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Functional Flavonoid Profiling and Combining Ability in Barley under Normal Conditions

Tao Yang[1], Hafiz Ghulam Muhu Din Ahmed[1,2*], Amjad Saeed[3], Amenah S. Alotaibi[4,5], Hakkı Akdeniz[6], Zeki Erden[7], Çağdaş Can Toprak[7], Exian Li[1*]

[1]Biotechnology and Germplasm Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming 650205, China

[2]Department of Plant Breeding and Genetics, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, 63100, Pakistan

[3]Institute of Forest Sciences, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, 63100, Pakistan

[4]Biodiversity Genomics Unit, Faculty of Science, University of Tabuk, Tabuk 71491, Saudi Arabia

[5]Department of Biology, Faculty of Science, University of Tabuk, Tabuk 71491, Saudi Arabia

[6]Iğdır University, Department of Agronomy, Faculty of Agriculture, 7600-Iğdır, Turkey

[7]Department of Field Crops, Faculty of Agriculture, Siirt University, Siirt, Turkey

[*]Corresponding authors. E-mail: ahmedbreeder@gmail.com, xiaogaogao4850@126.com

ABSTRACT

This study investigated the genetic architecture of functional flavonoid components in barley using a Line × Tester mating design under normal growing conditions. We evaluated 23 genotypes, comprising five lines, three testers, and 15 F_1 hybrids, for key functional compounds: catechin, myricetin, quercetin, kaempferol, and total flavonoids, quantified via High-Performance Liquid Chromatography (HPLC). Analysis of variance (ANOVA) revealed highly significant genetic variability ($p \leq 0.01$) among genotypes for all traits, indicating strong potential for genetic improvement. Both additive and non-additive gene actions significantly influenced flavonoid expression, with line × tester interactions being highly significant for all traits, underscoring the importance of specific combining ability (SCA). Parental genotype L4 consistently showed superior mean performance and highly significant positive General Combining Ability (GCA) effects across all traits, including catechin (0.072), quercetin (0.101), kaempferol (0.090), and total flavonoids (0.213), identifying it as the best general combiner. Tester T1 also demonstrated significant positive GCA effects, notably for total flavonoids (0.095) and kaempferol (0.062). Among hybrids, L4×T1 exhibited the highest mean values for catechin (1.51 mg/g DW), quercetin (1.18 mg/g DW), kaempferol (0.96 mg/g DW), and total flavonoids (4.58 mg/g DW), accompanied by strong positive SCA effects (e.g., kaempferol: 0.100, total flavonoids: 0.105), indicating favorable dominance and epistatic interactions. Dominance variance (σ^2D) consistently exceeded additive variance (σ^2A) for all traits. Broad-sense heritability (H2) was moderately high, ranging from 0.71 (quercetin) to 0.73 (total flavonoids), suggesting effective selection is feasible. These findings emphasize that hybrid breeding strategies effectively exploit both additive and non-additive gene actions for developing nutritionally enhanced barley cultivars.

Keywords: flavonoids, barley, heterosis, gene-action, heritability, genetics.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most ancient cereal crops cultivated globally for food, feed, and industrial purposes. As a staple food in many arid and semi-arid regions, barley holds a unique position among cereals due to its remarkable adaptability to diverse agro-climatic conditions and its nutritional and therapeutic value. In recent decades, barley has gained renewed attention owing to its rich profile of bioactive compounds, particularly flavonoids, which contribute significantly to human health (Pesaraklu et al., 2016; Feizi et al., 2020). These secondary metabolites have been linked to a wide range of health-promoting properties, including antioxidant, anti-inflammatory, anti-diabetic, and cardio-protective effects. Among the flavonoids present in barley, catechin, myricetin, quercetin, and kaempferol are of considerable interest due to their potent biological activity and their cumulative contribution to total

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flavonoid content (Han et al., 2018; Tokhetova et al., 2022; Huang et al., 2024).

In the context of growing concerns over chronic non-communicable diseases and lifestyle disorders, the role of diet in disease prevention and health promotion has become increasingly important. This shift in public health priorities has led to a surge in demand for functional foods - those that offer health benefits beyond basic nutrition. Barley, with its wide array of phytochemicals including β-glucans, tocols, and flavonoids, is now recognized as a functional grain with immense potential for value-added products (Kähkönen et al., 1999; Kim et al., 2003). Among these, flavonoids stand out as promising targets for nutritional enhancement due to their ability to scavenge reactive oxygen species and modulate various biochemical pathways. Their incorporation into the diet through barley-based foods can offer a natural and sustainable approach to improving health outcomes (Amer et al., 2012; Rao et al., 2020; Ahmed et al., 2025b).

