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RESEARCH ARTICLE

Multivariate analysis of barley (*Hordeum vulgare* L.) germplasm under temperate agro-climatic niches

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Abstract

Barley (*Hordeum vulgare* L.) is a resilient cereal crop with significant global importance, particularly in temperate regions, where it thrives under a range of climatic stresses. This study assessed genetic variability, trait associations and population structure among 51 barley genotypes under temperate conditions using an augmented block design during the rabi season 2021–2022 at Mountain Research Centre for Field Crops (MRCFC), Khudwani- Sher-e-Kashmir University of Agricultural Sciences and Technology (SKUAST) of Kashmir. Eight agronomically important traits namely, days to heading, days to maturity, plant height, tiller number per metre, spike length, grains per spike, 1000-grain weight and grain yield per plot, were evaluated using standard biometric and multivariate analytical approaches. Significant genetic diversity was observed, with high heritability and substantial genetic advance recorded for tiller number per meter and yield, indicating predominant additive gene action. Yield showed strong positive correlations with tiller number and grains per spike, highlighting their significance for selection. Path analysis confirmed the association pattern. Principal component analysis (PCA) identified 2 major components explaining almost 60 % of total variation, with plant height, tiller number per meter, grains per spike and yield being the dominant contributors. Clustering grouped genotypes into 5 clusters, with cluster V yielding highest. Large inter-cluster distances signified broad genetic divergence. Based on a combined selection index integrating PCA scores and yield performance, 6 superior barley genotypes were identified, among which IBYT-HI-2021-14, IBYT-HI-2021-12 and HUB113 exhibited the most consistent and outstanding agronomic performance under temperate conditions.

Keywords: analysis of variance; barley germplasm; Duncan's test; phenotypic and genetic variability; principal component analysis

Introduction

Barley (*Hordeum vulgare L.*) is one of the oldest cultivated cereal crops with considerable global importance due to its versatile applications. It is a self-pollinated diploid ($2n = 2x = 14$) species with an estimated genome size of 5 Gb (1). Rich in dietary fibre, particularly β-glucan, barley helps lower cholesterol and improving cardiovascular health (2). Recent data in human nutrition substantiates the importance of β-glucan-rich cereal meals in enhancing cardio-metabolic risk profiles, with barley-based products increasingly recognised as functional foods due to their soluble fibre content. A randomised crossover trial indicated beneficial effects of oat and barley flakes on blood lipids and glucose metabolism, underscoring the physiological significance of β-glucan-rich cereal therapies in adults (3). A recent synthesis of barley grain composition and processing applications underscores barley's significant potential for the creation of health-oriented functional meals, with β-glucan as a pivotal component enhancing this value proposition (4).

Beyond its nutritional value in various food products, such as soups, bread and health drinks, a hefty percentage of international barley production is used as livestock feed, providing vital nutrients for cattle and poultry (3, 5). From a feed perspective, barley is a strategically significant cereal ingredient; however, its wider utilisation, particularly in poultry systems, is limited by fluctuations in nutritive value and the presence of soluble non-starch polysaccharides that can diminish metabolic energy without proper management. A recent analysis delineates these constraints and presents practical alternatives, such as enzyme supplementation and cultivar selection, to enhance barley utilisation in poultry diets (5).

In India, barley cultivation covered over 628 thousand hectares, with a total production of 1.913 million tons and a productivity of 3.05 tons per hectare during the year 2023–2024 (6). Among the cereals, barley shows better adaptability to severe environments and can tolerate diverse abiotic stresses (7, 8). Current research suggests that wild barley cousins and traditional landraces serve as valuable repositories of genes for abiotic stress tolerance, encompassing resilience to drought and temperature variations and can be utilised for pre-breeding and genomic-assisted enhancement. A recent analysis highlights that these genetic resources offer effective methods to improve stress tolerance, while preserving agronomic performance (7).

However, under rainfed regions, such as northern hill region and other semiarid and arid environments, yield is affected by erratic weather during critical growth stages. Genetic variation is the foundation of plant breeding, which offers a wide range of genotypes that can be utilized to develop new crop varieties and advanced breeding materials (9). Given the complex nature of agronomic traits, understanding the genetic variability and trait interaction, present in the germplasm are of supreme significance for successful breeding (10). Barley, in particular, exhibits a great deal of morphological and genetic variation and systemic characterisation of genotypes with adequate documentation of idiosyncratic traits is vital for their effective utilisation (11, 12).

The genetic variety present in agricultural germplasm underpins effective selection and sustainable genetic enhancement, especially for intricate traits, such as yield and stress resilience. In barley, phenotypic and genetic diversity in agronomic parameters presents opportunity to find superior genotypes and to combine complementary qualities through breeding. Consequently, a thorough assessment of genetic variability and trait interrelationships among varied germplasm collections is crucial for formulating effective selection strategies tailored to specific agro-climatic conditions. A critical understanding of genetic variability for the traits under improvement is a prerequisite for the success of any breeding program. Correlation analysis also plays an important role in identifying associations among traits that inform selection strategies for simultaneous improvement of multiple traits. Similarly, studying genetic diversity in a germplasm aid in the progress of superior recombinants.

