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Deciphering Genetic Diversity and Gene Action in Barley Using Line × Tester Mating Design to Enhance Yield Attributes

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ABSTRACT Using a line × tester mating approach, the study sought to investigate the combining ability of barley (*Hordeum vulgare* L.) for important yield-related variables. For the majority of attributes, there were notable changes between the parents, hybrids, and their interactions, demonstrating the significant genetic diversity and efficacy of the selected breeding strategy. For parameters including plant height, spike length, grains per spike, tillers per plant, 1000-grain weight, and grain yield per plant, general combining ability (GCA) variants were greater than specialized combining ability (SCA) variances, suggesting the predominance of additive gene activity. Significant SCA effects, however, highlighted the importance of non-additive gene action for several characteristics and raised the possibility of heterosis exploitation. The crosses L5×T3 and L7×T4 exhibited significant positive SCA effects for grains per spike and grain yield per plant, whereas line L3 and tester T3 showed the strongest positive GCA effects for plant height and spike length, respectively. The findings underscore the significance of additive and non-additive gene activities in barley breeding and show that careful parent selection and hybridization can result in superior hybrids with higher yield potential.

Keywords*; Barley; Crosses; Yield; Gene; Traits; Genetics*

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INTRODUCTION Barley (*Hordeum vulgare* L.) is one of the oldest and most important cereal crops cultivated globally. It has played a pivotal role in human nutrition, agriculture, and industry for thousands of years. As a staple crop, barley is not only a significant source of food and feed but also an essential ingredient in brewing and malting industries. Its adaptability to diverse climatic conditions and soil types makes it a valuable crop for both temperate and semi-arid regions. The economic importance of barley is underscored by its use in various products, including malt for beer production, animal feed, and even health foods(H. G. M.-D. Ahmed et al., 2023; Khan et al., 2023; Sharma, Sharma, Joshi, & Sain, 2002; Singh, Singh, & Singh, 2002). Given the increasing global population and the need for sustainable agricultural practices, improving barley yield and quality is crucial for meeting future food security and economic demands (Amer, Eid, Elakhdar, & El-Shawy, 2012).

Despite its significance, barley production faces several challenges that impact yield and quality. Climate change, pest and disease pressures, and soil fertility issues are major factors influencing barley cultivation (H. G. M. D. Ahmed, Zeng, Yang, Fatima, & Faisal, 2024; Pawar & Singh, 2013; Prakash, Singh, & Saini, 2005). The increasing variability in weather patterns, including droughts and floods, has a direct impact on barley yield and quality. Additionally, diseases such as barley yellow dwarf virus and pests like aphids can severely affect crop performance. To address these challenges, breeders and researchers focus on enhancing barley's resilience and productivity through genetic improvement. Understanding the genetic basis of yield and related traits is essential for developing barley varieties that can withstand adverse conditions while maintaining high productivity (H. G. M.-D. Ahmed, M. Naeem, et al., 2024; Bornare et al., 2014; Potla, Bornare, Prasad, Prasad, & Madakemohekar, 2013).

The discovery and modification of genes linked to important agronomic properties is a necessary step in the genetic improvement of barley. Overall crop performance is mostly dependent on yield and yield-related parameters, including plant height, spike length, grains per spike, tillers per plant, 1000-grain weight, and grain yield per plant (Adriana, Madosa, Giancarla, & Ciulca, 2015; H. G. M.-D. Ahmed, Y. Zeng, et al., 2024; Patial, Pal, & Kumar, 2016). These traits are influenced by both additive and non-additive gene actions, which can be assessed through various genetic analyses. Additive gene action refers to the cumulative effect of individual genes on a trait, while non-additive gene action involves interactions between genes that result in different trait expressions in hybrids compared to their parents. Understanding the relative contributions of these gene actions helps in designing effective breeding strategies (Adriana et al., 2015; H. G. M.-D. Ahmed, Y. Zeng, et al., 2024; Patial et al., 2016).

