Harnessing Genetic and Environmental Interactions to Optimize Functional Traits in Barley Grass Powder

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ABSTRACT

This study evaluated 27 malt barley (Hordeum vulgare L.) varieties for key nutritional and functional components - total flavonoids, y-aminobutyric acid (GABA), alkaloids, and protein - in barley grass powder across contrasting winter and summer seasons. All two-row varieties, provided by the Yunnan Academy of Agricultural Sciences, were grown under a randomized complete block design in Kunming, China. Functional components were quantified using colorimetric and Kjeldahl methods, with data analyzed through ANOVA, Tukey's HSD, percent change calculations, biplots, and hierarchical clustering. Results showed highly significant genotypic, seasonal, and genotype \times season interaction effects for all traits. Concentrations of all components were higher in winter, reflecting enhanced biosynthesis under cooler temperatures. Varieties V5, V13, V14, V16, and V21 consistently exhibited high and stable functional profiles across seasons, while V3, V7, V9, V18, and V26 were more sensitive to seasonal changes. Alkaloid and protein levels showed notable seasonal sensitivity. Multivariate (Biplot, Heatmap) analyses revealed strong positive correlations among traits and clearly distinguished superior genotypes, highlighting their genetic stability. Lower coefficients of variation in winter suggested greater trait stability under cooler conditions. The study underscores the influence of genotype \times environment interactions on the functional quality of barley grass powder and identifies resilient genotypes suitable for functional food development. These findings provide a valuable foundation for breeding climate-resilient, nutritionally superior barley cultivars and promote the use of barley grass in health-oriented applications.

Keywords: flavonoids, GABA, alkaloids, protein, genotypes, stability.

INTRODUCTION

B arley (*Hordeum vulgare* L.) is one of the earliest domesticated cereal crops not only prized for its agronomic value but also for its nutritional quality and health benefits. Historically utilized as an animal feed, brewer, and food ingredient, barley has, in the last few years, been gaining more interest as a functional food crop, especially in the form of its young green shoots, barley grass (Filip, 2023, Huang et al., 2024). Barley grass is rich in bioactive compounds, such as flavonoids, γ -aminobutyric acid (GABA), alkaloids, and proteins, which are responsible for a range of physiological benefits like

antioxidant, anti-inflammatory, neuroprotective, and cardioprotective effects (Huang et al., 2024; Zeng et al., 2024).

The increasing consumer demand for functional foods with added health benefit over common nutrition has spurred research on crops like barley. Functional food ingredients like flavonoids are reported to have extremely high antioxidant capacity and a function of mitigating oxidative stressrelated diseases (Iannucci et al., 2021). Similarly, GABA, an amino acid that is not encoded into proteins, has crucial functions in neurotransmission and relief of stress, while dietary alkaloids are responsible for many kinds of pharmacological actions like

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antimicrobial, anti-diabetic activities (Deng et al., 2019; Zeng et al., 2024). Protein, which is a necessary nutrient, not only supports basic metabolism, but also supplies the nutritional improvement of functional foods. Therefore, the information regarding the factors regulating the deposition of such compounds in barley grass is of extreme importance to breeding programs to produce nutritionally improved and climate-adapted cultivars.

Barley grass powder is the optimum functional food that provides nutrients to cells within the human body and detoxifies cells; however, the functional compounds played a significant role towards the benefit to the individual's health. Barley grass powder, which is derived from young barley leaves, has been proved to be very helpful towards the healthy condition of the entire body due to the abundance of functional compounds, i.e., flavonoids, y-aminobutyric acid (GABA), alkaloids, and proteins. These compounds are responsible for numerous therapeutic activities, which are antioxidant, anti-inflammatory, and hypoglycemic activities, to regulate the progression of cardiovascular diseases like diabetes. diseases, and osteoporosis (Zeng et al., 2018; Baloch et al., 2024). GABA is particularly emphasized to induce calming effects and sleep improvement, and flavonoids are powerful antioxidants, which can produce anti-cancer effects and enhance immunity (Zeng et al., 2020; Kadege et al., 2024). Proteins present in barley grass further aid muscle function and overall nutrition, thus becoming an appropriate supplement to consume to enhance diet and prevent diseases (Filip, 2023; Raj et al., 2023). Barley grass can be one of the best functional foods to prevent chronic diseases and the best raw material of modern diet composition to innovate good health industry, and thus prove that flavonoids, Alkaloids, GABA, and protein mechanism of barley grass prevent and cure chronic diseases (Zeng et al., 2024). Barley grass powder, due to the synergistic effect of the above bioactive compounds. comes to be an effective functional food (Zeng et al., 2020; Mărginean et al., 2021).

