

Spectroscopic and Multivariate Analysis of Nutritional Traits and Anthocyanin Variation in Barley Genotypes

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ABSTRACT

This study evaluated the genetic diversity and nutritional potential of 195 barley genotypes obtained from the Biotechnology and Germplasm Resources Institute, Yunnan Academy of Agricultural Sciences, China, under a single-environment field trial at the Songming Research Station, Yunnan Province, China. Seven nutritional traits were assessed, namely, total anthocyanin content (mg g⁻¹), β-glucan content (%), fatty acid content (%), protein content (%), amylose content (%), starch content (%) and moisture content (%). Near-infrared spectroscopy (NIR) and pH differential methods were used for trait quantification. Analysis of variance (ANOVA) revealed highly significant genotypic differences for total anthocyanin, β-glucan, and fatty acids, with notable mean variation for protein (16.98-22.19%), amylose (16.01-21.98%), and starch (44.41-54.64%). The coefficient of variation (CV) ranged from 1.22% (moisture) to 34.44% (fatty acids), indicating substantial phenotypic diversity. Principal component analysis (PCA) extracted three significant components (eigenvalues >1: F1 = 2.61, F2 = 1.32, F3 = 1.08), cumulatively explaining 71.55% of the total variance. The first component (F1), contributing 37.32%, was heavily influenced by anthocyanin and protein, whereas F2 (18.85%) captured variation in fatty acids, amylose, and β-glucan. Biplot analysis identified superior genotypes (G150, G167, G170, G189) associated with multiple beneficial traits. Negative relationships were observed between starch and other quality traits such as protein and β-glucan from PCA vector results. The multivariate approach effectively differentiated genotypes with distinct nutritional profiles, underscoring the utility of PCA in trait reduction and ideotype selection. The study confirms the availability of broad genetic variability and provides a robust foundation for breeding nutrient-dense and functionally enhanced barley cultivars.

Keywords: diversity, anthocyanin, variability, genotypes, breeding, nutritional.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is a resilient cereal crop cultivated all over the world due to its tolerance to different agro-climatic conditions, comparatively short growth period, and comparatively high resistance to abiotic stresses such as drought and salinity. Barley has traditionally been used for animal feed, malting, and brewing. As more becomes known about its nutritional and functional characteristics, however, there is a growing interest in its use as a health-promoting whole grain in human food (Dang et al., 2022; Elouadi et al., 2023). Barley grains are rich in dietary fibre, particularly β-glucans together with proteins, vitamins, minerals, and other bioactive phytochemicals

such as flavonoids, phenolic acids, and anthocyanins. These phytoconstituents are credited with various health benefits such as regulation of glycemic response, improvement in lipid metabolism, and prevention of cardiovascular diseases and type 2 diabetes (Eticha et al., 2010; Ficco et al., 2014). These bioactive constituents have attracted increasing attention because of their potential roles in promoting gut health and preventing metabolic disorders (Chen et al., 2025). Barley is recognized as an important cereal crop with considerable nutritional value and diverse applications in food and feed systems (Ahmad et al., 2025).

Among the numerous functional compounds found in barley, water-soluble flavonoid pigments known as anthocyanins

have garnered considerable attention due to their antioxidant activity and capacity to improve human health. Anthocyanins are responsible for the blue, purple, and red pigmentations observed in certain barley genotypes and are known to scavenge free radicals, suppress oxidative stress, and possess anti-inflammatory and anti-carcinogenic activities (Han et al., 2018; Filip 2023). Notably, anthocyanins are also involved in plant defense systems, providing protection against ultraviolet radiation, pathogens, and environmental stresses. In barley, the pigments are localized in the pericarp and aleurone layers of the grain and are genotype-dependent, making them an important characteristic for the development of nutritionally upgraded and stress-resistant cultivars (Hosseinian et al., 2008; Iannucci et al., 2021; Baloch et al., 2024). Recent studies have demonstrated that the biosynthesis and accumulation of plant bioactive metabolites are regulated through complex molecular mechanisms, highlighting their importance for improving crop nutritional quality (Liu et al., 2019).

