

## ASSESSING FLAVONOID CONTENT IN BARLEY GENOTYPES: GENETIC CONTRIBUTIONS AND HYBRID POTENTIAL FOR NUTRITIONAL IMPROVEMENT

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**Abstract.** This study aimed to assess the genetic potential of barley genotypes for flavonoid content, focusing on catechins, myricetin, quercetin, kaempferol, and total flavonoids. A half-diallel mating design involving seven parental barley varieties was employed to create 21 hybrid combinations. The parental genotypes and their F<sub>1</sub> hybrids were evaluated for their flavonoid content, namely catechins, myricetin, quercetin, kaempferol and total flavonoids with significant variations observed in both the parental and hybrid populations. Among the parental genotypes, Yundamai2 exhibited the highest catechin content, while Baodamai6 and Supi4 Barley showed superior myricetin and total flavonoid content. Hybrid combinations, such as Hatixi Barley × S500 Barley, demonstrated high values for multiple flavonoid compounds, indicating the potential for heterosis and genetic improvement in flavonoid production. General combining ability (GCA) and specific combining ability (SCA) analysis revealed the genetic contributions of individual genotypes to flavonoid biosynthesis, with significant variation observed for each flavonoid compound. This research highlights the genetic potential of specific barley varieties and hybrid combinations for improving flavonoid content, which is essential for enhancing plant stress tolerance and nutritional quality.

**Keywords:** *barley, flavonoids, genotype, environment, GCA, SCA, hybrid*

### Introduction

Barley (*Hordeum vulgare* L.) represents a significant cereal crop on a global scale, recognized for its adaptability and nutritional benefits. This crop is predominantly grown

for its grain, which serves multiple purposes, including animal fodder, human consumption, and as a crucial ingredient in the manufacturing of beer and whiskey (Shupletsova et al., 2024). Barley is a very important crop in both developed and developing countries; thus, it holds a considerable economic value. In addition to its agronomical value, barley has also gained increasing interest due to its potential health-related benefits, which have been mainly linked to bioactive compounds, especially flavonoids. These polyphenolic compounds have been widely studied for their antioxidant, anti-inflammatory, anticancer, and antimicrobial properties. These metabolites are important research targets in both nutritional science and plant breeding programs due to their potential benefit to human health by reducing the risk of cardiovascular diseases, improving immune responses, and fighting against oxidative stress (Vaschenko et al., 2021; Fatemi et al., 2023; Wang et al., 2024).

Flavonoids represent one of the most represented groups of plant secondary metabolites, playing important roles in plants for protection against oxidative damage, pathogens, and herbivores. Barley flavonoids are produced via one of the best genetically and environmentally regulated metabolic pathways: the phenylpropanoid biosynthesis pathway (Abidi et al., 2015; Victoria et al., 2023). These flavonoids include catechins, myricetin, quercetin, and kaempferol, all of which are important in human health and potential resistance in barley against abiotic stresses. The antioxidant nature of flavonoids helps the plants cope with oxidative stress imposed by the abiotic stressors like drought, high temperatures, and salinity (Idehen et al., 2017). Enhanced flavonoid content in barley would, therefore, potentially be better performers under extreme conditions (Han et al., 2018; Kadege et al., 2024).

Recent research has indicated genetic diversity in flavonoid content among barley genotypes; it is therefore quantitatively variable across different genotypes. Such variability opens up avenues for the amelioration of barley through selective breeding programs. Improvement in flavonoid content not only improves nutritional quality in the grain but may also provide a basis for improvement against various stresses in the crop (Martínez et al., 2018; Kazimierczak et al., 2020). Breeding barley cultivars rich in flavonoids could be a part of the bio-fortification effort that will enhance health benefits in people consuming barley, while ensuring greater productivity of this crop facing variable environmental conditions. Improvement in both these respects requires knowledge about the operating genetic mechanism of flavonoid biosynthesis in barley (Bagues et al., 2021; Ge et al., 2021; Baloch et al., 2024).

The breeding of plants with diverse genetic backgrounds necessitates the use of varied breeding techniques in exploring the genetic diversity. Among various breeding techniques, diallel mating designs have turned out very effective in analyzing complicated traits (Iannucci et al., 2021; Kamara et al., 2021). Diallel crosses refer to making crosses between all possible pairs of parental lines. The breeders make use of this design for estimating the general combining ability and the specific combining ability of the parents (Xiao et al., 2017; Yang et al., 2019). GCA represents the additive genetic effects of one parent and usually gives the general genetic potential of the parent's contribution to the traits expressed in offspring (Saeed et al., 2024). SCA measures non-additive genetic effects due to interactions between parents (Katiyar et al., 2021; Kiani et al., 2021; Vaschenko et al., 2021). The study of GCA and SCA allows breeders to recognize superior parents and hybrids carrying desirable traits, such as high flavonoid content. These findings are indispensable in the development of better-barley cultivars with desirable nutritional and stress-resistance traits (Zeng et al., 2014; Zhu et al., 2015).

