

MULTI-REGIONAL STUDY OF GENOTYPE × ENVIRONMENT INTERACTIONS IN FLAVONOID ACCUMULATION OF BARLEY (*HORDEUM VULGARE* L.) GRAINS

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Abstract. Barley (*Hordeum vulgare* L.) is a nutritionally and functionally important crop, valued for its bioactive compounds, including flavonoids, which contribute to human health and crop resilience. This study evaluated the flavonoid content in grains of 15 barley genotypes grown in three regions of Yunnan Province: Kunming, Qujing, and Yuxi in China. Flavonoid analysis included catechin, myricetin, quercetin, kaempferol, and total flavonoids. Results revealed significant genotype- and region-specific variations in flavonoid accumulation. Yunpi 10 exhibited the highest catechin levels (65.78 µg/g) in Yuxi, while Yundamai 13YD-1 and Baodamai 17 showed the highest myricetin content (755.94 µg/g and 372.76 µg/g, respectively). Quercetin peaked in Yunpi 9 (38.45 µg/g) and kaempferol in Yunpi 11 (112.64 µg/g), both in Kunming. Total flavonoid content ranged from 47.53 to 490.45 µg/g, with Yundamai 13YD-1 reaching the highest level (805.16 µg/g) in Yuxi. Kunming and Yuxi regions generally supported higher flavonoid accumulation. Yundamai 13YD-1 and Baodamai 17, high in myricetin and total flavonoids, are promising for barley breeding programs. These findings highlight the influence of genetic and environmental factors on flavonoid biosynthesis in barley and identify promising genotypes for breeding programs focused on enhancing barley's nutritional and functional properties.

Keywords: *kaempferol, health, metabolites, traits, resistance, plant, genetics*

Introduction

Barley (*Hordeum vulgare* L.) is one of the most cultivated cereal crops, and one of the most important sources of nutrients. It is widely used in food, beverage, and livestock feed industries. Besides being consumed as a staple food, barley is recognized

for its functional components, mainly bioactive compounds such as flavonoids. Flavonoids are plant secondary metabolites with various beneficial human health impacts, such as antioxidant, anti-inflammatory, and anticancer activities (Pour-Aboughadareh et al., 2023; Huang et al., 2024). Plants from the genus *Hordeum*, mainly barley, have been studied for their flavonoid content, showing that flavonoid composition is highly variable among genotypes and growing environments. Due to the emerging interest in functional foods, improvement in the flavonoid content of barley is part of the current focus in agricultural research, mainly with regard to the variability of regional environmental factors and genotypes (Iannucci et al., 2021; Huang et al., 2024).

Flavonoids represent, especially in cereals like barley, an important constituent in the plant defense mechanism against numerous biotic and abiotic stresses. Part of the secondary metabolism of plants, these compounds are essential for regulating active oxygen species, modulating the immune responses of plants, and offering resistance to diseases caused by fungi, bacteria, and viruses. Quercetin, kaempferol, catechin, and myricetin flavonoids have been reported to enhance, in general, the ability of barley plants to resist stressors such as drought, disease, and UV radiation (Idehen et al., 2017). Increasing interest in flavonoids relates not only to their functions associated with crop resistance but also to possible health benefits for humans. Considering their well-documented antioxidant activity, flavonoids reduce oxidative damage, enhance immune function, and are supposed to protect from chronic diseases such as cardiovascular disease and cancer (Kazimierczak et al., 2020; Kumari et al., 2020). Therefore, the flavonoid profile of barley is very important for various reasons: not only in terms of increasing resistance, but also with respect to the nutritional quality of food products.

Extensive work on genotype and its effect on flavonoid biosynthesis has been conducted in various crops, one of which includes barley. It is the genetic machinery controlling the flavonoid accumulation in barley grains, though this is partly modified by environmental factors such as temperature, soil type, and geographical location (Fekadu et al., 2023; Hussain et al., 2024). Previous studies in barley showed a huge variation among the genotypes for flavonoid levels; some cultivars might produce more of these bioactive compounds, adding more nutritional value and possible health benefits (Fatemi et al., 2023; Ijaz et al., 2023). For example, in cereal crops such as wheat, there is often an indication that the genotypic selection might have a great influence over its flavonoid content—a breeding aspect of improvement in the nutritional and functional quality traits of crops (Han et al., 2018; Kadege et al., 2024). Understanding genotype-environment interactions will be critical to optimize barley breeding programs with respect to improving flavonoid content, a trait valued not only for its associated antioxidant activity but also for potentially beneficial nutritional effects.