The assessment of grain quality traits in breeding programs was previously conducted on the basis of time-consuming and labor-intensive chemical analysis. The new analytical techniques have introduced rapid, non-destructive, and economical means for cereal nutritional and functional quality determination. Near-Infrared Reflectance (NIR) spectroscopy has been an effective tool in such an application, allowing several grain quality characteristics to be quantified at once without the need for sophisticated sample preparation (Jin et al., 2022; Ahmed et al., 2025; Yang T. et al., 2025). NIR spectroscopy measures the absorption of near-infrared radiation by organic bonds in food constituents, producing spectral data which can be translated into chemical composition through calibration models. The technology is particularly relevant in high-throughput screening of breeding material, allowing real-time decision-making in genotype selection (Yang et al., 2020; Permatasari et al., 2025). Recent advances in plant genomics and molecular research have improved our

understanding of the genetic regulation of important agronomic traits, facilitating the characterization and utilization of valuable genetic resources for crop improvement (Abubakar et al., 2023; Shabbir et al., 2026).

Principal Component Analysis (PCA) has been one of the leading data reduction techniques in barley research in simplifying data complexity and identifying patterns of variation among multiple traits. For nutritional and phytochemical data such as anthocyanin content, protein, and fiber, PCA assists researchers in lowering the data dimensionality without sacrificing the inherent variability. This is especially valuable in genotype assessment, where the number of correlated variables can make straightforward interpretation difficult (Klem et al., 2019; Vrábl et al., 2023). Multivariate approaches, including correlation, principal component analysis, and cluster analysis, are effective for characterizing genetic diversity and identifying superior genotypes within crop germplasm collections (Almousa et al., 2026). In this study, PCA is employed in order to uncover underlying data structure to cluster similar genotypes with regard to their nutritional value and levels of anthocyanin, and it is easy to choose high-performing lines (Iannucci et al., 2021).

In the present work, PCA has a two-fold role: one, as a diagnostic tool to visually illustrate genetic and trait variation in genotypes; and two, as a strategic tool in the identification of drivers in grain quality variation. With the combination of PCA with spectral and biochemical information, the present work not only highlights the variation in barley genotypes but also provides a platform for multi-trait selection approaches to enhance nutritional quality as well as functional attributes. Such combined approaches are crucial to propel barley breeding programs for supplying enhanced cultivars for health-oriented food systems (Suriano et al., 2018; Meng et al., 2023). With growing consumer demand for functional foods, it is important to explore and tap the genetic potential of barley for health-conveying compounds. Evaluation of diverse plant characteristics provides

valuable information for identifying superior genetic resources and improving crop performance under different environmental conditions (Waleed et al., 2025). Screening genetically diverse barley genotypes for a wide range of grain quality traits - macronutrients and bioactives - can be enlightening for estimating underlying variation and facilitating development of nutritionally enhanced varieties (Son et al., 2016; Orth et al., 2024). Recent investigations on Romanian barley collections have confirmed the existence of substantial phenotypic diversity for agronomic, morphometric, and grain-quality traits, supporting the use of multivariate approaches for genotype characterization and parental selection in breeding programs (Vasilescu et al., 2022).

Assessment of genetic variability is essential for identifying superior genotypes and improving barley breeding programs (Sarwar et al., 2025). Advances in molecular and genetic research have enhanced our understanding of important plant traits associated with nutritional improvement (Khalid et al., 2025; Sarfraz et al., 2025). The present study complements previous evaluations of the same barley population (Yang X. et al., 2025) by applying multivariate analysis to characterize nutritional traits under a single-environment condition. The objective was to evaluate phenotypic variation among barley genotypes and identify promising lines with desirable nutritional characteristics for future breeding programs.