Tools such as Mahalanobis' D^2 compute the genetic divergence in germplasm, while principal component analysis (PCA) assists in identifying representative genotypes that capture the greatest genetic diversity, reducing the complex variance (13, 14). Recent barley research employs multivariate techniques, such GT biplots, PCA and hierarchical clustering, to analyse genotype–trait associations and to find prospective parental lines and high-yielding varieties across various germplasm panels. GT biplot and cluster analysis have been employed to elucidate connections among agro-morphological traits and to identify superior barley genotypes, underscoring the ongoing methodological significance of these methods for breeding decisions (15).

Notwithstanding the existence of many barley germplasm collections, there is a paucity of knowledge regarding the degree of genetic variability, trait interrelationships and population structure of barley genotypes assessed under the temperate agro-climatic conditions of the north-western Himalayan region. Specifically, thorough multivariate evaluations incorporating genetic variability metrics, correlation frameworks, PCA and cluster-based divergence are limited for barley germplasm suited to these conditions. Furthermore, the possible role of locally adapted and indigenous genotypes in enhancing the genetic diversity has not been adequately investigated. Rectifying these deficiencies is crucial for recognising optimal parental lines and formulating effective breeding strategies suited to temperate settings.

In this context, the present investigation was undertaken to evaluate 51 barley genotypes under temperate conditions with the objective of identifying promising genotypes with desirable trait combinations of yield and related agronomic traits for use in future breeding initiatives.

Materials and Methods

A barley germplasm collection of 51 genotypes, including both exotic and indigenous varieties provided by ICAR- Indian Institute of Wheat and Barley Research (IIWBR) Karnal under the All India Coordinated Research Project on Wheat and Barley, was evaluated during this study. The set included 45 entries along with six checks, namely, BH 946, HUB113, BHS 400, PL891, DWRB123 and DWRB137. These genotypes were selected and assessed for their variability for yield and yield-related traits.

Experimental site and environmental conditions

The field experiment was conducted during the rabi season of 2021–2022 at the experimental farm of the Mountain Research Centre for Field Crops (MRCFC), Khudwani, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Union Territory of Jammu and Kashmir, India. The experimental site is located at 33°70′ N latitude and 75°10′ E longitude, with an altitude of 1590 meters above mean sea level.

Experimental design and crop management

The experiment was laid out using an augmented block design, in which the 45 test genotypes were unreplicated, while the six control (check) varieties were replicated once in each of the five blocks, resulting in 5 replications per check genotype across the experimental layout. Augmented block designs are extensively utilized in early-generation and germplasm screening when seed availability is constrained, as replicated checks facilitate block adjustment and establish a foundation for comparing unreplicated test entries amidst field variation. Recent statistical research has elucidated the impact of control configurations and block structures on the accuracy of contrasts among checks, test entries and between checks and test entries, thereby reinforcing the appropriateness of augmented designs for unreplicated trials in plant breeding (16).

Each genotype was sown in a 2.5 m row with row-to-row spacing of 30 cm acclaimed crop management protocols and standard agronomic practices were followed. Observations were recorded on yield and yield attributing traits such as days to heading, days to maturity, plant height (cm), tiller number per meter, spike length (cm), grains per spike, 1000-grain weight (g) and yield per plot (g). The experimental field was prepared through conventional tillage practices, including one deep ploughing, followed by two harrowing to obtain a fine seedbed prior to sowing. Sowing was carried out manually during the recommended rabi planting window for the region, in the first fortnight of November 2021. The crop was grown under irrigated conditions and irrigation was applied uniformly to all plots at critical growth stages, including tillering, booting and grain filling, to avoid moisture stress and ensure optimal crop establishment and development.

Statistical and multivariate analyses

Statistical analyses were performed using R software (version 4.3.1) using the following packages: agricolae, psych, corrplot, factoextra and cluster. Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were estimated following standard biometric procedures. Phenotypic coefficient of variation and GCV were calculated as the ratio of the square root of phenotypic variance (σ^2p) and genotypic variance (σ^2g), respectively, to the trait mean and expressed as percentages. Broad-sense heritability (H^2) was computed as the ratio of genotypic variance to phenotypic variance ($H^2 = \sigma^2g / \sigma^2p$). Genetic advance (GA) was estimated using the formula $GA = k \times \sigma p \times H^2$, where k is the selection differential at 5 % selection intensity and genetic advance as a percentage of the mean (GA %) was calculated to facilitate comparison among traits.