Flavonoid biosynthesis in barley is complicated since it depends on too many factors, including genetic background, environment, and agricultural practices. Despite all the studies conducted on flavonoids in relation to benefits for human health and hardiness in plants, it is tough to outline the real genetic cause of flavonoids in barley, especially with respect to breeding approaches like diallel mating designs (Kamara et al., 2021; Jin et al., 2022; Shamyarira et al., 2022). In all, the genetics behind flavonoid accumulation in barley have been poorly investigated; thus, this calls for further studies in the identification of the key genes and regulation mechanisms. Besides that, environmental conditions for the growth of barley, such as water availability, temperature conditions, and soil type, will also be an important determinant for flavonoids level and hence evaluation of those traits under diverse environmental contexts (Fekadu et al., 2023; Nowak et al., 2023; Pour-Aboughadareh et al., 2023).

The present research investigation attempts to contribute to filling this gap in the research on genetic potentiation of flavonoid synthesis in barley using a half-diallel mating design. Seven barley genotypes in the diversity of flavonoid content were selected for the research with respect to the content of the individual flavonoids catechins, myricetin, quercetin, and kaempferol, and the total flavonoid content (Nowak et al., 2023; Friero et al., 2024). Thus, the major objectives of this study were to estimate GCA and SCA concerning those flavonoids' traits and to identify promising parents with high flavonoid content, besides hybrid combinations that may result in the production of superior offspring with increased flavonoids (Huang et al., 2024; Hussain et al., 2024). Therefore, the research is important for further elaboration of the genetic architecture of the production of flavonoids in barley, giving insights into further breeding programs aimed at the improvement of nutritional value and/or abiotic/biotic stress tolerance in barley (Nowak et al., 2024; Romayssa et al., 2024).

While this study is focused on an increase in flavonoids content in barley, it also may be of importance to get useful information on developing a new variety of barley-a nutritionally enhanced crop-to grow well under extreme conditions. In a world where agriculture faces challenges on a global scale-control factors like climate change, drought, and soil degradation-the development of more flavonoid-relevant barley varieties with better resilience is cardinal for assured food security and improved public health (Shupletsova et al., 2024; Wang et al., 2024). Ultimately, this work may contribute to the development of barley varieties possessing not only health benefits for the consumer but also improved tolerance to abiotic stresses, in view of more sustainable agriculture and food security under climatic change.

## Materials and methods

The experimental materials for testing general combining ability and specific combining ability consisted of seven parent varieties, as shown in *Table 1*. Using these seven varieties as parents, 21 hybrid combinations were generated through intercrossing in three blocks using a half-diallel mating design ( $1/2 \times 7 \times (7-1) = 21$  combinations) (*Table 2*). From December 2021 to March 2022, manual emasculation, hybridization, and crossbreeding were performed to produce F<sub>1</sub> seeds and seeds from the parent varieties. From October 2023 to April 2024, the parent varieties and their F<sub>1</sub> progeny were sown for evaluation.

**Table 1.** List of seven parental genotypes, sources and their row types

Code	Parent Name	Source	Row Type
1	Hatiexi	Yunnan Academy of Agricultural Sciences, Kunming, Yunnan	Two-row
2	Yundamai2	Yunnan Academy of Agricultural SciencesKunming, Yunnan	Two-row
3	Supi4	Yancheng Academy of Agricultural Sciences, Yancheng, Jiangsu	Two-row
4	Baodamai6	Baoshan Academy of Agricultural Sciences, Baoshan, Yunnan	Two-row
5	S500	Dali Academy of Agricultural Sciences, Dali, Yunnan	Two-row
6	Zhepi33	Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang	Two-row
7	E32380	Hubei Academy of Agricultural Sciences, Wuhan, Hubei	Two-row

**Table 2.** List of 21 cross combinations ( $F_1$  generations) used in this study

Code	Cross Combination	Code	Combination
1×2	Hatiexi × Yundamai2	3×4	Supi4 × Baodamai6
1×3	Hatiexi × Supi4	3×5	Supi4 × S500
1×4	Hatiexi × Baodamai6	3×6	Supi4 × Zhepi33
1×5	Hatiexi × S500	3×7	Supi4 × E32380
1×6	Hatiexi × Zhepi33	4×5	Baodamai6 × S500
1×7	Hatiexi × E32380	4×6	Baodamai6 × Zhepi33
2×3	Yundamai2 × Supi4	4×7	Baodamai6 × E32380
2×4	Yundamai2 × Baodamai6	5×6	S500 × Zhepi33
2×5	Yundamai2 × S500	5×7	S500 × E32380
2×6	Yundamai2 × Zhepi33	6×7	Zhepi33 × E32380
2×7	Yundamai2 × E32380		

### Experimental design

The materials were planted in the experimental field of the Yunnan Academy of Agricultural Sciences. The experiment followed a randomized block design with three replications. Each plot measured 2 m × 2 m, and identical treatments were applied to all plots. One week prior to sowing, the soil was deeply plowed to remove weeds. The row planting method was employed, with a sowing depth of 2–4 cm and a row spacing of 30 cm.