Environment and genotype are some of the principal factors that determine the nutritional value of barley grass. Genetic diversity supplies raw materials to be worked with through breeding and improvement, while the constituents such as temperature, intensity of light, and season affect the biosynthesis of the secondary metabolites and the primary compounds to a large extent (Zeng et al., 2018; Deng et al., 2019; Beleggia et al., 2021). The genotypeenvironment (G×E) interaction can stabilize or destabilize the expression of functional traits, which is the reason that such interactions should be dissected to pave the way for the reliable production of superior barley cultivars. Seasonal fluctuation, especially, has significant impacts on the metabolism of the plants. Decreasing temperatures increase the production of secondary metabolites such as flavonoids and alkaloids due to the activation of the stressresponse pathway (Zeng et al., 2018; Yang et al., 2020). Conversely, summer heat can disrupt biosynthetic pathways such that bioactive compounds of economic importance fail to accumulate to low levels. Given the problems of climate variability at the world scale, the process of barley genotype screening that has good functional quality irrespective of season gains more importance in sustainable agriculture and ensuring food availability (Ijaz et al., 2023; Zahoor et al., 2024).

Previous studies have emphasized the genetic variability of barley for several traits like the characteristics of the yield, the susceptibility to drought, and the nutritional composition (Beleggia et al., 2021; Filip, 2023; Shen et al., 2025). However, the studies specifically targeting the variability regarding functional component of barley grass powder, specifically under different seasonality, has been limited. Moreover, the studies previously have been focused on mature grain quality, while the unique nutritional traits of the barley grass, which is harvested at the young stage, remained comparatively less studied altogether (Klem et al., 2019; Shen et al., 2025). Multivariate analysis supported the findings too. Biplots indicated high, positive correlation between functional traits that the can he simultaneously enhanced. Hierarchical clustering efficiently grouped the genotypes into high- and low-performing clusters, presenting the breeder with a realistic platform to select candidate varieties to be translated into functional food production. Significantly, the lower winter coefficients of variation of all the traits indicated that the cooler climate enables the more stable expression of functional components, the issue of the highest salience to consistent commercial production (Martínez-Subirà et al., 2021; Zulfigar et al., 2024).

The use of genetic and environmental information to add to barley breeding programs becomes more appropriate under the double pressure of climate change and the increase of demand for foods based on health. Functional foods not only deliver preventable health benefits but are part of global initiatives to prevent non-communicable diseases through nutrition (Bunting, 2021; Iannucci et al., 2021). With its nutritional potential, barley grass is the green, ecofriendly answer to these problems. Its application requires the availability of genotypes that can produce high bioactive compounds under any climate or season. Besides breeding, this research has industrial importance. Natural health commodities like diet adjuncts and functional beverages are a rapidly growing market, and barley grass powder is a good fit for such applications (Meng et al., 2023; Huang et al., 2024). Consistency and functional ingredient quality can be attained with the use of strong barley genotypes, which can further help to enhance consumer trust and effectiveness of the products.

Future research possibilities involve more in-depth physiological, transcriptomic, and metabolomic investigations to account for the molecular foundations of trait stability and biosynthetic efficiency in barley grass. Key regulatory genes and biosynthetic pathways will be identified to continue enabling breeding programs directed towards the development of cultivars that are specialized in terms of agronomic traits as well as nutritional content (Yang et al., 2020; Huang et al., 2024). The existing research supports the strong impact of genotype, environment, and their interaction on the functional traits of barley grass powder. With the systematic evaluation and determination of stable, highperformance cultivars, we lay the platform for breeding programs connecting nutritional traits to sustainability in the environment. Such efforts will contribute not only to the development of climate-resilient functional foods but also to the broader goals of sustainable agriculture and global health promotion.

MATERIAL AND METHODS

Experimental materials and field design

The experimental materials (Table 1) comprised 27 barley (Hordeum vulgare L.) varieties provided by the Institute of Biotechnology and Germplasm Resources, Yunnan Academy of Agricultural Sciences, Kunming, Yunnan, China. Table 1 lists 27 malt barley varieties, all classified as tworow type, evaluated for various functional components extracted from barley grass powder across different seasons. Each variety is designated with a specific code (V1-V27) for reference in subsequent analyses. Field trials were conducted at the experimental research site in Dianyuan Town, Panlong District, Kunming City (elevation: 1,973 meters). The site was characterized by moderately fertile soil. The experiment was conducted in a three-replicated randomized complete-block design (RCBD). Three rows of each genotype were sown and 200 seeds were given to each. Each row was 2 meters in length and there was a 0.3-meter row-to-row spacing. Strip sowing was practiced, and regular agronomic practices were followed. Experiments were conducted across two seasons winter and summer. Thinning of the seedlings was carried out in the jointing stages. After harvesting, plant material from all treatments in the experiments was air dried, ground, packaged, and then stored in -20°C for future analysis.