Despite the nutritional promise of flavonoids, their accumulation in barley grains is influenced by both genetic and environmental factors. Previous studies have highlighted substantial variation in flavonoid content among barley genotypes, indicating the potential for genetic improvement. However, much of the existing work has focused either on environmental modulation or on individual genotype screening. Few studies have systematically dissected the genetic architecture governing these traits, especially under normal agronomic conditions that reflect practical cultivation environments. Understanding the genetic basis of flavonoid accumulation is crucial for breeding programs aimed at developing biofortified barley cultivars with enhanced nutraceutical value (Amer et al., 2012; Ahmed et al., 2025c).

To effectively improve such complex biochemical traits, it is essential to employ robust breeding strategies that can identify superior parents and cross combinations. The Line × Tester mating design is a powerful tool for evaluating the combining ability of genotypes and dissecting the nature of gene action controlling trait expression (Fekadu et al., 2023). This design enables the estimation of general combining ability (GCA), which reflects additive gene effects, and specific combining ability (SCA), which reflects non-additive gene effects such as dominance and epistasis. GCA is particularly important in selecting stable and superior parents, while SCA helps in identifying heterotic cross combinations that exhibit hybrid vigor in the germplasm. Such information is critical for efficient selection and hybrid development, particularly for quality traits like flavonoid content which are often polygenic in nature (Yang et al., 2025a; Yang et al., 2025b).

In this regard, the application of a Line × Tester design to evaluate the combining ability of barley genotypes for flavonoid content remains underexplored. While this design has been widely used for agronomic and yield-related traits in various crops, its use in functional trait improvement, such as antioxidant components in cereals, is relatively recent (Han et al., 2018; Feizi et al., 2020; Rao et al., 2020). Moreover, most previous studies have either not focused on biochemical traits or have been conducted under stress conditions, such as drought or salinity, which can significantly alter secondary metabolite profiles. There is a pressing need to assess the inherent genetic potential of barley genotypes for flavonoid biosynthesis under normal conditions, which provides a baseline for varietal selection and food quality improvement (Zhang et al., 2015; Ge et al., 2021).

Furthermore, there exists considerable genotypic variation in the content and composition of flavonoids in barley, which offers an untapped opportunity for breeding. Modern breeding programs are increasingly integrating nutritional and functional traits into selection indices, making it imperative to characterize and utilize the genetic diversity present in local and exotic germplasm. Selection based on GCA and SCA for flavonoid traits can guide the development of both high-yielding and nutritionally superior cultivars. Additionally, knowledge of heterosis for these traits can facilitate hybrid development for specialized markets, including health-conscious consumers and functional food industries (Amer et al., 2012; Yang et al., 2025a).

The present study was therefore undertaken to evaluate the genetic variability and combining ability of five barley lines and three testers, along with their fifteen F_1 hybrids, for key functional flavonoid components including catechin, myricetin, quercetin, kaempferol, and total flavonoids. These genotypes were evaluated under normal irrigated conditions using a randomized complete block design with three replications. The objectives of this research were to (i) assess the extent of genetic variability among parents and crosses for flavonoid traits; (ii) estimate general and specific combining ability effects to identify superior parents and cross combinations; (iii) analyse the magnitude of heterosis for functional traits; and (iv) provide a scientific basis for selecting potential parental lines for the development of nutritionally enriched barley cultivars.

This investigation is expected to contribute significantly to the understanding of genetic control over flavonoid biosynthesis in barley and support the development of functionally enhanced cultivars suitable for human consumption and health promotion (Mansour and Moustafa, 2016; Noreen et al., 2025). By identifying high-performing parental lines and cross combinations with desirable flavonoid profiles, this study also aims to lay the groundwork for future molecular studies and biofortification programs targeting secondary metabolite enrichment. Ultimately, integrating such quality-focused breeding strategies can help meet the dual challenge of ensuring food security and improving dietary health in a changing global context.

MATERIAL AND METHODS

The present study was conducted to evaluate the functional flavonoid components in barley grains under normal growing conditions using a Line × Tester mating design. A total of five genetically diverse barley genotypes were selected as female parents (lines), and three contrasting genotypes were used as male parents (testers), based on their variability in functional traits. Controlled hybridizations were performed using the crossing method to generate 15 F_1 hybrids (5 lines × 3 testers). Emasculation was carried out in the late afternoon by removing the immature anthers with fine forceps. Hand pollination followed the next morning using fresh pollen collected from the designated male parents, which was dusted onto the stigmas of the emasculated florets. The pollinated spikes were tagged, labelled, and immediately covered with pollination bags to ensure protection and avoid contamination. Upon physiological maturity, seeds from each successful cross were harvested separately and stored for further evaluation.

