

## COMBINING ABILITY ANALYSIS FOR MORPHOLOGICAL TRAITS IN BARLEY

KALYAR MNN<sup>1</sup>, ULLAH MA<sup>1</sup>, FAROOQ A<sup>2</sup>, KALYAR MHM<sup>1</sup>, SHAH KD<sup>2</sup>, ABBAS A<sup>3</sup>

<sup>1</sup>Department of Plant Breeding and Genetics, University of the Punjab Lahore, Pakistan

<sup>2</sup>Department of Plant Breeding and Genetics, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan

<sup>3</sup>National Nanfan Research Institute (Sanya), Chinese Academy of Agricultural Sciences, Sanya 572024, China

\*Correspondence author email address: [nuamankalyar@gmail.com](mailto:nuamankalyar@gmail.com)

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**Abstract** Barley (*Hordeum vulgare* L.) is an important cereal crop cultivated globally for its various uses in food, feed, and beverage industries. Developing high-yielding barley cultivars with desirable agronomic traits is crucial for meeting the growing demands of a rising global population. This study employed a line × tester mating design to evaluate the combining ability and genetic potential of diverse barley lines for key morphological characteristics. The research aimed to identify superior parental combinations and promising F<sub>1</sub> hybrids with high specific combining ability (SCA) for further breeding programs. A set of four diverse barley lines were crossed with three well-adapted testers. The resulting F<sub>1</sub> hybrids, along with their parental lines, were evaluated for various morphological traits, including plant height, days to headings, 1000 grains weight, spike length, grains per spike, and grain yield per plant. Data analysis revealed significant variation among genotypes for all evaluated traits. General combining ability (GCA) and SCA effects were estimated, and promising lines and hybrid combinations exhibiting high GCA and SCA for desirable traits were identified. The findings of this study provide valuable insights into the genetic basis of morphological traits in barley and highlight the potential of line × tester analysis in identifying superior parental combinations for breeding programs aimed at developing improved barley cultivars with enhanced agronomic performance.

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### Introduction

Barley (*Hordeum vulgare* L) is a multipurpose cereal crop that is grown all over the world for malt production, food, and feed. It is an important part of agriculture because of its nutritional value and capacity to adapt to a variety of settings (Ferioun et al., 2023). Barley plays an important role in human health because of its nutritional makeup, which includes high levels of dietary fibre, vitamins, minerals including manganese and selenium, and antioxidants (Rawat et al., 2023). These ingredients have been linked to several physiological advantages, such as better glycemic control, cholesterol level regulation, and improved digestive health. In Egypt, barley is the main grain crop grown on freshly recycled land as well as the Northwest Coast and the northern Sinai districts. Agriculture needs to produce barley varieties with higher yield potential. Cultivating early maturing barley varieties before cotton is planted is another way to increase grain production (Ovi and Shova, 2023). This will increase domestic barley production and lessen the gap

between barley production and consumption. It is important to produce superior barley cultivars with increased yield, improved quality features, and increased tolerance to biotic and abiotic stressors, given the growing world population and the pressing need for sustainable food production (Rebouh et al., 2023; Ullah et al., 2023). A complete understanding of genetic mechanisms, such as combining ability, is essential for the production of improved genotypes with increased yield potential before beginning a crop improvement program. A detailed evaluation of genetic data and a thorough understanding of combining ability, which informs parental selection, greatly raises the possibility of finding promising F<sub>1</sub> hybrids. To commercially exploit heterosis and isolate pure lines among the offspring of the heterotic hybrids, combining ability analysis helps identify parents with excess general combining ability (GCA) effects and cross combinations with high-specific combining effects (SCA). Plant breeders employ several tactics to accomplish this goal, and one

increasingly useful instrument in their toolbox is line tester analysis (Morales et al., 2022) for estimating general combining ability (GCA) (Zhang et al., 2022). This allows for the identification of the best parental combinations and the resulting F<sub>1</sub> hybrids, which have higher specific combining ability (SCA) (Han et al., 2020). Through the use of line×tester analysis, scientists can identify parental lines that show potential for hybridization and clarify the genetic basis of complex barley features.

The current study aimed to determine viable parental lines and testers as well as the corresponding F<sub>2</sub> populations by carefully assessing the effects of their general combining ability (GCA) and specific combining ability (SCA) (Mahmud et al., 2021). The purpose of this work was to quantify the genetic parameters linked to important production qualities in a subset of lines, making it easier to choose genotypes with the best GCA and SCA to incorporate into upcoming breeding initiatives (Melchinger et al., 2023). This study also aimed to clarify the underlying gene action and magnitude responsible for the phenotypic variance among the chosen lines and their derived F<sub>2</sub> populations that were observed.