The line × tester mating design is a well-established method for evaluating the combining ability of parental lines in breeding programs (Ali, Naeem, & Ahmed, 2024; Lal, 2018; Ram & Shekawat, 2017). This approach allows for the assessment of general combining ability (GCA) and specific combining ability (SCA) effects, which provide insights into the additive and non-additive genetic contributions to trait inheritance. GCA effects reflect the average performance of a parent across different crosses and are indicative of its overall genetic potential. In contrast, SCA effects highlight the performance of specific line × tester combinations, revealing the presence of interaction effects between parental lines (Ali, Naeem, Ghulam Muhu-Din Ahmed, et al., 2024; Sallam et al., 2018; Swati, Tiwari, Jaiswal, Kumar, & Goel, 2019). By analyzing GCA and SCA effects, breeders can identify the most promising lines and testers for developing high-yielding and resilient barley varieties. This method also aids in understanding the genetic mechanisms underlying trait expression and can guide the selection of parent lines with desirable attributes.

Previous studies on barley combining ability have provided valuable insights into the genetic basis of key traits (Khan, Yousaf, Ahmed, Fatima, & Alam, 2024; Kumari, Vishwakarma, & Singh, 2020). Research has demonstrated that both additive and non-additive gene actions contribute to the inheritance of traits such as plant height, spike length, and grain yield. For instance, studies have shown that plant height and spike length are often controlled by additive gene effects, while grains per spike and tillers per plant may involve significant non-additive interactions (Li et al., 2024; Saade et al., 2020). Research on combining ability in barley has also highlighted the importance of selecting appropriate parental lines for breeding programs. Lines with high GCA effects for yield-related traits are often chosen for developing hybrids with improved performance. Additionally, the identification of crosses with favorable SCA effects can lead to the development of superior hybrids with enhanced productivity (Mushtaq, Ahmed, & Zeng, 2024; Verma et al., 2021).

Using a line × tester mating strategy, the objective of this study is to assess the combining ability of different barley lines and testers for important yield-related variables. Estimating the effects of GCA and SCA on plant height, spike length, grains per spike, tillers per plant, 1000-grain weight, and grain production per plant are the study's particular goals. to choose the lines and testers with the best potential depending on how well they combine. To determine the nature of gene action (additive vs. non-additive) controlling these traits. To provide recommendations for breeding strategies that can enhance barley yield and related traits. By addressing these objectives, the study seeks to contribute to the advancement of barley breeding programs and the development of high-yielding varieties that can meet the demands of modern agriculture. By identifying lines and testers with favorable GCA and SCA effects, the study will aid in the selection of superior parental material for breeding programs (Bishnoi, Patial, Lal, & Verma, 2022; Zeng et al., 2024). The results will also help in understanding the relative contributions of additive and non-additive gene actions, guiding the development of effective breeding strategies.

MATERIAL AND METHODS

The study utilized 13 barley genotypes, consisting of 8 lines and 5 testers, all of which were two-rowed. These were chosen based on their distinct genetic backgrounds and their potential to exhibit variability in the traits of interest. The lines and testers were selected from existing germplasm collections, ensuring a broad representation of genetic diversity. 40 hybrids were produced by crossing these genotypes using a line x tester mating scheme. In the Rabi season of 2019–20, these hybrids were assessed in a Randomized Block Design with two replications at the University of Agriculture Faisalabad alongside the parent genotypes. The evaluation focused on combining ability and gene action for six traits: plant height (cm), spike length (cm), grains per spike, tillers per plant, 1000-grain weight (g), and grain yield per plant (g). ANOVA was conducted to determine the significance of differences among lines, testers, and their crosses for all measured traits. The analysis was performed, with a significance level set at $\alpha = 0.05$ and $\alpha = 0.01$. Combining ability analysis was carried out to estimate general combining ability (GCA) and specific combining ability (SCA) effects. GCA effects were estimated based on the performance of each line and tester across all crosses. SCA effects were calculated for each cross, reflecting the deviation from the expected performance based on GCA effects. The combining ability analysis followed the method outlined by Kempthorne (Kempthorne, 1957).