Field Evaluation and Experimental Design

The 23 genotypes, including five lines, three testers, and 15 F_1 hybrids, were evaluated under normal irrigated field conditions using a Randomized Complete Block Design (RCBD) with three replications. Each genotype was sown in a single row plot of three meters in length. A row-to-row spacing of 30 cm and plant-to-plant spacing of 60 cm were maintained to provide optimum growth space. Seeds were sown at a uniform depth of 2 to 4 cm using hand dibbling. Recommended agronomic practices including timely irrigation, hand weeding, and plant protection measures were uniformly applied to all plots throughout the growing season to ensure normal plant growth and development.

Trait Measurement and Biochemical Analysis

At maturity, grains from each genotype were harvested, cleaned, and sun-dried before being ground into fine powder using a laboratory grinder. Quantification of flavonoid components was performed using standard methanol-based extraction procedures followed by High-Performance Liquid Chromatography (HPLC) analysis. The functional components measured included catechin, myricetin, quercetin, kaempferol, and total flavonoids (calculated as the sum of individual flavonoids). All biochemical analyses were carried out under controlled laboratory conditions to ensure accuracy and reproducibility of the results (Kähkönen et al., 1999; Kim et al., 2003).

Statistical Analysis

All collected data were subjected to statistical analysis using standard procedures. Analysis of variance (ANOVA) was performed to detect significant differences among parental genotypes and their F_1 hybrids. Line × Tester analysis was conducted following Kempthorne's method (Kempthorne, 1957) to estimate the General Combining Ability (GCA) effects of lines and testers and the Specific Combining Ability (SCA) effects of crosses. Types of gene action (Additive, dominance) and heritability calculated. All statistical analyses were carried out using R software and Microsoft Excel. Graphical visualizations, including bar charts and heatmaps, were generated to support data interpretation and illustrate trait performance across genotypes.

RESULTS AND DISCUSSION

The Line × Tester ANOVA for functional components in barley revealed highly significant differences ($p \leq 0.01$) among genotypes for all traits, indicating a substantial presence of genetic variability in the studied material (Table 1). The significant mean squares for genotypes for catechin, myricetin, quercetin, kaempferol, and total flavonoids suggest that selection and hybridization can be effectively utilized for improving these traits. These findings align with previous reports by (Yang et al., 2025a; Yang et al., 2025b), who also observed significant genotypic differences for flavonoid content in cereal crops, highlighting the genetic potential for enhancement through breeding.

The significant differences among parents and crosses for most traits further emphasize the importance of both additive and non-additive gene actions in governing these phytochemicals. Notably, crosses showed higher mean squares than parents, implying the involvement of dominance or epistatic interactions in hybrid performance. The comparison between parents and crosses was significant for most traits, particularly for myricetin, quercetin, and total flavonoids, which indicates the occurrence of heterosis - a phenomenon also reported by (Brueggeman, 2024; Güngör et al., 2024) in cereal crop. Partitioning the cross components into lines, testers, and line × tester interactions revealed that both parental groups contributed significantly to trait expression. Lines exhibited significant variation for most traits, such as catechin and myricetin, while testers contributed significantly to all traits, especially catechin ($p \leq 0.01$). This indicates the presence of additive gene effects contributed by both lines and testers, supporting the findings of (Yang et al., 2013; Xiao Ya et al., 2017), who emphasized the importance of parental GCA in secondary metabolite enhancement.

The line × tester interaction was highly significant for all traits, suggesting a major role of non-additive gene action in the expression of functional compounds like quercetin and kaempferol. This pattern points to the importance of specific combining ability (SCA) in identifying superior hybrid combinations with enhanced biochemical traits. These results are in accordance with the studies of (Huang et al., 2024), who reported significant SCA effects in cereal crops for quality-related traits. The non-significant replication effect across all traits suggests experimental consistency and the reliability of results. Moreover, the low error mean square values reinforce the precision of the experimental setup and support the biological significance of the observed variations. The results demonstrate that both additive and non-additive gene actions are involved in the inheritance of flavonoid components in barley. The significant Line × Tester effects suggest that hybrid breeding strategies can be effectively employed to enhance the accumulation of functional compounds, supporting the development of nutritionally rich and health-promoting barley cultivars.

