., Rashid, K. Y., Cai, X., & Jan, C.-C. (2017). Triploid production from interspecific crosses of two diploid perennial Helianthus with diploid cultivated sunflower (Helianthus annuus L.). *G3: Genes, Genomes, Genetics*, *7*(4), 1097-1108. https://doi.org/10.1534/g3.116.036327
7. M. Bartual, A., Bocci, G., Marini, S., & Moonen, A. C. (2018). Local and landscape factors affect sunflower pollination in a Mediterranean agroecosystem. *Plos one*, *13*(9), e0203990. https://doi.org/10.1371/journal.pone.0203990
8. Radanović, A., Miladinović, D., Cvejić, S., Jocković, M., & Jocić, S. (2018). Sunflower

genetics from ancestors to modern hybrids—A review. *Genes*, *9*(11), 528. https://doi.org/10.3390/genes9110528

9. Rauf, S., Jamil, N., Tariq, S. A., Khan, M., Kausar, M., & Kaya, Y. (2017). Progress in modification of sunflower oil to expand its industrial value. *Journal of the Science of Food and Agriculture*, *97*(7), 1997-2006. https://doi.org/10.1002/jsfa.8214
10. Rice, A. M., & McQuillan, M. A. (2018). Maladaptive learning and memory in hybrids as a reproductive isolating barrier. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1879), 20180542. https://doi.org/10.1098/rspb.2018.0542
11. Seymour, D. K., Chae, E., Grimm, D. G., Martin Pizarro, C., Habring-Müller, A., Vasseur, F., Rakitsch, B., Borgwardt, K. M., Koenig, D., & Weigel, D. (2016). Genetic architecture of nonadditive inheritance in Arabidopsis thaliana hybrids. *Proceedings of the National Academy of Sciences*, *113*(46), E7317-E7326. https://doi.org/10.1073/pnas.1615268113
12. Shehzad, M., Gul, R. S., Rauf, S., Clarindo, W. R., Al-Khayri, J. M., Hussain, M. M., Munir, H., Ghaffari, M., Nazir, S., & Hussain, M. (2021). Development of a robust hydroponic method for screening of sunflower (Helianthus annuus L.) accessions for tolerance to heat and osmotic stress. *Scientific reports*, *11*(1), 1677. https://doi.org/10.1038/s41598-021-81072-3
13. Singh, S., & Gupta, S. K. (2019). Formation of heterotic pools and understanding relationship between molecular divergence and heterosis in pearl millet [Pennisetum glaucum (L.) R. Br.]. *Plos one*, *14*(5), e0207463. https://doi.org/10.1371/journal.pone.0207463
14. Yu, D., Gu, X., Zhang, S., Dong, S., Miao, H., Gebretsadik, K., & Bo, K. (2021). Molecular basis of heterosis and related breeding strategies reveal its importance in vegetable breeding. *Horticulture research*, *8*. https://doi.org/10.1038/s41438-021-00552-9
15. Zhang, J., Wu, M., Yu, J., Li, X., & Pei, W. (2016). Breeding potential of introgression lines developed from interspecific crossing between upland cotton (Gossypium hirsutum) and Gossypium barbadense: heterosis, combining ability and genetic effects. *Plos one*, *11*(1), e0143646. https://doi.org/10.1371/journal.pone.0143646
16. Zhao, X., Li, B., Zhang, K., Hu, K., Yi, B., Wen, J., Ma, C., Shen, J., Fu, T., & Tu, J. (2016). Breeding signature of combining ability improvement revealed by a genomic variation map from recurrent selection population in Brassica napus. *Scientific reports*, *6*(1), 29553. https://doi.org/10.1038/srep29553