FLAVONOID VARIABILITY IN BARLEY GENOTYPES FOR FUNCTIONAL FOOD AND SUSTAINABLE BREEDING

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Abstract. Barley (*Hordeum vulgare*) is increasingly recognized for its functional properties, particularly its flavonoid content, which contributes to various health benefits including antioxidant, anti-inflammatory, and anticancer effects. This study evaluates the genetic diversity of flavonoid content across 20 barley genotypes, focusing on key compounds including Catechin, Myricetin, Quercetin, Kaempferol, and total flavonoids. The experiment was conducted using a Randomized Complete Block Design (RCBD) with three replications. Chromatography (HPLC) was used to measure the flavonoid content, and data were analyzed using Analysis of Variance (ANOVA), Least Significant Difference (LSD) test, and Principal Component Analysis (PCA). Significant genotypic differences were observed in all flavonoid content, with Myricetin, Quercetin, and total flavonoids showing the highest levels of variability. Genotypes V2, V4, and V1 exhibited the highest Catechin content, making them promising for the antioxidant properties of barley. For Myricetin, V18 and V7 were the best performers, while V4 and V1 demonstrated high Quercetin content. Kaempferol content was highest in V18, indicating its potential for both health and plant defense benefits. Total flavonoid content was dominated by V1 and V4, highlighting their suitability for breeding programs aimed at improving barley's nutritional and functional attributes. PCA identified the primary components influencing flavonoid variability, with the first two components explaining 81.16% of the total variation. The study concludes that the genotypes V1, V4, V7 and V18 exhibit superior flavonoid profiles, which are valuable for future barley breeding programs focused on enhancing both health-promoting properties and disease resistance.
Keywords: *catechin, myricetin, nutrition, genetics, antioxidants, chromatography*

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Introduction

Barley (*Hordeum vulgare*) is one of the most important cereal crops worldwide and is cultivated for various uses, including food, feed, and malt production. Interest in the functional properties of barley has increased in recent years due to its nutritional value and potential health benefits, particularly its flavonoid content (Iannucci et al., 2021; Shvachko et al., 2021). Flavonoids are a diverse group of polyphenolic compounds known for their antioxidant, anti-inflammatory, anticancer, and cardiovascular benefits. Among the numerous flavonoids found in barley, catechin, myricetin, quercetin, and kaempferol are particularly notable due to their bioactive effects on human health and their potential role in enhancing plant resistance to environmental stressors including disease and abiotic stress (Loskutov and Khlestkina, 2021; Meng et al., 2023).

The variability of different barley genotypes in flavonoid content contributes to an immense scope for breeding programs, which not only improve nutritional quality but also enhance tolerance against different biotic and abiotic stresses. Knowledge regarding factors controlling flavonoid biosynthesis in barley is essential in the selection of high-flavonoid varieties which could be incorporated into further breeding programs (Han et al., 2018; Nowak et al., 2023). Present research is targeted at establishing the flavonoid content of 20 barley genotypes in respect of catechin, myricetin, quercetin, and kaempferol concentrations and that of total flavonoids. The aim of the research was to establish the genetic diversity of the abovementioned flavonoids in studied genotypes, thus enabling the use of such genotypes for breeding new cultivars with better functional and nutritional quality (Derakhshani, 2019; Huang et al., 2024).

The secondary metabolite flavonoids synthesized via the phenylpropanoid pathway depend on genetic and environmental factors for their biosynthesis. Previous studies show that flavonoids accumulated in plants are not genetically predetermined but result from the interaction among environmental factors like light, temperature, soil, and water (Mohamed et al., 2010; Yang et al., 2019). In barley, environmental factors strongly influence the expression of flavonoid biosynthesis genes, thereby controlling the specific accumulation of flavonoids. A full understanding of G × E interaction will be hence important for selecting those barley varieties which possess stable and high flavonoid content under different growing conditions (Yang et al., 2013; Geng et al., 2022).

In addition to the genetic and environmental aspects, the antioxidant properties of flavonoids make them of particular interest in barley due to their potential health benefits. Catechin, for example, has been widely studied for its antioxidant activity, which helps to neutralize free radicals in the body and reduce oxidative stress (Han et al., 2018). Myricetin and quercetin also have anti-inflammatory, anti-cancer, and cardio-protective effects. Another most important flavonoid, kaempferol, was associated with the reduction of oxidative damage and was regarded as a powerful natural compound to improve human health. The accumulation of these flavonoids in barley grains will hence lead to increased health properties of barley-based products and therefore its manipulation becomes very important for functional foods development (Peukert et al., 2013; Friero et al., 2024).

