NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro; doi.org/10.59665/rar4278

ROMANIAN AGRICULTURAL RESEARCH, NO. 42, 2025
First Online: July, 2025. DII 2067-5720 RAR 2025-196

Differential Flavonoid Accumulation and Drought Response among Barley Genotypes

Tao Yang[1], Hafiz Ghulam Muhu Din Ahmed[1,2*], Muhammad Irfan Akram[3], Amenah S. Alotaibi[4,5], Zeki Erden[6], Çağdaş Can Toprak[6], Changjiao Ke[1*], Yawen Zeng[1*]

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

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

[3]Department of Entomology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur 63100, Pakistan

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

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

[6]Department of Field Crops, Faculty of Agriculture, Siirt University, Siirt 56100, Turkey

[*]Corresponding authors. E-mail: ahmedbreeder@gmail.com, changjiaoke123@163.com, zengyw1967@126.com

ABSTRACT

This study evaluated flavonoid composition and inter-trait relationships in twelve barley (*Hordeum vulgare* L.) genotypes under normal (T_1) and drought (T_2) conditions using a randomized complete block design (RCBD) with three replications in Yunnan, China. High-performance liquid chromatography (HPLC) quantified five key flavonoids: catechin, myricetin, quercetin, kaempferol, and total flavonoids. Significant genotypic variation was observed for all traits ($p<0.05$), with stronger expression under drought. Catechin ranged from 2.66 to 6.28 mg/g, peaking in genotype G3 under T_2, while total flavonoids (TFC) remained stable (T_1: 31.95 mg/g; T_2: 31.86 mg/g), with G3 reaching 37.51 mg/g under stress. Correlation analysis revealed strong positive associations among flavonoids ($r = 0.81$-0.96), particularly under drought, indicating a coordinated antioxidant response. Biplot explained 95.50% (T_1) and 96.08% (T_2) of total variance, effectively distinguishing high-performing genotypes. Biplot analysis identified G3, G5, and G11 as consistently superior in flavonoid accumulation, while G2, G4, and G9 showed poor performance. Genotype × treatment interaction was highly significant, reflecting genotype-specific metabolic shifts under drought. The intensification of trait correlations and stability of elite lines under stress highlight their utility for biofortification and climate-resilient breeding. These findings support flavonoid-based selection strategies for enhancing both nutritional quality and stress tolerance in barley breeding programs targeting functional food and arid-environment adaptation.

Keywords: flavonoids, barley, drought, genotypes, antioxidants.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is considered one of the most important cereal crops globally, cultivated for food, feed, and brewing worldwide. It is the fourth most produced cereal crop in the world after wheat, maize and rice, and plays an important role in food security and agricultural economies around globe (Tyagi et al., 2020; Brueggeman, 2024). However, barley productivity can be negatively impacted by environmental stresses, such as drought, which is expected to increase due to global climate change. Drought stress can restrict water availability and reduces crop yield and quality by affecting physiological processes. Therefore, it is important to appreciate the biochemical and physiological impacts of drought on barley to provide knowledge for developing resilient cultivars to adapt to changing climates (Khodaeiaminjan and Bergougnoux, 2021; Baltacıer and Acar, 2024).

A critical adaptive reaction of plants in response to drought stress, is the production and accumulation of secondary metabolites, such as flavonoids. Flavonoids are an extensive group of polyphenols that are ubiquitous in plants, and are known to be anti-oxidants, anti-microbials, and photoprotective (Al-Khayri et al., 2022). Flavonoids are an essential biochemical

---

Received 30 May 2025; accepted 29 July 2025.

pathway for relieving oxidative stress via the scavenging of free radicals and stabilizing cellular membranes as a result of reactive oxygen species (ROS) induced by drought (Ahmed et al., 2025b). For example, in barley and numerous cereals, flavonoids play a role in plant defense, regulation of plant growth, and signalling pathways for environmental stress. Due to their multifunctional roles, flavonoids are of interest both in terms of agronomy, but also in the nutritional and health benefits they bring to human diets through their anti-inflammatory and cardioprotective properties (Patil et al., 2024; Yang et al., 2025a).

Among the many different types of flavonoids, the major flavonoids we will explore consist of catechin, myricetin, quercetin, and a type of flavonoid known as kaempferol. The main reason for their predominance is due to their versatile compounds that possess considerable antioxidant activities as free radical scavengers and for their relatively high levels of presence in barley grains and leaves (Ahmed et al., 2025a; Yang et al., 2025b). Catechin, a type of flavan-3-ol, has a strong capacity for free radical scavenging activity, alongside its purported role in assisting with the detoxing of reactive oxygen species (ROS) during states of abiotic stress (Patil et al., 2024). Both myricetin and quercetin are plant-based flavanols that have been reported to protect plants from exposure to ultraviolet (UV) radiation and pathogen invasion by acting as UV-light protectants if exposed to adequate light energy, while functioning kaempferol assists with directing the growth of plants while also allowing for some protection and tolerance to stress (Al-Khayri et al., 2022). The biosynthesis of flavonoids (i.e., catechin, myricetin, quercetin, and kaempferol) are interrelated as they utilize the same phenylpropanoid pathway, hence levels of flavonoids often vary with the emergence of environmental factors, offering a range of biochemical markers that can be used to study the response of plants to biotic and abiotic stress (Appiah et al., 2023; Kamal et al., 2023).