**Materials and Methods**

The present study was conducted in the field at the Department of Plant Breeding and Genetics University of the Punjab Lahore during the growing season 2022-23. The Genetic material was composed of Baladi 16, Mariout, Sahara 93, Rihane 03 (females). And the testers Tadmor, Barke, WI2291 (males). The F<sub>1</sub> hybrid was developed by using lines×testers (4×3) design. The lines and testers were crossed to produce 12 F<sub>1</sub> hybrids. In the first growing season lines and testers were grown. At the anthesis stage, the crosses were made between lines and testers to obtain F<sub>1</sub> hybrids. In the next growing season, lines, testers, and hybrids were grown separately and comparison was made between them having different parameters like Plant height, Days to heading, spike length, Grains per spike, 1000 grains weight, and Grain yield/plant. The plant height was measured by a Stadiometer from the base of the stem to the top of

the plant. The spike length was measured by measuring tape. 1000 grains weight was also measured by taking 1000 grains and calculating by weight balance. The total grain yield of every plant was also calculated. After calculating all the parameters, the general combining ability (GCA) and specific combining ability were also calculated by formulating an Excel sheet.

**Results and Discussion**

An analysis of key parameters in barley breeding has revealed intriguing patterns and potential implications for crop improvement. Days to heading, a crucial trait determining the timing of flowering and maturity, exhibited significant variation across genotypes, parental lines, and their crosses (Table 1). This indicates a strong genetic influence on this trait, allowing breeders to target specific heading times based on environmental conditions or desired agronomic characteristics. Similarly, plant height and spike length both are important determinants of yield and lodging resistance, showed significant differences across all levels of analysis. This suggests substantial genetic variability in these traits, offering breeders opportunities to manipulate plant architecture for improved performance and resilience (Ober et al., 2021). The parameter 1000 grains weight, a key component of yield, displayed significance only at the genotype level. This implies that inherent differences exist among wheat varieties in terms of grain weight, but parental combinations and crosses did not significantly alter this trait. This finding could be attributed to the complex genetic control of grain weight, involving multiple genes and interactions, or environmental factors playing a more substantial role. Interestingly, grain yield per plant, the ultimate measure of productivity, showed significant variation across genotypes, parents, and crosses. This indicates a combined influence of genetic and environmental factors on grain yield. Breeding efforts can focus on selecting for favorable genotypes and parental combinations, as well as optimizing cultivation practices to maximize yield potential (Scott et al., 2020).

**Table 1: Mean square values of all parameters of 7 parents and 12 F<sub>2</sub> populations were evaluated by linex tester analysis (4×3) of Barley during the year 2023-24**

Source of Variation (SOV)	Days to Heading	Plant Height	Spike Length	Grain per Spike	1000 Grains Weight	Grain Yield/Plant
Replications	0.47	0.51	0.013	0.47	0.22	0.13
Genotypes	22.45*	40.32*	0.73*	14.63	8.68*	4.94*
Parents	31.71*	47.84*	0.80*	14.86	9.51	5.25*
Crosses	19.34*	39.71*	0.75*	15.52	8.80	5.12*
P*C	1.17	1.93	0.14	3.48	2.42	1.10
L (Lines)	47.58	103.55	2.07	40.25	25.79	15.73
T (Testers)	33.25	60.17	0.97	22.75	9.30	4.30
L*T	0.58	0.97	0.01	0.75	0.15	0.10
Error	5.29	8.19	0.20	5.29	2.58	0.72

**General Combining Ability (GCA)**

L2 and L4 have positive GCA effects, suggesting they would contribute to earlier heading in hybrids. L3 has