RESULTS AND DISCUSSION

The analysis of variance for combining ability (Table 1) revealed significant differences among parents, hybrids, and lines × testers for most of the traits studied. Specifically, the parents exhibited significant variation for all traits, with the exception of spike length, indicating the presence of substantial genetic diversity. The hybrids also showed significant variation for all traits, suggesting the effectiveness of the line × tester mating design in generating variability. The comparison between parents and hybrids demonstrated highly significant differences for all traits, confirming the superiority of hybrids over their parents in terms of yield-related attributes (Bornare et al., 2014; Lal, 2018; Swati et al., 2019).

To ascertain the type of gene activity governing these qualities, the variances of the general combining ability (GCA) and the specific combining ability (SCA) were also calculated. For plant height, spike length, grains per spike, tillers per plant, 1000-grain weight, and grain yield per plant, the GCA variations were greater than the SCA variances, suggesting that additive gene action is primarily responsible for determining these attributes. For every characteristic, the GCA/SCA ratio was smaller than 1, indicating that additive gene action predominates (EL-BAWAB, 2003; Pawar & Singh, 2013; Sharma et al., 2002; Verma et al., 2021). Plant height, spike length, grains per spike, tillers per plant, 1000-grain weight, and grain yield per plant show substantial variations between parents and hybrids. These observations imply that these qualities are governed by genetic variables that may be efficiently altered by selective breeding. The significant GCA effects for most traits indicate that additive genetic variance plays a crucial role, making these traits amenable to improvement through selection. The higher GCA variances relative to SCA variances and the GCA/SCA ratio below 1 for all traits confirm the predominance of additive gene action (Adriana et al., 2015; Ram & Shekawat, 2017; Sharma et al., 2002; Singh et al., 2002).

Certain variables, such spike length and grains per spike, show strong SCA effects, indicating that non-additive gene activity also plays a role in the transmission of these features. This emphasizes how barley breeding efforts may be able to take advantage of heterosis. Both additive and non-additive genetic effects are present when lines, testers, and their interactions differ significantly from one another; the latter is essential to hybrid performance (EL-BAWAB, 2003; Prakash et al., 2005; Sallam et al., 2018). These findings imply that the inheritance of important yield-related features in barley is influenced by both additive and non-additive gene activities, with additive effects predominating. Thus, choosing parents with strong GCA effects may result in the creation of superior barley hybrids with higher potential yields. The results further highlight the significance of hybrid breeding approaches that take use of additive and non-additive gene effects in order to optimize barley production and associated attributes (Adriana et al., 2015; Prakash et al., 2005; Swati et al., 2019; Verma et al., 2021).

Performance of Lines and Testers

The mean performance of the barley lines and testers for yield and yield-related traits is presented in Table 2. Among the lines, L5 exhibited the greatest plant height (101.2 cm), while L4 had the shortest height (76.5 cm). Spike length varied across the lines, with L1 having the longest spike (9.0 cm) and L8 the shortest (7.4 cm). Regarding grains per spike, L3 had the highest number of grains (74), while L5 had the lowest (22). For tillers per plant, L8 recorded the highest number (14.7), whereas L4 had the fewest (11.8). The 1000-grain weight ranged from 57.3 g in L8 to 41.1 g in L6. In terms of grain yield per plant, L7 performed the best with 11.2 g, and L2 the lowest with 7.9 g. Among the testers, T3 stood out with the greatest plant height (102.4 cm) and grains per spike (62). T2 had the shortest plant height (85.8 cm) and the fewest grains per spike (20). T5 showed the lowest 1000-grain weight (35.6 g) and grain yield per plant (7.6 g), while T4 had the highest grain yield per plant (10.5 g).

The analysis of mean performances among the barley lines and testers reveals significant variation across the studied traits. The observed differences in plant height, spike length, grains per spike, and other yield-related traits among the lines and testers highlight the genetic diversity within the parent material (Amer et al., 2012; Bornare et al., 2014; Ram & Shekawat, 2017). The notable performance of L7 for grain yield per plant suggests that it is a promising line for breeding programs aimed at improving yield. Similarly, the high number of grains per spike observed in L3 indicates its potential for contributing to increased grain production (Kumari et al., 2020; Swati et al., 2019).