MATERIAL AND METHODS

A total of 195 barley genotypes were evaluated during the 2023 growing season at the Songming Research Station of the Yunnan Academy of Agricultural Sciences, Yunnan Province, China. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Each plot consisted of a 2-meter row with 100 seeds per row and 0.30 m spacing. The field site was uniform in soil fertility and equipped with proper irrigation and drainage. Standard

local agronomic practices were followed. Seven nutritional traits were assessed. After sun-drying, 30 g grain samples were collected per genotype. One portion was ground, sieved, dried, and stored at low temperature, while the other remained unground. Near-infrared spectroscopy (NIR) was performed using 44 cm² standard dishes. Powdered or whole grain samples were leveled and compacted before scanning. Three replicates were analyzed per sample, and the average was recorded. Calibration models for moisture, amylose, starch, β -glucan (BG), Fatty acids (FA), and protein were developed using wet chemistry reference data. Models with $R^2 > 0.85$ and residual predictive deviation (RPD) > 2.5 confirmed reliable predictive performance (Martínez-Subirà et al., 2021; Raj et al., 2023). NIR spectral data were exported for further analysis. Total anthocyanin content (Tac) was quantified using the pH differential method (Hosseinian et al., 2008). Statistical analyses included ANOVA to evaluate genotypic effects (Steel and Torrie, 1960). Principal component analysis (PCA) was performed to identify the major sources of variation among genotypes and to characterize relationships among nutritional traits. PCA has been widely employed in barley germplasm characterization and diversity studies because of its ability to reduce data dimensionality and facilitate the identification of superior genotypes with desirable trait combinations (Rizza et al., 2018; Vasilescu et al., 2022).

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) revealed the genotypic difference in 195 barley genotypes for all the traits under study, which verified the high genetic diversity of the population. The total anthocyanin content differed significantly ($p < 0.01$) with a mean square value of 53.47 and suggested the presence of varying anthocyanin accumulation capacities in genotypes. These findings agree with (Ficco et al., 2014; Meng et al., 2023), who also noted high variation in the anthocyanin content of barley germplasm

because of interactions between genetics and the environment. Moisture content also exhibited significant genotypic differences ($p < 0.05$), indicating that genetic factors contributed to the observed variation among barley genotypes. However, the low coefficient of variation ($CV = 1.25\%$) suggested relatively limited phenotypic variability compared with the other quality traits evaluated. This finding indicates that moisture content was comparatively stable across genotypes, although significant genetic effects were still present. Similar observations have been reported in barley, where grain moisture content was influenced by both genotype and post-harvest conditions (Suriano et al., 2018; Raj et al., 2023). Amylose and starch content also showed significant differences ($p < 0.05$ and $p < 0.01$, respectively), which suggest genotypic control over starch composition. These findings agree with (Elouadi et al., 2023), which emphasized genetic control of amylose and starch deposition in cereal crops. The analysis also showed very significant differences for β -glucan ($p < 0.01$), fatty acid composition ($p < 0.01$), and protein composition ($p < 0.05$), showing that these qualities are genetically influenced to a great extent. The significant variation in β -glucan composition highlights the genetic diversity available for improving this important nutritional trait in barley. The difference in protein and fatty acid composition is consistent with observations reported by (Verardo et al., 2015), showing the need for genotype selection for nutritional improvement in barley breeding programs. In general, the large genotypic effects across traits are evidence that there exists widespread genetic diversity, which is desirable for selection and improvement in nutritionally dense lines of barley. The comparatively low mean squares of error for the majority of the traits also contribute to the robustness of observed differences. The results demonstrate the considerable potential to use this barley population for the identification and selection of superior genotypes possessing enhanced nutritional quality, functional attributes, and adaptation to sustainable agricultural production systems.

Summary statistics of studied traits across 195 barley genotypes

Summary statistical values of the seven nutritional and functional characteristics in 195 barley genotypes indicate significant variability, reflecting a rich pool of genetic diversity Table 2. Total anthocyanin content varied from 0.28 to 1.61 mg/g with a mean of 0.74 mg/g and high coefficient of variation (CV) of 32.43%, reflecting high genotype differences for this characteristic. Such wide variation concurs with Liang et al. (2021), citing high genotypic effect on anthocyanin biosynthesis in barley grains. Likewise, β -glucan content varied widely (3.7-10.72%) with a mean of 6.81% and CV of 22.32%, concurs with Iannucci et al. (2021), who reported wide variation in β -glucan among barley lines, reflecting its potential in functional food uses. Fatty acid content revealed maximum variability ($CV = 34.44\%$), varying from 0.37 to 1.73%, suggesting variability in pathways of lipid metabolism between genotypes. The findings are in conformity with (Eticha et al., 2010), who emphasized fatty acid diversity as a key character to enhance nutritional quality of barley. Conversely, moisture content exhibited the lowest variability among all evaluated traits ($CV = 1.22\%$), indicating a relatively stable expression across the studied genotypes. Despite the limited phenotypic variation, ANOVA revealed significant genotypic differences ($p < 0.05$), suggesting that both genetic and environmental factors contributed to moisture content variation. Similar trends have been reported in barley grain quality studies, where moisture content generally showed lower variability than other nutritional traits (Piasecka et al., 2017).