Code	Variety Name	Туре	Row	Code	Variety Name	Туре	Row
V1	Yunpi 9	Malt Barley	2	V15	Yunjing 2	Malt Barley	2
V2	Yunpi 10	Malt Barley	2	V16	Yunpi 18	Malt Barley	2
V3	Yunpi 11	Malt Barley	2	V17	Yunpi 20	Malt Barley	2
V4	Aisigan 4	Malt Barley	2	V18	Tengyunmai 4	Malt Barley	2
V5	Yunpi 7	Malt Barley	2	V19	Yunpi 21	Malt Barley	2
V6	Yunpi 5	Malt Barley	2	V20	Yunpi 22	Malt Barley	2
V7	Yanmai 2	Malt Barley	2	V21	Yunpi 3	Malt Barley	2
V8	Aaoxuan 3	Malt Barley	2	V22	Kunpi 4	Malt Barley	2
V9	Yunpi 2	Malt Barley	2	V23	Aisi82gan1	Malt Barley	2
V10	Yunpi 4	Malt Barley	2	V24	Yunpi 24	Malt Barley	2
V11	Kunpi 2	Malt Barley	2	V25	Yungong 1	Malt Barley	2
V12	Yunpi 12	Malt Barley	2	V26	Yungong 2	Malt Barley	2
V13	Yunyumai 1	Malt Barley	2	V27	Aisi 500	Malt Barley	2
V14	Yunpi 15	Malt Barley	2				

Table 1. List of 27 malt barley studied varieties for various functional components extracted from barley grass powder evaluated in different seasons

Analysis of nutritional and functional components in barley

The assays of nutritional and functional constituents - total flavonoids, y-aminobutyric acid (GABA), alkaloid, and protein content were conducted in the Key Laboratory of the Institute of Biotechnology and Germplasm Resources of Yunnan Academy of Agricultural Sciences. Barley grass powders were determined in triplicate (three biological replicates) and averaged. Flavonoid was analyzed by using a method of aluminum nitrate colorimetric according to the approach of Zhao Chunyan et al. (2011). GABA content was measured following the method of (Inatomi and Slaughter, 1975). Alkaloid content was quantified using the method described by Han Shuyun (2010). Protein content was estimated using the Kjeldahl method. For protein estimation, 1.0000 ± 0.0001 g of the powdered sample was placed into a 250 mL digestion tube. Two Kjeldahl catalyst tablets (equivalent to 7 g K₂SO₄ and 0.8 g CuSO₄·5H₂O) and 12 mL of concentrated H₂SO₄ were added. Digestion was performed at 420°C for 2 hours using an 8400 Automatic Kjeldahl Nitrogen Analyzer. After cooling, titration was conducted, and the titrant concentration was accurate to four decimal places. A blank test was included with each batch of samples. Protein content (%) was calculated using the formula:

Protein (%) = Nitrogen (%) \times 6.25

where:

T = Volume (mL) of HCl used in sample titration;

B = Volume (mL) of HCl used in blank titration;

N = Normality of HCl (accurate to four decimal places).

Statistical analysis

All physiological data were exposed to analysis of variance (ANOVA) technique (Steel and Torrie, 1960) by using statistic 8.1. Post-hoc mean comparison test (Tukey's HSD) following the ANOVA for each key traits were conducted to highlighting statistically significant differences among genotypes (Tukey, 1949). Reduction percentages were performed to conclude the performance of genotypes based on the seedling traits in normal and water deficit conditions. The percent change for each trait was calculated using the formula: Percent Change (%) = [(Summer – Winter) / Winter] 100. Biplot, Hierarchical clustering Х heatmaps and dendrograms analysis were performed using R studio 4.1.3 (Team, 2020) with the support of the factoextra, ggplot2, and pheatmap packages for visualization and clustering analysis (Kassambara and Mundt, 2016; Kolde, 2019).

RESULTS AND DISCUSSION

ANOVA indicated highly significant (p<0.01) variation among the genotypes of the 27 barley varieties under study regarding all the functional constituents, i.e., flavonoids, GABA, alkaloids, and protein, isolated from barley grass powder (Table 2). Highly significant genotypic effects indicate the wide genetic variation among the barley genotypes with the potential to select the superior genotypes with enhanced nutritional levels at the vegetative stage. It is reasonable to expect the variation to be due to the intrinsic variation between the biosynthetic pathways and the expression of the genes to produce the secondary metabolites. Similar, (Deng et al., 2019; Elouadi et al., 2023), documented appreciable variability of barley at early nutritional growth phases in and phytochemical composition. Seasonal variation was significant statistically under all the characteristics, which indicated that the biosynthesis and deposition of the metabolites under barley grass varied under winter and summer season conditions. In particular, alkaloid (MS=920.61*) and protein (MS=812.4**) showed strong seasonality responses, which mean that abiotic stress, light intensity, and temperature under varying seasons manage metabolic activity. These findings align with (Iannucci et al., 2021),

who indicated that the season greatly affected the phenolics, GABA, and the protein content of cereal grasses. Besides, the occurrence of significant genotype \times season (G \times S) interactions on the four components indicates that the varieties responded variably under different environments. The interaction indicates the importance of multi-environment testing to determine stable, high-performing lines that maintain constant high expression of functional compounds. Flavonoid and GABA content variation between seasons may be due to abiotic-induced secondary metabolism since the compounds are generally involved in defence processes and antioxidant activities (Klem et al., 2019). Protein content interaction was also found to be significant, reflecting the fact that some varieties possess enhanced nitrogen assimilation and amino acid metabolism capabilities under varied environments. Genotype, season, and genotype \times season interaction, which greatly impact the functional composition of barley grass powder, emphasize specific breeding programs and environmental management. These results can be applied towards the production of functional foods through the screening of varieties with elevated levels of compounds having health benefits, such as flavonoids, GABA, alkaloids, and proteins, to be offered as green foods and nutraceuticals (Son et al., 2016; Huang et al., 2024).