The testers also displayed considerable variability, with T3 showing superior performance in both plant height and grains per spike. The high 1000-grain weight and grain yield per plant observed in T4 suggest that it could be an effective parent in hybrid combinations aimed at enhancing these traits. These findings underscore the importance of selecting parents with desirable traits for use in hybridization programs. The significant variation observed in key yield components such as grains per spike, tillers per plant, and grain yield per plant suggests that these traits could be effectively improved through targeted breeding strategies (Lal, 2018; Pawar & Singh, 2013; Sallam et al., 2018). The performance of the lines and testers indicates the potential for developing high-yielding barley hybrids by combining the strengths of different parent lines (Amer et al., 2012).

General Combining Ability (GCA) Effects

As shown in Table 3, the estimations of general combining ability (GCA) impacts for barley lines and testers show clear differences in yield and yield-related variables. L3 showed the strongest positive GCA effects for both spike length (0.62) and plant height (8.60) among all the lines, indicating that it might be a donor for both characteristics. In contrast, L6 displayed the most substantial negative GCA effects across several traits, including plant height (-9.05) and grain yield per plant (-1.25), indicating its potential to contribute to the improvement of these characteristics through breeding. While lines L4 and L8 exhibited positive GCA effects for tillers per plant (5.85 and 0.31, respectively), lines L2 and L5 showed substantial positive effects for grain yield per plant (0.97 g and 7.95 g, respectively). T3 had the most positive GCA impact (6.75) for plant height among the tests, whereas T5 displayed the greatest negative effect (-7.52) for 1000-grain weight. T4 had favorable GCA effects for spike length (0.43) and grain production per plant (0.79), indicating its potential for enhancing these characteristics. T2 had significant negative GCA effects for plant height (-1.50) and grain yield per plant (-0.37).

The analysis of GCA effects reveals that certain lines and testers have a clear potential to enhance specific traits in barley breeding programs. Line L3, with its positive GCA effects on plant height and spike length, can be leveraged to increase the size and structural attributes of barley plants. However, its negative effects on grain yield highlight a trade-off that needs to be managed in breeding strategies. On the other hand, Line L6, which consistently shows negative GCA effects across multiple traits, might be valuable in breeding programs aimed at reducing undesirable characteristics (Lal, 2018; Ram & Shekawat, 2017). The testers also demonstrated variability in their GCA effects. T3's positive impact on plant height suggests its suitability for breeding programs focused on taller barley plants. In contrast, T5's negative effect on 1000-grain weight could indicate its role in reducing grain size, which may be undesirable for some breeding objectives (Adriana et al., 2015; Patial et al., 2016). Notably, T4's positive effects on grain yield and spike length suggest its potential as a valuable contributor to enhancing these traits in barley populations. The distinct GCA effects observed across lines and testers underscore the importance of selecting appropriate parental material for achieving specific breeding goals. The variability in GCA effects can guide the selection of parent lines and testers to maximize desirable traits and achieve balanced improvements in barley yield and related characteristics (Bornare et al., 2014; Potla et al., 2013).

Specific Combining Ability (SCA) Effects

In this study, Table 4 presents the significant specific combining ability (SCA) effects and mean values for various barley crosses. The crosses L5×T3, L3×T4, and L7×T3 showed notably negative SCA effects for plant height, with values of -11.45, -10.72, and -8.05, respectively, and mean heights of 86, 74.8, and 69.2. These crosses demonstrate a potential for shorter plant stature, which could be advantageous in certain breeding scenarios. For spike length, crosses L7×T4 and L3×T3 exhibited positive SCA effects of 2.20 and 0.88, respectively, with mean spike lengths of 10.7 and 9.9, suggesting their potential to enhance this trait.

In terms of grains per spike, the cross L5×T3 had the highest positive SCA effect of 29.88 grains, with a mean value of 80.5 grains per spike, indicating its strong potential for increasing grain number. The cross L7×T4 also showed significant improvement with an SCA effect of 25.67 grains and a mean of 80.3 grains. Regarding tillers per plant, L7×T4 and L7×T3 were the most significant, with SCA effects of 6.40 and 6.15 tillers and mean values of 24.5 and 21, respectively.