The ability of barley for the synthesis of flavonoids is commanded mainly by genetic and environmental factors. Several works reported considerable variation in the flavonoid contents between various barley genotypes. Some genotypes accumulate large amounts of this bioactive important class of compounds, whereas others accumulate low amounts. Environmental factors associated with temperature, soil type, water availability, and altitude heavily influence the flavonoid production in barley (Rao et al., 2020; Chaieb et al., 2021). Since flavonoids are linked with disease resistance, their accumulation is all the more important from the point of view of sustainable agriculture, influenced by both genetic and environmental factors. The objective of this work was to investigate the response variation among different barley genotypes under a wide range of diverse

environmental conditions with respect to flavonoid production and their effects on disease resistance. Elucidation of the genotype \times environment interaction and its consequences on flavonoid biosynthesis could lead to a better-informed breeding strategy in identifying those genotypes that would show high flavonoid content and resistance against crop diseases (Mierziak et al., 2014; Holub et al., 2019; Victoria et al., 2023).

The contents of five major flavonoid compounds, such as catechin, myricetin, quercetin, kaempferol, and total flavonoids in grains, were different among the barley varieties within various regions of Yunnan. Currently, there are no reports on the variation in the said substance regarding barley varieties or at the regional level. The objective of the current paper is therefore to evaluate and analyze the content of five major flavonoid compounds from barley grains in different regions, which reflects the variation in flavonoids among barley varieties and regional variation in its content (Iannucci et al., 2021; Nowak et al., 2024). The objective of the experiment was to screen out the germplasm resources with high contents of catechin, myricetin, quercetin, kaempferol, and total flavonoids from barley varieties cultivated in three regions of Yunnan Province, namely Kunming, Qujing, and Yuxi. This study discusses the influence of genotype, environment, and genotype \times environment interaction on the content of five major flavonoid compounds, averages of different genotype performance under varied environments, and their sensitivity and stability analysis. It will provide a very important genetic basis not only for the development of functional barley germplasm and new variety breeding but also for the breeding of barley varieties with high contents of functional components and optimization of barley cultivation in various regions.

Materials and methods

The key characteristics and sources of the 15 barley varieties used in this study are shown in *Table 1*. All the genotypes are spring-type, hulled, two-row commercial cultivars, mainly grown for malting.

Table 1. List of studied 15 barley genotypes, their types and used for malting purpose

Sr.No.	Parent name	Source	Hulled type
1	Yunpi 9	Yunnan Academy of Agricultural Sciences	Two-row
2	Yunpi 10	Yunnan Academy of Agricultural Sciences	Two-row
3	Yunpi 11	Yunnan Academy of Agricultural Sciences	Two-row
4	Fengdamai 7	Dali Academy of Agricultural Sciences	Two-row
5	Fengdamai 8	Dali Academy of Agricultural Sciences	Two-row
6	Kunpi 1	Kunming Academy of Agricultural Sciences	Two-row
7	Kunpi 2	Kunming Academy of Agricultural Sciences	Two-row
8	S-4	Midu County Seed Management Station	Two-row
9	Yunpi 12	Yunnan Academy of Agricultural Sciences	Two-row
10	Yunpi 13	Yunnan Academy of Agricultural Sciences	Two-row
11	Baodamai 17	Baoshan Academy of Agricultural Sciences	Two-row
12	Yundamai 13YD-1	Yunnan Academy of Agricultural Sciences	Two-row
13	ChuB11-618	Chuxiong Academy of Agricultural Sciences	Two-row
14	Kunpi 3	Kunming Academy of Agricultural Sciences	Two-row
15	Jingdamai 2	Qujing Academy of Agricultural Sciences	Two-row

Experimental location

The experiment was conducted at three locations in Yunnan Province, China: Kunming, Qujing, and Yuxi. Kunming is situated at an altitude of 1891 m, with geographic coordinates of 25°05'N latitude and 102°40'E longitude. The soil physicochemical properties include a pH of 6.74, organic matter content of 32.36 g/kg, hydrolyzable nitrogen of 132.15 mg/kg, available phosphorus of 25.81 mg/kg, and available potassium of 221.35 mg/kg. During the growing season, the average temperature is 15°C, with an annual rainfall of 950 mm and an average relative humidity of 70%. Qujing is located at an altitude of 1900 m, with coordinates 25°53'N latitude and 103°35'E longitude. The soil properties include a pH of 6.32, organic matter content of 41.53 g/kg, hydrolyzable nitrogen of 163.36 mg/kg, available phosphorus of 15.32 mg/kg, and quick-acting potassium of 208.16 mg/kg. The climatic conditions during the growing season include an average temperature of 14°C, annual precipitation of 624 mm, and relative humidity of 65%. Yuxi, positioned at an altitude of 1638 m with coordinates 24°22'N latitude and 102°32'E longitude, has soil with a pH of 6.08, organic matter content of 24.38 g/kg, hydrolyzed nitrogen of 115.46 mg/kg, available phosphorus of 28.52 mg/kg, and available potassium of 186.74 mg/kg. The growing season is characterized by an average temperature of 20°C, annual rainfall of 991 mm, and relative humidity of 75%.