Table 2. Mean	squares from a	analysis of varian	ce (ANOVA)	for various f	functional c	components	extracted
from barley	grass powder	in 27 malt barley	varieties eva	luated across	s winter and	d summer se	asons

Sources of Variation	DF	Flavonoids	γ-aminobutyric acid	Alkaloids	Protein
Replications	2	44.21	12.62	15.35	9.02
Varieties	27	31.91**	16.77**	17.61**	88.43**
Seasons	1	21.51*	29.21**	920.61*	812.4*
Varieties * Seasons	27	18.01*	21.98**	18.42*	62.13**
Error	104	3.13	6.38	4.15	3.74
Total	161				

* = significance at 0.05 level; ** = highly significance at 0.01 level.

Descriptive statistics of functional components

Descriptive statistics of the functional compounds obtained using barley grass powder indicated obvious seasonality among the 27

malt barley varieties (Table 3). Flavonoid, γ -aminobutyric acid (GABA), alkaloid, and protein content tended to be higher in the winter season than the summer season. Mean flavonoid content was significantly higher in

winter (137.51 mg/100g) than summer (91.13 mg/100g), indicating higher secondary metabolite accumulation at low temperature. This is supported by (Beleggia et al., 2021; Bunting, 2021), who reported that lowtemperature conditions in winter stimulate the phenylpropanoid pathway, leading to increased flavonoid biosynthesis in young cereal grasses. Similarly, GABA content also 180.37 peaked during winter (average mg/100g), which could be attributed to cold-induced activates stress that the GABA shunt pathway, promoting GABA accumulation as a protective response (Klem et al., 2019; Shabbir et al., 2024).

Protein and alkaloid content showed the same profile, with higher mean values under winter (107.56 mg/100g, and 30.56%, respectively) than under summer (96.09 mg/100g, and 27.51%). Increase of alkaloids under winter can be accounted for alkaloidstress-responsive inducing secondary metabolism. since alkaloids typically take part in abiotic stress factor defense mechanisms of plants (Piasecka et al., 2017; Pham-Khanh et al., 2024). Higher barley grass protein under winter growth, however, higher nitrogen assimilation, reflects alongside higher metabolism efficiency. under lower growth temperatures. This supports the earlier findings of (Iannucci et al., 2021) that barley grass harvested at early spring has higher nutritional value, such as proteins and phytochemicals.

In terms of variability, the coefficient of variation (CV) of all the components was smaller under winter (from 8.05% to 9.58%) compared to the summer (11.25%) to 14.82%), indicating greater stability of the traits under cooler climates. This smaller variability under winter can be an indication uniformity of physiological of higher response of the varieties due to smaller variability of the environment (environmental variability). Conversely, the greater CVs of summer, specifically GABA (14.82%) and flavonoids (13.16%),indicate greater genotype \times environment interaction under hot climates, which can cause destabilization of the process of metabolite accumulation. This observation suggests the necessity of the choice of varieties with stable trait performance under diversified climates (Zeng et al., 2018, 2024).

Winter was the more appropriate season for higher accumulation and stability of expression of functional compounds' of barley grass powder under this study. Results support the extent of targeting early winter sowing time to be followed under optimal applications. functional food Genotypic differences among seasons, along with the effects of the observed environments, indicate the necessity of combined physiological, biochemical, and breeding approaches to evolve high-quality barley cultivars to be under nutraceutical applications used (Piasecka et al., 2017; Raj et al., 2023).

Parameters	Seasons	Flavonoids	γ-aminobutyric acid	Alkaloids	Protein
Minimum	Winter	100.55	139.21	90.00	26.16
IVIIIIIIIIIIII	Summer	60.31	76.10	75.31	22.71
Movimum	Winter	180.59	202.69	124.35	34.87
Maximum	Summer	137.89	160.37	121.99	33.27
A viene de	Winter	137.51	180.37	107.56	30.56
Average	Summer	91.13	113.72	96.09	27.51
Variance	Winter	738.64	446.48	152.96	6.12
variance	Summer	575.75	720.87	216.71	9.57
Standard Deviation	Winter	13.18	14.53	9.37	2.47
Standard Deviation	Summer	11.99	16.85	11.72	3.09
Coefficient of veriation	Winter	9.58	8.05	8.71	8.10
	Summer	13.16	14.82	12.20	11.25

Table 3. Descriptive statistics of various functional components extracted from barley grass powder in 27 malt barley varieties across winter and summer seasons

