

## Analysis and Comprehensive Evaluation of Key Quantitative Traits of Qingke Barley

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### ABSTRACT

**Qingke barley (*Hordeum vulgare* L. var. *nudum*), a staple cereal cultivated on the Qinghai-Tibet Plateau, is an important source of both nutrition and functional compounds. Comprehensive evaluation of its phenotypic variation is essential for germplasm utilization and breeding improvement. This study measured the contents of five nutritional functional components and three yield-related grain traits across 188 Qingke barley accessions. A series of systematic analyses including descriptive statistics analysis, correlation analysis, cluster analysis, and principal component analysis (PCA) were conducted. The results revealed substantial phenotypic variation among accessions across all measured traits. Correlation analysis indicated significant associations between grain traits and nutritional functional components, as well as among the different nutritional functional components. Cluster analysis categorized the germplasm resources into four distinct groups with characteristic profiles. PCA successfully condensed the eight traits into three principal components (PCs), accounting for 72.314% of the variance, with the PC1 primarily reflecting yield potential. A comprehensive evaluation system based on the PCs identified the top ten elite accessions, and most of the top 30 ranked accessions belonged to Group IV, characterized by high yield and high  $\gamma$ -aminobutyric acid (GABA) content. This study provided a direct foundation and theoretical guidance for the precision breeding of high-yield, high-quality, and functional specialized Qingke barley varieties.**

**Keywords:** Qingke barley, comprehensive evaluation, nutritional functional components, yield potential.

### INTRODUCTION

**Q**ingke barley (*Hordeum Vulgare* Linn. var. *nudum* Hook. F), commonly known as hulless barley, is a cultivated barley variety characterized by the natural separation of the lemma and palea from the caryopsis during harvest (Guo et al., 2020; Lu et al., 2025). As a staple food crop, forage, and brewing substrate on the Qinghai-Tibet Plateau of China, it has been cultivated for approximately 3500 years (Zeng et al., 2018), mainly distributed in Tibet, Qinghai, Sichuan, Gansu, and Yunnan (Obadi et al., 2021). Qingke barley exhibiting great ability to resist adversities such as drought, salinity,

low temperature, and impoverished soils, as well as excellent characteristics such as a short growth cycle, strong adaptability, stable yield performance, and easy cultivation (Liang et al., 2017; Kan and Hong, 2020). These attributes establish it as a distinctive genetic resource for both fundamental research and crop improvement.

Qingke barley is nutritionally superior to other cereals and has gained increasing attention for its nutritional functional ingredients that offer validated health benefits (Guo et al., 2020; Obadi et al., 2021). It contains the highest  $\beta$ -glucan content among cereal crops, which is 50-fold higher than wheat (Ni et al., 2021).  $\beta$ -glucan possesses

functions such as regulating blood glucose and cholesterol, anti-obesity, antioxidant, anti-inflammatory, and anticancer effects (Guo et al., 2018, 2020). Qingke barley from the Qinghai-Tibet Plateau is known to contain higher level of  $\gamma$ -aminobutyric acid (GABA) content (Cao et al., 2010), which plays an effective role in lowering blood pressure, enhancing immunity, inhibiting cancer cell proliferation, and improving intelligence (Techo et al., 2019; Zeng et al., 2020). Flavonoids, an important class of polyphenols, exhibiting antioxidant, anticancer, antibacterial, anti-inflammatory, and blood glucose-lowering properties (Han et al., 2018; Yang et al., 2019), are approximately three times more abundant in whole-grain Qingke barley than in common barley (Yang et al., 2015). Qingke barley has higher protein content (~11.31%) and contains all eight essential amino acids, with notably higher lysine and tryptophan levels than those in wheat, rice, and maize (Yan et al., 2016). Qingke barley also contains other beneficial components, such as vitamins, phytosterols, tocopherols, and minerals (Guo et al., 2020; Obadi et al., 2021), making it a promising crop for developing staple, popular, and high-value-added products that meet consumer demands for nutrition and functionality.

Grain yield-related traits have long been a central focus in crop genetic improvement. Integrating yield potential and nutritional composition is crucial for advancing Qingke barley breeding. Grain size, quantified by thousand-kernel weight, grain length, width, and thickness (Hong et al., 2024), significantly influences both yield potential and grain quality (Luan et al., 2025). Zhao et al. (2022) demonstrated that barley yield improvement is largely driven by increased thousand-kernel weight. Moreover, barley grain size is closely correlated with the starch-to-protein ratio within the kernel (Agu et al., 2007; Watt et al., 2020). A multidimensional evaluation framework combining both yield and nutritional traits provides a robust foundation for selecting high-yielding, nutritionally superior Qingke barley germplasm resources.

Plant germplasm resources harbor extensive genetic variation within a species, developed through long-term natural

domestication and artificial selection, serving as the foundation for breeding innovation (Lhundrup, 2019). Phenotypic traits offer a simple, comprehensive, and intuitive representation of germplasm characteristics (Tian et al., 2024), and their comprehensive evaluation is critical for elucidating the specificity and diversity of crop germplasm, also playing a vital role in breeding programs for enhanced food quality and nutrition (Wang et al., 2025). To date, numerous studies have conducted comprehensive evaluations to identify elite germplasm resources across a wide range of species, from crops such as rice, cotton, and legumes to economic trees and fruits including apple and *Camellia oleifera* (Maji and Shaibu 2012; Ullah et al., 2020; Chen et al., 2023; Tian et al., 2024; Wang et al., 2025). However, comprehensive evaluations of Qingke barley focusing on agronomic and nutritional traits have reported in only few studies (Liu et al., 2024; Lu et al., 2025).