Table 1. Mean square values from Line × Tester (L × T) ANOVA for functional components in barley

Source of Variation	df	Catechin	Myricetin	Quercetin	Kaempferol	Total Flavonoids
Replications	2	0.021^{ns}	0.019^{ns}	0.024^{ns}	0.015^{ns}	0.032^{ns}
Genotypes	22	0.945^{**}	1.102^{**}	0.876^{**}	0.612^{**}	2.342^{**}
Parents	7	0.752^{*}	0.864^{*}	0.698^{*}	0.481^{*}	1.897^{*}
Crosses	14	1.082^{*}	1.221^{**}	0.992^{*}	0.703^{*}	2.587^{**}
Parents vs Crosses	1	0.804^{ns}	1.043^{**}	0.711^{*}	0.498^{*}	2.115^{**}
Lines	4	0.658^{*}	0.793^{*}	0.623^{*}	0.407^{ns}	1.552^{**}
Testers	2	0.417^{**}	0.526^{*}	0.411^{*}	0.296^{*}	1.131^{**}
Line × Tester	8	1.236^{**}	1.408^{**}	1.115^{**}	0.792^{*}	2.843^{**}
Error	44	0.018	0.021	0.02	0.014	0.031

*ns = non-significant; * = Significant at p≤0.05; ** = Highly significant at p≤0.01.*

Mean performance of parental genotypes and their crosses

The heatmap provides a comprehensive visual overview of the variation in functional components, including catechin, myricetin, quercetin, kaempferol, and total flavonoids among parental barley genotypes and their respective hybrids (Figure 1). The intensity of the colors represents the concentration levels of each trait, with darker shades indicating higher values. This allows for the rapid identification of superior genotypes and cross combinations. The distinct variations in color patterns across genotypes and traits highlight a high degree of genetic diversity, which is essential for effective selection. Furthermore, the heatmap reveals trait-specific combining abilities, where certain genotypes excel in accumulating specific flavonoid compounds. Overall, the heatmap simplifies complex numerical data into an easily interpretable format, supporting informed decision-making in the selection of high-performing parental lines and hybrids for enhanced functional quality in barley (Pesaraklu et al., 2016; Feizi et al., 2020).

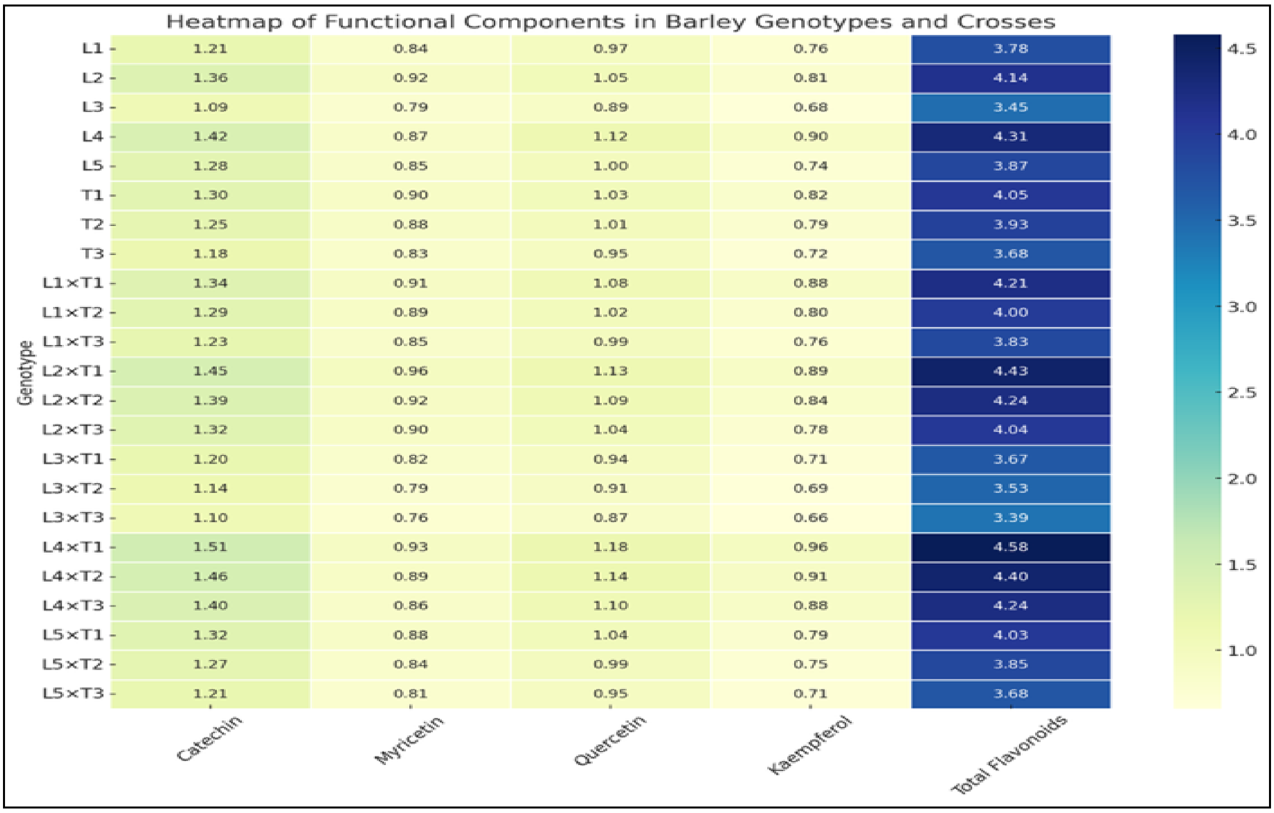
Among parents, genotype L4 consistently exhibited the highest values for catechin [1.42 Milligrams per gram of dry weight (mg/g DW)], quercetin (1.12 mg/g DW), kaempferol (0.90 mg/g DW), and total flavonoids (4.31 mg/g DW), indicating its superior potential for contributing to functional quality (Figure 2). These findings align with those of (Adriana et al., 2015; Ahmed et al., 2025c), who reported significant genotypic influence on phenolic and flavonoid contents in barley and other cereals. In contrast, L3 and T3 showed the lowest mean values across all traits, confirming their weaker functional profiles. However, their utility may lie in specific combinations rather than standalone performance. The high performance of T1 among testers, particularly for myricetin (0.90 mg/g DW) and total flavonoids (4.05 mg/g DW), suggests it can serve as a promising male parent. These variations among parents reflect the presence of both additive and non-additive gene actions, a concept also supported by (Noreen et al., 2025) in their studies on cereal quality traits.