### Flavonoids content determination

The harvested seeds of barley parents and  $F_1$  generations were dried and ground. Approximately 1 g of the ground sample was subjected to ultrasonic extraction with a methanol solution for 30 minutes, followed by filtration. The filtrate was evaporated, and the residue was dissolved in double-distilled water. After extraction with ethyl acetate, the aqueous phase was evaporated, and the residue was dissolved in 10 mL of methanol to a constant volume. The solution was filtered through a 0.45 µm membrane filter. The content of catechins, myricetin, quercetin, and kaempferol in the samples was determined by high-performance liquid chromatography (HPLC) using an YMC-Pack ODS AM-303 (5 µm, 250 mm × 4.6 mm i.d.) column. The mobile phase consisted of A—0.1% glacial acetic acid aqueous solution and B—acetonitrile with gradient elution. The flow rate was 0.8 mL/min, and detection was conducted at 280 nm. The injection volume was 10 µL. The contents of catechins, myricetin, quercetin, and kaempferol in the samples were calculated based on retention time and peak area.

The data on catechins, myricetin, quercetin, kaempferol, and total flavonoid contents in barley grains for parent and their F<sub>1</sub> generation were analyzed using DPS and Excel software, along with genetic analysis software. The genetic analysis was conducted using the plant quantitative trait using combining ability model (NCII design). Least significance difference (LSD) applied for the pairwise comparisons among the means of genotypes (Box, 1980). Combining ability analysis for the parent and F<sub>1</sub> materials was performed following the Griffing method (Griffing, 1956). This analysis provided estimates of general combining ability (GCA), specific combining ability (SCA), additive variance, dominance variance, genetic variance, environmental variance, phenotypic variance, and the genetic determination coefficient.