the most negative GCA, likely contributing to the later heading (Table 2). Similar to DH, L2 and L4 show positive GCA (taller plants), while L3 contributes to shorter plants. L4 has the highest positive GCA for longer spikes, while L3 contributes to shorter spikes. L4 stands out again with the highest positive GCA, suggesting it would increase the number of grains per spike. Conversely, L3 shows the most negative effect. L4 has the highest positive GCA, contributing to higher grain weight, while L3 tends to decrease it. L4 consistently shows the highest positive GCA across most traits, indicating it is the most promising line for improving grain yield. L3 consistently shows negative GCA effects, suggesting it might not be ideal for improving yield. The assessment of General Combining Ability (GCA) has provided valuable insights into the potential of different Barley lines for breeding improved varieties (Katiyar et al., 2021). GCA, a measure of a parent's average performance across various crosses, serves as a crucial indicator of its overall breeding value. In this study, line L2 demonstrated a notably high GCA value for days to heading, a trait of significant agronomic importance. This finding suggests that L2 possesses the genetic potential to contribute to the development of wheat varieties with desirable early heading characteristics. Early heading can be advantageous in regions with short growing seasons or where terminal heat or drought stress is prevalent, allowing crops to mature before these stresses become severe. Consequently, incorporating L2 into breeding programs could lead to the creation of varieties that exhibit improved adaptation to specific environmental conditions and yield stability (Liliane and Charles, 2020). Similarly, line L4 exhibited a high GCA value for plant height, another key trait influencing barley productivity. Optimal plant height is crucial for balancing yield potential with lodging resistance, and the ability of plants to withstand strong winds and rain without collapsing. The high GCA value of L4 suggests its potential to contribute to breeding efforts aimed at optimizing plant height, ultimately enhancing yield and reducing crop losses due to lodging. Conversely, lines L1 and L3 demonstrated negative GCA values across multiple traits. This indicates that these lines, on average, underperformed in various cross combinations, suggesting a lower overall breeding value. While these lines might possess specific desirable traits, their negative GCA

values raise concerns about their potential to consistently contribute favorable characteristics to offspring (El Hanafi et al., 2022). Therefore, it is prudent to exclude L1 and L3 from further breeding efforts, focusing resources on more promising lines like L2 and L4. The results of this GCA analysis have important implications for Barley breeding programs. By identifying lines with high GCA values, breeders can prioritize their use as parents in crosses, increasing the likelihood of developing superior varieties. This approach not only saves valuable time and resources but also enhances the efficiency and effectiveness of breeding efforts (Ullah et al., 2024). However, it's important to acknowledge that GCA is just one aspect of a complex breeding process. While high GCA values indicate a line's general potential, its performance in specific crosses can vary due to the influence of Specific Combining Ability (SCA). SCA reflects the unique interactions between specific parental combinations, leading to deviations from the expected average performance based on GCA. Therefore, a comprehensive breeding strategy should consider both GCA and SCA to maximize genetic gain and develop varieties with the desired combinations of traits (Yu et al., 2020). Furthermore, it's crucial to validate the performance of selected lines through extensive field trials across diverse environments (Swarup et al., 2021). While GCA provides valuable insights into a line's average performance, its actual performance can be influenced by various environmental factors, including soil type, rainfall patterns, and temperature fluctuations. Field trials allow breeders to assess the stability and adaptability of promising lines under real-world conditions, ensuring that the selected varieties perform optimally across different agricultural settings. In conclusion, this GCA analysis serves as a valuable guide for wheat breeders, highlighting the potential of lines L2 and L4 for developing improved varieties with desirable early heading and plant height characteristics. However, it's essential to consider this analysis within the broader context of a comprehensive breeding strategy that incorporates SCA assessment, field trials, and other relevant factors. By leveraging this multi-faceted approach, barley breeders can accelerate the development of high-yielding, climate-resilient, and economically viable barley varieties to meet the growing global demand for food (Lopes, 2023).

**Table 2: General combining ability effects among lines and testers for various traits were evaluated in lines 4×3 and tester mating design of Barley during the year 2023-24**

Parental Genotypes	Days to Heading (DH)	Plant Height (PH)	Spike Length (SL)	Grain per Spike (Tp)	1000 Grains Weight	Grain Yield/Plant (GYLD)
<b>Lines</b>						
<b>L1</b>	-0.58	-1.11	-0.19	-0.75	-0.67	-0.65
<b>L2</b>	1.08	0.78	0.06	0.58	0.64	0.29
<b>L3</b>	-2.92	-3.88	-0.50	-2.42	-1.96	-1.35
<b>L4</b>	2.42	4.20	0.63	2.58	1.98	1.71

Testers						
T1	0.42	0.97	0.14	0.67	0.33	0.26
T2	1.42	1.59	0.19	0.92	0.67	0.43
T3	-1.83	-2.56	-0.33	-1.58	-1.00	-0.68

**Specific Combining Ability (SCA)**

The present study delved into the potential of various barley crosses (lines × testers) by evaluating their Specific Combining Ability (SCA). SCA, a critical genetic parameter, quantifies the deviation of a specific hybrid's performance from the expected average of its parental lines (Fonseca, 2021). A high positive SCA value indicates that a hybrid surpasses the average of its parents for a particular trait, a hallmark of desirable hybrid vigor or heterosis. Several hybrid combinations emerged as exceptional candidates, showcasing high positive SCA values for key agronomic traits. The crosses L1×T1, L3×T3, and L4×T3 consistently demonstrated superior plant height, suggesting their potential to develop high-yielding wheat varieties with lodging resistance (Table 3). L1×T2 displayed a significantly higher 1000-grain weight, a crucial yield component. This finding highlights the potential to increase grain yield and improve the overall economic value of barley production. L1T3 and L3T2 exhibited increased grains per spike, directly contributing to higher yields. These crosses could revolutionize barley productivity in regions where grain yield is a major constraint (Begna, 2020).