In this study, the contents of five nutritional functional components [ $\beta$ -glucan, total flavonoids, GABA, protein, and resistant starch (RS)] and three yield-related traits (thousand-kernel weight, grain length, and grain width) in 188 Qingke barley accessions were measured and statistically analyzed to elucidate the genetic diversity of germplasm. And comprehensive evaluation was conducted using correlation analysis, cluster analysis, and principal component analysis (PCA) to identify elite Qingke barley germplasms. This study aims to provide a robust foundation for the scientific evaluation of Qingke barley resources and the identification of promising germplasm candidates for breeding new varieties that meet the nutritional expectations of consumers.

## MATERIAL AND METHODS

### Plant materials

A total of 188 Qingke barley germplasm resources, including landraces and bred lines, mainly from major production regions in China and some from overseas, were used in this study (Table S1). All materials were cultivated in Nanying Village, Dianyuan Town, Panlong District, Kunming City, Yunnan

Province (25°15'29.54"N, 102°52'20.72"E, altitude 1945.2 m) in November 2023. Each accession was planted in a single 0.5-m row with 0.25-m row spacing. Mature grains were harvested in May 2024, sun-dried naturally, and milled into powder for subsequent analysis.

### Measurement of phenotypic traits

Grain  $\beta$ -glucan content was determined using a mixed linkage  $\beta$ -Glucan assay kit [K-BGLU, AACC Method 32-23.01, AOAC Method 995.16, Megazyme Int. (Wicklow, Ireland)]; total flavonoids content by the  $\text{NaNO}_2$ - $\text{Al}(\text{NO}_3)_3$  colorimetric method (Zhao et al., 2010); GABA content by the colorimetric method described by Zhao et al. (2009); RS content by the modified Goñi method (Goñi et al., 1996); the Kjeldahl method was used to determine the protein content (GB/T 5009.5-2010, Yang et al., 2017). All measurements were performed in three replicates. Three grain traits were obtained using an SC-G automatic seed testing instrument (Hangzhou Wanshen, China).

### Data analysis

Descriptive statistics including the mean, standard deviation (sd) and coefficient of variation (CV), along with the Shannon-Wiener genetic diversity index ( $H'$ ) of the eight phenotypic traits were calculated by R software (version 4.4.1.). Correlation analysis was conducted using Origin Pro 2021. Systematic cluster analysis was conducted using ggtree in R 4.4.1, euclidean distances were calculated by the hclust function, and the Ward.D2 method was used for hierarchical clustering.

A comprehensive evaluation was performed based on PCA according to the method described by Tian et al. (2024). Phenotypic data were first standardized using the scale function in R (v 4.4.1). PCA was then applied to the standardized dataset, and PCs with eigenvalues  $>1$  and cumulative variance contribution rates  $\geq 70\%$  were extracted. According to the eigenvalues and the loading matrix, eigenvectors were calculated and used as the coefficient of each trait index to construct the linear equation for each PC. The ratio of the variance contribution rate of each PC to the cumulative variance contribution rate of the extracted PCs was used as the weight coefficient to obtain the comprehensive F total value.

## RESULTS AND DISCUSSION

### Diversity analysis of phenotypic traits

Phenotypic variation analysis of eight traits across 188 Qingke barley germplasms (Table 1) revealed significant diversity, with the CV ranging from 8%-53%. GABA content, total flavonoids content, and thousand-kernel weight showed high CVs exceeding 20% (53%, 22%, and 24%, respectively), indicating abundant variation for these traits and suggesting significant potential for genetic improvement. Conversely, grain length with low CV value (8%), suggesting relatively stable genetic characteristics. The  $H'$  of all eight traits exceeded 1.00, varied between 1.78 and 2.05 (Table 1), indicating high genetic diversity. Frequency distributions are shown in Figure 1. All traits followed a normal distribution except GABA content.

Table 1. Diversity analysis of phenotypic traits in Qingke barley germplasm resources

| Traits                     | min    | max    | median | mean   | sd     | CV   | $H'$ |
|----------------------------|--------|--------|--------|--------|--------|------|------|
| $\beta$ -Glucan (%)        | 3.23   | 6.90   | 4.69   | 4.68   | 0.71   | 0.15 | 1.93 |
| Total flavonoids (mg/100g) | 244.88 | 909.89 | 541.52 | 533.34 | 115.32 | 0.22 | 1.84 |
| GABA (mg/100g)             | 2.79   | 47.64  | 15.04  | 15.94  | 8.46   | 0.53 | 1.82 |
| Protein (%)                | 9.66   | 18.11  | 14.07  | 13.97  | 1.82   | 0.13 | 2.04 |
| RS (%)                     | 5.40   | 9.82   | 7.18   | 7.26   | 0.85   | 0.12 | 1.89 |
| Thousand-kernel weight (g) | 16.46  | 51.11  | 33.86  | 34.03  | 8.25   | 0.24 | 2.10 |
| Grain length (mm)          | 5.54   | 9.19   | 7.25   | 7.29   | 0.61   | 0.08 | 1.78 |
| Grain width (mm)           | 2.29   | 3.78   | 3.19   | 3.17   | 0.34   | 0.11 | 2.05 |