Experimental design

Each site had 45 experimental plots that measure 1 m \times 1 m replicated thrice. The experiment was laid out in a randomized block arrangement. The varieties of barley used in the experiment were 15 in number and were grown in the 45 plots. The same treatment was applied to each plot. Land preparation involved deep plowing one week before sowing and removal of weeds. The method of sowing followed was row planting; the row spacing was 30 cm and the depth of sowing varied from 2-4 cm. Fertilization was carried out in two stages. As a base fertilizer, 225 kg/ha of urea (46% N) and 600 kg/ha of ordinary superphosphate (16% P₂O₅) were applied to enhance soil fertility. Additionally, a tillering-stage fertilizer application included 225 kg/ha of urea (46% N) to support vigorous growth and tiller development. Irrigation was conducted using a sprinkling irrigation system, ensuring uniform water distribution across the field to meet crop water requirements. For pest management, imidacloprid was applied at a rate of 15 g/ha to control aphid infestations and minimize potential damage to the barley plants.

Contents determination

Barley seeds from different regions were collected to determine catechin, myricetin, quercetin, kaempferol and total flavonoid contents. The extraction and quantification were conducted using an optimized ultrasonic-assisted extraction method followed by high-performance liquid chromatography (HPLC) analysis. For extraction, barley seeds were finely ground, and 1.0 g of the powdered sample was weighed into a conical flask with a stopper. Methanol (10 mL) was added, maintaining a solvent-to-sample ratio of 1:10 (w/v). The mixture was tightly sealed and subjected to ultrasonic extraction for 30 min at an ultrasonic power of 90 W. HPLC analysis was performed using a gradient elution method. The mobile phase consisted of solvent A (0.1% acetic acid aqueous solution) and solvent B (acetonitrile). The gradient elution program was as follows: 0–5 min, 10% A;

5–10 min, 20% A; 10–17 min, 40% A. The flow rate was set at 0.8 mL/min, with a detection wavelength of 280 nm and an injection volume of 10 μ L. These conditions ensure precise quantification of flavonoid content in the barley samples.

Data analysis

Data on catechins, myricetin, quercetin, kaempferol, and total flavonoid contents were collected for statistical analysis. A three-way factorial ANOVA under a randomized complete block design (RCBD) was used to assess the interaction effects of genotypes across different regions. Genetic variance, environmental variance, and genotype \times environment interaction variance was estimated to evaluate the influence of genetics and environment on the levels of catechins, myricetin, quercetin, kaempferol, and total flavonoids in barley grains. Post hoc LSD comparisons were further applied to compare the different varieties across the studied regions (Box, 1980).

Results and discussion

Analysis of variances for genotype-environment interaction

Results of variance analysis for catechin, myricetin, quercetin, and kaempferol contents from the grains of 15 varieties (genotypes) at 3 locations (environments) are presented in *Table 2*. ANOVA showed that there were significant differences among these active ingredients. Variance analysis for catechin content showed very highly significant effects due to genotype, environment, and genotype \times environment interaction. The most profound contribution was due to genotypic variation, followed by G \times E interaction, while environmental variation had the lowest contribution. This could be interpreted that genetic predisposition is of major importance in the determination of catechin content while genotypic-environmental interaction presented much more importance than environmental conditions (Pour-Aboughadareh et al., 2023; Huang et al., 2024).

Table 2. Analysis of variance of flavonoids contents in 15 varieties of barley across 3 regions

Compound	Block	Environment	Genotype	Genotype \times Environment
Catechin	1.56842*	175.6321**	433.5263**	198.3542**
Myricetin	3.12174	8.40216**	15.52967**	4.52812*
Quercetin	12.09842*	1062635**	1689020**	548623**
Kaempferol	4.72698*	5384.756**	8397.521**	6312.514**
Total flavonoids	2.56742	10.0387**	21.8251**	4.23281**

* Indicates that the F-value test reached a significant level, and ** indicates that the F-value test reached a highly significant level

Additionally, in terms of myricetin content, highly significant effects of both genotype and environment, as well as their interaction, were observed. Most variation was contributed by genotypic variation, followed by variation in environments. The effect of G \times E interaction is rather small. This suggests that myricetin content is controlled mainly genetically, while there is an important contribution from

environmental aspects (Fekadu et al., 2023; Hussain et al., 2024). ANOVA of quercetin content revealed highly significant variance due to genotype, environment, and G \times E interaction. Their order of magnitude was genotypic variance > G \times E interaction > environmental variance, indicating that quercetin content is under tight genetic control, and genotype \times environment interactions are more important compared to the environment itself (Iannucci et al., 2021; Huang et al., 2024).

In the kaempferol content, significant variance appeared for genotype, environment, and G \times E interaction. The highest magnitude of genetic variances was followed by significant environmental effects, but the role of G \times E interaction was minor. It means that the accumulation of kaempferol is driven mostly by genetic reasons, but the environmental component is not negligible. The ANOVA showed that the total flavonoid content had highly significant variations due to genotype, environment, and G \times E interaction in the decreasing order. Therefore, it may be concluded that the total flavonoid content is under the primary control of genetics with a relatively high participation of environmental factors (Vaschenko et al., 2021; Shamuyarira et al., 2022).