Characterization of varieties

Performance of the 27 malt barley varieties manifested strong genotypic variation between the summer season and the winter season based on the proof of the test of Tukey's HSD (p≤0.05) presented in Table 4 and 5. Varieties that are indicated with "a", such as V5, V13, V14, V16, and V21, exhibited the highest flavonoid, GABA, alkaloid, and protein levels in the two seasons, indicating that the varieties are more metabolically stable and climate change tolerant. These highest-performing varieties further manifested the least decrease of the bioactive constituent, indicating the varieties are more functional foods appropriate all through the year. On the contrary, varieties indicated with "d", such as V3, V7, V9, V18, and V26, presented the lower values with the greatest decreases for all the constituents, indicating the highest susceptibility to heat stress and climate change. Statistical differentiation of the means invariably through the use of letters ensures the validity of the results, which supports the genotype \times environment interaction status of the barley varieties with constant functional traits under fluctuating climates (Suriano et al., 2018; Huang et al., 2024).

Relative performances of the 27 malt barley varieties under winter to summer condition exhibited notable genotypic variability in the retention of the functional constituents flavonoids and γ -aminobutyric acid (GABA) (Table 4). These reductions were least percent-wise in the case of V13, V14, V16, V21, and V5, the highest-performing varieties. Genotype V21 exhibited the least reduction in the case of GABA content (-18.25%) and maintained high flavonoid (169.31 mg/100g under winter; 129.82 mg/100g under summer) and GABA (196.18 mg/100g under winter; 160.37 mg/100g under summer) levels, reflective of superior metabolic efficiency and adaptability towards the external condition. V16 also yielded high levels with only reductions of flavonoids and GABA, which are -24.91% and -20.24%, respectively. These high-performance varieties likely harbor superior genetic potential to continue biosynthesis of the secondary metabolites under heat or stress due to likely superior antioxidant defense systems and the operation of conditionresponsive genes (Baik and Ullrich, 2008; Zeng et al., 2020).

Conversely, the lowest five varieties - V9, V3, V18, V7, and V26 - registered the greatest decline in flavonoids and GABA. Notably, V9 registered a -43.83% decline in flavonoids and -50.37% in GABA, the greatest among all varieties, which suggests poor heat tolerance and stress tolerance. The extreme decline in these metabolites in summer implies that these varieties are environmentally sensitive, possibly due to impaired enzymatic activity in the phenylpropanoid and GABA-shunt pathways, which are heat-sensitive (Vrábl et al., 2023; al., 2024). Moreover, Wang et low biosynthetic efficiency and impaired antioxidant responses may be the cause of the decline in the levels of functional component in these varieties under summer conditions.

The large range in percent change between varieties indicates the large genotype \times environment interaction and the need to choose varieties with stable expression of health-promoting compounds over seasons. Varieties V21, V16, and V13 are good choices for functional food production, particularly for grass powder-based products for uniform nutritional quality. These results are consistent with (Suriano et al., 2018), who highlighted the genetic background in influencing the phytochemical composition of barley under different environmental conditions. In addition, choosing varieties with little seasonal degradation of bioactive compounds is essential for perennial functional food processing and breeding climate-resilient cultivars for climate-smart agriculture (Son et al., 2016; Martínez-Subirà et al., 2021).

The evaluation of 27 malt barley lines for protein and alkaloid content in barley grass powder over two contrasting seasons revealed high genotypic variability in percent change, reflecting unequal stability under diverse environmental conditions (Table 5). Top-performing lines, V13, V16, V21, V14, and V5, reflected minimal seasonal decline, reflecting high genetic resistance and

metabolic stability. Notably, V13 experienced the lowest loss in alkaloids (-1.14%) and also the lowest loss in protein content (-4.58%), demonstrating high biosynthetic ability even in extreme summer temperatures. Similarly, V16 and V21 experienced low loss in alkaloids (-1.42% and -1.31%, respectively) and moderate retention of proteins, indicating their viability for use in functional foods. These varieties are most likely to have effective nitrogen metabolism and stable secondary metabolite pathways, which remain active despite environmental stresses (Han et al., 2018; Deng et al., 2019). On the other hand, the lowest five performers, V3, V7, V26, V18, and V9, demonstrated drastic seasonal drops, especially in alkaloids (range -15.14% to -16.36%) and protein (-13.50% to -19.56%). The V18 experienced the most drastic drop in protein (-19.56%), which suggests that high summer temperatures had a major impact on protein biosynthesis or encouraged degradation. This may be due to stress-downregulation of amino acid biosynthesis routes or increased protease activity upon heat stress (Meng et al., 2023). In addition, the drastic decrease in alkaloids in these varieties suggests faulty functioning in the biosynthetic pathways associated with defense-related secondary metabolites due to impaired stress-responsive gene expression or enzyme inhibition upon heat stress conditions (Yang et al., 2020).

highlight These results genotype environment interaction to be one of the crucial factors in determining the nutritional profile of barley grass, especially in seasonal variation. Variety selection of V13, V16, V21, V14, and V5 ensures high and consistent yield of bioactive substances, hence desirable for functional food processing and production of green powder all year round. The variation observed is in agreement with previous reports by (Son et al., 2016, Iannucci et al., 2021), who reported season effect on barley bioactive and highlighted genotype's role in controlling thermal tolerance and retention of metabolites.