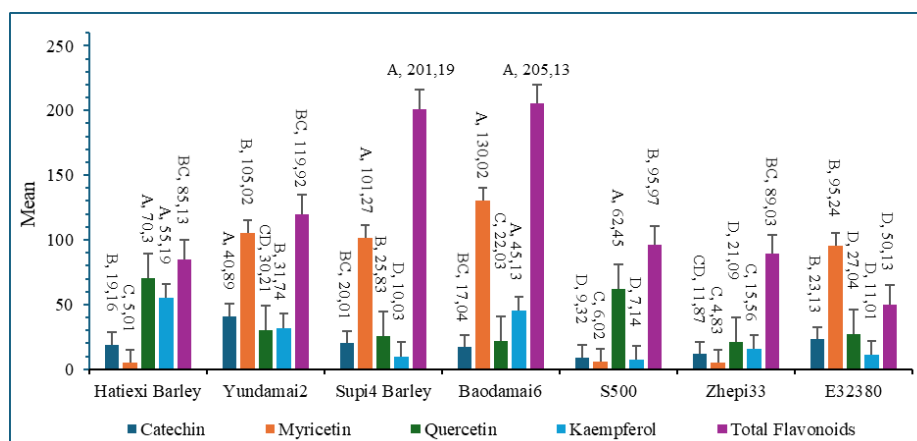
## Results and discussion

### *Mean performance of Parental genotypes and their cross combinations*

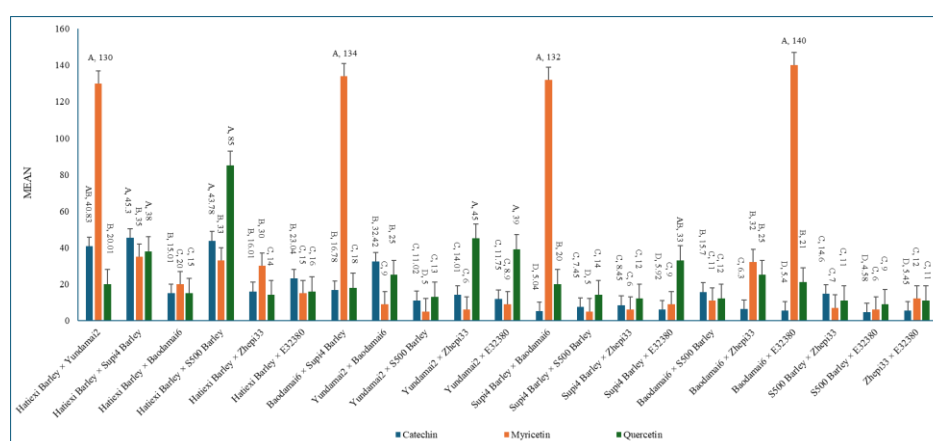
Catechin is one of the most important flavonoids, giving stress tolerance and antioxidant activity, which has been considered important in breeding programs concerning improvement in barley resilience and overall grain quality. In the present study, *Figure 1* presented the mean flavonoid content (including catechin, myricetin, quercetin, kaempferol, and total flavonoids) in seven barley genotypes. The graph displayed the concentrations of each flavonoid compound, with letters above the bars indicating statistically significant differences between genotypes as determined by an LSD test ( $P < 0.05$ ). Error bars, represented the standard deviation of the mean. Large differences in catechin content were observed among the parental genotypes. Highest value of catechin was noted in Yundamai2 (40.89  $\mu\text{g/g}$ ), followed by E32380 (23.13  $\mu\text{g/g}$ ) and Supi4 Barley (20.01  $\mu\text{g/g}$ ), while it was low in S500 (9.32  $\mu\text{g/g}$ ). Catechin levels varied widely among the crosses (*Figure 1*). The *Figure 2* displayed the average concentrations of three flavonoid compounds (catechin, myricetin, and quercetin) measured in 21 different cross combinations of 7 barley genotypes. The data is presented as a clustered bar chart, where each cluster represented a specific barley cross combination. Within each cluster, there are three bars, each corresponding to the mean value of one of the three flavonoids: catechin (blue), myricetin (orange), and quercetin (green). The letters above the bars represent groups that are statistically significant, as determined by the Least Significant Difference (LSD) test at a significance level of  $P < 0.05$ . Bars sharing the same letter do not show significant differences, while bars with different letters indicate statistically significant variations in their means. The highest catechin content was observed in Hatiexi Barley  $\times$  Supi4 Barley (45.3  $\mu\text{g/g}$ ) and Hatiexi Barley  $\times$  S500 Barley (43.78  $\mu\text{g/g}$ ). In contrast, Supi4 Barley  $\times$  E32380 (5.92  $\mu\text{g/g}$ ), Baodamai6  $\times$  E32380 (5.4  $\mu\text{g/g}$ ), and Zhepi33  $\times$  E32380 (5.45  $\mu\text{g/g}$ ) had the lowest catechin content (*Figure 2*). Catechin is one of the major flavonoids with antioxidant properties, which results in causing stress tolerance and health in plants. As great variations in catechin occur among different crosses and particularly higher values among such combinations as Hatiexi Barley  $\times$  Supi4 Barley and Hatiexi Barley  $\times$  S500 Barley, this trait is a prospective donor for improving stress resistance in barley, previously scientist reported similar findings (Martínez et al., 2018; Kamara et al., 2021).

Myricetin It is an important active trait in plant defense mechanisms and stress adaptation and, hence, a key trait in the improvement of barley for overcoming environmental challenges (Abidi et al., 2015). The myricetin contents showed apparent variation, with the highest in Baodamai6, 130.02  $\mu\text{g/g}$ , followed by Yundamai2

(105.02  $\mu\text{g/g}$ ) and Supi4 Barley (101.27  $\mu\text{g/g}$ ); by contrast, the lowest values were 4.83  $\mu\text{g/g}$  in Zhepi33 and 5.01  $\mu\text{g/g}$  in Hatiexi Barley, respectively (*Figure 1*). Among them, the hybrids from Baodamai6  $\times$  E32380 had the highest contents of 140  $\mu\text{g/g}$ ; Supi4 Barley  $\times$  Baodamai6 hybrids were second with contents of 134  $\mu\text{g/g}$ , and Hatiexi Barley  $\times$  Yundamai2 had 130  $\mu\text{g/g}$ . Yundamai2  $\times$  S500 Barley and Supi4 Barley  $\times$  S500 Barley showed the lowest contents at five  $\mu\text{g/g}$  each (*Figure 2*). Myricetin is one of the main compounds that have been reported for high antioxidant activities. This could indicate the prospect of using the combinations Baodamai6  $\times$  E32380 and Supi4 Barley  $\times$  Baodamai6 in the selection and breeding programs aimed at improving the resistance level and metabolic activity of barley (Fekadu et al., 2023; Pour-Aboughadareh et al., 2023; Nowak et al., 2024).



**Figure 1.** This figure shows the mean values of individual flavonoids (catechin, myricetin, quercetin, and kaempferol) and the total flavonoid content in seven barley genotypes: Hatiexi, Yundamai2, Supi4, Baodamai6, S500, Zhepi33, and E32380. Error bars represent the standard deviation of the mean. Mean values for each flavonoid compound in each genotype are shown. Letters above the bars indicate significantly different groups based on the Least Significant Difference (LSD) test at  $P < 0.05$ .