Correlation research among flavonoids and total flavonoid levels will help elucidate their regulated coordination and the opportunity for combining them in a breeding context. Previous research suggests that flavonoid content is often positively correlated to drought tolerance indices in cereals, indicating a functional role in mitigating stress conditions (Piasecka et al., 2020; Shrestha et al., 2024). In addition to correlation analysis, biplot analysis also offers an effective multivariate method to visualize these interrelationships and the genotype-trait associations in two-dimensional space. Biplots accomplish this by plotting both traits and genotypes at the same time so that genotypes with multiple flavonoids in the higher range can be identified. In addition to visually integrating into denial e each trait, breeders can also identify groups of traits that co-vary and which genotype performs the best and more stable across changing conditions (Güngör et al., 2023; Güngör et al., 2024). Using both correlation and biplot analysis provides a better definition of complex trait architecture and can help identify an elite barley line with more desirable flavonoid profiles and resilience in a stress environment (Feizi et al., 2020; Karahan and Akgün, 2020).

The purpose of this study is to assess the changes in important flavonoids: catechin, myricetin, quercetin, kaempferol, and total flavonoids, in twelve barley genotypes tested under normal and drought stress conditions. By using ANOVA, descriptive statistics, correlation analyses, and biplot analysis, this study will quantify the effect of drought on flavonoid accumulation, identify differences in flavonoid profiles among genotypes, explore relationships between flavonoid compounds and total flavonoids, and visualize genotype assessment and trait relationships for easy selection. The results will establish a biochemical basis of choosing barley genotypes that have enhanced antioxidant capacity and resilient to drought stress, and that could lead to the production of sustainable barley quality despite climate variability.

MATERIAL AND METHODS

The current study was designed to determine the flavonoid content of twelve barley (*Hordeum vulgare* L.) genotypes both under normal conditions and under drought-stressed conditions. The experiment was completed using a Randomized Complete Block Design (RCBD) with three replications in each treatment. The experiment was conducted at experimental stations in Yunnan Province, China. The sowing method used was row planting, with 30 cm row spacing and a sowing depth of 2-4 cm. Fertilization was done in two stages. Two irrigation treatments were applied, where normal irrigation (T_1) was applied for the entirety of the growing season, while drought stress (T_2) was the withholding of water at critical growth stages during the growing season (Farooq et al., 2009), including tillering and grain filling, to simulate water-limited conditions. Each genotype was planted in both treatments with three replications in a total of 72 experimental units (12 genotypes × 2 treatments × 3 replications).

Grain samples were harvested at physiological maturity, air-dried and ground into a fine powder using a laboratory mill. The samples were then stored at -20ºC in sealed containers until conducting a biochemical analysis (Kähkönen et al., 1999). For individual flavonoids quantification, including Catechin, Myricetin, Quercetin, and Kaempferol, a 1.0 g of ground grain was extracted with 10 mL of 80% methanol. The mixed samples were vortexed for 5 minutes, then incubated at room temperature for 2 hours. The samples were then centrifuged at 10,000 rpm for 15 minutes, and the supernatant was filtered with a 0.45 µm syringe filter for chromatographic analysis. Individual flavonoids were quantified using High-Performance Liquid Chromatography (HPLC) with a C18 reverse-phase column and UV detector (Kim et al., 2003). The mobile phase was a gradient elution of solvent A (0.1% formic acid in water) and solvent B (acetonitrile) that was optimized for flavonoids separation. Detection was made at 280 nm; Catechin, Myricetin, Quercetin, and Kaempferol standard solutions (Sigma-Aldrich) were used to determine calibration curves. The results are reported in mg per g dry weight.

The Total Flavonoid Content (TFC) was estimated through the aluminum chloride colorimetric method (Chang et al., 2002). An amount of 0.5 mL of extract was combined with 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 4.3 mL of distilled water. The total mixture was incubated for 30 minutes at room temperature and the absorbance was recorded at 415 nm using UV-Visible spectrophotometer. Quercetin was utilized to establish the standard curve and TFC was reported as mg of Quercetin Equivalent (QE) per g of dry weight of grain.

All data gathered were statistically analyzed with the appropriate Analysis of Variance (ANOVA) for a factorial RCBD. Means were compared using the Least Significant Difference (LSD) test and significance was determined at the 5% probability level (Steel and Torrie, 1960). Significance was reported for genotype, treatment and genotype x treatment interactions. Further multivariate analysis such as correlation biplot analysis were conducted after models were fitted to assess performance trends across genotypes. All statis tical analysis were conducted using Statistix, and R software.

RESULTS AND DISCUSSION

The analysis of variance for genotypes is given in Table 1, which demonstrates that for most flavonoid traits, such as myricetin, kaempferol, and total flavonoid content, differences among the genotypes were significant at a p<0.01 level, whereas catechin and quercetin differences were significant at a p<0.05 level. This suggests that considerable genetic variability exists among barley genotypes, which is necessary for an effective selection and breeding program aimed at processing higher flavonoid concentration. Similar results were noted by (Shrestha et al., 2024) showing significant genetic differences in phenolic content among cereal genotypes, affirming that breeding offers a novel avenue for improving these bioactive traits.

The treatment factor and its effect (normal vs. drought stress) were found to be significant ($p<0.05$) for all traits, thus establishing that drought stress had an effect on flavonoid biosynthesis in barley. This finding is consistent with previous work done by (Piasecka et al. 2020; Yang et al., 2025a), who confirmed that abiotic stress could induce the accumulation of secondary metabolites (including flavonoids) in plants and established that accumulation of metabolites could act as a defense mechanism. The significant genotype × treatment (G × T) interaction, especially highly significant for all traits except total flavonoids (significant at $p<0.05$), indicates that the flavonoid synthesis response to drought stress varies by genotype. The G×T is also pertinent for observing some genotypes with more stable or improved flavonoid profiles under stress conditions, as also seen in barley (Friero et al., 2024). This provides insights for breeding stress resilient barley genotypes with increased nutraceutical value.