Amylose and starch contents revealed moderate variability (CVs of 7.35% and 3.93%, respectively), varying from 16.01 to 21.98% for amylose and 44.41 to 54.64% for starch. The findings concur with Klem et al. (2019), who reported starch and its fractions to be both under environmental and genetic control. Such variation is important because starch characteristics are closely associated with nutritional functionality and digestibility (Tong et al., 2026). Protein content ranged

from 16.98% to 22.19%, averaging 19.13%, with a CV of 6.48%, representing moderate genotypic variability. This is in accordance with (Martínez-Subirà et al., 2021; Farooq et al., 2025), who noted that protein concentration in barley grains can be increased through selective breeding. Overall, the range of variation in traits observed verifies the potential of genetic improvement in nutritional quality of barley. The wide phenotypic ranges and acceptable levels of variability support that this group of germplasm is definitely worth using in breeding programs for enhanced grain quality and functional properties across a range of agro-climatic conditions.

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) revealed high variance among the 195 barley genotypes for the traits of interest. Eigenvalues in principal component analysis (PCA) above 1 based on Kaiser's criterion are statistically significant and explain more variance than one original variable and are therefore kept for interpretation (Kaiser, 1960). Three principal components - F1 (2.61), F2 (1.32), and F3 (1.08) - in Table 3 have eigenvalues above 1, and collectively explain a cumulative 71.55% of the total variance among the 195 barley genotypes. This guarantees that most trait variance is satisfactorily explained within the three components. The first principal component (F1) accounted for 37.32% of the total variance, i.e., it accounted for the highest percentage of trait variance (Figure 1). F2 and F3 accounted for 18.85% and 15.37%, respectively. The first three components accounted for the cumulative 71.55% of the total phenotypic variation, i.e., the majority of the information contained in the original traits was accounted for in these components. Hosseinian et al. (2008) and Eticha et al. (2010) also stated a similar result and described that the first PCs accounted for the majority of the variance in nutritional and biochemical traits in barley. Since the scree plot reveals a sharp decline after the third component (the so-called "elbow point"), the inclusion of more

components (F4 to F7) explains increasingly less the variation of traits. Combined, the components explain an additional 28.45% for a total cumulative of 100%. The visual trend corroborates the usefulness of the application of PCA to simplify dimension and classify genotypes according to complex traits. The sharp decline in eigenvalues after F3 and the leveling off of cumulative variance indicate that the first three principal components adequately summarize the variation in the dataset with minimal loss of information. This is in line with findings by (Eticha et al., 2010; Verardo et al., 2015; Dang et al., 2022) in wheat and barley, where the initial principal components with eigenvalues >1 were capable of capturing the majority of the phenotypic and biochemical trait variation.

The fourth (F4) and fifth (F5) factors contributed an additional 12.16% and 9.49%, cumulatively explaining 93.20% of variance, their eigenvalues were below 1. Therefore, only the first three principal components were retained for interpretation according to Kaiser's criterion. The relatively lower contributions of F6 (5.08%) and F7 (1.72%) indicate diminishing returns in variance explanation after the fifth factor. These results confirm the presence of large genetic variation between the genotypes and provide a firm statistical foundation for the identification of improved lines for most of the traits. The contribution of each constituent suggests that traits like anthocyanin, β -glucan, amylose, protein, and starch probably loaded predominantly on the first PCs - which is consistent with similar interpretations by (Meng et al., 2023; Orth et al., 2024), who emphasized PCA as a useful method for trait reduction and genotype clustering in barley breeding programs. In addition, the high cumulative variance also implies the possibility of ideotype identification with a combination of a number of desirable traits that can be useful for deliberate improvement for nutritional quality, functional foods, and climate resilience crops. Our results confirm (Piasecka et al., 2017; Raj et al., 2023), who emphasized the applicability of PCA in crop

improvement initiatives in the detection of prospective genotypes on the basis of multivariate expression of traits.