Interest in the genetic background to flavonoid accumulation in barley has been very strong in recent years. Several works have been published which have discussed the involvement of genes on the pathway to flavonoid biosynthesis: for instance, PAL, CHS, and F3'H genes. These genes provide the key intermediates in the biosynthetic pathway of flavonoids. The amount of flavonoids produced by each step in the pathway will be dependent on the level of expression of these flavonoid biosynthesis genes. In addition, it also explains that the presence of alleles of these genes in some barley genotypes may

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account for the variation among the genotypes with respect to flavonoid content (Hambira, 2010; Derakhshani et al., 2020).

The importance of flavonoids in human nutrition and health motivated the screening of barley varieties with high contents of those compounds that can be used in the production of functional foods having specific health benefits. Moreover, barley is an important model cereal for the elucidation of the genetic control of flavonoids biosynthesis, providing knowledge that can be extended to other crops, specially those with related health benefits (Katiyar et al., 2021; Raj et al., 2023). By selecting genotypes with higher flavonoid content, breeders can enhance the nutritional value of barley and improve its suitability for health-conscious consumers.

These results make a valuable contribution to the understanding of the genetic basis of flavonoid accumulation in barley and form useful insights in designing future breeding aimed at the improvement of nutritional and functional quality in barley (Fatemi et al., 2023; Ijaz et al., 2023). Development of high-flavonoid genotypes will help not only the barley industry, which will produce value-added barley-based products with enhanced health benefits, but also the scientific community in general, since the genetic regulation of bioactive compounds is an area of active research in the realm of functional genomics (Niazi et al., 2022; Rawat et al., 2023).

The present study has pointed out the genetic diversity in flavonoid content among barley genotypes and underlined their potentials for improving the nutritional and health-promoting properties of barley by breeding. Thus, this paper identifies genotypes bearing superior flavonoid profiles, providing perspectives for developing barley varieties that can give enhanced health benefits to the consumer with environmental sustainability. Further studies at the genetic level are required in barley for flavonoid biosynthesis and environmental factors influencing flavonoid accumulation so that full use can be made of barley as a functional foods crop.

Materials and methods

The experiment was conducted using a Randomized Complete Block Design (RCBD) with three replications at the experimental site of Yunnan academy of agricultural sciences, China. Each replication consisted of 45 experimental plots, each measuring 1 m × 1 m. A total of 20 barley varieties were planted across these plots, and the same treatments were applied uniformly to each plot. These all six-row barley varieties were selected based on their diverse genetic backgrounds, agronomic importance, and determines the variations in flavonoid content to ensure consistency in the research background and assess their potential for high and stable flavonoid accumulation. Only six-rowed barley genotypes were used in this study, as they are the primary feed barley type with high yield potential, ensuring consistency in the research background and aligning with the study objectives. For land preparation, deep plowing was done one week prior to sowing, followed by weed removal. Row planting was employed with a row spacing of 30 cm, and sowing depth ranged from 2 to 4 cm (*Table 1*).

Contents determination

Barley seeds collected from different replication were dried and ground. Approximately 1 g of the ground sample was extracted using ultrasonic treatment with a methanol solution for 30 minutes. The extract was then filtered, and the filtrate was evaporated under reduced pressure. The resulting residue was dissolved in double-

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distilled water and subjected to three extractions with ethyl acetate. After evaporating the water phase, the residue was re-dissolved in 10 mL of methanol and filtered using a 0.45 μm membrane filter. The contents of Catechin, Myricetin, Quercetin, and Kaempferol were determined by High-Performance Liquid Chromatography (HPLC) using a YMC-Pack ODS AM-303 column (5 μm, 250 mm × 4.6 mm i.d.). The mobile phases consisted of A—0.1% ice acetic acid aqueous solution and B—ethyl acetonitrile, with gradient elution. The flow rate was set at 0.8 mL/min, and the detector wavelength was 280 nm. An injection volume of 10 μL was used for each sample. The content of each flavonoid compound in the samples was calculated based on peak retention times and areas.