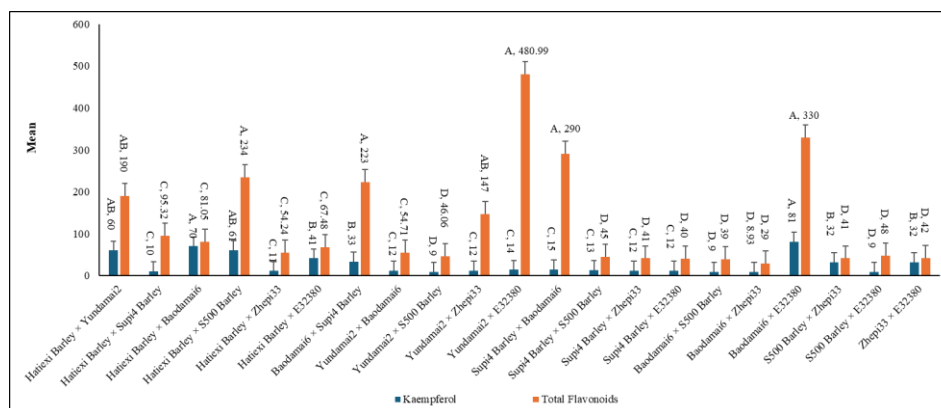
**Figure 2.** This figure shows the mean values of catechin, myricetin, quercetin content in 21 cross combinations of 7 barley genotypes. Error bars represent the standard deviation of the mean. Mean values for each flavonoid compound in each cross are shown. Letters above the bars indicate significantly different groups based on the Least Significant Difference (LSD) test at  $P < 0.05$ .

Quercetin takes part in plant metabolism and gives substantial resistance to plants; thus, it helps in selecting those genotypes of barley with higher flavonoid content that are of better nutritional and adaptive properties (Ge et al., 2021; Shupletsova et al., 2024). In relation to quercetin, the highest value was measured for the Hatiexi Barley variety-70.3 µg/g, followed by the S500 variety-62.45 µg/g (*Figure 1*). The lowest contents of quercetin were determined in the varieties Zhepi33 (21.09 µg/g) and Baodamai6 (22.03 µg/g). For quercetin, this oscillated between the highest-85 µg/g in Hatiexi Barley × S500 Barley down to 45 µg/g in Yundamai2 × Zhepi33 and 39 µg/g in Yundamai2 × E32380, while the lowest quercetin content was documented in S500 Barley × E32380 and Baodamai6 × S500 Barley with 9 µg/g and 12 µg/g, respectively. Quercetin plays a very important role in the adaptation of plants to stress and has health-related benefits linked with its nature, an antioxidant. Crosses like Hatiexi Barley × S500 Barley, and Yundamai2 × Zhepi33 with high quercetin content could therefore be promising genetic combinations for further enhancement of flavonoid traits in barley (Shamuyarira et al., 2022; Friero et al., 2024).

The improvement of plant growth regulation and abiotic stress tolerance by kaempferol thus plays an important role in developing barley varieties with enhanced flavonoid accumulation and improved environmental adaptability (Kazimierczak et al., 2020). Kaempferol was highest in Hatiexi Barley (55.19 µg/g) and Baodamai6 (45.13 µg/g), while S500 showed the lowest kaempferol content with a value of 7.14 µg/g (*Figure 1*). The *Figure 3* displayed the average concentrations of two flavonoid compounds (kaempferol and total flavonoids) measured in 21 different cross combinations of 7 barley genotypes. The data is presented as a clustered bar chart, where each cluster represented a specific barley cross combination. Within each cluster, there are two bars, each corresponding to the mean value of one of the two flavonoids: kaempferol (blue), total flavonoids (orange). Letters above the bars denote statistically significant groups based on the Least Significant Difference (LSD) test at a significance level of  $P < 0.05$ . The highest kaempferol content was found in Baodamai6 × E32380 (81 µg/g), followed by Hatiexi Barley × Baodamai6 (70 µg/g) (*Figure 3*). These were followed by lower values from Baodamai6 × Zhepi33 and S500 Barley × E32380 at 8.93 and 9 µg/g, respectively. Kaempferol is one of the flavonoids that participate in abiotic stress resistance and regulation of plant growth. A higher content hence showed that Baodamai6 × E32380 and Hatiexi Barley × Baodamai6 could be used to enhance drought tolerance and flavonoid accumulation in barley (Kiani et al., 2021; Nowak et al., 2024; Shupletsova et al., 2024).

Total flavonoid content also represents an important index that reflects accumulation antioxidant capacity, which can be used to distinguish superiors with more tolerant nutritional quality in barley genotypes (Kiani et al., 2021). In this paper, a wide variation in total flavonoid content was observed in the tested parental genotypes. Baodamai6 showed the highest total flavonoid content of 205.13 µg/g, while Supi4 Barley attained 201.19 µg/g, and Yundamai2 had 119.92 µg/g. On the other hand, the lowest total flavonoid content is E32380, which is 50.13 µg/g, followed by Zhepi33, 89.03 µg/g as presented in *Figure 1*. Considering total flavonoids, there is great variation among the crosses. The cross Yundamai2 × E32380 showed a maximum value of total flavonoids, which is 480.99 µg/g, followed by Baodamai6 × E32380, 330 µg/g and Supi4 Barley × Baodamai6, with the mean values of 290 µg/g. The lowest total flavonoid content was those observed for Supi4 Barley × Zhepi33 41µg/g, S500 Barley × Zhepi33 41µg/g, and Supi4 Barley × E32380 40µg/g as observed in *Figure 3*. Flavonoids are an important

index of the total antioxidant potentials that plants possess. These ultra-high values in the Yundamai2  $\times$  E32380 and Baodamai6  $\times$  E32380 are indicative of their better genetic potentials for improvements in flavonoid biosynthesis, hence making them potential candidates in barley improvement programs with reference to stress tolerance and nutritional quality (Iannucci et al., 2021; Katiyar et al., 2021; Huang et al., 2024).