Table 1. Analysis of variances for studied traits under normal and drought conditions

Source of variation	df	Catechin	Myricetin	Quercetin	Kaempferol	Total Flavonoids
Replication	2	35.3	46.12	41.13	51.04	49.3
Genotype (G)	11	73.3*	82.12**	79.51*	93.04**	95.04**
Treatment (T)	1	19.58*	21.58*	29.7*	33.05*	31.05*
G × T	11	122.88**	132.08**	142.48**	102.3**	98.05*
Error	46	12.76	1.03	9.03	15.13	17.13
Total	71	31.08				

Note: indicates significance (*) at the 0.05 probability level ($p<0.05$); indicates high significance (**) at the 0.01 probability level ($p<0.01$).

Flavonoid Trait Variability in 12 Barley genotypes

The presented descriptive statistics for the flavonoid traits of barley genotypes in normal and drought conditions (Table 2) give an indication of the environmental and genetic variability of secondary metabolite accumulation. The average and across-environment values for catechin were consistent across environments (4.43 mg/g) and this phenomenon may indicate catechin is less sensitivity to drought stress, is more stable genetically than some of the other flavonoids quantified. However, there was a finite drop in the minimum value of catechin under drought (2.35 mg/g vs 3.03 mg/g), indicating some genotypes showed drought stress response suppression. Interestingly, the maximum value of catechin under drought stress (6.28 mg/g) markedly exceeded the maximum value under normal conditions (5.61 mg/g), indicating some genotypes demonstrated considerable upregulation. This study's results regarding catechin support the findings of (Karahan and Akgün, 2020; Baltacıer and Acar, 2024) that moderate stress can enhance flavonoid synthesis as part of the plants adaptive defense mechanism.

The average content of myricetin slightly decreased in drought conditions (2.3 mg/g vs 2.41 mg/g), and the minimum value also fell under drought (0.68 mg/g), suggesting myricetin may exhibit some degrees of sensitivity to drought in some genotypes. Nevertheless, the maximum value increased (3.87 mg/g vs 3.48 mg/g), suggesting that there may be genotypic differences in drought responsiveness, similar to the observations of (Patil et al., 2024; Shrestha et al., 2024), that reported abiotic stress differentially regulating individual flavonoid pathways depending on genotype and severity. There was a similar response for quercetin, which had a slight decrease in mean content at drought (3.34 mg/g vs. 3.43 mg/g), but a higher maximum (5.09 mg/g vs 4.42 mg/g). The variation for quercetin, measured by the coefficient of variation (CV), also increased at drought (19.76% vs 17.18% in normal conditions), indicating a greater range of genetic response. This is consistent with findings presented by (Friero et al., 2024) that indicated during drought conditions, growth patterns show greater phenotypic variability, providing an increased opportunity for selection of drought-resilient genotypes with greater nutritional value.

For kaempferol, there was a slight decrease in mean under drought (2.42 mg/g vs 2.5 mg/g), but an increased amount of variability (CV increase from 12.81% to 16.92%). The minimum value decreased significantly at drought (1.18 mg/g vs 2.0 mg/g), whereas the maximum value increased (3.48 mg/g vs 2.92 mg/g) again indicating that induction is dependent on genotype under stress. This supports predictions about the induction of the phenylpropanoid pathway under abiotic stress and regulatory flexibility (Appiah et al., 2023; Ahmed et al., 2025b). The average total flavonoid content remained fairly consistent, averaging 31.95 mg/g (31.86 mg/g on drought), with very minor variation in the minimum and maximum values from both environments. The drought treatment elicited a slightly higher standard deviation and CV, indicating a slightly higher variability between genotypes. These results are in agreement with (Shrestha et al., 2024), who concluded that total flavonoid content is a stable trait, not significantly affected by environmental variability and primarily governed by genotype.

In the present study, we also observe that the descriptive statistics yield information suggesting that most of the average values for traits decreased slightly under drought stress, while the broader ranges and variability of the higher maximum values under drought shows that there is a subset of these genotypes that appear to be increasing flavonoid biosynthesis as a response to stress. These results align with previous studies (Khodaeiaminjan and Bergougnoux, 2021; Patil et al., 2024; Yang et al., 2025b) that discuss the role of flavonoids in plant defense and their dynamic regulation under abiotic stress. These observations provide useful information regarding the identification of elite genotypes for developing functional foods and in breeding programs focused on stress tolerance in barley.

Table 2. Descriptive Statistics of studied traits under normal and drought conditions in 12 barley genotypes

Parameters	Environment	Catechin	Myricetin	Quercetin	Kaempferol	Total Flavonoids
Minimum	Normal	3.03	1.58	2.66	2	26.9
	Drought	2.35	0.68	1.78	1.18	26.13
Maximum	Normal	5.61	3.48	4.42	2.92	37.28
	Drought	6.28	3.87	5.09	3.48	37.51
Average	Normal	4.43	2.41	3.43	2.5	31.95
	Drought	4.43	2.3	3.34	2.42	31.86
Standard deviation	Normal	0.83	0.47	0.59	0.32	3.30
	Drought	0.87	0.45	0.66	0.41	3.64
Coefficient of variation	Normal	18.8	19.49	17.18	12.81	10.32
	Drought	19.65	19.54	19.76	16.92	11.42

Flavonoid Variations in genotypes across Conditions

The examination of mean values and LSD groupings for the five flavonoids (catechin, myricetin, quercetin, and kaempferol) and total flavonoids across the two conditions (normal and drought stress) in the 12 genotypes of barley indicated substantial genotypic variability, indicating that flavonoid biosynthesis is under strong genetic control (Tables 3). The results further suggest that differences between genotypes should not be attributed to random chance or pre-existing bias but are real differences and are therefore a representation of inherent phenotypic expression and genetic variation of potential biosynthetic activity (Han et al., 2018).