The loading plot of the first two principal components (F1 and F2) that explain 56.18% of the overall variation between the analyzed traits in 195 barley genotypes shows clear relationships between the quality parameters that varied (Figure 2). The F1 axis explaining 37.32% of the variation chiefly separates the traits such as protein and total anthocyanin on the positive axis from starch and moisture on the negative axis. Specifically, protein content is strongly positively correlated with F1, indicating that the genotypes with higher protein make a significant contribution to this factor. Starch content is strongly negatively correlated with F1, indicating that this relationship is the reverse with protein and total anthocyanin; this is consistent with the majority of the work on cereal grain composition in which high starch is accompanied by low protein (Verardo et al., 2015; Son et al., 2016; Yang X. et al., 2025). The F2 axis, yielding an additional 18.85% of variance, is highly associated with fatty acids, amylose, and β -glucan. These are positioned in the upper quadrants and are positively correlated with each other. For instance, the strong association and narrow angle of the amylose and β -glucan vectors suggest that these two carbohydrates are positively correlated, as confirmed by previous studies on their co-occurrence and importance in barley quality. Total anthocyanin is also positively correlated with F2 but less so than with fatty acids, amylose, and β -glucan. Besides, the plot also reveals some significant inverse relationships. Starch content is inversely related with fatty acid, amylose, β -glucan, and total anthocyanin, since their vectors have opposite signs. Similarly, moisture content is inversely related with nearly all other traits, particularly fatty acid, amylose, β -glucan, and total anthocyanin, since increased content of moisture is associated with decreased content of these precious compounds (Dang et al., 2022; Meng et al., 2023). Our findings of negative correlation between starch and β -glucan, for example, are in agreement with

earlier literature in terms of competitive biosynthesis mechanisms or dilution effects during grain development. The almost 90-degree relationship between protein and fatty acid tells us that these two traits are independently varying in the genotypes of interest, a situation that can be supported by studies on the genetic control of different biochemical pathways in barley. This close scrutiny of the loading plot is informative of the complex interaction of these quality characters in barley, an investigation of interest to breeding programs for improving specific nutritional or industrial qualities (Suriano et al., 2018; Yang et al., 2020; Raj et al. 2023).

The biplot in Figure 3, which is both genotypes and traits combined and explains 56.18% of the total variation in the first two principal components (F1: 37.32%, F2: 18.85%), offers an integrated perspective of the relationships between 195 barley genotypes and quality traits examined. Genotypes located in the direction of a trait vector have high values for the concerned trait. For example, genotypes like G167, G159, and G162 have high values for fatty acid content because they are located in the direction of the 'Fatty Acid' vector. Similarly, genotypes like G170, G189, and G150 also appear to have higher amylose and β -glucan content, clustering close to these respective character vectors (Eticha et al., 2010; Iannucci et al., 2021). This clustering suggests that these genotypes could be good sources for breeding programs aimed at enhancing these specific nutritional factors. Here, our results are in agreement with work suggesting specific genotypes to be superior for specific quality traits, which is typically related to their genetic origin and to their interaction with the environment. Conversely, genotypes on a direction opposite to a trait vector possess lower values of the same trait. For example, genotypes in the lower-left quadrant, like G146 and G114, have high negative correlations with fatty acid, amylose, β -glucan, and total anthocyanin, but positive correlations with starch and moisture. This negative correlation between starch and other desirable components like

protein, fatty acids, and β -glucan is a general feature of cereal grains, which is generally explained in terms of the redistribution of resources during grain filling (Jin et al., 2022; Elouadi et al., 2023).

Genotypes like G106 have high association with high protein content, in the direction of the 'Protein' vector, and thus have potential in application for high-protein breeding of barley. Some genotypes appeared over the majority of the traits, indicating general nutritional quality. G79 exhibited the highest level of anthocyanin and protein content; G90 exhibited the highest level of amylose, starch, and β -glucan; G146 exhibited the highest level of moisture and starch; G85 appeared in both moisture and fatty acid traits; and G189 exhibited the highest levels of amylose, starch, and β -glucan (Meng et al., 2023; Vrábl et al., 2023).