For 1000-grain weight, the cross L3×T3 showed the highest positive SCA effect of 13.20, with a mean value of 75.2, indicating its potential to improve grain size. L5×T4 and L5×T3 also demonstrated notable positive SCA effects of 7.55 and 7.40, respectively. In grain yield per plant, L5×T3 achieved the highest SCA effect of 4.80, with a mean yield of 15.8 per plant, suggesting its strong potential for increasing yield. L7×T4 and L7×T3 also displayed significant positive effects with SCA values of 4.02 and 3.90, respectively.

The analysis of SCA effects highlights several promising barley crosses with significant impacts on key yield and yield-related traits. The crosses L5×T3, L3×T4, and L7×T3, despite their negative SCA effects on plant height, demonstrate considerable potential for reducing plant stature, which may be desirable for certain agronomic practices. The positive SCA effects observed in L7×T4 and L3×T3 for spike length suggest their utility in improving spike characteristics, potentially leading to better overall plant architecture and productivity.

For grains per spike, the crosses L5×T3 and L7×T4 stand out with high positive SCA effects, reflecting their potential to increase grain number significantly. This trait is crucial for improving yield and overall productivity in barley. Additionally, the significant SCA effects on tillers per plant observed in L7×T4 and L7×T3 indicate their potential to enhance tillering, which is beneficial for achieving higher plant density and better yield (Sallam et al., 2018; Swati et al., 2019). The SCA effects for 1000-grain weight reveal that the cross L3×T3 has a strong potential for increasing grain size, which could positively impact overall yield quality. The performance of L5×T4 and L5×T3 also highlights their ability to improve grain weight, which is crucial for achieving desirable grain quality. Finally, the positive SCA effects observed for grain yield per plant in L5×T3, L7×T4, and L7×T3 underscore their potential to significantly boost yield. This improvement in yield performance is a key goal in breeding programs aimed at enhancing barley productivity (Bishnoi et al., 2022; Verma et al., 2021). The SCA effects indicate that these specific crosses hold promise for enhancing various traits in barley. Selecting and utilizing these crosses in breeding programs can lead to substantial improvements in plant height, spike length, grain number, tillering, grain weight, and yield, ultimately contributing to more productive and efficient barley cultivation (Kumari et al., 2020; Saade et al., 2020).

Table 1: Analysis of variance for combining ability for yield and yield related traits of barley

Source of variation	**Df**	**Plant height (cm)**	**Spike length (cm)**	**Grains Per spike**	**Tillers per plant**	**1000-grain weight (g)**	**Grain yield per plant (g)**
Parents (P)	12	142.85*	1.29*	749.12**	2.81*	104.15**	2.73**
Hybrids (H)	39	202.84**	2.07*	411.78**	34.98*	237.14**	11.21*
P vs. H	1	1.79	2.27**	1401.25**	49.32**	3361.84**	130.14**
Lines	7	470.92**	1.64	870.45*	78.63**	584.22*	6.24
Testers	54	389.41	4.12*	320.39	49.52	290.71	28.32*
Lines x Testers	28	107.34**	1.92**	305.47**	21.88*	147.65*	9.74*
Error	52	8.37	0.27	5.62	3.22	18.45	0.42
GCA effects		25.04	0.09	23.07	3.17	23.35	0.61
SCA effects		48.62	0.81	151.89	9.15	62.39	4.58

GCA/SCA		0.497	0.087	0.148	0.351	0.358	0.119

*,** Significant at 5% and 1% level, respectively

Table 2: Mean performances of Lines and Testers for yield and yield related traits of barley