Table 4. Performance of 27 malt barley varieties for functional components (Flavonoids and γ -Aminobutyric Acid) extracted from barley grass powder, across winter and summer seasons, including percent change from winter to summer. Genotypic means were compared using Tukey's HSD test; means followed by different letters indicate significant differences at $p \leq 0.05$.

N 7 · · ·		Flavonoids		γ-aminobutyric acid			
varieties	Winter	Summer	%Changes	Winter	Summer	%Changes	
V1	147.22c	94.61b	-35.74	187.58b	111.5c	-40.56	
V2	150.87b	95.17b	-36.92	190.95b	108.75c	-43.05	
V3	108.99d	63.67d	-41.58	149.96d	76.1e	-49.25	
V4	110.21c	69.64cd	-36.82	168.8c	94.71d	-43.89	
V5	175.64a	137.89a	-21.49	198.17a	151.13a	-23.74	
V6	114.33c	85.54b	-25.18	169.03c	101.62c	-39.88	
V7	104.23d	63.49d	-39.09	139.32d	77.21e	-44.59	
V8	150.03b	93.67b	-37.56	189.84b	99.03d	-47.84	
V9	107.69d	60.5d	-43.83	153.34d	76.1e	-50.37	
V10	109.84c	74.7c	-31.99	164.15c	104.96c	-36.06	
V11	109.09c	70.51c	-35.36	166.18c	92.64d	-44.25	
V12	108.53c	73.33c	-32.44	166.76c	99.66d	-40.24	
V13	170.21a	131.11a	-22.97	199.08a	159.5a	-19.88	
V14	175.73a	133.7a	-23.92	197.24a	151.18a	-23.35	
V15	108.35c	73.95c	-31.74	161.88c	101.61c	-37.23	
V16	180.59a	135.6a	-24.91	199.16a	158.85a	-20.24	
V17	151.71b	94.37b	-37.79	195.95b	116.8c	-40.39	
V18	106.64d	60.68d	-43.10	144.73d	77.53e	-46.43	
V19	154.23b	95.28b	-38.22	199.79a	113.07c	-43.40	
V20	161.62b	97.65b	-39.58	200.83a	133.51b	-33.52	
V21	169.31a	129.82a	-23.32	196.18a	160.37a	-18.25	
V22	154.05b	95.36b	-38.10	195.89b	115.35b	-41.12	
V23	163.39b	98.28b	-39.85	202.69a	150.89b	-25.56	
V24	158.63b	99.49b	-37.28	197.4a	118.28c	-40.08	
V25	145.37c	90.98b	-37.42	199.44a	122.81b	-38.42	
V26	100.55d	60.31d	-40.02	139.21d	77.26e	-44.50	
V27	115.71c	81.34b	-29.71	196.41a	120.12b	-38.84	

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Table 5. Performance of 27 malt barley varieties for functional components (Alkaloids and Protein),extracted from barley grass powder, across winter and summer seasons, including percent changefrom winter to summer. Genotypic means were compared using Tukey's HSD test;means followed by different letters indicate significant differences at $p \le 0.05$.

Maniatian		Alkaloids		Protein			
varieties	Winter	Summer	%Changes	Winter	Summer	%Changes	
V1	111.2b	99.96c	-10.10	30.74b	27.77b	-9.65	
V2	110.75b	98.52c	-11.04	30.41b	27.94b	-8.14	
V3	90.12c	75.38d	-16.36	26.49c	22.71d	-14.25	
V4	100.09b	89.26c	-10.82	29.1c	25.65c	-11.86	
V5	123.19a	114.82a	-6.79	33.65a	32.61a	-3.11	
V6	101.68b	88.92c	-12.55	29.31c	26.13c	-10.87	
V7	90c	75.31d	-16.32	26.16c	22.88d	-12.55	
V8	111.84b	96.49c	-13.72	31.02b	27.85b	-10.20	
V9	90.61c	76.85d	-15.18	26.56c	22.98d	-13.50	
V10	93.01c	83.18c	-10.57	28.68c	25.82c	-9.96	
V11	97.46c	84.86c	-12.93	28.62c	25.55c	-10.73	
V12	94.02c	82.85c	-11.89	28.5c	25c	-12.29	
V13	122.91a	121.51a	-1.14	34.87a	33.27a	-4.58	
V14	124.35a	119.67a	-3.76	34.34a	32.21a	-6.20	
V15	90.64c	80.68c	-10.99	28.35c	24.33c	-14.20	
V16	123.75a	121.99a	-1.42	33.57a	30.81a	-8.24	
V17	113.02b	98.62c	-12.75	31.24b	28.04b	-10.24	
V18	90.97c	77.19d	-15.14	28.28c	22.75d	-19.56	
V19	116.96b	101.46b	-13.25	32.04b	28.57b	-10.82	
V20	117.37b	102.89b	-12.34	32.29b	29.53b	-8.53	
V21	122.63a	121.03a	-1.31	34.69a	32.28a	-6.96	
V22	112.61b	99.9c	-11.29	31.05b	28.42b	-8.46	
V23	118.16b	101.2b	-14.35	32.44b	29.43b	-9.28	
V24	115.01b	101.49b	-11.76	31.62b	28.64b	-9.44	
V25	115.3b	101.74b	-11.76	31.99b	29.4b	-8.08	
V26	91.36c	76.7d	-16.04	28.03c	24.1d	-14.04	
V27	115 18b	101.85b	-11 57	31 02b	28 17h	-9 20	