In both conditions, the genotypes G3, G5, and G11 continually showed higher values for all four flavonoid groups. Under normal conditions, G3 had the highest catechin value (5.61 mg/g, group 'a'), while under drought stress its value increased to 6.28 mg/g, while still maintaining the high catechin rank. G5 and G11 followed closely in both conditions, further indicating stability for flavonoid accumulation. Conversely, genotypes G2, G4, and G9 consistently demonstrated the lowest values and fell in the lowest LSD groups, indicating low or limited biosynthetic activity and low potential for an antioxidative response to oxidative stress. These are in agreement with previous studies conducted by Ma et al. (2014) and Wang et al. (2024), which demonstrated that flavonoid biosynthesis can be expressed depending on the genotype and vary under conditions of abiotic stress. When looking at myricetin, G5 and G11 were the top two genotypes across both environments overall. G11 performed the best with a value of 3.87 mg/g under drought (group 'a'). G3 was also among the top genotypes although it was just slightly lower overall. G9, G4 and G2 performed the worst for this trait as previously demonstrated for catechin and confirmatory of the low antioxidants of these genotypes. These results agree with (Chaieb et al., 2021), who discussed the crucial benefits of myricetin as a part of the plant's oxidative defense systems, particularly for environmentally stressed systems.

A similar trend was also observed under the Quercetin levels. In both the drought and irrigated environments, G5, G11, and G3 continued to be ranked the top, with a range of values 4.42-5.09 mg/g that were significantly higher than the G4 and G2 which showed consistently low values. This support the ranking pattern presented by (Fekadu et al., 2023), who reported elite genotypes were able to keep producing flavonoids as an adaptive mechanism of stress. The relationship between kaempferol content followed the same trend when group 5 (G5), group 3 (G3) and group 11 (G11) were on top at 3.30-3.48 mg/g under drought and group 9 (G9), group 4 (G4) and group 2 (G2) at the bottom. These results support the multifunctional role of kaempferol, as it is known to scavenge reactive oxygen species (ROS) and has the potential to indicate that the superior genotypes possess greater antioxidant machinery under water-limited conditions (Rao et al., 2020) showed similar findings.

In terms of total flavonoids, G3 again accummulated the highest under both normal (37.28 mg/g) and drought (37.51 mg/g) conditions, followed closely by G5 and G11. Consistently, G9, G2, and G4 grouped the lowest in all environments suggesting a lower capacity in flavonoids to respond under stress conditions. These results are in align with Ma et al. (2014) and Kiani et al. (2021) realizing that genotypes that can sustain high flavonoids under drought conditions are more resilient to stress. The fact that genotypes in LSD groups are consistently ranked under normal and drought conditions means that both statistical reliability and biological relevance can be assumed. Based on both normal and drought conditions, G3, G5, and G11 are strongly validated as elite donor lines for breeding to enhance flavonoid content to achieve nutrition and stress-resilience objectives. In contrast, G2, G4, and G9 underperformed in all conditions. This suggests that these genotypes are not likely appropriate for environments with abiotic stress pressures. Overall, the current study supports significant inter-genotypic differences in flavonoid traits in barley. This also complements previous reports from (Pour-Aboughadareh et al., 2023) who showed that drought-tolerant genotypes tend to accumulate higher levels of secondary metabolites that provide oxidative balance for physiological hardiness to drought exposure. These identified genotypes may provide a valuable resource for biofortification and functional food development in climate induced stress scenarios.

Table 3. Mean values and LSD groupings of 12 barley genotypes under normal and drought conditions for the evaluated traits

Genotype	Environments	Catechin	Myricetin	Quercetin	Kaempferol	Total Flavonoids
G1	Normal	4.77 cd	2.16 d	3.00 e	2.61 abc	32.83 bc
	Drought	5.00 cd	2.43 c	2.58 f	2.47 cd	32.44 c
G2	Normal	3.12 g	1.86 e	2.88 f	2.02 c	27.31 d
	Drought	2.37 g	1.10 fg	2.17 g	1.51 fg	26.76 e
G3	Normal	5.61 a	3.07 b	4.15 b	2.87 ab	37.28 a
	Drought	6.28 a	3.39 ab	4.65 b	3.43 a	37.51 a
G4	Normal	3.03 g	1.82 e	2.66 g	2.00 c	27.72 d
	Drought	2.41 g	1.11 fg	1.78 h	1.39 g	27.18 e
G5	Normal	5.37 ab	3.48 a	4.37 a	2.92 a	35.78 ab
	Drought	5.67 ab	3.71 a	5.09 a	3.48 a	36.40 ab
G6	Normal	4.61 de	1.94 de	3.05 de	2.67 abc	33.86 bc
	Drought	4.92 de	2.14 de	2.65 f	2.86 bc	33.80 bc
G7	Normal	4.12 f	2.21 d	3.03 e	2.65 abc	31.04 c
	Drought	4.28 f	2.40 c	3.05 e	2.70 bc	30.72 cd
G8	Normal	4.97 bc	2.62 c	3.75 c	2.65 abc	32.39 bc
	Drought	5.39 bc	2.21 cd	3.45 d	2.19 de	32.22 c
G9	Normal	3.29 g	1.58 f	2.96 f	2.12 c	26.90 d
	Drought	2.35 g	0.68 g	2.37 g	1.18 g	26.13 e
G10	Normal	4.39 e	2.22 d	3.66 c	2.38 bc	31.12 c
	Drought	4.43 ef	1.86 e	3.97 c	1.95 ef	31.61 cd
G11	Normal	5.02 bc	3.48 a	4.42 a	2.84 ab	35.91 ab
	Drought	5.33 bc	3.87 a	4.93 a	3.30 a	36.28 ab
G12	Normal	4.86 cd	2.50 c	3.26 d	2.23 c	31.24 c
	Drought	4.69 de	2.73 bc	3.40 d	2.62 cd	31.22 cd