In the present work, the content of catechin, myricetin, quercetin, and kaempferol was analyzed in the grains of 15 barley varieties. The results showed that catechin, myricetin, quercetin, and kaempferol contents differed significantly among most varieties, but the contents of total flavonoids did not differ significantly. Among these, myricetin and quercetin were only high in some varieties, with trace or absence in other varieties (Martínez et al., 2018; Iannucci et al., 2021; Baloch et al., 2024). Varieties with relatively stable flavonoid content, with the selection of pairwise varying combinations of high and low, the crossing with each other of quercetin-containing varieties and those containing no quercetin, analyzing flavonoid content in hybrid offspring, can be further supported theoretically for a genetic study of barley flavonoids (Han et al., 2018; Fekadu et al., 2023).

The analysis of variance revealed that flavonoid accumulation in barley grains is significantly influenced by genetic, environmental, and genotype \times environment (G \times E) interaction effects, though their relative contributions varied among different flavonoid compounds. Catechin content was predominantly controlled by genetic factors, as indicated by the highest genotypic variance, while G \times E interactions played a substantial role, suggesting that specific genotypes respond differently to environmental conditions. Similarly, quercetin and kaempferol exhibited strong genetic control, with highly significant G \times E interactions, reinforcing the importance of selecting stable genotypes with consistently high flavonoid content across diverse environments (Vaschenko et al., 2021; Shamuyarira et al., 2022). In contrast, myricetin and total flavonoid content showed relatively lower G \times E interaction effects, implying that their accumulation was more influenced by genetic predisposition rather than environmental variability. The lower contribution of environmental variance across all compounds suggests that while environmental factors influence flavonoid biosynthesis, genetic factors play a dominant role in determining their levels. These findings highlight the necessity of integrating both genetic selection and environmental optimization strategies in breeding programs aimed at enhancing flavonoid-rich barley varieties (Holub et al., 2019; Huang et al., 2024).

Performance of 15 barley genotypes in different regions for Flavonoid Compounds

The present study deals with the contents of five major flavonoid components such as catechin, myricetin, quercetin, kaempferol, and total flavonoids among the 15 varieties representative of three distinct regions of Yunnan Province, namely, Kunming, Qujing,

and Yuxi (Figs. 1–3). These figures illustrate the flavonoid content profiles of 15 barley varieties from the Kunming, Qujing and Yuxi region, with each variety represented by a distinct color. The concentric circles represent different flavonoid compounds in barley grains from the respective region. The innermost circle corresponds to catechin, the second circle to myricetin, the third to quercetin, the fourth to kaempferol, and the outermost circle represents the total flavonoid content. The mean concentration values for each flavonoid compound are shown for each barley variety. This figure provides a comparative overview of the flavonoid composition across the barley varieties, facilitating the assessment of differences in individual and total flavonoid contents within the Yuxi region. The findings in these figures showed that genetic and regional variation in flavonoid contents underlines the potential of barley functional breeding for nutritional quality improvement and disease resistance (Yang et al., 2019).

Catechin content

It is clear that the three regions differ in catechin content. In Kunming, Yunpi 10 has the highest concentration of 44.65 µg/g, followed by Yunpi 9 and Yunpi 11, having a concentration of 9.12 and 10.56 µg/g, respectively (Fig. 1). Qujing showed that Yunpi 10 was still at the top, having the highest catechin level of 24.5 µg/g, while Yunpi 9 had a value of 8.74 µg/g and Yunpi 11 11.35 µg/g (Fig. 2). Lastly, Yuxi still kept Yunpi 10 at the top, having the highest catechin content of 65.78 µg/g, far above the rest, including Yunpi 9 with 11.02 µg/g and Yunpi 11 with 36.48 µg/g (Fig. 3). From this overall trend, it is clear that Yunpi 10 yields a high level of catechin across the three regions and therefore would be a great variety for use in breeding a catechin-enriched barley antioxidant useful in crop production and human nutrition (He et al., 2012; Yang et al., 2019; Deng et al., 2021).

Myricetin content

Myricetin is one among the powerful antioxidants showing striking regional variation with anti-inflammatory activities. Among them, Fengdamai 7 and Kunpi 2 had the highest myricetin contents in Kunming, followed by 132.58 and 125.42 µg/g, respectively (Fig. 1). The rest of the varieties, Yunpi 9 and S-4, did not show detectable myricetin content. Baodamai 17 and Yundamai 13YD-1 were the high myricetin-producing varieties in Qujing, with concentrations of 278.13 and 198.85 µg/g, respectively. These were significantly higher when compared to Fengdamai 7 and Yunpi 10, which recorded 0 µg/g each (Fig. 2). The highest values for Yuxi were from Baodamai 17 and Yundamai 13YD-1 at 372.76 and 755.94 µg/g, respectively, among the highest values among the three regions (Fig. 3). This indicates that the two tested varieties, Baodamai 17 and Yundamai 13YD-1, would be a potential candidate in a breeding program with the purpose of improving myricetin content in barley. These two varieties had the highest content across the region and hence could be used to potentially improve the antioxidant property of barley (Kim et al., 2007; Yang et al., 2019).