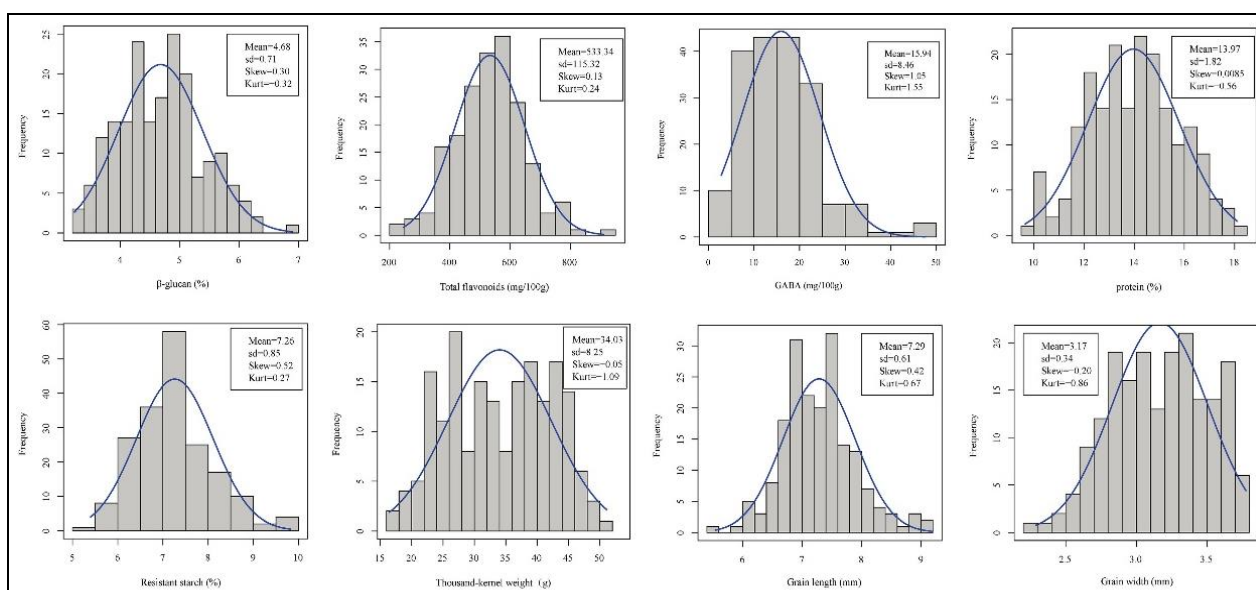


Figure 1. Histograms of the frequency distribution of phenotypic traits

### Correlation analysis of phenotypic traits

The associations among phenotypic traits were estimated by Pearson correlation coefficient analysis. The results (Figure 2) revealed strong correlations between grain traits and nutritional functional component contents. For example, thousand-kernel weight showed highly significant positive correlations with  $\beta$ -glucan and GABA contents, but a highly significant negative correlation with total flavonoids content. Grain length was significantly positively correlated with  $\beta$ -glucan and protein contents, and highly significantly positively correlated with GABA and RS contents.

Grain width exhibited highly significant positive correlations with  $\beta$ -glucan and GABA contents, but a highly significant negative correlation with total flavonoids content and a significant negative correlation with protein content. Significant correlations were also observed among several nutritional functional components. Total flavonoids content was highly significantly negatively correlated with  $\beta$ -glucan content, but highly significantly positively correlated with protein content. Furthermore, protein content showed a highly significant positive correlation with GABA content.

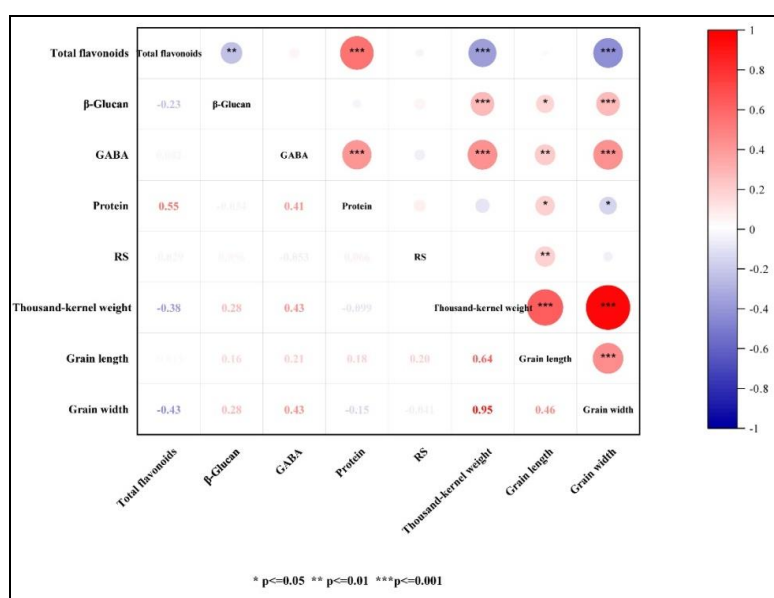


Figure 2. Correlation analysis of phenotypic traits in germplasm resources

### Cluster analysis of phenotypic traits

Hierarchical cluster analysis based on eight phenotypic traits classified the 188 Qingke barley accessions into four distinct groups (Figure 3). The mean values of the eight phenotypic traits for each cluster are shown in Table 2. Group I contained 49 accessions, accounting for 26.1% of the germplasm resources, characterized by the highest total flavonoids and protein contents, moderate RS content, but comparatively lower levels of  $\beta$ -glucan and GABA, and

small grain size. Group II comprised 50 accessions (26.6%) with moderate values for all eight traits. Group III included 28 accessions (14.9%), characterized by the highest  $\beta$ -glucan content and plump grains, but the lowest contents of total flavonoids, protein, and RS. Group IV was the largest group with 61 accessions (32.4%), exhibiting the highest GABA content, along with intermediate levels of other nutritional functional components and large grains.