Correlation Analysis

Analysis of the relationships among flavonoid traits across 12 barley genotypes tested in both normal and drought conditions suggested highly significant and positive correlations, implying coordinated regulation and/biosynthetic linking of the metabolites (Figure 1). Such relationships are of utmost importance for understanding metabolic strategies barley have to adapt to the environment under abiotic stress factors, like drought, that affect flavonoid metabolism (Yang et al., 2013; Pour-Aboughadareh et al., 2023). Under normal conditions, catechin had a strong and significant correlation with myricetin (r = 0.81), quercetin (r = 0.79), and kaempferol (r = 0.87), suggesting they might have a common biosynthetic pathway or mechanism of regulatory control. Under drought stress, these same associations were heightened, with catechin-myricetin (r = 0.88) and catechin-kaempferol (r = 0.90) corrosion values increased. The strength of these association were consistent with (Fekadu et al., 2023; Yang et al., 2025a), where they reported drought stress maximized synchronization of flavonoid synthesis in response to an antioxidative stress response of the plant. Under the previously noted conditions (normal and drought), results showed a significant relationship between myricetin and quercetin (r = 0.94 under normal, r = 0.87 under drought), corroborating their designation as being closely related in the flavonol subclass. High correlations were also noted with kaempferol and strongly with other traits, especially under drought (r = 0.96 with myricetin, r = 0.79 with quercetin), suggesting plants increase multiple flavonoid branches simultaneously under water stress to maintain redox homeostasis. The observations further support previous work by (Appiah et al. 2023; Ahmed et al. 2025b), who reported these antioxidant metabolites are collectively upregulated in cereals under environmental stress.

Total flavonoids were significantly correlated with all individual components in both environments, the coefficients were also very strong (r = 0.94-0.95 with catechin, r = 0.84-0.93 with myricetin, r = 0.81-0.85 with quercetin and r = 0.94-0.95 with kaempferol) also confirming that total flavonoid variability is essentially tied to these four flavonoid compounds, also previously noted in barley (Huang et al., 2020; Pour-Aboughadareh et al., 2023; Patil et al., 2024). The slightly stronger correlations under drought suggest a better synchronized metabolic response during drought, probably in response to enhancing ROS scavenging ability during drought. The present correlation findings not only confirm the biosynthetic and functional relationships among flavonoid compounds in barley, but also show that drought stress enhances those inter-trait associations which indicates a strong metabolic defense network is present. These results are in agreement with previous research conducted on plant secondary metabolism and stress physiology (Yang et al., 2013; Rao et al., 2020; Yang et al., 2025a), and emphasize the merit of using multi-trait selection approaches in breeding programs aimed at developing drought tolerance.

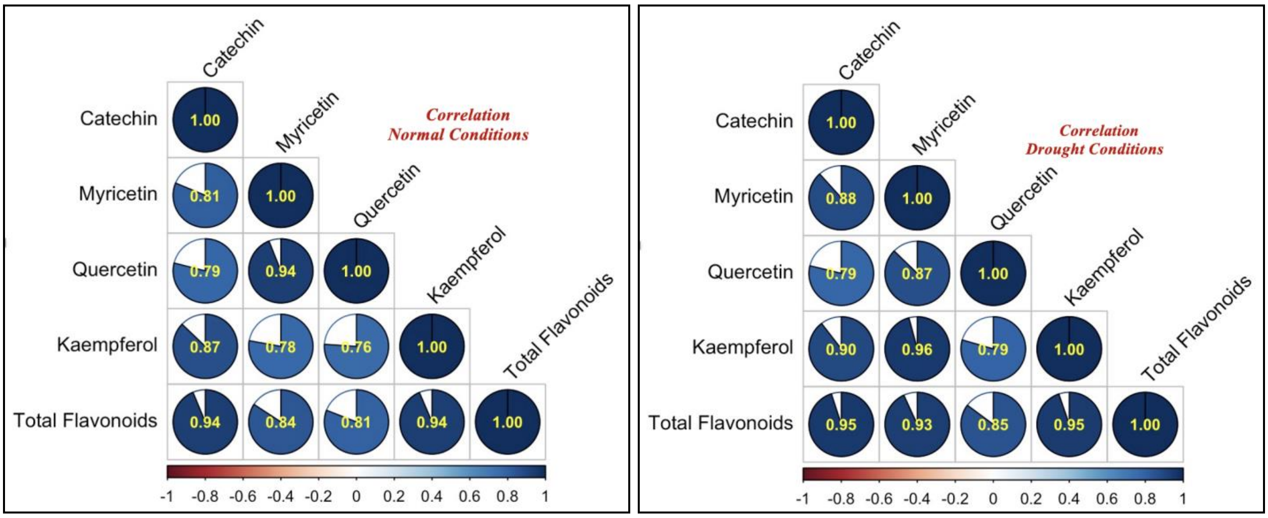

Figure 1. Correlation among studies traits under normal and drought conditions in 12 barley genotypes

Biplot Analysis

In particular, biplot analysis is a graphical method for expressing multivariate relationships between genotypes and traits, which helps to find the best genotypes that exhibit favourable flavonoid accumulation, which may vary in different environments. The biplot shown in Figure 2 describes the relationships of a selected set of 12 barley genotypes (G1-G12) with the measured traits in both normal and drought environments. The first two principal components (F1 and F2) explained 95.50% of the total variation under normal conditions (F1: 87.84% and F2: 7.65%) and 96.08% of the variability under drought conditions (F1: 91.08% and F2: 5.00%). The proportion of variance explained by the two principal components demonstrates that PCA captures most of the variation in the dataset in both environments, which echoes previous work in which PCA was used to assess the variation in complex biochemical traits in cereals (Eticha et al., 2010; Huang et al., 2020).