Parents	**Plant height (cm)**	**Spike length (cm)**	**Grains Per spike**	**Tillers per plant**	**1000-grain weight (g)**	**Grain yield per plant (g)**
L1	82.1	9	36	13.8	47.3	8.4
L2	85.2	7.9	27	13.2	44.7	7.9
L3	83.8	8.5	74	13.3	41.2	8.1
L4	76.5	7.8	64	11.8	42.8	9.9
L5	101.2	8.8	22	14.2	50.9	8.7
L6	93.6	8.7	28	12.8	41.1	8.7
L7	94.7	8.6	32	12.4	54.1	11.2
L8	77.3	7.4	26	14.7	57.3	8.5
T1	82.8	8	24	12.3	56.2	8.6
T2	85.8	6.5	20	11.2	54.1	7
T3	102.4	6.8	62	14.3	56	9.4
T4	83.1	7.3	56	13.2	45.2	10.5
T5	86.7	7.7	58	13.8	35.6	7.6

Table 3: Estimates of GCA effects for Lines and Testers for yield and yield related traits of barley

Parents	**Plant height (cm)**	**Spike length (cm)**	**Grains Per spike**	**Tillers per plant**	**1000-grain weight (g)**	**Grain yield per plant (g)**
L1	-3.10**	-0.18	-5.67*	0.72	5.38*	-0.18
L2	1.45*	-0.43*	-3.80**	5.85**	-0.23	0.97**
L3	8.60**	0.62**	12.72**	-1.58*	-2.74**	-0.3
L4	2.80*	0.07	16.98*	0.21	-4.30**	0.3
L5	7.33**	-0.53**	-7.12**	0.52	7.95**	-0.42*
L6	-9.05**	0.31*	-4.21**	-3.78**	-15.12*	-1.25*
L7	-9.72**	-0.23	-3.63**	-2.04**	0.95	-0.43**
L8	1.93**	0.31*	-5.50**	0.31	8.21*	1.24**
SE ±	0.66	0.13	0.53	0.48	1.19	0.19
T1	-6.35**	0.43**	-4.60*	1.34**	0.25	1.85**
T2	-1.50**	-0.28*	-4.35**	1.42**	2.55*	-0.37**
T3	6.75**	-0.56**	1.15*	0.72	2.54*	-0.89**
T4	2.50**	-0.28*	6.18**	-0.65	2.02*	0.79*
T5	-1.25**	0.64**	1.50**	-2.78*	-7.52**	-1.40**
SE ±	0.47	0.12	0.37	0.35	0.89	0.13

Table 4: SCA effects of significant crosses with their Mean values

Traits	**Best cross with significant SCA effects**	**SCA effects**	**Mean Values**
Plant height (cm)	L5×T3	-11.45**	86
	L3×T4	-10.72**	74.8
	L7×T3	-8.05**	69.2
	L5×T4	-7.95**	64
Spike length (cm)	L7×T4	2.20**	10.7
	L3×T3	0.88**	9.9
	L5×T3	0.81**	9.6
	L3×T4	0.65*	10.3
Grains per spike	L5×T3	29.88**	80.5

	L7×T4	25.67**	80.3
	L3×T4	12.65**	62.5
	L5×T4	3.30**	53.5
Tillers per plant	L7×T4	6.40**	24.5
	L7×T3	6.15**	21
	L3×T4	3.35**	20.5
	L5×T4	3.15**	24.8
1000-grain weight (g)	L3×T3	13.20**	75.2
	L5×T4	7.55**	78.8
	L5×T3	7.40**	76.3
	L7×T4	6.70**	77.9
Grain yield per plant	L5×T3	4.80**	15.8
	L7×T4	4.02**	16.9
	L7×T3	3.90**	15.6
	L3×T4	2.98**	15.3

CONCLUSION

This study provides valuable insights into the genetic mechanisms governing yield and related traits in barley, revealing that additive gene action predominates for most traits, while non-additive effects contribute significantly to specific traits such as grains per spike and spike length. The identification of lines and testers with desirable GCA effects, alongside crosses with significant SCA effects, offers promising avenues for improving barley yield through targeted breeding strategies. The results underscore the potential for developing high-yielding barley hybrids by combining the strengths of parent lines with complementary traits, ultimately contributing to the advancement of barley breeding programs focused on enhancing productivity and yield stability.

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