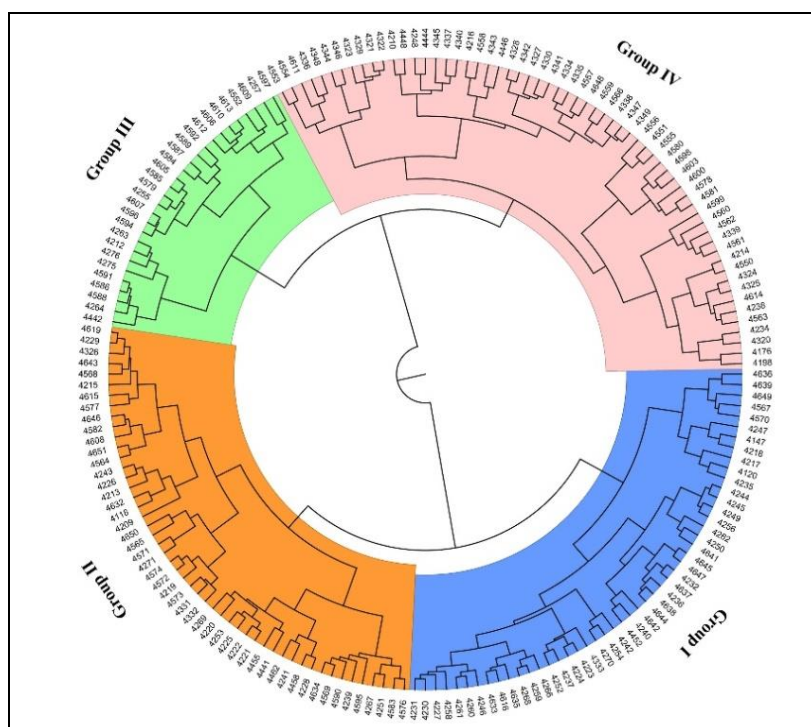


Figure 3. Cluster analysis of 188 Qingke barley germplasm resources

Table 2. Phenotypic characteristics of four groups of Qingke barley germplasms

| Group     | Number of germplasms | Total flavonoids | $\beta$ -Glucan | GABA   | Protein | RS    | Thousand-kernel weight | Grain length | Grain width |
|-----------|----------------------|------------------|-----------------|--------|---------|-------|------------------------|--------------|-------------|
| Group I   | 49 (26.1%)           | 624.908          | 4.363           | 11.537 | 14.707  | 7.104 | 24.145                 | 6.833        | 2.762       |
| Group II  | 50 (26.6%)           | 502.609          | 4.652           | 13.056 | 13.471  | 7.503 | 30.661                 | 7.098        | 3.059       |
| Group III | 28 (14.9%)           | 455.004          | 5.279           | 15.947 | 12.648  | 6.534 | 42.075                 | 7.149        | 3.542       |
| Group IV  | 61 (32.4%)           | 520.940          | 4.668           | 21.829 | 14.391  | 7.526 | 41.027                 | 7.870        | 3.409       |

### Principal component analysis of phenotypic traits

The first three PCs, with eigenvalues of 2.871, 1.787, and 1.128, were extracted, collectively explaining 72.314% of the total variance (Table 3). The first PC (PC1) with a contribution rate of 35.884%, exhibited high loadings for thousand-kernel weight, grain

length, and grain width, indicating that the PC1 primarily reflects grain size. PC2 with a contribution rate of 22.333%, exhibited high loadings for protein, total flavonoids, and GABA. PC3 contributing 14.097%, was predominantly characterized by RS. PC2 and PC3 primarily reflect the comprehensive nutritional quality of Qingke barley grains.

Table 3. Principal component analysis of the phenotypic traits of 188 Qingke barley germplasm resources

| Traits                           | Loading matrix |        |        |
|----------------------------------|----------------|--------|--------|
|                                  | 1              | 2      | 3      |
| Total flavonoids                 | -0.486         | 0.700  | 0.022  |
| $\beta$ -Glucan                  | 0.401          | -0.161 | 0.300  |
| GABA                             | 0.475          | 0.585  | -0.357 |
| Protein                          | -0.107         | 0.893  | 0.046  |
| RS                               | 0.054          | 0.088  | 0.872  |
| Thousand-kernel weight           | 0.969          | 0.040  | -0.058 |
| Grain length                     | 0.645          | 0.345  | 0.344  |
| Grain width                      | 0.936          | -0.048 | -0.159 |
| Eigenvalue                       | 2.871          | 1.787  | 1.128  |
| Contribution rate (%)            | 35.884         | 22.333 | 14.097 |
| Cumulative contribution rate (%) | 35.884         | 58.217 | 72.314 |

### Comprehensive Evaluation of Qingke barley germplasm resources

Eigenvectors were obtained by dividing the loading values (Table 3) by the square

root of their eigenvalues. These eigenvectors served as coefficients in the following linear equations to calculate each principal component (PC) score:

$$F_1 = -0.29x_1 + 0.24x_2 + 0.28x_3 - 0.06x_4 + 0.03x_5 + 0.57x_6 + 0.38x_7 + 0.55x_8 \quad (1)$$

$$F_2 = 0.52x_1 - 0.12x_2 + 0.44x_3 + 0.67x_4 + 0.07x_5 + 0.03x_6 + 0.26x_7 - 0.04x_8 \quad (2)$$

$$F_3 = 0.02x_1 + 0.28x_2 - 0.34x_3 + 0.04x_4 + 0.82x_5 - 0.05x_6 + 0.32x_7 - 0.15x_8 \quad (3)$$

where  $x_1$ - $x_8$  represent the standardized values of total flavonoids,  $\beta$ -glucan, GABA, protein, RS, thousand-kernel weight, grain length, and grain width, respectively.

For these three PCs, the weight coefficients were 0.4962, 0.3088, 0.1950, respectively. The F total value of each material was calculated by the following equation:

$$F_{\text{total}} = 0.4962F_1 + 0.3088F_2 + 0.1950F_3 \quad (4)$$

A higher F total value indicates superior comprehensive quality. The F total value of the 188 Qingke barley germplasms ranged from -2.397 to 2.247. The top 10 resources

with the highest F total value were listed in Table 4 and could be used as candidate materials for breeding varieties with superior comprehensive quality.