Biplot (Genotype*Traits) analyses

The biplots analysis (Figures 1A and 1B) revealed the distribution of 27 malt barley varieties based on four major functional components - flavonoids, γ -aminobutyric acid content (GABA), alkaloids, and protein extracted from barley grass powder under winter season and summer season conditions. Under winter season, the first principal component (F1) and the second principal component (F2) explained 97.48% of the total variance (F1: 94.29%, F2: 3.19%), while under summer season, the total variance was 98.28% (F1: 96.54%, F2: 1.74%). The high explained variance in both the seasons attested to the robustness and reliability of the principal component analysis (PCA) to describe the multidimensional trait data. In both the biplots, flavonoids, GABA, alkaloids, and protein vectors were near to other, indicating strong positive each intercorrelation among these traits. This was consistent with the previous observation by (Islam et al., 2021; Yang et al., 2025), who reported strong interrelationship among functional and nutritional contents of barley grass under varied environmental conditions.

During winter season, varieties V13, performed V14. V16. V21. and V5 better, clustering near the positive side of F1 along the direction of the vectors of traits. These varieties had higher accumulations of flavonoids, GABA, alkaloids, and protein. Similarly, during summer season, varieties V13, V14, V16, V21, and V5 were the best. Interestingly, V13 and V14 (synonyms for V13 and V14) had better functional profiles in both seasons, which showed genetic stability and strength for functional trait accumulation. The consistent performance of these varieties was in line with previous reports of (Martínez-Subirà et al., 2021; Huang et al., 2024), who reported some barley lines with stable production of bioactive compounds under different temperature conditions.

higher functional The component accumulation in the highest-yielding varieties could be attributed to more efficient secondary metabolite biosynthetic pathways and stressconditioned physiological processes, as suggested by (Beleggia et al., 2021; Fatemi et al., 2023). Higher summer temperatures could also have further enhanced GABA and flavonoid biosynthesis, confirming the hypothesis that environmental signals control secondary metabolite production in cereals (Piasecka et al., 2017; Iannucci et al., 2021).

While, during summer and winter analysis, varieties V9, V3, V18, V7, and V26 had consistently lower levels of functional components, located on the negative side of F1 and far from the trait vectors. Such consistent poor performance during the seasons revealed inherent genetic constraints in their metabolic ability. The same kind of consistent results of low bioactive compound producers among the varieties of barley were documented by (Raj et al., 2023; Yang et al., 2025), and they attributed such traits as a consequence of insufficient activation of biosynthetic pathways under environmental stresses.

Overall, the biplot patterns of the two seasons separated the high genetic diversity of malt barley varieties in trial for the functional and nutritional traits. The anticipated clustering of the good varieties in the vicinity of the vectors showed a potential improving the varying functional for components through directional selection and breeding schemes. Lack of statistically significant difference in the genotype's position during the seasons showed the relative stability of the functional traits, thereby further showing the viability of the selected varieties for producing functional foods and biofortification programs. The results agreed with earlier research conducted by (Klem et al., 2019; Elouadi et al., 2023), who showed the application of cereal crop genetic diversity in their nutritional quality improvement under contrasting environmental conditions.



Figure 1. Biplot analysis (varieties × traits) of 27 malt barley varieties based on functional components (Flavonoids, γ-Aminobutyric Acid, Alkaloids, and Protein) extracted from barley grass powder: (Figure A) during the winter season, and (Figure B) during the summer season.

Hierarchical clustering heatmaps and dendrograms

The heatmaps and hierarchical clustering dendrograms (Figures 2A and 2B) revealed the diversity among 27 malt barley varieties (V1-V27) according to their functional component profiles - i.e.,

flavonoids, γ -aminobutyric acid (GABA), alkaloids, and protein contents - both in winter and summer seasons. These multivariate analyses captured well the biochemical diversity and underlying genetic differences among the varieties. During the winter season (Figure 2A), the clustering divided the varieties into two broad categories. The first cluster, which was mostly shaded in darker green, consisted of V13, V14, V16, V21, and V5, with uniformly high levels of accumulation of the functional components studied. These varieties exhibited superior profiles in all traits, as indicated by their best performance status in previous biplot analyses. Their greater abundance of secondary metabolites and proteins might have been due to greater activity of biosynthetic pathways and superior physiological adaptations, consistent with patterns reported by Klem et al. (2019), Elouadi et al. (2023), Lavrukhina et al. (2024) in barley and related cereals.