These genotypes are most suitable for integrated breeding strategies to develop multi-nutrient enriched barley varieties. These advanced multi-trait genotypes are production and nutrigenomic assets, integral barley improvement strategies for health-conscious consumers and industrial applications. The genotypic profile also captures the trade-offs in breeding; superior genotypes for high protein are not necessarily the best for other quality traits like β -glucan or amylose, a finding consistent with the often observed negative genetic correlations among different quality traits in crops. The biplot is thus an effective tool to identify superior genotypes for specific end-use and to disentangle the complex genetic architecture of barley quality traits, enabling more efficient breeding (Eticha et al., 2010; Han et al., 2018).

Table 1. Mean sum of squares values form ANOVA for studied traits for 195 barely genotypes

Sources of Variation	df	Total anthocyanin	Moisture	Amylose	Starch	β -glucan	Fatty acid	Protein
Replication	2	278.36	91.53	198.45	365.72	2.63	1.317	0.724
Genotype	194	53.47**	143.82*	387.91*	48.93**	27.51**	467.203**	14.37*
Error	388	7.514	30.27	176.82	10.11	3.98	8.932	7.49
Total	584							

Significance levels are denoted as * $p < 0.05$, ** $p < 0.01$, ns = non-significant.

Table 2. Summary Statistics of studied traits in 195 barley genotypes

Parameters	Total anthocyanin	Moisture	Amylose	Starch	β -glucan	Fatty acid	Protein
Minimum	0.28	11.06	16.01	44.41	3.7	0.37	16.98
Maximum	1.61	11.83	21.98	54.64	10.72	1.73	22.19
Mean	0.74	11.43	18.63	49.93	6.81	0.9	19.13
Variances	0.06	0.02	1.89	3.85	2.31	0.1	1.56
Standard Deviation	0.24	0.14	1.37	1.96	1.52	0.31	1.25
CV	32.43	1.22	7.35	3.93	22.32	34.44	6.48

Table 3. Variability in studied attributes among 195 barley genotypes

Variability	F1	F2	F3	F4	F5	F6	F7
Eigenvalue	2.61	1.32	1.08	0.85	0.66	0.36	0.12
Variability (%)	37.32	18.85	15.37	12.16	9.49	5.08	1.72
Cumulative (%)	37.32	56.18	71.55	83.71	93.20	98.28	100

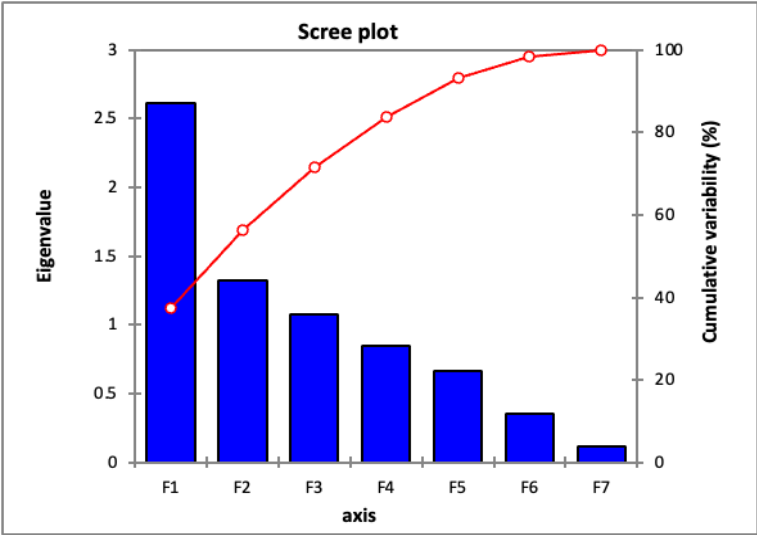


Figure 1. The scree plot illustrates the distribution of eigenvalues and cumulative variability explained by the principal components derived from the analysis of seven traits across 195 barley genotypes