Table 1. *List of studied 20 barley varieties, their source and row-types*

Varieties	Pedigree	Source	Types
V1	11YD-9	Yunnan Academy of Agricultural Sciences	6 -row
V2	Yunsimai 1	Yunnan Academy of Agricultural Sciences	6-row
V3	Yunsimai 2	Yunnan Academy of Agricultural Sciences	6-row
V4	Yunsimai 3	Yunnan Academy of Agricultural Sciences	6-row
V5	09-J20	Baoshan Academy of Agricultural Sciences	6-row
V6	Feng 03-39	Dali Academy of Agricultural Sciences	6-row
V7	Chu B09-172	Chuxiong Academy of Agricultural Sciences	6-row
V8	Lindamai 3	Lincang Academy of Agricultural Sciences	6-row
V9	Yunsimai 4	Yunnan Academy of Agricultural Sciences	6-row
V10	Yunsimai 5	Yunnan Academy of Agricultural Sciences	6-row
V11	Yunsimai 7	Yunnan Academy of Agricultural Sciences	6-row
V12	Chu B11-585	Chuxiong Academy of Agricultural Sciences	6-row
V13	Chuandamai 12259	Sichuan Academy of Agricultural Sciences	6-row
V14	Yundamai 15YD-5	Yunnan Academy of Agricultural Sciences	6-row
V15	Yunsimai 11	Yunnan Academy of Agricultural Sciences	6-row
V16	Chu B14-1	Chuxiong Academy of Agricultural Sciences	6-row
V17	Wansi 14008	Anhui Academy of Agricultural Sciences	6-row
V18	Baodamai 13BJ-20	Baoshan Academy of Agricultural Sciences	6-row
V19	Baodamai 16-J6	Baoshan Academy of Agricultural Sciences	6-row
V20	Fengsimai 2	Dali Academy of Agricultural Sciences	6-row

Data analysis

The significance and average values of Catechin, Myricetin, Quercetin, Kaempferol, and Total flavonoids content were computed according to the different variety categories. Analysis of variance (ANOVA) was performed to determine the significance of differences in each trait among genotypes from the recorded data. The analysis was performed using Statistix 8.1, with a significance threshold set at $p < 0.05$ and $p \leq 0.01$. (Steel et al., 1986). The Least Significant Difference (LSD) test was applied for multiple comparisons among genotypes (Box 1980). Additionally, Principal Component Analysis (PCA) (Jolliffe, 2002) was performed to assess the diversity among the 20 barley varieties. Principal Component Analysis (PCA) was performed to identify trait relationships, detect patterns, and highlight key contributors to variability among the studied genotypes.

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Results and discussion

Variability of flavonoids contents in 20 barley genotypes

The ANOVA results (*Table 2*) reveal significant differences among the 20 barley genotypes for flavonoid content, with the source of variation being partitioned into replication, varieties, and error. The varieties demonstrated highly significant effects ($p \leq 0.01$) for Myricetin, Quercetin, Kaempferol, and Total flavonoids, as well as significant effects ($p \leq 0.05$) for Catechin, indicating that genetic variation among the genotypes plays a critical role in determining flavonoid levels. The mean squares for varieties were notably higher compared to the error for all flavonoids, particularly for Myricetin (6139.26) and total flavonoids (12849.8), highlighting substantial genetic differences. Conversely, the replication effects were minimal, suggesting consistency across experimental replicates. The relatively low error variance for Catechin (11.17) and Quercetin (16.561) further supports the reliability of the observed genotypic differences. Overall, the results underline the genetic diversity in flavonoid content among the barley genotypes, offering potential for targeted breeding and selection for higher flavonoid accumulation (Yang et al., 2013; Iannucci et al., 2021).

Table 2. *Analysis of variances for flavonoid consents in 20 barley genotypes*

Source	DF	Catechin	Myricetin	Quercetin	Kaempferol	Total Flavonoids
Replication	2	15.01	201.03	95.76	129.31	304.86
Varieties	19	531.346*	6139.26**	993.06**	1199.05**	12849.8**
Error	38	11.17	577.17	16.561	256.46	1646.6
Total	59					

**= highly significance ($p \leq 0.01$), * = significance ($p \leq 0.05$)

The LSD test revealed significant differences in catechin content among 20 genotypes (*Table 3*). Significant differences among the mean values in *Table 3* are indicated by uppercase letters (A, B, C) based on the LSD comparison test. Means sharing the same letter within a column are not significantly different, while different letters denote statistically significant variations among the barley varieties. Genotypes V2 (47.104), V4 (45.204), and V1 (42.894) were the highest performers, grouped as "A," indicating superior catechin accumulation. These genotypes may enhance nutritional and antioxidant properties. V8 (28.697) ranked second in group "B," followed by V6 (24.074) and V5 (22.901) in groups "BC" and "C," showing intermediate performance. Genotypes V7 (18.654), V3 (17.214), V16 (16.134), and V19 (14.994) clustered in groups "CD" and "DE," while V10 (14.244), V9 (13.181), and V11 (12.287) were in groups "DEF" and "EFG," indicating moderate performance. The lowest catechin values were recorded for V14 (9.104), V13 (7.144), V17 (7.084), V15 (6.484), V12 (6.244), V18 (6.014), and V20 (4.964), which fell into groups "FGH," "GH," and "H." These results highlight the variability among genotypes, with V2, V4, and V1 showing promising potential for high catechin content. Catechin's antioxidant and health benefits make these genotypes valuable for future breeding programs aimed at improving barley's health properties. The lower-performing genotypes may require further investigation to enhance their catechin content (Graton et al., 2024; Kukoeva et al., 2024).