In both scenarios, the primary dimension, represented by the horizontal axis (F1), is useful for differentiating genotypes based on their compositional levels of Total Flavonoids, Catechin, Kaempferol, Quercetin, and Myricetin. Genotypes that are on the right side of either biplot - G3, G5, and G11 - display consistently elevated values of the traits in both environments. Under normal conditions, G3 has increased Total Flavonoids, Catechin, and Kaempferol; G5 has higher Quercetin and Myricetin. Under drought conditions, G5 and G11 had higher Quercetin, while G3 had higher Myricetin, Total Flavonoids, Kaempferol, and Catechin. Thus, the results are indicative of these genotypes having a robust biochemical profile with limited variation between the different environments. Our results align with those of (Xiao Ya et al., 2017; Huang et al., 2020; Rao et al., 2020;), who reported that genotypes with a high antioxidant capacity due to genetics maintain or increase flavonoids under stressed environments.

On the other hand, genotypes G2, G4, and G9, which occupy the left side of both biplots, exhibit consistently reduced levels of the traits measured, thus they are expected to be the least favorable as bioactive compound accumulators. This lower accumulation of bioactive compounds under dry stress may have been due to reduced antioxidant defense ability and/or not inducing the activity of biosynthetic pathways (Xiao Ya et al., 2017; Feizi et al., 2020; Al-Khayri et al., 2022). Genotypes G1, G6, G7, G8, and G12 are situated close to the origin under both conditions, which indicates they exhibited when averaged moderate or average levels of traits demonstrating little variance from the mean; G1, G6, G7, G8, and G12 exhibited moderate or average adaptations.

The direction and angles between vectors of traits also provide insight into relationships between the traits. In both environments, the acute angles between Total Flavonoids, Catechin, Kaempferol, and Myricetin indicate strong relationships between the traits that were indicative of either strong positive relationships (i.e., high in one trait is typically high in others), or some complication thereof. These relationships may be stronger under drought conditions, as can be inferred by the closer clustering of the vectors of the observed relationships. Our results corroborate the findings of (Han et al., 2018; Patil et al., 2024), who both showed that drought stress and other stress conditions provoked co-induction of several flavonoids (that are necessary for elevating and combating oxidative stress).

Quercetin has a positive relationship with other traits but appears to follow a slightly different trajectory, particularly under drought stress, suggesting it is also a driving factor in the separation of genotypes. This unique behavior could be attributed to Quercetin's strong antioxidant and radical scavenging activities, which are often upregulated under water-deficit conditions as part of the protective response from plants (Eticha et al., 2010; Fekadu et al., 2023). The contrasting arrangement of the genotypes, including the high performing genotypes G3, G5, and G11 compared to the low performing G2, G4, and G9, is clear in both biplots, and even more pronounced under drought stress. This divergence suggests that one or more of the high performing genotypes (G5 and G11) could enhance their bioactive compound profile as a physiological response to water deficit. This finding follows previous studies, which emphasized the upregulation of secondary metabolite production as a central drought tolerance strategy in barley and other cereal crops (Güngör et al., 2023; Güngör et al., 2024). Such conduct emphasizes the flexible adaptability of these genotypes to function as dual-purpose lines that either enhance nutrition or withstand stress. Therefore, the PCA biplot is a strong visual method for identifying potential elite genotypes with enhanced capacity for bioactive compounds under optimal and stress conditions. The stable and high performance of G3, G5, and G11 across environments makes a strong case for their use as parental lines for programs that will enhance barley for both health-beneficial phytochemicals and adaptation to drought environments.

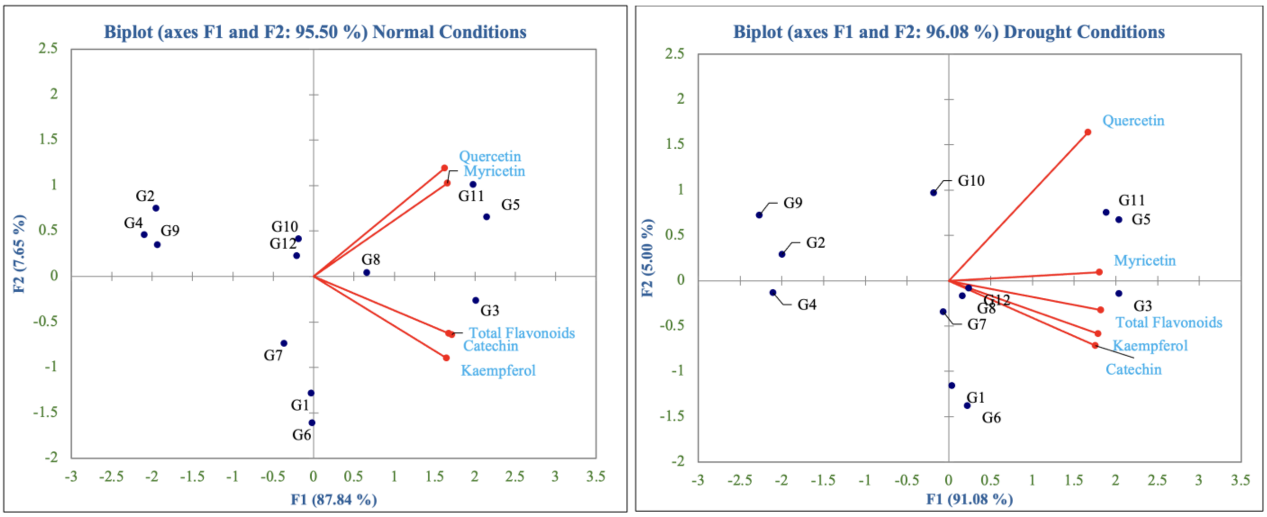

Figure 2. Biplot of studied traits under normal and drought conditions in 12 barley genotypes