Conversely, the second cluster, which comprised mostly magenta to pink colors, contained varieties such as V9, V3, V18, V7, and V26, with reduced flavonoid, GABA, alkaloid, and protein content. These varieties were always ranked the lowest in all the traits. Their negative accumulation profiles may be due to genetic restriction in secondary metabolite biosynthesis or reduced physiological response under winter conditions, as also suggested by Mărginean et al. (2021), Li et al. (2023), Meng et al. (2023). Notably, intracluster variation was observed, which suggested that even within major groups, individual varieties possessed certain strengths in different components. For example, some varieties possessed relatively high flavonoid content but moderate GABA content, suggesting partial independence in the genetic control of these processes. This observation was consistent with the results of Deng et al. (2019), who suggested incomplete genetic linkage between flavonoid and GABA synthesis in barley germplasm. Furthermore, the dendrogram structure suggested that varieties with similar functional profiles clustered together, suggesting the potential for selection of complementary parents in breeding programs for the improvement of multiple bioactive traits in combination, as promoted by Mărginean et al. (2021).

Concordantly, summer season analysis (Figure 2B) also supported trends of observed genotype performance. The heatmap clearly showed segregation of varieties according to the intensity of their functional contents. Varieties V13, V14, V16, V21, and V5 were tightly clumped and uniformly high in flavonoids, GABA, and protein. These varieties probably had genetic potential for enhanced secondary metabolite biosynthesis, which would have been enhanced under summer environmental stresses of elevated temperatures and UV radiation. Studies by Piasecka et al. (2017), Filip (2023), Khalid et al. (2024) also showed that abiotic stresses trigger biosynthesis of flavonoid and other phenolic compounds as a result of their antioxidant property. Furthermore, GABA accumulation, which is typically involved in was greater under such relief. stress conditions. Contrary to these, lines such as V9, V3, V18, V7, and V26 contained lower functional component contents even during the summer season, all of which fell in the low-performance category. Such lines may lack the genetic machinery for high metabolite accumulation or possess poor physiological plasticity under summer stress conditions. This was in agreement with earlier research by Iannucci et al. (2021), Islam et al. (2021), who emphasized that environment and genetic background play an important role in the accumulation of the bioactive compound in barley grass. The clustering patterns during the two seasons had strong intercorrelations of the functional traits, whereby genotypes with high flavonoids were also characterized with high GABA and protein content. This co-regulation can be indicative of integrated metabolic pathways triggered by genotype-environment interactions, as proposed by Islam et al. (2021), Yang et al. (2025).

In the current experiment, the dendrograms and heatmaps by seasons yielded global insights into the biochemical and genetic variability in the malt barley varieties. Repeated recognition of high-performing varieties (V13, V14, V16, V21, and V5) indicated their future use in improvement for greater nutraceutical and functional quality of barley. Simultaneously, recognition of poorly performing varieties gave scope for improving them in targeted ways or with targeted agronomic intervention. Such findings corroborated earlier literature stressing the paramount role of genotype-environment interactions to determine the composition of the functional component in barley (Son et al., 2016; Yang et al., 2020).



Figure 2. Dendrogram of 27 malt barley varieties based on functional components (Flavonoids, γ-Aminobutyric Acid, Alkaloids, and Protein) extracted from barley grass powder: (**Figure A**) Winter season, and (**Figure B**) Summer season. Varieties are labelled by numbers 1-27, corresponding to V1-V27.

CONCLUSIONS

This study demonstrated significant genotypic and environmental influences on the accumulation of flavonoids, GABA, alkaloids, and protein in barley grass powder from 27 two-row malt barley varieties. Highly significant genotype, season, and genotype \times season interactions highlighted the complex genetic and environmental regulation of functional components. Varieties V13, V14, V16, V21, and V5 consistently exhibited superior performance, making them strong candidates for functional food development and breeding for enhanced nutraceutical value. Seasonal effects. particularly alkaloid and protein levels, impacting reflected metabolic responses to temperature and light intensity. Winter cultivation favored higher and more stable functional compound accumulation, suggesting cooler conditions support enhanced metabolite biosynthesis. Multivariate analyses, including biplots and hierarchical clustering, effectively separated highand low-performing genotypes, providing practical tools for selection. Strong positive correlations among traits indicated the potential for simultaneous improvement through targeted breeding. This research offers valuable insights into selecting resilient barley varieties for year-round functional grass production, supporting food security and sustainability. Future studies should investigate the molecular mechanisms behind trait stability and validate these findings across broader environments. Overall, this work reinforces the importance of integrating genetic and environmental strategies in barley improvement and identifies stable, high-value genotypes for functional food applications. Leveraging these genetic resources will facilitate the development of climate-resilient, healthpromoting barley cultivars, meeting the growing demand for sustainable, nutritionfocused agriculture.

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