Table 4. F total value of the top 10 germplasm resources

| Germplasm number | Total flavonoids | $\beta$ -Glucan | GABA  | Protein | RS   | Thousand-kernel weight | Grain length | Grain width | Principal component score |       |        | $F_{\text{total}}$ | Ranking |
|------------------|------------------|-----------------|-------|---------|------|------------------------|--------------|-------------|---------------------------|-------|--------|--------------------|---------|
|                  |                  |                 |       |         |      |                        |              |             | F1                        | F2    | F3     |                    |         |
| 4320             | 645.76           | 5.85            | 17.96 | 16.66   | 7.32 | 47.60                  | 9.04         | 3.52        | 2.710                     | 2.164 | 1.204  | 2.247              | 1       |
| 4551             | 285.10           | 5.08            | 33.48 | 15.48   | 7.34 | 51.11                  | 8.23         | 3.65        | 3.841                     | 0.695 | -0.297 | 2.063              | 2       |
| 4346             | 509.72           | 5.38            | 15.72 | 14.37   | 9.17 | 47.74                  | 8.32         | 3.71        | 2.821                     | 0.494 | 2.353  | 2.011              | 3       |
| 4344             | 496.78           | 4.72            | 11.23 | 13.20   | 9.56 | 49.02                  | 8.58         | 3.66        | 2.705                     | 0.039 | 2.778  | 1.896              | 4       |
| 4234             | 671.63           | 4.89            | 17.20 | 16.23   | 7.89 | 46.49                  | 8.84         | 3.38        | 1.885                     | 2.221 | 1.365  | 1.887              | 5       |
| 4555             | 288.46           | 4.46            | 39.01 | 16.10   | 7.26 | 48.69                  | 7.91         | 3.63        | 3.392                     | 1.180 | -0.975 | 1.857              | 6       |
| 4322             | 432.13           | 5.71            | 20.19 | 14.06   | 7.86 | 43.09                  | 8.99         | 3.39        | 2.817                     | 0.407 | 1.542  | 1.824              | 7       |
| 4550             | 515.39           | 5.71            | 32.81 | 16.88   | 7.58 | 40.25                  | 8.06         | 3.35        | 2.081                     | 2.048 | 0.389  | 1.741              | 8       |
| 4214             | 579.16           | 5.65            | 34.35 | 17.78   | 7.36 | 38.51                  | 7.81         | 3.41        | 1.739                     | 2.618 | -0.029 | 1.666              | 9       |
| 4345             | 658.52           | 4.92            | 14.18 | 13.71   | 7.83 | 49.52                  | 8.45         | 3.67        | 2.357                     | 0.878 | 1.030  | 1.641              | 10      |

Qingke barley is a characteristic and advantageous industry in the Qinghai-Tibet plateau, serving as a geographical emblem and cultural symbol, and playing an essential role in the local agricultural economy. Systematic evaluation of Qingke barley germplasm resources is fundamental to their effective utilization, enabling the discovery of superior accessions and providing critical support for genetic improvement.

Genetic variation is pivotal for germplasm innovation and breeding programs (Jordan et al., 2011). CV reflects the degree of dispersion of phenotypic traits, and is significantly positively correlated with phenotypic divergence and genetic diversity (Zhang et al., 2022). A CV exceeding 10% generally indicates abundant variation and substantial genetic diversity among different germplasm resources (Li et al., 2023), providing a broad base for the comparison and selection of distinctive materials. In this study, variation analysis of eight phenotypic traits across 188 Qingke barley accessions revealed that seven traits exhibited CV values greater than 10%. Furthermore, the data distribution shown in Figure 1 illustrates a broad phenotypic range across all traits, underscoring a high trait variation.  $H'$  value also serves as a robust index of genetic diversity (Chen et al., 2023). The high  $H'$  values indicate substantial genetic variability in all eight traits, providing a rich resource for breeding selection. In summary, the Qingke barley materials investigated in this study exhibit rich phenotypic variation and substantial genetic diversity, making them well-suited for systematic evaluation and for breeding programs targeting high nutritional quality and high yield.

Correlation analysis is a key approach for deciphering the inherent relationships among complex traits and crucial for establishing coordinated breeding strategies. In this study, significant correlations were detected between grain traits and nutritional functional component contents. Specifically,  $\beta$ -glucan and GABA contents showed significant positive correlations with thousand-kernel weight, grain length, and grain width, suggesting the potential for simultaneously

improving these nutritional components while breeding for larger grain size and higher yield. However, thousand-kernel weight and grain width were significantly negatively correlated with total flavonoids content, indicating a trade-off between grain size and flavonoids accumulation. Notably, existing reports on the relationships between  $\beta$ -glucan content and yield-related traits are inconsistent. For example, Li et al. (2022) reported a significant negative correlation between  $\beta$ -glucan content and yield in naked barley from Jiangsu Province, while Rey et al. (2009) found a modest positive correlation in barley, Eticha et al. (2010) observed no significant correlation between  $\beta$ -glucan content and yield or thousand-kernel weight in naked spring barley. These discrepancies may be attributed to differences in genetic backgrounds and growing environments.

Significant correlations were also found among different nutritional functional components. Consistent with Liu et al. (2024), our study showed a highly significant positive correlation between protein and GABA contents. We also found that total flavonoids content was highly significantly negatively correlated with  $\beta$ -glucan content and positively correlated with protein content. For malting applications, high  $\beta$ -glucan may lead to insufficient degradation of cell walls, reducing extraction yield (Geng et al., 2024). Therefore, among our 188 accessions, those with low  $\beta$ -glucan content are promising for brewing, as they ensure high extraction yield while contributing high flavonoids levels to enhance the nutritional quality of the final product.