CONCLUSIONS

This research establishes considerable genotypic diversity in flavonoid biosynthetic pathways across twelve barley genotypes under control and drought conditions. The use of high-resolution HPLC profiling indicated that drought conditions induced greater variability of traits while also inducing differential secondary metabolite expression. The genotypes G3, G5, and G11 were consistently among the highest for catechin, myricetin, quercetin, kaempferol, and total flavonoid content (TFC), with G3 producing the highest levels of TFC in both the control and drought conditions. The other genotypes G2, G4, and G9 demonstrated limited accumulation of flavonoids and adaptability to drought. The results also highlighted the significant interaction between genotype x treatment, supporting the hallmarks of dynamic regulation of flavonoid pathways in response to changing environmental conditions. The pairwise correlations of flavonoids, particularly myricetin-kaempferol and catechin-myricetin, under drought conditions suggested possible synergistic regulation of those chemicals. Total flavonoid content produced strong correlations with all individual chemicals, supporting TFC as an overall indicator trait. The multivariate PCA and biplot statistical analyses confirmed distinct separations of high-performing genotypes, thus supporting their biochemical traits and potential utility in breeding programs to develop cultures that are improved in phytochemical content and more resilient to drought. The enhanced coordination of flavonoid biosynthetic pathways under drought conditions supports their role in regulating redox homeostasis and contributing to adaptive plasticity. Based on this evidence, all findings support future recommendation of advancing flavonoid traits as biochemical marker traits in climate-smart breeding approaches for barley developed for stress resistance and phytochemical good agronomic practices**.**

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (Grant No.32160453). This research was supported by the Research, Development, and Innovation Authority (RDIA) - Kingdom of Saudi Arabia under grant number (13445-Tabuk-2023-UT-R-3-1-SE).

REFERENCES

Ahmed, H., Yang, X., Akram, M., Iqbal, R., AL-Ghamdi, A., AL Farraj, D., Zeng, Y., 2025a. *Deciphering variability and heterosis in barley for sustainable yield potential*. Applied Ecology and Environmental Research, 23(3): 4443-4456.

Ahmed, H.G.M.D., Iqbal, R., Yang, X., Yang, J., Pu, X., Li, X., Yang, L.E., Zeng, Y., 2025b. *Bioactive Compounds in Barley Modulating Glycemic Response: Implications for Diabetes Management*. Natural Product Communications, 20(4): 1934578X251336985.

Al-Khayri, J.M., Sahana, G.R., Nagella, P., Joseph, B.V., Alessa, F.M., Al-Mssallem, M.Q., 2022. *Flavonoids as potential anti-inflammatory molecules: A review*. Molecules, 27(9): 2901.

Appiah, M., Abdulai, I., Schulman, A.H., Moshelion, M., Dewi, E.S., Daszkowska-Golec, A., Bracho-Mujica, G., Rötter, R.P., 2023. *Drought response of water-conserving and non-conserving spring barley cultivars*. Frontiers in Plant Science, 14: 1247853.

Baltacıer, G., and Acar, O., 2024. *Determination of the Short-Term Drought Stress Tolerance of Three Barley Varieties Using Physiological and Biochemical Changes*. Cumhuriyet Science Journal, 45(3): 471-477.

Brueggeman, R.S., 2024. *Barley*. Agrios' Plant Pathology, Elsevier: 679-684.

Chaieb, N., Labidi, S., Chiab, A.K., Ali Idoudi, Z.B., Jeddi, F.B., Ben-Hammouda, M., 2021. *Natural Mycorrhization, Mineral Uptake, Total Polyphenols and Total Flavonoids of Oat as Affected by Tillage Practices under Rainfed Conditions*. Asian Plant Research Journal, 8(4): 105-115.

Chang, C.-C., Yang, M.-H., Wen, H.-M., Chern, J.-C., 2002. *Estimation of total flavonoid content in propolis by two complementary colorimetric methods*. Journal of Food and Drug Analysis, 10(3), Article 3.

Eticha, F., Grausgruber, H., Berghoffer, E., 2010. *Multivariate analysis of agronomic and quality traits of hull-less spring barley (Hordeum vulgare L.)*. Journal of Plant Breeding and Crop Science, 2(5): 81-95.

Feizi, M., Solouki, M., Sadeghzadeh, B., Fakheri, B., Mohammadi, S.A., 2020. *Evaluation of drought tolerance indices for barley landraces under irrigated and dry conditions*. Bioscience Journal, 36(5): 1518-1527.

Fekadu, W., Mekbib, F., Lakew, B., Haussmann, B.I., 2023. *Genotype× environment interaction and yield stability in barley (Hordeum vulgare L.) genotypes in the central highland of Ethiopia*. Journal of Crop Science and Biotechnology, 26(2): 119-133.

Friero, I., Macià, A., Romero, M.-P., Romagosa, I., Martínez-Subirà, M., Moralejo, M., 2024. *Unlocking Phenolic Potential: Determining the Optimal Grain Development Stage in Hull-Less Barley Genotypes with Varying Grain Color*. Foods, 13(12): 1841.

Güngör, H., Çakır, M.F., Dumlupınar, Z., 2023. *GGE biplot analysis of genotype by environment interaction of barley cultivars*. Revista de la Facultad de Agronomía de la Universidad del Zulia, 40(2), e234021.