Quercetin content

Quercetin content is relatively low in all regions. Among the cultivars in Kunming, Yunpi 9 has the highest content of 38.45 µg/g, followed by Fengdamai 7 (0 µg/g) and S-4 with 35.64 µg/g (Fig. 1). Qujing has modest levels of quercetin, with Yunpi 9 containing the highest at 13.89 µg/g (Fig. 2). Other clones have none, including

Fengdamai 7 (0 $\mu\text{g/g}$) and S-4 (0 $\mu\text{g/g}$). In Yuxi, Yunpi 9 has the moderate quercetin content (15.78 $\mu\text{g/g}$), and none of the varieties could overpass this threshold by a large margin (Fig. 3). The low level of quercetin across all regions might suggest that it is not the major flavonoid in these cultivars of barley; its presence in Yunpi 9 across all regions does, however, hold good promise for breeding targeted to increase quercetin concentration (Zeng et al., 2014; Zhang et al., 2021).

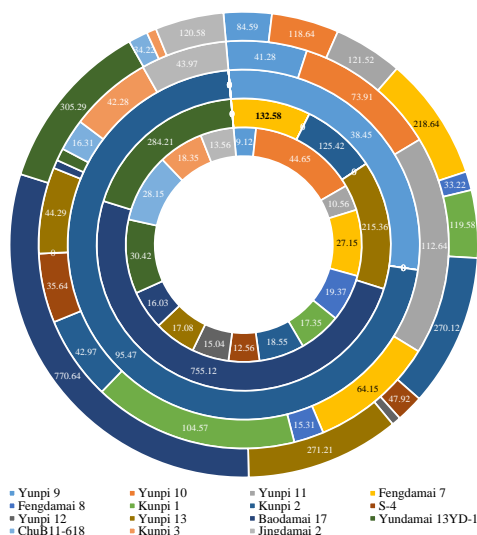


Figure 1. Different colors represent the 15 barley varieties. In this figure, the first (inner) circle represents catechin, the second circle represents myricetin, the third circle represents quercetin, the fourth circle represents kaempferol, and the fifth (outer) circle represents total flavonoid content in barley grains from different varieties in the Kunming region. The mean values for each variety are shown for the respective flavonoid compounds

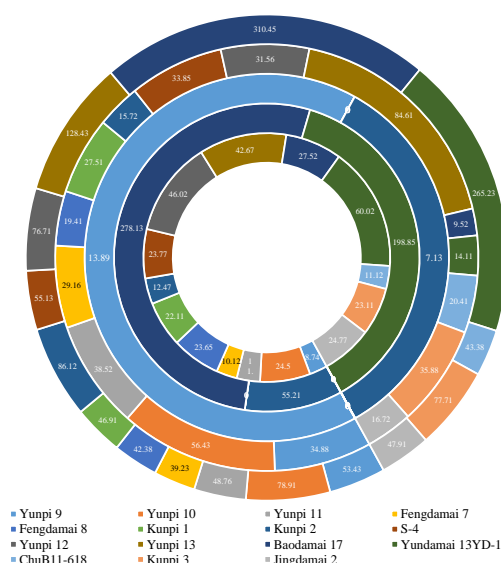


Figure 2. Different colors represent the 15 barley varieties. In this figure, the first (inner) circle represents catechin, the second circle represents myricetin, the third circle represents quercetin, the fourth circle represents kaempferol, and the fifth (outer) circle represents total flavonoid content in barley grains from different varieties in the Qujing region. The mean values for each variety are shown for the respective flavonoid compounds

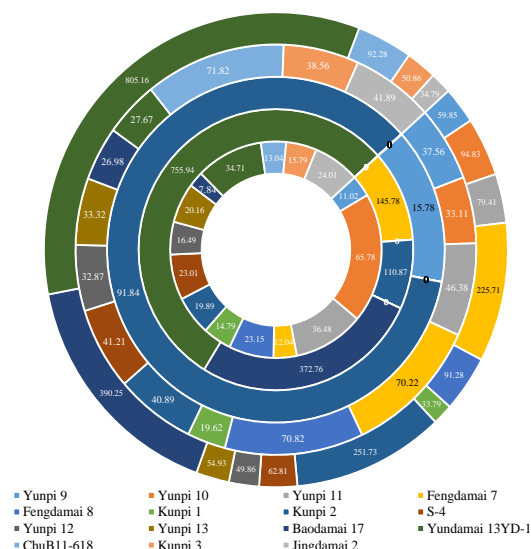


Figure 3. Different colors represent the 15 barley varieties. In this figure, the first (inner) circle represents catechin, the second circle represents myricetin, the third circle represents quercetin, the fourth circle represents kaempferol, and the fifth (outer) circle represents total flavonoid content in barley grains from different varieties in the Yuxi region. The mean values for each variety are shown for the respective flavonoid compounds