Cluster analysis classifies germplasms into distinct groups based on phenotypic traits, providing a basis for resource management and selection of breeding materials (Ullah et al., 2020). Our study categorized 188 Qingke barley into four groups. Group I comprising germplasms with highest levels of total flavonoids and protein along with moderate RS content, stands out as optimal materials for producing high antioxidant, protein-rich functional foods. Group II showing intermediate values for all traits, serves as ideal “universal” parents for hybridization

and a crucial resource for maintaining population genetic diversity. Groups III and IV are characterized by superior grain size, coupled with uniquely high content of a specific functional component -  $\beta$ -glucan and GABA, respectively. This ideal combination of “plump grains” and “elevated specific nutritional functional component” positions them as direct breeding materials for developing specialized Qingke barley varieties: “high-yield & high- $\beta$ -glucan” and “high-yield & high-GABA” types.

PCA reduces the dimensionality of data by condensing multiple highly correlated traits into a few uncorrelated PCs while preserving main variation information of the original dataset, serving as a key tool for streamlining the trait selection in germplasm evaluation (Yano et al., 2019). It is widely applied in germplasm classification and evaluation. For instance, Derbew (2020) performed PCA on eight quantitative traits of 22 naked barley accessions, extracting three PCs that cumulatively explained ~78% of the total variation. Similarly, Lu et al. (2025) condensed 16 agronomic traits of 58 Qingke barley accessions into six PCs, explaining 73.062% of the variation. In this study, eight traits were summarized into three PCs with a cumulative contribution rate of 72.314%, accounting for the major variation within the dataset. Among these, PC1 primarily represents grain size, closely related to yield potential. As the component with the highest contribution rate (35.884%), PC1 objectively reflects that yield-related traits as the key contributor to phenotypic diversity. Moreover, the top-ranked accessions (e.g., top ten) in the comprehensive evaluation generally exhibited high PC1 scores ( $F_1$  values) and large-grain characteristics (Table 4), indicating that yield potential is the primary consideration when selecting accessions with superior comprehensive traits.

Notably, most of the top 30 ranked accessions belonged to Group IV, which is characterized by high yield potential and superior GABA content, concurrently exhibiting intermediate levels of other nutritional functional components, achieving a good balance among all traits.

Consequently, accessions from Group IV are ideal candidates for breeding programs that aim to simultaneously enhance yield and core health functions, thereby meeting the demand for high-performance functional crops.

## CONCLUSIONS

We performed a systematic analysis of five core nutritional functional component contents and three yield-related traits across 188 Qingke barley accessions. The results revealed a substantial phenotypic variation, providing a rich resource for breeding. Correlation analysis uncovered a series of important associations among traits, providing clear direction and identifying critical trade-offs for multi-trait breeding. Cluster analysis classified the germplasms into four distinct groups: a high-flavonoids and high-protein type, a balanced type, a high-yield and high- $\beta$ -glucan type, and a high-yield and high-GABA type, offering a clear genetic resource for targeted breeding. Subsequent comprehensive evaluation ranked all materials, and most accessions within Group IV exhibited superior comprehensive performance, identifying them as suitable parental materials for breeding high-yield and high-quality Qingke barley varieties.

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## SUPPLEMENTARY MATERIAL

Table S1. Accession details of Qingke barley materials used in this study.

| Accession ID | Accession Name     | Origin  | Accession ID | Accession Name        | Origin  |
|--------------|--------------------|---------|--------------|-----------------------|---------|
| 4118         | Qu Qu Mai          | Yunnan  | 4241         | FB10-4-1              | Qinghai |
| 4120         | Duan Mang Da Mai   | Yunnan  | 4242         | Yang Da Mai           | Yunnan  |
| 4147         | Hua Gou Mi Da Mai  | Yunnan  | 4243         | Mi Da Mai             | Yunnan  |
| 4176         | Yun Da Mai 12YD-4  | Yunnan  | 4244         | Chang Mang Luo Da Mai | Yunnan  |
| 4198         | Yun Da Mai 12 Hao  | Yunnan  | 4245         | Mi Da Mai             | Yunnan  |
| 4209         | Chuan Da Mai 12259 | Sichuan | 4246         | Mang Shui Mi Da Mai   | Yunnan  |
| 4210         | Dian Qing 1 Hao    | Yunnan  | 4247         | Gou Mang Mi Da Mai    | Yunnan  |
| 4212         | Jiu Ge             | Yunnan  | 4248         | Hei Qing Ke           | Yunnan  |
| 4213         | Zang Qing 25       | Tibet   | 4249         | Nuo Qing Ke           | Yunnan  |
| 4214         | Zang Qing 690      | Tibet   | 4250         | Qing Ke Mai           | Yunnan  |
| 4215         | Xi Ma La 22 Hao    | Tibet   | 4251         | Shang Jiang Qing Ke   | Yunnan  |
| 4216         | Kun Lun 12 Hao     | Qinghai | 4252         | Ba Shi Tian Qing Ke   | Yunnan  |
| 4217         | Kun Lun 13 Hao     | Qinghai | 4253         | Dong Wang Bai Qing Ke | Yunnan  |
| 4218         | Bei Qing 7 Hao     | Qinghai | 4254         | Wen Quan Qing Ke      | Yunnan  |
| 4219         | Bei Qing 8 Hao     | Qinghai | 4255         | Bai Da Mai (2)-1      | Yunnan  |
| 4220         | Gan Qing 4 Hao     | Gansu   | 4256         | Mi Da Mai             | Yunnan  |
| 4221         | Gan Qing 5 Hao     | Gansu   | 4257         | Di Tou Mai            | Yunnan  |
| 4222         | A Qing 5 Hao       | Sichuan | 4258         | Mi Da Mai             | Yunnan  |
| 4223         | A Qing 6 Hao       | Sichuan | 4259         | Mi Da Mai             | Yunnan  |
| 4224         | Kang Qing 7 Hao    | Sichuan | 4260         | Luo Da Mai            | Yunnan  |
| 4225         | Dong Qing 8 Hao    | Tibet   | 4261         | Qing Ke               | Yunnan  |
| 4226         | Guo Luo            | Tibet   | 4262         | Bai Qing Ke           | Yunnan  |
| 4227         | 891                | Tibet   | 4263         | Bai Qing Ke           | Yunnan  |