**Figure 3.** This figure shows the mean values of kaempferol and the total flavonoid contents in 21 cross combinations of 7 barley genotypes. Error bars represent the standard deviation of the mean. Mean values for each flavonoid compound in each cross are shown. Letters above the bars indicate significantly different groups based on the Least Significant Difference (LSD) test at  $P < 0.05$

Among the parental genotypes, Baodamai6 and Supi4 Barley showed higher total flavonoid content and most of the individual flavonoid compounds and hence are outstanding genotypes for flavonoid accumulation. On the other hand, S500 and Zhepi33 showed relatively lower values for most of the traits. Among the combinations, Yundamai2  $\times$  E32380, Baodamai6  $\times$  E32380 and Supi4 Barley  $\times$  Baodamai6 showed the significantly higher total flavonoid contents hence possess greater potential for flavonoid accumulation. While both Hatiexi Barley  $\times$  S500 Barley and Hatiexi Barley  $\times$  Yundamai2 also exhibited high values of individual flavonoid compounds, the value of most other traits was generally lower in crosses with Zhepi33 and S500 Barley. Such variation suggests that the crosses have great genetic potential for flavonoid improvement in barley breeding programs (Vaschenko et al., 2021; Pour-Aboughadareh et al., 2023; Shupletsova et al., 2024).

### General combining ability (GCA) and significance analysis of flavonoids content

As shown in Table 3, the general combining ability (GCA) for the flavonoid compounds, including catechins, myricetin, quercetin, kaempferol, and total flavonoids, exhibited highly significant differences across the seven barley varieties. Among these, the highest GCA for catechin content was observed in 'Yundamai2', with a value of 9.12. For myricetin content, the highest GCA was found in 'Baodamai6' and 'Supi4 Barley', with values of 80.15 and 74.92, respectively, and no significant difference was observed between these two varieties. The highest GCA for quercetin content was found in 'Hatiexi Barley' and 'S500 Barley', with values of 58.20 and 56.77, respectively, and again, no significant difference was observed between these two varieties. 'Baodamai6' (5.12) and 'Hatiexi Barley' (3.13) had relatively high GCA for kaempferol, only 'Supi4 Barley'



(Table 3) was significantly different from the other six genotypes, including these two. For total flavonoids content, the highest GCA was observed in 'Supi4 Barley' and 'Baodamai6', with values of 69.78 and 65.34, respectively, and no significant difference was found between the two varieties. Among these flavonoid compounds, myricetin and total flavonoids content showed higher general combining ability, indicating a greater affinity for these compounds in the parent varieties (Iannucci et al., 2021; Kamara et al., 2021).

**Table 3.** General combining ability effects of flavonoids compounds

Variety	Catechin (GCA)	Myricetin (GCA)	Quercetin (GCA)	Kaempferol (GCA)	Total Flavonoids (GCA)
Hatiexi Barley	1.82 bB	-58.41 cC	58.20 aA	3.13 aA	-8.65 cdBC
Yundamai2	9.12 aA	27.43 bB	-20.43 cdCD	1.83 aA	11.46 bB
Supi4 Barley	1.01 bBC	74.92 aA	-5.10 bB	-7.54 bB	69.78 aA
Baodamai6	-0.82 bcBC	80.15 aA	-22.90 cC	5.12 aA	65.34 aA
S500	-6.29 dD	-70.24 cC	56.77 aA	-0.82 aA	-22.47 dC
Zhepi33	-3.56 cCD	-65.23 cC	-31.78 dD	0.61 aA	-99.80 eD
E32380	1.47 bB	23.72 bB	-32.14 dD	-0.54 aA	-4.34 bcBC

In this table, the GCA effects for the flavonoid compounds—catechins, myricetin, quercetin, kaempferol, and total flavonoids—are presented for each of the seven barley varieties. Using the Griffing method, significant differences are indicated by lowercase letters (at the 5% level) and uppercase letters (at the 1% level) following each GCA value, with the parental genotypes showing distinct levels of combining ability for each flavonoid compound

The large magnitude of GCA for flavonoid compounds in the seven varieties depicted genetic potential for flavonoids synthesis. In addition, the highest GCA for catechin was found for 'Yundamai2', which showed the strongest genetic contribution to the trait. 'Baodamai6' and 'Supi4 Barley' possessed superior GCA for myricetin, indicating that the compound played a crucial role in enhancing antioxidant activity. The GCA for quercetin was high for 'Hatiexi Barley' and 'S500 Barley', while the contents of kaempferol were preponderant in 'Baodamai6' and 'Hatiexi Barley'. Hence, higher GCA for myricetin and total flavonoids might express an additive genetic effect and could be suitable for selection in breeding programs (Katiyar et al., 2021; Shamuyarira et al., 2022).