Kaempferol content

The concentration of kaempferol also showed a high variation. In Kunming, Yunpi 11 (112.64 µg/g) exhibited the highest kaempferol content, followed by Kunpi 1 (104.57 µg/g) and S-4 (35.64 µg/g) (Fig. 1). In Qujing, varieties of Yunpi 10 (56.43 µg/g) and Baodamai 17 (9.52 µg/g) had the most remarkable kaempferol contents, while most cultivars showed low values, including Yunpi 9 (Fig. 2). In Yuxi, Fengdamai 7 (70.22 µg/g) and Yunpi 11 (46.38 µg/g) exhibited a higher kaempferol content, and Yunpi 9 and Yunpi 12 have middle contents (Fig. 3). Being an important flavonoid for antioxidant and anti-inflammatory activities, the above genotypes, such as Yunpi 11, Yunpi 10, and Fengdamai 7, were considered the most important candidates for the breeding program to enhance kaempferol content in barley. Similar findings reported by some scientists (Xiao Ya et al., 2017; Han et al., 2018; Kumari et al., 2020).

Total flavonoids content

Total flavonoid content, which sums up the contents of catechin, myricetin, quercetin, and kaempferol, varies greatly among all barley varieties. The highest total flavonoid concentrations in Kunming were detected in Kunpi 2 and Fengdamai 7, with high values of 270.12 µg/g and 218.64 µg/g, respectively, thus showing great potential in breeding high-quality barley with high functional components (Fig. 1). The highest flavonoids were detected in Qujing from Baodamai 17 and Yundamai 13YD-1, with values of 310.45 µg/g and 265.23 µg/g, respectively (Fig. 2). Lastly, Yundamai 13YD-1 exhibited a total flavonoid content of 805.16 µg/g in Yuxi; the next highest, Baodamai 17 and Kunpi 2, had 390.25 µg/g and 251.73 µg/g, respectively (Fig. 3). It follows that interregionally, several are high performers; varieties such as Yundamai 13YD-1 and

Baodamai 17 should be regarded as the main resources for breeding high flavonoid concentration barley. This is the best candidate for increasing the general functional quality of barley through breeding. These results supported by the previously findings (Mierziak et al., 2014; Zhu et al., 2015; Desta et al., 2024).

Comparison of differences in flavonoid compounds in barley grains from different varieties

In the present study, it can be found from *Table 3* that the catechin content of barley grains ranged from 9.63 to 44.98 µg/g among the investigated varieties. Large variation was observed within these varieties. The catechin concentrations of Yunpi 10 and Yundamai 13YD-1 were remarkably higher than those of the other varieties; the differences were statistically significant. Their contents in these two varieties were far higher when compared with the other genotypes.

Table 3. *Flavonoids contents in barley grain of 15 barley varieties and their mean values across different regions using LSD multiple comparison method*

Varieties	Catechin (µg/g)	Myricetin (µg/g)	Quercetin (µg/g)	Kaempferol (µg/g)	Total flavonoids (µg/g)
Yunpi 9	9.63 hG	0	22.71 bB	37.91 eE	65.96 deC
Yunpi 10	44.98 aA	0	0	54.48 bB	97.46 cdeBC
Yunpi 11	19.46 fEF	0	0	65.85 aA	83.23 cdeBC
Fengdamai 7	16.44 gF	92.79 bB	0	54.51 bB	161.19 bcBC
Fengdamai 8	22.06 dD	0	0	35.18 fF	55.63 deC
Kunpi 1	18.08 fEF	0	0	50.57 cC	66.76 cdeC
Kunpi 2	16.97 fgF	97.17 bB	64.81 aA	33.19 fF	202.66 bB
S-4	19.78 eE	0	0	36.9 dD	55.29 deC
Yunpi 12	25.85 cC	0	0	21.48 gG	47.53 eC
Yunpi 13	26.64 cC	71.79 bB	0	54.07 bB	151.52 bcdBC
Baodamai 17	17.13 fgF	468.67 aA	0	13.59 iI	490.45 aA
Yundamai 13YD-1	41.72 bB	413 aA	0	16.05 hH	458.56 aA
ChuB11-618	17.44 gF	0	0	36.18 fF	56.63 deC
Kunpi 3	19.08 fEF	0	0	38.91 eE	48.53 eC
Jingdamai 2	20.78 eE	0	0	34.19 fF	67.76 cdeC

Small letter indicated at 5% significance level and Capital letter indicated at 1% significance level

The myricetin content values of all analyzed cultivars varied between 0 and 468.67 µg/g, with relatively large differences being detected among them. In comparison with other varieties, such as Yunpi 13, Kunpi 2, and Fengdamai 7, Yundamai 13YD-1 and Baodamai 17 showed a significantly higher content of myricetin. These varieties showed highly significant differences. No significant difference was observed either between Yundamai 13YD-1 and Baodamai 17 or between Yunpi 13, Kunpi 2, and Fengdamai 7. Myricetin could not be detected in a number of varieties.