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Table 3. *Mean values of flavonoids contents and their significance using LSD comparison test in 20 barley varieties with their standard deviation (SD) values for each trait*

Catechin			Myricetin			Quercetin			Kaempferol			Total Flavonoids		
V2	47.104	A	V18	141.98	A	V4	89.984	A	V18	82.984	A	V1	247.10	A
V4	45.204	A	V7	135.98	A	V10	46.984	B	V1	61.984	AB	V4	233.16	A
V1	42.894	A	V1	119.98	AB	V2	42.984	B	V3	51.317	BC	V7	208.61	AB
V8	28.697	B	V12	93.32	B	V11	40.984	B	V4	46.317	BCD	V18	183.33	ABC
V6	24.074	BC	V8	51.65	C	V15	32.317	C	V12	34.651	CDE	V6	152.67	BCD
V5	22.901	C	V2	37.98	CD	V8	25.984	CD	V19	32.984	CDE	V12	144.71	BCDE
V7	18.654	CD	V4	34.98	CD	V17	22.317	DE	V6	32.984	CDE	V2	140.06	CDEF
V3	17.214	DE	V5	27.98	CD	V1	22.234	DE	V11	30.984	CDE	V3	129.17	CDEFG
V16	16.134	DE	V17	24.65	CD	V12	21.984	DE	V7	28.317	CDE	V8	115.06	DEFGH
V19	14.994	DE	V3	21.98	CD	V14	20.317	DEF	V10	22.984	DE	V10	82.58	EFGHI
V10	14.244	DEF	V6	17.98	CD	V18	19.651	DEF	V9	18.317	E	V11	79.05	EFGHI
V9	13.181	DEF	V9	15.32	CD	V7	18.984	EF	V13	13.984	E	V5	74.06	FGHI
V11	12.287	EFG	V16	9.98	D	V3	17.984	EFG	V8	13.984	E	V19	67.95	GHI
V14	9.104	FGH	V11	9.45	D	V6	16.984	EFG	V14	12.984	E	V17	65.73	GHI
V13	7.144	GH	V15	8.98	D	V16	16.317	EFGH	V15	12.984	E	V9	62.09	HI
V17	7.084	GH	V10	8.65	D	V13	15.984	EFGH	V5	12.984	E	V15	59.99	HI
V15	6.484	H	V19	7.98	D	V5	15.984	EFGH	V2	11.984	E	V13	55.16	HI
V12	6.244	H	V13	7.05	D	V9	13.984	FGH	V16	9.984	E	V14	49.52	HI
V18	6.014	H	V14	6.98	D	V19	11.984	GH	V20	9.984	E	V16	48.72	HI
V20	4.964	H	V20	6.98	D	V20	9.984	H	V17	9.484	E	V20	31.92	I
±SD	± 12.68		±SD	± 8.51		±SD	± 6.72		±SD	± 7.03		±SD	± 11.01	

Significant differences among the mean values in Table 3 are indicated by uppercase letters (A, B, C) based on the LSD comparison test. Means sharing the same letter within a column are not significantly different, while different letters denote statistically significant variations among the barley varieties