Among the hybrids, L4×T1 emerged as the top-performing cross, surpassing all combinations for catechin (1.51 mg/g DW), quercetin (1.18 mg/g DW), kaempferol (0.96 mg/g DW), and total flavonoids (4.58), followed closely by L4×T2 and L2×T1 (Figure 3). The superior hybrid performance indicates strong specific combining ability (SCA mg/g DW), particularly in combinations involving high-performing parents like L4 and T1. These observations suggest that dominance or epistatic gene interactions play a significant role in determining functional traits, as previously demonstrated in the works of (Kumari et al., 2020; Fekadu et al., 2023).

Interestingly, the L2×T1 hybrid also showed high values for catechin (1.45 mg/g DW), quercetin (1.13 mg/g DW), and total flavonoids (4.43 mg/g DW), demonstrating favorable hybrid vigor, as its performance exceeded that of both parents. This supports the potential of heterotic breeding strategies for enhancing phytochemical content, corroborated by (Adriana et al., 2015; Huang et al., 2024), who highlighted similar heterotic effects in barley crosses for secondary metabolites.

The relatively moderate performance of L5×T1 and L2×T2 for total flavonoids and individual compounds further validates the significance of both parental GCA and cross-specific SCA. Meanwhile, the lower-performing crosses, particularly those involving L3 (e.g., L3×T3), exhibited reduced values across all traits, reflecting the limited potential of these combinations for functional enhancement.

The mean performance data confirms substantial genetic variation among genotypes and crosses, offering clear opportunities for trait improvement through parental selection and hybridization. The superiority of specific crosses, particularly those involving L4 and L2, emphasizes the value of exploiting both additive and non-additive genetic components in functional food breeding. Notably, genotypes such as L4, L2×T1, and L4×T1 exhibited darker hues across most traits in heatmap, signifying their potential as promising candidates for functional breeding and improvement programs. These findings support the strategic development of high-flavonoid barley cultivars with potential nutraceutical benefits, in line with earlier research by (Ge et al., 2021), advocating for biofortification through genetic approaches.



Heatmap of Functional Components in Barley Genotypes and Crosses

Genotype	Catechin	Myricetin	Quercetin	Kaempferol	Total Flavonoids
L1	1.21	0.84	0.97	0.76	3.78
L2	1.36	0.92	1.05	0.81	4.14
L3	1.09	0.79	0.89	0.68	3.45
L4	1.42	0.87	1.12	0.90	4.31
L5	1.28	0.85	1.00	0.74	3.87
T1	1.30	0.90	1.03	0.82	4.05
T2	1.25	0.88	1.01	0.79	3.93
T3	1.18	0.83	0.95	0.72	3.68
L1×T1	1.34	0.91	1.08	0.88	4.21
L1×T2	1.29	0.89	1.02	0.80	4.00
L1×T3	1.23	0.85	0.99	0.76	3.83
L2×T1	1.45	0.96	1.13	0.89	4.43
L2×T2	1.39	0.92	1.09	0.84	4.24
L2×T3	1.32	0.90	1.04	0.78	4.04
L3×T1	1.20	0.82	0.94	0.71	3.67
L3×T2	1.14	0.79	0.91	0.69	3.53
L3×T3	1.10	0.76	0.87	0.66	3.39
L4×T1	1.51	0.93	1.18	0.96	4.58
L4×T2	1.46	0.89	1.14	0.91	4.40
L4×T3	1.40	0.86	1.10	0.88	4.24
L5×T1	1.32	0.88	1.04	0.79	4.03
L5×T2	1.27	0.84	0.99	0.75	3.85
L5×T3	1.21	0.81	0.95	0.71	3.68

Figure 1. Heatmap of Mean Performance of Parental Genotypes and Their Crosses for Functional Components in Barley. This heatmap visualizes the mean performance of barley parental genotypes and their crosses across five functional components. The color-coded representation enables rapid identification of genotypes exhibiting higher or lower concentrations of catechin, myricetin, quercetin, kaempferol, and total flavonoids, facilitating the selection of superior lines for nutritional enhancement and breeding programs.