## ROMANIAN AGRICULTURAL RESEARCH

| Accession ID | Accession Name                          | Origin   | Accession ID | Accession Name       | Origin  |
|--------------|-----------------------------------------|----------|--------------|----------------------|---------|
| 4228         | Dong Qing 15 Hao                        | Tibet    | 4264         | You Mang Bai Qing Ke | Yunnan  |
| 4229         | Dong Qing 17 Hao                        | Tibet    | 4266         | Zao Shu Bai Qing Ke  | Yunnan  |
| 4230         | Dong Qing 11 Hao                        | Tibet    | 4267         | 80 Tian Qing Ke      | Yunnan  |
| 4231         | Dong Qing 16 Hao                        | Tibet    | 4268         | Duan Bai Qing Ke     | Yunnan  |
| 4232         | Yun Qing 2 Hao                          | Yunnan   | 4269         | Zi Qing Ke (Huang)   | Yunnan  |
| 4234         | 11YD-8                                  | CIMMYT   | 4270         | Ruan Ke Da Mai       | Yunnan  |
| 4235         | Bei Qing 4 Hao                          | Yunnan   | 4271         | Mi Da Mai            | Yunnan  |
| 4236         | Gan 94-19-1                             | Yunnan   | 4275         | Di Qing 3 Hao        | Yunnan  |
| 4237         | 03--2                                   | Yunnan   | 4276         | Zhou Qu Qing Ke      | Gansu   |
| 4238         | Huang Chang Guang Da Mai                | Yunnan   | 4320         | ATAHUALPA            | ICARDA* |
| 4239         | Zi Qing Ke                              | Yunnan   | 4321         | CBSS05Y00293S        | ICARDA  |
| 4240         | Luo Da Mai                              | Beijing  | 4322         | CBSS05Y00294S        | ICARDA  |
| Accession ID | Accession Name                          |          |              | Origin               |         |
| 4323         | CBSS04B00111S-13M-0Y-0M-2Y-0M-0AP       |          |              | ICARDA               |         |
| 4324         | CBSS01Y00890T-A-0Y-4M-1M-1Y-0M-0AP      |          |              | ICARDA               |         |
| 4325         | CBSS04Y00047S -20Y-3M-0Y-0M-0Y-0AP      |          |              | ICARDA               |         |
| 4326         | CBSS04Y00017S-1Y-1M-0Y-0M-0Y-0AP        |          |              | ICARDA               |         |
| 4327         | ICB02-0487-57AP-0AP-0AP-0AP             |          |              | ICARDA               |         |
| 4328         | CBSS01Y00891T-Z-0Y-2M-1M-2Y-0M-0AP      |          |              | ICARDA               |         |
| 4329         | CBSS05Y00293S-25Y-0M-0Y-0M-3AP          |          |              | ICARDA               |         |
| 4330         | ICB02-0487-97AP-0AP-0AP-0AP             |          |              | ICARDA               |         |
| 4331         | CBSS04B00030S-5M-0Y-0M-1Y-0M-0AP        |          |              | ICARDA               |         |
| 4332         | CBSW01WM00121T-0TOPY-4M-1Y-1M-1Y-0M-0AP |          |              | ICARDA               |         |
| 4333         | CBSS04M00214S-5M-0Y-0M-0Y-1M-0AP        |          |              | ICARDA               |         |
| 4334         | ICB02-0488-16AP-0AP-0AP-0AP             |          |              | ICARDA               |         |
| 4335         | CBSS 04 Y 00048S-23Y-2M-0Y-0M-0Y-0AP    |          |              | ICARDA               |         |
| 4336         | ICB03-0213-27AP-0AP                     |          |              | ICARDA               |         |
| 4337         | ICB03-0226-18AP-0AP                     |          |              | ICARDA               |         |
| 4338         | ICB03-0244-22AP-0AP                     |          |              | ICARDA               |         |
| 4339         | ICB03-0276-10AP-0AP                     |          |              | ICARDA               |         |
| 4340         | ICB03-0375-11AP-0AP                     |          |              | ICARDA               |         |
| 4341         | ICP03-0451-2AP -0AP                     |          |              | ICARDA               |         |
| 4342         | CBSS01Y00432S-0Y-2M-1M-1Y-0MP           |          |              | ICARDA               |         |
| 4343         | ICB02-0481-0AP-4TR-9AP-0AP              |          |              | ICARDA               |         |
| 4344         | ICB06-1155-4AP-0AP                      |          |              | ICARDA               |         |
| 4345         | ICB06-1179-11AP-0AP                     |          |              | ICARDA               |         |
| 4346         | ICB06-1183-20AP-0AP                     |          |              | ICARDA               |         |
| 4347         | ICB06-1242-19AP-0AP                     |          |              | ICARDA               |         |
| 4348         | ICB06-1243-37AP-0AP                     |          |              | ICARDA               |         |
| 4349         | ICBO1-1145-1AP-10AP-0AP                 |          |              | ICARDA               |         |
| Accession ID | Accession Name                          | Origin   | Accession ID | Accession Name       | Origin  |
| 4441         | Yun Ke 4 Hao                            | Yunnan   | 4588         | 2018 Qing 35         | Tibet   |
| 4442         | Yun Ke 1 Hao                            | Yunnan   | 4589         | 2018 Qing 36         | Tibet   |
| 4444         | Hatiexi Tuihua Erleng                   | Yunnan   | 4590         | Zang Qing 2000       | Tibet   |
| 4446         | Hiproly                                 | Ethiopia | 4591         | Su La Qing 3472      | Tibet   |
| 4448         | Prokŭ pkŭ r                             | Poland   | 4592         | Su La Qing 3453      | Tibet   |
| 4452         | Hong Qingke                             | unknown  | 4594         | Yun Ke MF17-28       | Yunnan  |
| 4455         | Liu Leng Qingke                         | unknown  | 4595         | Yun Ke MF17-32       | Yunnan  |
| 4458         | Chun Qingke                             | unknown  | 4596         | Dong Wang Zi Qingke  | Yunnan  |
| 4462         | Lao Damai                               | unknown  | 4597         | Di Qing 6 Hao        | Yunnan  |
| 4550         | 0406-17                                 | Gansu    | 4598         | Bao Damai 16-J11     | Yunnan  |
| 4551         | 917-1-1-2-2                             | Gansu    | 4599         | Bao Damai 16BJ-7     | Yunnan  |
| 4552         | QB27                                    | Tibet    | 4600         | Yun Ke MF17-26       | Yunnan  |
| 4553         | QB16                                    | Tibet    | 4603         | Di Qing 7 Hao        | Yunnan  |
| 4554         | QB14                                    | Tibet    | 4605         | Kun Lun 14 Hao       | Qinghai |
| 4555         | QTB11                                   | Tibet    | 4606         | Kun Lun 15 Hao       | Qinghai |
| 4556         | QTB13                                   | Tibet    | 4607         | 14YN-686             | Qinghai |
| 4557         | QTB25                                   | Tibet    | 4608         | 14YN-785             | Qinghai |