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From *Table 3*, the data for Myricetin showed great disparities in the values across varieties from V1 to V20, and their classification was performed using letters from A to D. Myricetin is a flavonoid with antioxidant and anti-inflammatory activities and thus showed interesting variation in the genotypes. This was highest in V18 (141.98) and V7 (135.98) followed by group A. The varieties are well outshining all the rest, therefore, establishing highly significant variations. The high values of these genotypes can, therefore, present the best antioxidant potential and should, therefore, be targeted by breeders developing new varieties of these crops for better nutritional value and health benefits (Yang et al., 2019; Iannucci et al., 2021). V1 (119.98) followed in group AB, showing slightly lower but still elevated values, indicating a moderate level of Myricetin content. V12 (93.32) was placed in group B, representing a moderate level of Myricetin, further emphasizing the presence of genetic variability within the varieties. The V8 (51.65) stood alone in group C, reflecting a notable decrease compared to group B. Varieties V2, V4, V5, V17, V3, V6, and V9, forming group CD, exhibited intermediate values ranging from 37.98 to 15.32, demonstrating variability but without clear separation between them. Finally, varieties V16 to V20, in group D, recorded the lowest values, spanning from 9.98 to 6.98, indicating minimal Myricetin levels among these genotypes. This observed grouping pattern, accentuated by different performance differences with soft decay from A to D, probably reflects variation in underlying reasons including growth conditions, genetic factors, and other environmental aspects affecting the biosynthesis of Myricetin. Considering the value of Myricetin regarding health and its role in the prevention of oxidative stress and minimizing chronic disease risk, the present findings provided an insight that high-performance genotypes would be very helpful for further study and genetic improvement through barley breeding (Beta and Camire, 2018; Martínez-Subirà et al., 2020).

Table 3 shows the significant variation in Quercetin content among 20 barley varieties (V1 to V20) that were grouped into groups (A to H). V4 showed the highest value (89.984) in group A with high potential for antioxidant activity and disease resistance. Group B consisted of V10 (46.984), V2 (42.984), and V11 (40.984) with moderate levels of Quercetin. V15 (32.317) was placed in group C, with a further decline, while groups CD and DE comprised V8, V17, V1, and V12 with intermediate values (22.317–19.651). The gradual decline in the content of Quercetin was observed in groups EF, EFG, and EFGH from V7 to V5 with the lowest levels in V9 (13.984), V19 (11.984), and V20 (9.984) in groups FGH, GH, and H. The results indicate highly significant variation in Quercetin content, which may be genetically, environmentally, or treatment-controlled factors that determine its accumulation. Elite genotypes like V4 may be useful for breeding programs aimed at enhancing the nutritional and medicinal value of barley (Oluwajuyitan et al., 2021; Xiong et al., 2022).

Table 3 presents the Kaempferol content in 20 varieties of barley (V1 to V20), statistically grouped. V18, with the highest content of Kaempferol, was 82.984 and hence fell into group A, maybe due to its genetic or environmental advantage of higher synthesis of Kaempferol, highly beneficial for health and plant defense. V1 belonged to group AB with 61.984, performing high but lower than V18. V3 and V4, belonging to groups BC and BCD, respectively, showed medium contents of 51.317 and 46.317, respectively. V12, V19, V6, V11, and V7, belonging to group CDE, had intermediate values from 34.651 to 28.317. This would mean that they have rather medium health benefits. Variety V10, grouped in DE, had a further decline in Kaempferol content, with 22.984. The remaining varieties, ranging from V9 to V17, belonged to group E and showed the lowest

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values ranging from 18.317 to 9.484 with low variability. These might be those genotypes which have low production of Kaempferol or need specific conditions for higher accumulation (Rani et al., 2024). The overall trend was that from group A to E, there was a clear decline, indicating the varying levels of performance. High-yielding genotypes such as V18 may be useful for breeding programs aimed at improving nutritional and disease resistance traits in barley. Breeders can substantially improve the nutritional value of barley by selecting genotypes with higher flavonoid content, meeting the desires of health-conscious consumers and, to some extent, alleviating worldwide malnutrition (Zeng et al., 2020; Rawat et al., 2023).

Table 3 presents the immense variability in the total flavonoids content of the 20 barley varieties (V1 to V20) that were classified from A to I. Since flavonoids are important for both plant defense and nutritional value, as well as playing an important role in antioxidant activity and several health-promoting benefits, V1 (247.10) and V4 (233.16) from group A are likely the best candidates to improve nutritional quality in barley breeding programs. The next highest was measured for V7 (208.61), in class AB, showing a little bit lower but still appreciable content. For the subsequent class, ABC, this gradual decrease followed with the samples V18 (183.33), showing a moderate flavonoid content. Classes BCD to CDEF were shown as from V6 (152.67) through V2 (140.06), therefore representing moderate levels and being more suitable in breeding when considering a balance with agronomic characteristics (Liaqat et al., 2024; Kadege et al., 2024). Further reduction was observed from V3 (129.17) to V8 (115.06), classified as groups CDEFG and DEFGH. Lower and relatively similar values, conferring moderate health benefits, were obtained in the genotypes of groups EFGHI and FGHI, including V10 (82.58) to V5 (74.06). The lowest flavonoid content was obtained in V19 (67.95) to V16 (48.72), while the few differences among them were not statistically significant. Lastly, the lowest content was recorded by V20 (31.92) of group I. Such is indicative of the influence of genetic, environmental, or even treatment factors on flavonoid variation, hence making V1 and V4 attractive prospects toward further breeding efforts (Wijekoon et al., 2022; Liaqat et al., 2024).