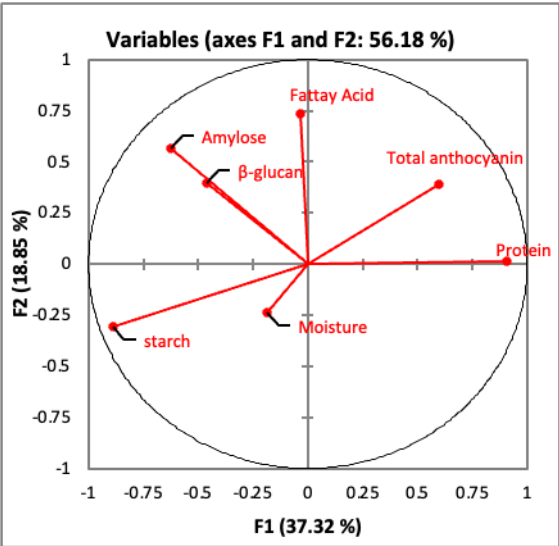


Figure 2. Traits Vector Loading plot for studied traits in 195 barley genotypes

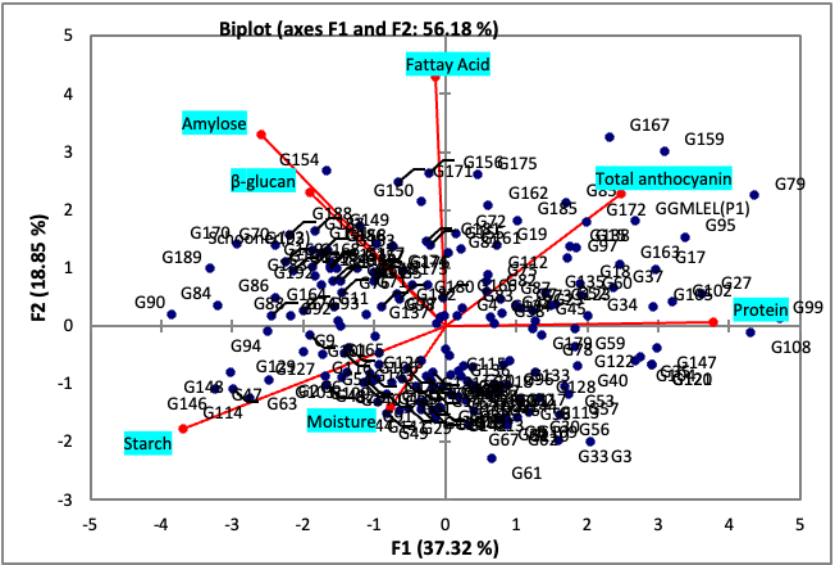


Figure 3. Biplot (Genotypes X Traits) for studied traits in 195 barley genotypes

CONCLUSIONS

The extensive phenotypic and multivariate evaluation of 195 barley genotypes revealed genetic variability for key nutritional and functional traits. Significant genotypic differences were detected for total anthocyanin, β -glucan, fatty acid, protein, amylose, and starch contents, demonstrating the existence of valuable diversity that can be exploited in barley improvement programs. The wide phenotypic ranges observed for anthocyanin, β -glucan, fatty acids, and protein highlight the potential of the studied germplasm as a source of desirable traits for enhancing grain nutritional quality. Principal Component Analysis (PCA) effectively described trait complexity in three principal components (F1-F3) accounting for 71.55% cumulative variation. F1 was primarily associated with protein content and anthocyanin, while F2 was focused on variation in β -glucan, amylose, and fatty acids, which were strongly positively correlated. It is important to note that biplot and loading plot graphics enabled identification of elite genotypes such as G150, G167, G170, and G189 with desirable nutritional profiles. The negative relationships were observed between starch and several quality-related traits, particularly protein and β -glucan, provide valuable information for balancing trait improvement in breeding programs. In contrast, the relatively low variability observed for moisture content indicated a more stable expression of this trait across the evaluated genotypes. This study reinforces the power of multivariate tools in characterizing trait networks and clustering genotypes with complex trait architectures. The findings highlight the potential of integrating biochemical profiling with advanced statistical methods to identify barley ideotypes suitable for health-promoting food products and resilient agricultural systems. Ultimately, this work provides a scientific framework for utilizing genetic diversity to develop next-generation barley cultivars tailored for nutrition-sensitive breeding and functional food innovation.

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