Güngör, H., Türkoğlu, A., Çakır, M.F., Dumlupınar, Z., Piekutowska, M., Wojciechowski, T., Niedbała, G., 2024. *GT Biplot and Cluster Analysis of Barley (Hordeum vulgare L.) Germplasm from Various Geographical Regions Based on Agro-Morphological Traits*. Agronomy, 14(10): 2188.

Han, Z., Zhang, J., Cai, S., Chen, X., Quan, X., Zhang, G., 2018. *Association mapping for total polyphenol content, total flavonoid content and antioxidant activity in barley*. BMC Genomics, 19: 1-10.

Huang, H., Gao, X., Li, Y., Tian, P., Nima, Y., Laba, Z., Ci, Z., Wei, X., Qu, J., Guan, W., 2020. *Content analysis of vitamins, dietary fibers and amino acids in a wide collection of barley (Hordeum vulgare L.) from Tibet, China*. Bioinformation, 16(4): 314.

Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.-P., Pihlaja, K., Kujala, T.S., Heinonen, M., 1999. *Antioxidant activity of plant extracts containing phenolic compounds*. Journal of Agricultural and Food Chemistry, 47(10): 3954-3962.

Kamal, G.M., Liaquat, A., Noreen, A., Sabir, A., Saqib, M., Khalid, M., Iqbal, R., 2023. *Phytochemical Profile of Cereal Grains*. Cereal Grains, CRC Press: 177-193.

Karahan, T., and Akgün, I., 2020. *Selection of barley (Hordeum vulgare) genotypes by GYT (genotype× yield× trait) biplot technique and its comparison with GT (genotype× trait)*. Applied Ecology and Environmental Research, 18(1): 1347-1359.

Khodaeiaminjan, M., and Bergougnoux, V., 2021. *Barley Grain Development during Drought Stress: Current Status*. Cereal Grains, 1: 195.

Kim, D.-O., Chun, O.K., Kim, Y.J., Moon, H.-Y., Lee, C.Y., 2003. *Quantification of polyphenolics and their antioxidant capacity in fresh plums*. Journal of Agricultural and Food Chemistry, 51(22): 6509-6515.

Patil, J.R., Mhatre, K.J., Yadav, K., Yadav, L.S., Srivastava, S., Nikalje, G.C., 2024. *Flavonoids in plant-environment interactions and stress responses*. Discover Plants, 1(1): 1-19.

Piasecka, A., Sawikowska, A., Kuczyńska, A., Ogrodowicz, P., Mikołajczak, K., Krajewski, P., Kachlicki, P., 2020. *Phenolic metabolites from barley in contribution to phenome in soil moisture deficit*. International Journal of Molecular Sciences, 21(17): v6032.

Pour-Aboughadareh, A., Barati, A., Gholipoor, A., Zali, H., Marzooghian, A., Koohkan, S.A., Shahbazi-Homonloo, K., Houseinpour, A., 2023. *Deciphering genotype-by-environment interaction in barley genotypes using different adaptability and stability methods*. Journal of Crop Science and Biotechnology, 26(5): 547-562.

Rao, S., Santhakumar, A.B., Chinkwo, K.A., Blanchard, C.L., 2020. *Investigation of phenolic compounds with antioxidant activity in barley and oats affected by variation in growing location*. Cereal Chemistry, 97(4): 772-782.

Shrestha, A., König, T., Meikle, L.A., Westhoff, P., Erban, A., Stich, B., 2024. *Morphological, physiological and metabolic responses of diverse* *barley inbreds to dry down and moderate drought stress*. bioRxiv: 2024.2005. 2021.595183.

Steel, R.G.D., and Torrie, J.H., 1960. *Principles and procedures of statistics*. McGraw-Hill Book Company, New York, Toronto, London.

Tyagi, V., Jacob, S.R., Gupta, K., Brahmi, P., 2020. *Status of introduction and conservation in barley (Hordeum vulgare L.)*. Journal of Cereal Research, 12(1): 13-18.

Wang, J., Yao, L., Hao, J., Li, C., Li, B., Meng, Y., Ma, X., Si, E., Yang, K., Zhang, H., 2024. *Growth Properties and Metabolomic Analysis Provide Insight into Drought Tolerance in Barley (Hordeum vulgare L.)*. International Journal of Molecular Sciences, 25(13): 7224.

Xiao Ya, X.Y., Du Juan, D.J., Yang XiaoMeng, Y.X., Pu XiaoYing, P.X., Zeng YaWen, Z.Y., Yang Tao, Y.T., Yang JiaZhen, Y.J., Yang ShuMing, Y.S., Chen ZhiYuan, C.Z., 2017. *Analysis of functional ingredients in barley grains from different regions between southwest China and ICARDA*. Southwest China Journal of Agricultural Sciences, 30(8): 1700-1706.

Yang, T., Zeng, Y.-W., Pu, X.-Y., Du, J., Yang, S.-M., 2013. *Correlation analysis of functional components of barley grain*. Advance in Barley Sciences, Proceedings of 11th International Barley Genetics Symposium, Springer: 199-207.

Yang, T., Jing, X., Ahmed, H., Akram, M., Iqbal, R., Al-Ghamdi, A., Al Farraj, D., Zeng, Y., 2025a. *Multi-Regional Study of Genotype X Environment Interactions in Flavonoid Accumulation of Barley (Hordeum Vulgare L.) Grains*. Applied Ecology and Environmental Research, 23(2): 3805-3820.

Yang, T., Jing, X., Ahmed, H., Akram, M., Iqbal, R., Alghamdi, A., Al Farraj, D., Zeng, Y., 2025b. *Assessing Flavonoid Content in Barley Genotypes: Genetic Contributions and Hybrid Potential for Nutritional Improvement*. Applied Ecology and Environmental Research, 23(2): 3677-3690.