Among 67 cultivated and 156 Tibetan wild barley genotypes, substantial flavonoid variability was unraveled and at higher concentration and genetic diversity in Tibetan wild barley (Han et al., 2018). The variability thus justifies sustainable breeding for functional foods featuring improved phenolic compounds in barley (Eid et al., 2024). Previously, the total flavonoid content reported in the varieties of barley ranged from 0.41 to 0.55 mg/100 g by Yiblet et al. (2024). The analysis of total flavonoid content in the 20 varieties of barley showed variations of great significance; hence, the genotypes were V1-247.10, V4-233.16, V7-208.61, and V18-183.33. These are the best performing and promising candidates for high flavonoid content in future breeding programs. These varieties possess a high value regarding the improvement of nutritional and functional values of barley. On the other hand, the minimum flavonoid content of varieties were V9 (13.984), V14 (19.651), and V20 (31.92), hence not suitable for inclusion in barley breeding programs focused on functional components.

Principal component analysis (PCA)

PCA results (*Table 4*) showed the contribution of four main factors (F1-F4) explaining the total variability in the flavonoids' composition of the studied 20 genotypes of barley. The first principal component, F1, had the highest eigenvalue value of 2.717 and explained 54.35% of the total variability; therefore, this principal component explains the

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most important proportion of variation among the flavonoid traits under consideration. The variance explained by the second one, F2, was 26.81%, while the first two factors explained 81.16% cumulatively, suggesting these two factors are the most vital for describing the dataset under study. On the other hand, the other two main factors, F3 and F4, contributed 9.57% and 9.27%, respectively, to give a total cumulative variability of 100%. In this respect, the results reflect that these two first components summarize most of the variation in flavonoids' composition and therefore give the major contributors to differentiate the barley varieties according to their flavonoid composition (Yang et al., 2013; Thabet et al., 2022).

Table 4. *Principal Component Analysis of flavonoid content in 20 barley varieties*

Variability	F1	F2	F3	F4
Eigenvalue	2.717	1.341	0.479	0.463
Variability (%)	54.348	26.814	9.571	9.266
Cumulative %	54.348	81.163	90.734	100.000

Figure 1 showed the Scree Plot resulting from the PCA of the flavonoids' contents of 20 varieties of barley, showing eigenvalues and the explained variance in a cumulative way. Most of the variation is explained by PC1 and PC2, while the contributions of PC3 and PC4 were very small. As it becomes clear that the curve is leveling off after the fourth component, this points to the first two PCs carrying the main data variability. The rapid drop of the eigenvalues after PC2 verifies the minimum contribution of further components. The results here will henceforth give an indication that PC1 and PC2 are significant towards interpreting flavonoids profile and trait selection for breeding programs in developing improved flavonoid content across barley varieties. PCA simplifies such a dataset and retains necessary information for practical purposes. Previously experiment conducted by scientists (Yang et al., 2013) and they identified 14 barley accessions with high total flavonoid content (>195 mg/100 g), highlighting variability among genotypes. This variability supports sustainable breeding efforts for functional foods, enhancing barley's nutritional value and potential health benefits.

Catechin and Quercetin are highly positively correlated, going in the same direction, which stipulates that those barley varieties that contain high catechin will also have quercetin in relatively high levels (*Figure 2*). Myricetin and kaempferol were negatively correlated; when myricetin is high, kaempferol is lower. The total flavonoids vector was positioned between catechin and quercetin, indicating in barley that the total flavonoid content is most influenced by these two compounds. The results indicate the complexity of the flavonoid profile in barley as determined by genetics and environment. These related flavonoid compounds stipulate that catechin and quercetin are the major determinants of flavonoid variation and might also be useful markers in breeding (Hambira, 2010; Graton et al., 2024). The unusual positioning of myricetin testifies to a particular role assigned probably by some genetic or environmental influence. The moderate contributions of total flavonoids and Kaempferol further underline that flavonoid biosynthesis pathways are complex and may present useful insights for functional foods production in barley (Chaieb et al., 2021; Yiblet et al., 2024).