| Accession ID | Accession Name    | Origin       | Accession ID | Accession Name              | Origin  |
|--------------|-------------------|--------------|--------------|-----------------------------|---------|
| 4558         | Gan Qing 9 Hao    | Gansu        | 4609         | 1021-1                      | Qinghai |
| 4559         | Gan Qing 8 Hao    | Gansu        | 4610         | 1216-1                      | Qinghai |
| 4560         | 2013WJ/207        | Xingjiang    | 4611         | Bei Qing 1309-2             | Qinghai |
| 4561         | 2013J/193         | Xingjiang    | 4612         | Chai Qing 1 Hao             | Qinghai |
| 4562         | Long Ke Mai 4 Hao | Heilongjiang | 4613         | 14-702                      | Qinghai |
| 4563         | Long Ke Mai 5 Hao | Heilongjiang | 4614         | 14YN-787                    | Qinghai |
| 4564         | Yan Shi Luo 1 Hao | Jiangsu      | 4615         | 14-831                      | Qinghai |
| 4565         | R1                | Tibet        | 4616         | BQ15061-1                   | Qinghai |
| 4566         | R2                | Tibet        | 4619         | A Qing 7 Hao                | Sichuan |
| 4567         | 04-2894-1         | Tibet        | 4632         | Bai Damai (2)-2             | Yunnan  |
| 4568         | 09-5024-13        | Tibet        | 4633         | Bai Damai (2)-6             | Yunnan  |
| 4569         | 12-10556          | Tibet        | 4634         | Bai Damai (2)-7             | Yunnan  |
| 4570         | 12-893            | Qinghai      | 4635         | Bai Damai (2)               | Yunnan  |
| 4571         | 12-940            | Qinghai      | 4636         | Chang Mang Mi Damai         | Yunnan  |
| 4572         | 1216-1            | Qinghai      | 4637         | Kai Bao Mai                 | Yunnan  |
| 4573         | 1021-1            | Qinghai      | 4638         | Zao Shu Damai               | Yunnan  |
| 4574         | Huang Qing 1 Hao  | Gansu        | 4639         | Fu Heng Mi Damai            | Yunnan  |
| 4576         | Gan Qing 6 Hao    | Gansu        | 4641         | Chang Hei Qingke            | Yunnan  |
| 4577         | Gan Qing 7 Hao    | Gansu        | 4642         | Song Hua Mai                | Yunnan  |
| 4578         | CQK-02            | Tibet        | 4643         | Hei Bai Qingke              | Yunnan  |
| 4579         | CQK-03            | Tibet        | 4644         | Za Jiao Qingke              | Yunnan  |
| 4580         | CQK-05            | Tibet        | 4645         | Yang Mao Qingke             | Yunnan  |
| 4581         | 16001             | Tibet        | 4646         | Gan De La Qingke            | Yunnan  |
| 4582         | 16002             | Tibet        | 4647         | Huang Chang Gou Luo Er Leng | Yunnan  |
| 4583         | CH88009           | Tibet        | 4648         | Luo Hei Damai               | Yunnan  |
| 4584         | 14-5186           | Tibet        | 4649         | Gou Mang Luo Damai          | Yunnan  |
| 4585         | 14-3492           | Tibet        | 4650         | Xian Luo Damai              | Yunnan  |
| 4586         | 13-9139           | Tibet        | 4651         | Guang Mang Xian Damai       | Yunnan  |
| 4587         | 13-5171-7         | Tibet        |              |                             |         |

\*ICARDA - International Center for Agricultural Research in the Dry Areas.