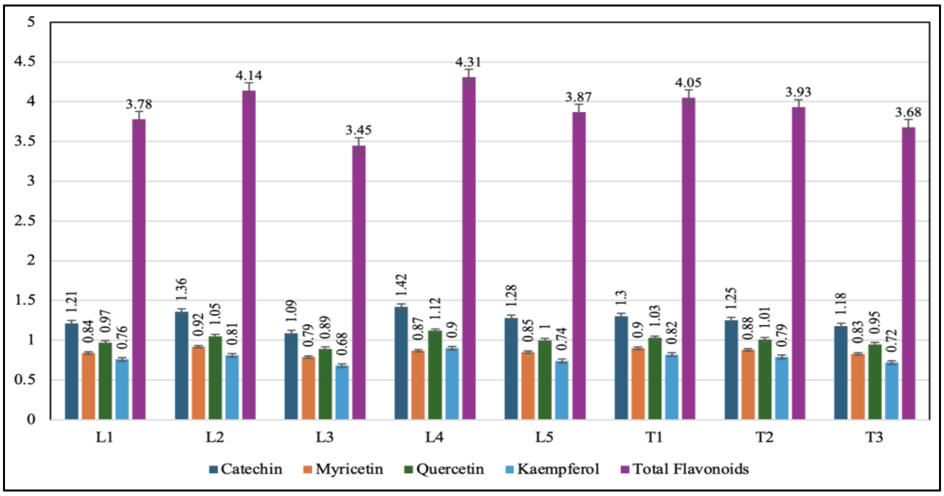


Figure 2. Mean performance of five functional flavonoid components - catechin, myricetin, quercetin, kaempferol, and total flavonoids - in barley parental genotypes (L1-L5) and testers (T1-T3) under normal growing conditions. Error bars represent the standard error of the mean for each component.

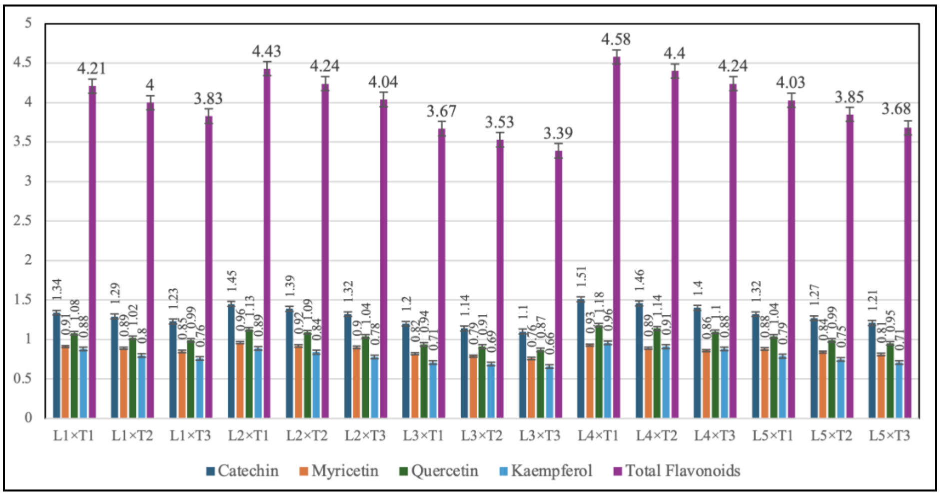


Figure 3. Mean performance of five functional flavonoid components - catechin, myricetin, quercetin, kaempferol, and total flavonoids - in barley 15 crosses under normal growing conditions. Error bars represent the standard error of the mean for each component.

Combining Ability effects

The analysis of General Combining Ability (GCA) and Specific Combining Ability (SCA) effects for functional components in barley provides critical insights into the nature of gene action and the potential of parental lines in hybrid development (Table 2). GCA effects revealed that parent L4 consistently demonstrated highly significant positive contributions across all traits, including catechin (0.072*), quercetin (0.101**), kaempferol (0.090**), and total flavonoids (0.213**), making it the best general combiner. This highlights the predominance of additive gene effects, which are fixable and heritable across generations, and supports earlier findings by (Sultan et al., 2016; Tokhetova et al., 2022; Ahmed et al., 2025a), who emphasized the importance of GCA in selecting parents for enhanced phytochemical traits.