For the quercetin content, the range was 0 to 64.81 µg/g among the varieties for this parameter. There were large differences in the quercetin content among the studied genotypes. The content of quercetin was significantly higher in Yunpi 9 and Kunpi 2 than that in the other varieties, although it was undetectable in the rest of the barley genotypes, which further proves the selective accumulation of quercetin within these

two varieties. The content of kaempferol ranged from 13.59 to 65.85 µg/g, with very significant differences among the investigated varieties of barley. Yunpi 11 and Fengdamai 7 had the highest content of kaempferol and were significantly different from the other varieties investigated. Others showed relatively lower contents of kaempferol, though there were significant differences among them.

The total flavonoid (TF) content varied between 47.53 and 490.45 µg/g, with highly significant differences at a 1% probability level among different varieties of barley. The TF of Yundamai 13YD-1 and Baodamai 17 was significantly higher than those of Yunpi 13, Kunpi 2, Fengdamai 7, Yunpi 11, and Yunpi 10. Yundamai 13YD-1, Baodamai 17, and Kunpi 2 contained a higher TF concentration than Yunpi 12, S-4, Kunpi 1, Yunpi 10, and Yunpi 9. Yundamai 13YD-1 and Baodamai 17 did not have any significant variance in TF, showing almost the same profile for their accumulation.

These results pointed out that there are tremendous differences in barley grain flavonoid composition among different cultivars and underlined the paramount role of genetic background and environmental conditions for flavonoid accumulation (Jin et al., 2022; Eid et al., 2023). In other words, a few of the var. Yundamai 13YD-1 and var. Baodamai 17 can be regarded as promising sources, especially when considering myricetin and total flavonoids.

Comparison of flavonoid compounds in barley grains across different regions

As can be seen from *Table 4*, large variation was observed with respect to flavonoids in three regions: Kunming, Qujing, and Yuxi. Catechin contents ranged from 21.52 to 27.58 µg/g, and its distribution showed large regional variation, ranking Qujing > Yuxi > Kunming, which reflected that the environmental condition in Qujing was more suitable for its biosynthesis (Dyulgerova et al., 2016; Yang et al., 2018). The concentrations of myricetin ranged from 120.42 to 302.44 µg/g, showing great variations in the different regions. Higher levels of myricetin were detected in Kunming and Yuxi, showing more favorable environmental factors in these two regions for the production of myricetin. Quercetin contents exhibited wide regional variations, from 9.52 to 65.24 µg/g. Its highest level was detected in Kunming, followed by Yuxi and Qujing; this revealed that environmental conditions relative to quercetin synthesis in Kunming were much better than in the rest of the areas under study.

Table 4. *Flavonoids contents in barley grain in the different regions and average values of 15 barley varieties using LSD multiple comparison method*

Compound	Kunming	Qujing	Yuxi
Catechin (µg/g)	21.52 bB	27.58 aA	24.85 cC
Myricetin (µg/g)	302.44 aA	120.42 bB	275.91 aA
Quercetin (µg/g)	65.24 aA	9.52 cC	52.93 bB
Kaempferol (µg/g)	46.79 aA	33.25 cC	39.87 bB
Total flavonoids (µg/g)	210.83 aA	105.41 bB	193.49 aA

Small letter indicated at 5% significance level and Capital letter indicated at 1% significance level

The concentration of kaempferol ranged from 33.25 to 46.79 µg/g. Very highly significant differences were observed among regions. The highest content of kaempferol

was found in Kunming, followed by Yuxi and Qujing, indicating that the environmental conditions of Kunming were very fit for producing kaempferol. Total flavonoids in flowers were between 105.41 and 210.83 $\mu\text{g/g}$; great significant differences existed among regions. These data also showed that, while the environments of Kunming and Yuxi favored higher total flavonoid accumulation than in Qujing, the overall favorable effects of environmental conditions in Kunming and Yuxi on the whole biosynthesis process were underlined. This therefore may indicate that regional environmental factors could contribute to great changes in the flavonoid profiles of barley by regulating both the quality and quantity of flavonoid compounds (Ge et al., 2021; Desta et al., 2024).

Such large variations among the three regions reveal that flavonoids are dependent on genotype and environment for their final concentration. While some cultivars, like Yundamai 13YD-1 and Baodamai 17, have performed consistently across all the regions, other genotypes have varied region-wise for flavonoid content. This variation, with respect to flavonoid content, indicates that environmental factors like soil conditions, climate, and farming practices might also participate in regulating the expression of flavonoid biosynthesis pathways. $G \times E$ interaction studies will be of immense help in optimizing cultivation areas of barley for high flavonoid production in different ecological zones (Huang et al., 2024).