### *Specific combining ability analysis of flavonoids compounds in barley*

From Table 4, it is evident that there are significant variations in the specific combining ability (SCA) of flavonoid compounds—catechins, myricetin, quercetin, kaempferol, and total flavonoids—across different hybrid combinations. Several hybrid combinations exhibit relatively high SCA, notably Hatiexi Barley × Yundamai2, Hatiexi Barley × Supi4 Barley, Hatiexi Barley × S500 Barley, and Baodamai6 × E32380. These combinations indicate a high level of heterosis for total flavonoid compounds. Specifically, the combination of Hatiexi Barley × Yundamai2 showed the highest SCA for myricetin, reaching 160.22. For the combination of Hatiexi Barley × Supi4 Barley, the SCA for catechins and quercetin are high, at 24.11 and 91.26, respectively. The Hatiexi Barley × S500 Barley combination showed the highest SCA for catechins, quercetin, and total flavonoids, with values of 14.64, 343.77, and 378.15, respectively. Finally, the combination of Baodamai6 × E32380 demonstrates the highest SCA for myricetin, kaempferol, and total flavonoids, with values of 698.23, 33.17, and 734.38,

respectively. These results indicate that myricetin, quercetin, and total flavonoids exhibit the highest specific combining ability, suggesting that these compounds have substantial hybrid vigor in different barley combinations (Kamara et al., 2021; Vaschenko et al., 2021; Hussain et al., 2024).

**Table 4.** *Specific combining ability effects of flavonoids in barley*

Combination	Catechins (SCA)	Myricetin (SCA)	Quercetin (SCA)	Kaempferol (SCA)	Total Flavonoids (SCA)
Hatiexi Barley × Yundamai2	5.72 bB	160.22 aA	-6.33 cC	5.89 bB	165.1 aA
Hatiexi Barley × Supi4 Barley	24.11 aA	19.56 bB	91.26 aA	-21.04 cC	113.93 aA
Hatiexi Barley × Baodamai6	-4.97 dD	-130.92 dE	-42.15 cC	16.87 aB	-162.83 dD
Hatiexi Barley × S500 Barley	14.64 aA	11.99 bB	343.77 aA	9.89 bB	378.15 aA
Hatiexi Barley × Zhepi33	-1.07 dD	9.42 bB	-59.88 dD	-30.12 cC	-81.88 cC
Hatiexi Barley × E32380	-8.74 dD	-87.36 dD	-65.18 dD	13.91 aB	-147.37 dD
Baodamai6 × Supi4 Barley	7.13 bB	50.51 bB	-12.31 cC	2.77 bB	48.18 bB
Yundamai2 × Baodamai6	8.09 bB	-142.12 dE	8.21 bB	-1.12 cC	-129.94 dD
Yundamai2 × S500 Barley	-23.47 dE	14.83 bB	-61.08 dD	-27.13 cC	-98.34 cC
Yundamai2 × Zhepi33	-6.11 dD	27.92 bB	26.13 bB	-2.38 cC	47.44 bB
Yundamai2 × E32380	1.88 cC	-39.45 cC	22.98 bB	-27.92 cC	-43.51 cC
Supi4 Barley × Baodamai6	0.36 cC	65.92 bB	1.12 bC	-22.66 cC	38.58 bB
Supi4 Barley × S500 Barley	-15.14 dE	-28.72 cC	-17.56 cC	-17.82 cC	-79.24 cC
Supi4 Barley × Zhepi33	1.67 cC	-43.25 cC	-7.33 cC	-18.67 cC	-66.58 cC
Supi4 Barley × E32380	-4.76 dD	-175.04 dE	14.85 bB	-12.19 cC	-178.19 dD
Baodamai6 × S500 Barley	7.35 bB	-141.89 dE	-2.03 cC	10.13 aB	-127.46 dD
Baodamai6 × Zhepi33	-8.47 dD	54.63 bB	13.77 bB	-31.02 cC	28.87 bB
Baodamai6 × E32380	-8.92 dD	698.23 aA	13.91 bB	33.17 aA	734.38 aA
S500 Barley × Zhepi33	2.45 cC	10.68 bB	-39.58 cC	12.78 aB	-14.03 cC
S500 Barley × E32380	-4.89 dD	-84.72 dD	-50.33 dD	-3.94 cC	-141.08 dD
Zhepi33 × E32380	-11.6 dE	-86.55 dD	22.98 bB	16.45 aB	-61.17 cC