L2×T3 and L4×T2 showed early heading, a trait valuable in environments with short growing seasons or where terminal heat or drought stress is prevalent. Early heading varieties can escape these stresses, ensuring stable yields even under challenging conditions. L2×T1 showed a remarkable increase in the number of grains per spike, a strong indicator of

high yield potential. This cross could be instrumental in developing barley varieties that meet the growing global demand for food. While several crosses like L2×T2, L3×T1, and L4×T1, exhibited negative SCA values across multiple traits. This indicates that these hybrids underperformed compared to their parental lines. Consequently, these crosses were deemed unsuitable for further breeding efforts, saving valuable resources and time (Bohra et al., 2020).

The results of this SCA analysis provide valuable insights for barley breeders. The study streamlines the breeding process by identifying promising hybrids and discarding underperforming ones, accelerating the development of high-yielding, climate-resilient, and economically viable barley varieties (Worku et al., 2020). However, it is essential to acknowledge that SCA is just one piece of the puzzle. While a high SCA value suggests a hybrid's potential, its actual performance in the field can be influenced by various environmental factors, including soil type, rainfall patterns, and temperature fluctuations. Therefore, thorough field trials are crucial to validate the performance of these promising hybrids under real-world conditions (Baumfeld Andre et al., 2020). Moreover, it is important to consider General Combining Ability (GCA) alongside SCA. GCA represents the average performance of a parent in various hybrid combinations and provides insights into its overall breeding value. A comprehensive breeding strategy should consider both SCA and GCA to maximize genetic gain and develop superior barley varieties (Medimagh and Mansouri).

**Table 3: SCA of hybrids all parameters**

F <sub>2</sub> Population	Days to Heading (DH)	Plant Height (PH)	Spike Length (SL)	Grain per Spike (Tp)	1000 Grains Weight	Grain Yield/Plant (GYLD)
L1×T1	0.58	0.45	0.02	0.00	0.02	0.01
L 1×T 2	-0.42	0.03	-0.03	-0.25	0.04	0.01
L 1×T 3	-0.17	-0.48	0.02	0.25	-0.06	-0.01
L 2×T 1	-0.08	0.49	0.09	0.67	0.31	0.28
L 2×T 2	-0.08	-0.42	-0.06	-0.58	-0.24	-0.13
L 2×T 3	0.17	-0.07	-0.04	-0.08	-0.07	-0.15
L 3×T 1	-0.08	-0.25	-0.04	-0.33	-0.09	-0.06
L 3×T 2	-0.08	-0.17	0.01	0.42	-0.04	0.00
L 3×T 3	0.17	0.42	0.03	-0.08	0.13	0.06
L 4×T 1	-0.42	-0.69	-0.07	-0.33	-0.23	-0.22
L 4×T 2	0.58	0.56	0.08	0.42	0.23	0.12
L 4×T 3	-0.17	0.14	-0.01	-0.08	0.00	0.11

**Conclusion**

In conclusion, the analysis of barley traits reveals a complex interplay between genetics and environment. The significant genetic influence on days to heading, plant height, and spike length presents opportunities

for targeted breeding to optimize these traits for specific environments and agronomic goals. While 1000-grain weight may be less responsive to direct genetic selection, focusing on parental combinations and environmental factors can still lead to

improvements. The significant variation in grain yield per plant, influenced by both genetics and environment, underscores the potential for substantial yield enhancement through a combination of strategic breeding and optimized cultivation practices. These findings provide a valuable roadmap for future barley breeding programs aimed at developing high-yielding, adaptable, and resilient varieties to address the challenges of global food security.

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#### **Declarations**

##### **Data Availability statement**

All data generated or analyzed during the study are included in the manuscript.

##### **Ethics approval and consent to participate**

Not applicable

##### **Consent for publication**

Not applicable

##### **Funding**

Not applicable

##### **Conflict of Interest**

Regarding conflicts of interest, the authors state that their research was carried out independently without any affiliations or financial ties that could raise concerns about biases.