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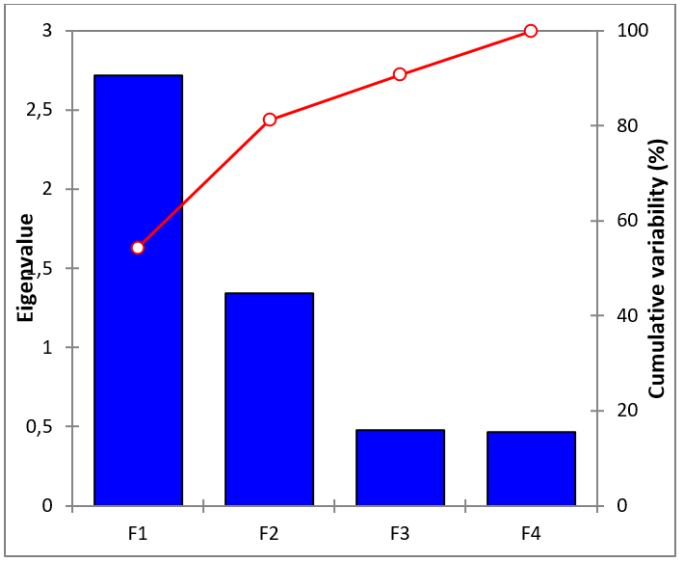

Figure 1. *Scree plot obtained from Principal component analysis of flavonoid content in 20 barely varieties*

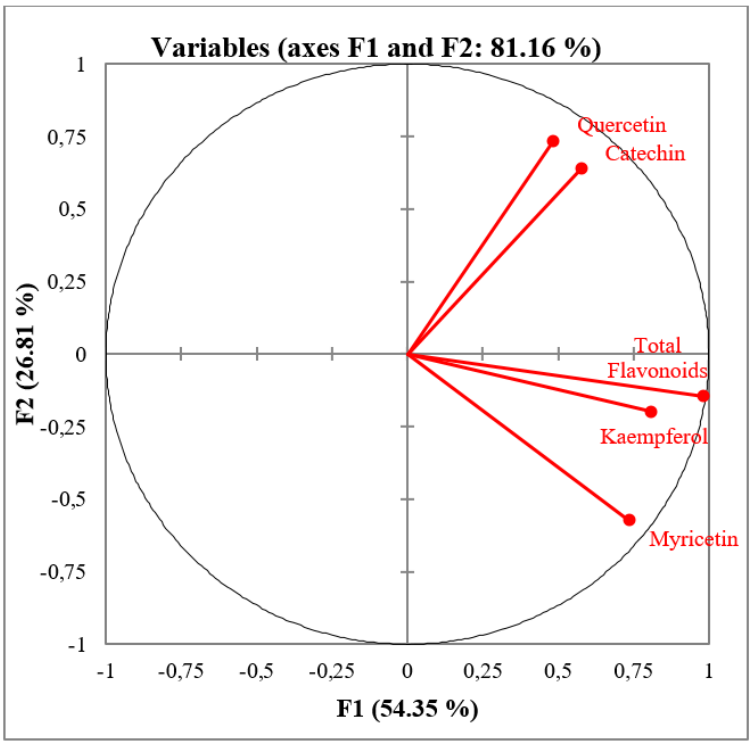

Figure 2. *Vector plot (traits observation) of flavonoid content (Catechin, Myricetin, Quercetin, Kaempferol, Total Flavonoids)*

In the present study, Biplot (*Figure 3*) on the 20 varieties (V1-V20) and five flavonoid traits, namely catechin, myricetin, quercetin, kaempferol, total flavonoids using principal component analysis. Clustering among the blue dots of varieties showed similarities among their flavonoid profile and therefore genetic or environmental factors caused these

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similarities in flavonoid content among tested varieties. From vectors, it can be seen that catechin and quercetin are highly positively correlated, and thus a high level of catechin relates to a high level of quercetin. Similarly, myricetin and kaempferol were highly negatively correlated with opposite directions and therefore high myricetin content may relate to low content of kaempferol. An earlier study has calculated the genetic variation of flavonoid content in 16 recombinant inbred lines of barley and showed high heritability and also significant correlation with the antioxidant activity. The identified better genotype like L1997 and L3005 may enhance functional foods properties and support the sustainable breeding process (Iannucci et al., 2021).