The results of this study provide a reference for selection in breeding programs for nutritional and functional quality improvement in barley. For example, Yundamai 13YD-1, Baodamai 17, Kunpi 2, and Yunpi 10 possess favorable high flavonoid contents, especially myricetin, kaempferol, and total flavonoids, which could be considered as sources in developing new varieties with better antioxidant activities and disease resistance. On the other hand, low flavonoid contents in some varieties, like Kunpi 1 and Jingdamai 2, indicate that genotypes of such low flavonoid content should be modified by breeding or genetic means to improve their respective flavonoid compositions (Liang et al., 2022). The performance of barley genotypes in terms of flavonoid content varies significantly across different environments, with some genotypes consistently exhibiting high flavonoid accumulation, while others perform well only in specific regions. Yunpi 10 demonstrated the highest catechin content across all three regions—Kunming (44.65 $\mu\text{g/g}$), Qujing (24.5 $\mu\text{g/g}$), and Yuxi (65.78 $\mu\text{g/g}$)—indicating its genetic stability in catechin production regardless of environmental conditions. Similarly, Baodamai 17 and Yundamai 13YD-1 consistently ranked as top performers for myricetin content, particularly excelling in Yuxi (372.76 $\mu\text{g/g}$ and 755.94 $\mu\text{g/g}$, respectively), suggesting that their myricetin accumulation is enhanced in higher-altitude conditions (Mierziak et al., 2014; Tang et al., 2024).

Conversely, flavonoid content in some genotypes appeared to be region-specific. For instance, Yunpi 9 exhibited the highest quercetin concentration in Kunming (38.45 $\mu\text{g/g}$), yet its levels were significantly lower in Qujing (13.89 $\mu\text{g/g}$) and Yuxi (15.78 $\mu\text{g/g}$), implying that quercetin synthesis in this genotype is influenced by environmental factors. Similarly, Yunpi 11 displayed superior kaempferol accumulation in Kunming (112.64 $\mu\text{g/g}$) but showed only moderate performance in other regions. Total flavonoid analysis further reinforced these patterns, with Yundamai 13YD-1 achieving the highest content in Yuxi (805.16 $\mu\text{g/g}$), while Kunpi 2 and Fengdamai 7 led in Kunming (270.12 $\mu\text{g/g}$ and 218.64 $\mu\text{g/g}$, respectively). These findings suggest that while certain genotypes maintain consistently high flavonoid levels across regions,

others exhibit environment-dependent variations, emphasizing the need for region-specific breeding strategies (Liang et al., 2022).

Flavonoid accumulation in barley is significantly influenced by regional environmental factors, including temperature, soil type, and altitude, which collectively impact plant metabolism and secondary metabolite biosynthesis. Higher altitudes, such as those in Yuxi, are often associated with increased UV radiation, which has been shown to enhance flavonoid production as a protective response against oxidative stress (Zoratti et al., 2014; Qaderi et al., 2023). Similarly, temperature plays a crucial role in regulating enzyme activity involved in flavonoid biosynthesis, with cooler temperatures generally favoring higher flavonoid accumulation (Jaakola and Hohtola, 2010; Jaakola, 2013). The observed variations in catechin, myricetin, and kaempferol content among barley genotypes across Kunming, Qujing, and Yuxi may thus be attributed to differences in temperature regimes and exposure to environmental stressors. Soil composition also exerts a significant influence on flavonoid levels, particularly through nutrient availability and pH balance. Qujing, known for its relatively fertile soil with moderate organic matter, may contribute to enhanced nutrient uptake, thereby supporting the synthesis of specific flavonoids such as myricetin and quercetin. Conversely, Yuxi's soil, which may be more nutrient-deficient or alkaline, could induce mild stress conditions that stimulate flavonoid accumulation as a defense mechanism (Mierziak et al., 2014; Tang et al., 2024). These findings align with previous research demonstrating that environmental stressors, including soil characteristics and climatic conditions, modulate flavonoid biosynthesis in crops, highlighting the importance of selecting region-specific barley genotypes for enhanced functional properties and nutritional benefits.

Conclusion

This study highlights the strong influence of genetic and environmental factors on flavonoid accumulation in barley, providing valuable insights into genotype × environment interactions affecting key bioactive compounds. Significant differences in flavonoid content were observed among genotypes across Kunming, Qujing, and Yuxi. Notably, Yunpi 10 exhibited the highest catechin content across all regions, making it a promising candidate for breeding programs focused on enhancing antioxidant properties. Yundamai 13YD-1 and Baodamai 17 demonstrated superior myricetin accumulation, while Yunpi 9 and Yunpi 11 showed high quercetin and kaempferol concentrations, respectively. Total flavonoid content peaked at 805.16 µg/g in Yundamai 13YD-1 (Yuxi), reinforcing the role of regional conditions in optimizing flavonoid biosynthesis. Beyond the studied regions, these high-performing genotypes hold significant potential for breeding stress-resistant barley varieties and developing functional food products rich in bioactive compounds. Selecting and cultivating flavonoid-rich barley could enhance nutritional value and confer health benefits, while also improving plant resilience to environmental stresses. Future research should explore the adaptability of these genotypes in diverse agro-climatic zones to facilitate their broader utilization in breeding programs aimed at improving both the functional and agronomic traits of barley.

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