In this table, the SCA effects for the flavonoid compounds—catechins, myricetin, quercetin, kaempferol, and total flavonoids—are presented for each of the seven barley varieties. Using the Griffing method, significant differences are indicated by lowercase letters (at the 5% level) and uppercase letters (at the 1% level) following each SCA value, with the cross combinations showing distinct levels of combining ability for each flavonoid compound

The wide variations noticed in SCA of flavonoid compounds among hybrid combinations suggest the involvement of non-additive gene effects and heterosis. High SCA values noticed in several combinations, viz. Hatiexi Barley × Yundamai2, Hatiexi Barley × Supi4 Barley, Hatiexi Barley × S500 Barley, and Baodamai6 × E32380, bring maximum flavonoid content by hybridization. Most notably, high SCA for catechins, myricetin, and total flavonoids belonged to Hatiexi Barley × S500 Barley, while for myricetin and total flavonoids; it belonged to Baodamai6 × E32380. Such heterosis presents a great opportunity for breeders in extending these into more practical applications of breeding towards better antioxidant properties in various barley cultivars (Kamara et al., 2021; Fekadu et al., 2023).

### *Genetic parameter analysis of flavonoids in barley*

As shown in *Table 5*, the dominant variance for flavonoids, including catechins, myricetin, quercetin, kaempferol, and total flavonoids content, reached highly significant levels. This suggests that these compounds can be effectively utilized in barley breeding programs to produce hybrid vigor. Besides, genetic and environmental variances for those compounds were highly significant, which indicated that the main factor responsible for their expressions is genetic (Bashir et al., 2023; Younus et al., 2024). Among them, myricetin and total flavonoids displayed the highest genetic determination, having 98.92 and 98.11%, respectively, showing that these compounds fall under the influence of more genetic factors than environmental similar findings reported by many scientists (Fekadu et al., 2023; Nowak et al., 2023). Following these, quercetin, kaempferol, and catechins had genetic determination values of 92.12%, 82.23%, and 76.43%, respectively. This indicates that these traits are strongly influenced by genetic factors, although they may also be more responsive to environmental conditions (Ali, 2024; Mahmood, 2024). The high heritability values for myricetin and total flavonoids suggest that these traits are particularly promising for selection in barley breeding programs aimed at enhancing flavonoid content. These results were align with the study of previously scientists (Bagues et al., 2021; Iannucci et al., 2021; Romayssa et al., 2024). Additionally, the significant dominance variance observed for most flavonoid compounds further underscores the potential for hybrid vigor in these traits.

**Table 5.** *Genetic parameters of flavonoids in barley*

<b>Genetic Parameter</b>	<b>Catechin</b>	<b>Myricetin</b>	<b>Quercetin</b>	<b>Kaempferol</b>	<b>Total Flavonoids</b>
Additive Variance	16.2032	0.00	1201.12	0.00	0.00
Dominant Variance	130.3056**	47000.4221**	8055.7732**	462.1592**	58000.2548**
Genetic Variance	146.4175**	47000.4221**	9205.5547**	462.1592**	58000.2548**
Environmental Variance	33.5914	512.1945	105.2321	101.3261	1150.325
Phenotypic Variance	179.7889**	47512.6166**	8315.0053**	563.4853**	59150.5801**
Heritability	76.43%	98.92%	92.12%	82.23%	98.11%

The estimate of the different parameters such as additive, dominant, genetic, and environmental variances allows one to form an idea about the relative role of genetic and environmental factors in the expression of these traits (Ijaz et al., 2023; Zeng et al., 2024). High estimates of heritability for myricetin (98.93%) and total Flavonoids (98.05%) reveal high genetic control and little interference due to the environment. Thus, other flavonoids like quercetin, kaempferol, and catechins exhibited high heritability and hence hold promise for selection during breeding. Previously scientist reported similar findings (Katiyar et al., 2021; Fekadu et al., 2023; Huang et al., 2024).

### **Conclusion**

These findings have brought out enormous genetic variation in flavonoid content among parental genotypes and their hybrid combinations. Some of these genotypes, specifically Yundamai2, Baodamai6, and Supi4 Barley, have obtained high flavonoid contents and hence are good candidates for further use in breeding programs concerning stress tolerance and improved antioxidant properties. Hybrid combinations, especially with Hatiexi Barley, resulted in high heterosis and SCA, hence indicating improved

flavonoid biosynthesis with hybrid vigor. GCA estimates supported the fact that these parents contributed a lot to the variation in different flavonoid compounds, hence confirming the presence of additive genetic effects for the studied traits. Overall, these results give an idea about the underlying genetic architecture for flavonoid production in this crop and form a basis for the development of barley varieties that harbor superior flavonoid contents-a very important feature for agricultural productivity and human health. Future breeding efforts can leverage these insights to enhance barley's nutritional profile and stress resilience.

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