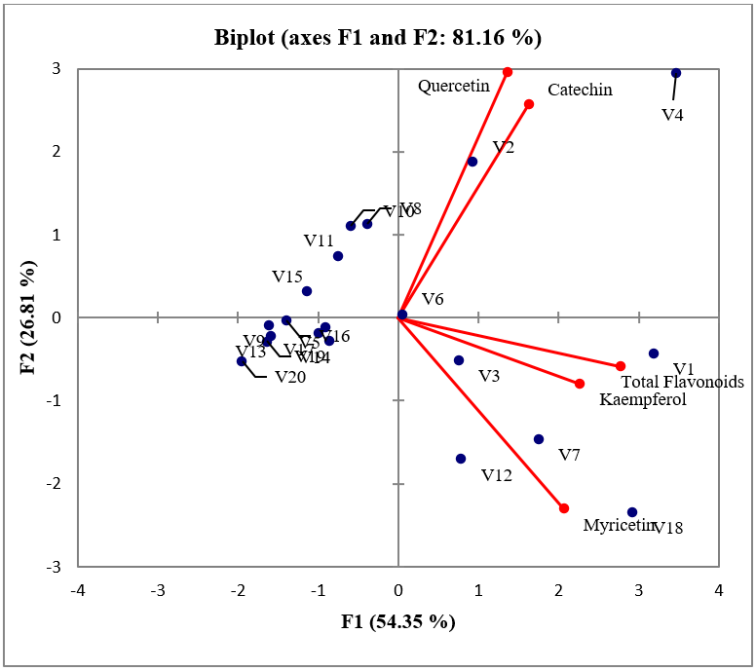

Figure 3. *Biplot of 20 barley varieties (V1-V20) and studied traits (Catechin, Myricetin, Quercetin, Kaempferol, Total Flavonoids) obtained from Principal Component analysis*

The position of each variety in relation to the vectors of traits captures its flavonoid profile; varieties closer to the vectors of catechin and quercetin probably contain more of these compounds. Such patterns show the complicated interactions between flavonoid compounds and point to possible genetic and environmental factors affecting flavonoid variability in barley. Generally speaking, higher contents of flavonoids like catechin, myricetin, quercetin, and kaempferol, and total flavonoids, are preferable in nutritional studies for their various health-promoting properties (Yang et al., 2013; Han et al., 2018; Yiblet et al., 2024). Varieties positioned closer to the respective vectors for Catechin and Quercetin will contain more of these compounds according to the results obtained from the biplot analysis. Thus, varieties V1, V2, and V4, being the nearest ones to the respective vectors, would be regarded as promising candidates to have a high content of Catechin and Quercetin. Contrasting with Myricetin, varieties farther away from the Myricetin vector assumed lower levels of Myricetin; thus, V1, V2, and V4 may be suitable when one wants a low Myricetin content. With Kaempferol, the varieties lying near the vector of Kaempferol would contain higher levels of this compound. So that V3, V12, and V18 were relatively close to the Kaempferol vector and could be considered

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promising candidates with a high amount of Kaempferol content. V9, V14, and V20 showed the poorest performance and are therefore not suitable for functional components in a breeding program for barley (Baloch et al., 2024). Finally, varieties that fall close to the total flavonoids vector can be assumed to contain high total flavonoid content. Thus, following the biplot, V1, V4, V7, and V18 could be the best candidate for a high value of total flavonoid content.

Conclusion

The findings of this study underscore the significant genotypic variability in flavonoid content among the 20 barley genotypes evaluated. The analysis revealed that flavonoid content, including Catechin, Myricetin, Quercetin, Kaempferol, and total flavonoids, varied considerably, with certain genotypes standing out for their superior nutritional and functional potential. Specifically, genotypes V1, V4, and V7 showed promising levels of Catechin and Myricetin, which are known for their antioxidant and anti-inflammatory properties. Genotypes V1 and V4 exhibited the highest total flavonoid content, suggesting their potential for improving the overall nutritional profile of barley. Additionally, V18 demonstrated the highest Kaempferol levels, indicating its usefulness for both health benefits and plant defense. The PCA results provided further insights into the genetic diversity among the genotypes, highlighting the importance of the first two principal components (F_1 and F_2) in explaining most of the variability in flavonoid content. These findings emphasize the need for targeted breeding programs that focus on enhancing specific flavonoid compounds, particularly Catechin, Myricetin, and total flavonoids, in barley. Genotypes with higher flavonoid content, such as V1, V4, V7 and V18, could be selected for breeding efforts aimed at improving barley's nutritional and medicinal properties, offering significant potential for both health-conscious consumers and sustainable agricultural practices. Future studies should further explore the environmental and genetic factors influencing flavonoid accumulation to optimize barley breeding strategies for enhanced disease resistance and nutritional quality.

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Competing interest. The authors declare no competing interests.

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