Conversely, L3 exhibited significant negative GCA values for all traits, suggesting its poor general combining potential and limited usefulness in functional trait improvement. Among testers, T1 showed positive and significant GCA effects for all traits, particularly total flavonoids (0.095**) and kaempferol (0.062*), confirming its value as a promising male parent. These findings align with the observations of (Zhang et al., 2015; Tokhetova et al., 2022), who reported that testers with strong GCA effects contribute significantly to trait enhancement through additive genes. The SCA effects of crosses further reinforced the role of non-additive gene action. Crosses such as L4×T1, L2×T1, and L1×T1 exhibited highly significant positive SCA values across multiple traits. For instance, L4×T1 recorded strong SCA effects for kaempferol (0.100**) and total flavonoids (0.105**), indicating the presence of dominance and epistatic interactions. This non-additive gene expression can be exploited through heterosis breeding, as supported by (Amer et al., 2012), who emphasized the importance of specific parental combinations to maximize hybrid performance in secondary metabolite content.

Interestingly, the top-performing crosses in terms of mean performance, such as L4×T1 and L2×T1, also showed superior SCA effects, suggesting that both additive and non-additive gene actions are influencing the inheritance of functional components. This is consistent with the dual nature of gene action reported by (Mansour and Moustafa, 2016), indicating that a balanced approach using both GCA and SCA is essential for effective genetic improvement. On the other hand, crosses involving L3, such as L3×T3 and L3×T2, showed non-significant and negative SCA effects across all traits, reaffirming the poor combining ability of this line and its limited hybrid potential. These results echo the conclusions of (Pesaraklu et al., 2016), who reported that hybrids with low-performing parents and insignificant SCA often result in poor phenotypic expression.

In the present study, demonstrates that L4 and T1 are the most promising general combiners for improving catechin, quercetin, kaempferol, and total flavonoid content in barley, while crosses like L4×T1 and L2×T1 hold significant promise due to their strong SCA effects. These findings provide a solid foundation for designing barley breeding programs aimed at enhancing functional food qualities and are well-supported by existing research on combining ability and flavonoid enrichment strategies.

Table 2. General Combining Ability (GCA) Effects of Parents and Specific Combining Ability (SCA) Effects of Crosses for Functional Components in Barley

Parents	Catechin	Myricetin	Quercetin	Kaempferol	Total Flavonoids
L1	-0.113**	-0.034ns	-0.018ns	-0.027ns	-0.079*
L2	-0.012ns	0.048*	0.032ns	0.016ns	0.115**
L3	-0.260**	-0.134**	-0.119**	-0.098**	-0.240**
L4	0.072*	0.051*	0.101**	0.090**	0.213**
L5	-0.134**	-0.073*	-0.030ns	-0.016ns	-0.046*
T1	-0.036ns	0.039*	0.059*	0.062*	0.095**
T2	-0.090*	-0.042*	-0.024ns	-0.030ns	-0.031ns
T3	-0.149**	-0.113**	-0.125**	-0.129**	-0.172**
Crosses	***Catechin***	***Myricetin***	***Quercetin***	***Kaempferol***	***Total Flavonoids***
L1×T1	0.085 *	0.090 *	0.115 *	0.120 *	0.155 ns
L1×T2	0.015 ns	0.055 ns	0.025 ns	0.015 ns	0.095 ns
L1×T3	0.005 ns	0.015 ns	0.010 ns	0.010 ns	0.055 ns
L2×T1	0.115 **	0.100 *	0.090 *	0.085 *	0.135 *
L2×T2	0.065 *	0.020 ns	0.030 ns	-0.005 ns	0.055 ns
L2×T3	0.010 ns	0.025 ns	0.020 ns	-0.015 ns	0.010 ns
L3×T1	-0.015 ns	-0.035 ns	-0.030 ns	-0.045 ns	-0.080 ns
L3×T2	-0.055 ns	-0.045 ns	-0.035 ns	-0.045 ns	-0.065 ns
L3×T3	-0.065 ns	-0.055 ns	-0.055 ns	-0.050 ns	-0.075 ns
L4×T1	0.100 **	0.045 *	0.060 *	0.100 **	0.105 **
L4×T2	0.075 *	-0.015 ns	0.015 ns	0.015 ns	0.035 ns
L4×T3	0.030 ns	-0.030 ns	-0.010 ns	-0.020 ns	0.005 ns
L5×T1	0.040 ns	-0.005 ns	0.015 ns	0.010 ns	0.070 ns
L5×T2	0.015 ns	-0.025 ns	-0.015 ns	-0.015 ns	-0.025 ns
L5×T3	-0.015 ns	-0.030 ns	-0.025 ns	-0.025 ns	-0.035 ns

*Significance Levels: ns = non-significant; * * = Significant at p≤0.05; ** = Highly significant at p≤0.01.*

Heritability and Types of Gene Action

The estimates of genetic parameters for functional components in barley, including additive variance (σ^2A), dominance variance (σ^2D), broad-sense heritability (H^2), and degree of dominance, provide critical insights into the underlying genetic mechanisms controlling these traits (Table 3). Across all traits studied - catechin, myricetin, quercetin, kaempferol, and total flavonoids, the dominance variance (σ^2D) consistently exceeded the additive variance (σ^2A), indicating a greater contribution of non-additive gene effects. The degree of dominance for all traits was greater than one, ranging from 1.73 (quercetin) to 1.98 (kaempferol), clearly suggesting over-dominance as the prevailing type of gene action. Such over-dominance implies that hybrid combinations may outperform their parents due to favourable heterotic effects, which is highly beneficial for trait enhancement through hybrid breeding strategies. Broad-sense heritability (H^2) values were moderately high for all traits, with the highest observed for total flavonoids (0.73), followed closely by catechin (0.72) and quercetin (0.71). These moderate to high heritability estimates suggest that a substantial portion of the phenotypic variation is genetically controlled, and thus, selection can be effective in later generations. This aligns with findings from (Pesaraklu et al., 2016; Tokhetova et al., 2022; Fekadu et al., 2023), who reported similar heritability ranges for bioactive compounds in cereals and emphasized their usefulness in genotype selection and trait improvement. Specifically, the dominance of over-dominance gene action for catechin (degree = 1.78) and total flavonoids (degree = 1.75) points to the potential of exploiting heterosis for maximizing the accumulation of these beneficial metabolites. These results are in agreement with those of (Chang et al., 2002; Zymogliad et al., 2021; Al-Khayri et al., 2022), who reported non-additive gene action and high heterotic response for phenolic and flavonoid content in barley and wheat.

Furthermore, the significant over-dominance for kaempferol (degree = 1.98) and myricetin (degree = 1.91) reflects the complex genetic regulation of these traits and supports previous reports by (Patial et al., 2018), who observed similar dominance patterns in polyphenolic traits of barley. The combination of moderately high heritability and over-dominance gene action also suggests that selection in later segregating generations or reciprocal recurrent selection strategies would be effective for improving these traits. The genetic parameter estimates highlight that non-additive gene effects (over-dominance) are predominant in controlling the expression of functional flavonoid components in barley. While heritability estimates support the feasibility of selection, the over-dominant nature of gene action strongly favors the development and utilization of heterotic hybrids. These findings are consistent with earlier studies in cereals and provide a robust foundation for future breeding programs aimed at enhancing the nutraceutical and functional value of barley.

Table 3. Estimates of Additive Variance (σ^2A), Dominance Variance (σ^2D), Broad-Sense Heritability (H^2), Degree of Dominance, and Type of Gene Action for Functional Components in Barley

Trait	σ^2A	σ^2D	H^2	Degree of Dominance
Catechin	0.0053	0.0084	0.72	1.78
Myricetin	0.0034	0.0062	0.65	1.91
Quercetin	0.004	0.006	0.71	1.73
Kaempferol	0.0037	0.0073	0.66	1.98
Total Flavonoids	0.0077	0.0118	0.73	1.75

CONCLUSIONS

This genetic study decisively reveals substantial genetic variability among barley genotypes for key flavonoid components: catechin, myricetin, quercetin, kaempferol, and total flavonoids. Line × Tester ANOVA confirmed that both additive and non-additive gene actions significantly influence these traits, with line × tester interactions being highly significant. Parental line L4 emerged as the best general combiner, exhibiting superior mean performance and consistently high positive General Combining Ability (GCA) effects, such as 0.213 for total flavonoids. Tester T1 also showed strong positive GCA, notably 0.095 for total flavonoids. These parents offer excellent potential for transmitting desirable functional traits. Among hybrids, L4×T1 was exceptional, recording the highest mean total flavonoid content (4.58) and strong positive Specific Combining Ability (SCA) effects (e.g., 0.105 for total flavonoids). This highlights significant dominance and epistatic interactions, crucial for maximizing hybrid performance. Genetic parameter estimates showed dominance variance (σ^2D) consistently exceeded additive variance (σ^2A), with degrees of dominance ranging from 1.73 to 1.98, indicating over-dominance. While broad-sense heritability was moderately high (up to 0.73), the predominant over-dominance strongly advocates for hybrid breeding strategies. These findings underscore that exploiting both additive and non-additive gene actions is paramount for developing nutritionally enhanced, high-flavonoid barley cultivars.

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