To improve analytical reproducibility in augmented design trials, adjusted means and related summaries can be calculated using specialised R implementations for augmented randomised complete block designs, offering standardized workflows for ANOVA, mean adjustment and genetic parameter summaries (17). Replication of the control varieties across blocks enabled estimation of block effects and adjustment of test genotype means, thereby facilitating valid statistical comparisons among genotypes within the augmented design framework.

Results

Descriptive statistics

Field evaluation for eight agronomic traits across 51 barley genotypes showed considerable variation in agronomic performance (Table 1). Days to heading and days to maturity recorded narrow ranges (158–166 and 197–207 days, respectively), with standard deviations of 2.3 and 2.5, indicating low variability. While plant height had a mean of 83.5 cm, showing considerable variation, ranging from 47 to 108 cm and a relatively high standard deviation of 11.4, suggesting significant morphological diversity among genotypes. Tiller number per meter varied widely, ranging from 52 to 298 with a mean and standard deviation of 158.3 and 51, respectively. This reflects substantial variability and thus higher potential for effective selection. Spike length (6.2–10.6 cm) also showed moderate variation, indicating scope for improvement in spike architecture. Grain per spike (23.5–54.6) with a mean of 41.8, 1000-grain weight (mean = 48.1 g) and yield per plot (mean = 308.8 g) also exhibited wide ranges. Skewness and kurtosis values indicated near-normal distributions for most traits, except grain per spike and tiller per meter, which showed moderate skewness.

Analysis of variance

Analysis of variance (ANOVA) revealed significant differences among the 51 barley genotypes for most of the traits except for tiller number per meter, 1000 GW (g) and yield per plot (g) confirming the presence of substantial genetic variation (Table 2). Genotype effects were significant for days to heading ($p = 0.05$), days to maturity ($p = 0.01$), spike length ($p = 0.01$), grains per spike ($p = 0.01$) and plant height ($p = 0.01$), indicating strong genetic control and their potential for effective selection. The lack of statistical significance for tiller number per meter, 1000-grain weight and yield per plot does not necessarily indicate limited biological relevance of these traits. In augmented block designs, test genotypes are unreplicated and trait expression is strongly influenced by environmental variability, which can inflate error variance and reduce the power of significance testing for complex quantitative traits. As a result, highly variable traits such as tiller number and yield may fail to reach statistical significance despite exhibiting wide phenotypic ranges and substantial differences among genotypes.

Genetic variability parameters

The analysis of genetic variability parameters revealed substantial variability among barley genotypes for all measured traits (Table 3). The mean values ranged from 161.27 days to heading to 308.8 g for

Table 1. Descriptive statistics for eight agronomic and yield-related traits measured in 51 barley (*Hordeum vulgare*) genotypes evaluated under temperate conditions during the rabi season 2021–2022

Trait	Mean	SD[1]	Min[2]	Max[3]	Skewness	Kurtosis
Days to heading (75 %)	161.3	2.3	158	166	0.63	2.5
Days to maturity (75 %)	201.6	2.5	197	207	0.41	2.36
Plant height (cm)	83.5	11.4	47	108	-0.52	3.85
Tiller number per meter	158.3	51	52	298	0.04	2.61
Spike length (cm)	8.4	0.8	6.2	10.6	0.02	3.63
Grain spike^{-1}	38.5	11.5	23.5	54.6	-0.08	1.18
1000 GW (g)	48.1	3.7	40.8	55.6	0.09	2.36
Yield per plot (g)	308.8	86	165	610	1.25	5.47

[1] Standard deviation, [2] minimum and [3] maximum.

Table 2. ANOVA for eight agronomic and yield-related traits in 51 barley (*Hordeum vulgare*) genotypes evaluated using an augmented block design under temperate conditions

Source	DF[2]	MS[1]							
		Days to heading (75 %)	Days to maturity (75 %)	Plant height (cm)	Tiller number per meter	Spike Length (cm)	Grain /spike	1000 GW (g)	Yield per plot (g)
Genotype	50	6.59*	7.85**	173.65**	2962.53 ns	0.94**	191.84**	16.45ns	8572.64 ns
Block	1	3.27 ns	0.067 ns	98.82 ns	3270.82 ns	0.171ns	1.87ns	0.038ns	1926.67 ns
Residuals	23	2.78	2.66	38.31	1780.61	0.28	10.76	8.89	5064.49

[1] Mean squares are shown for genotype, block and residual effects. Significance levels are indicated as $*p \leq 0.05$ and $**p \leq 0.01$, while ns denotes non-significant effects, [2] degrees of freedom.

Table 3. Estimates of genetic variability parameters for eight agronomic and yield-related traits in 51 barley (*Hordeum vulgare*) genotypes grown under temperate agro-climatic conditions

Trait	**Mean**	**PCV[1]**	**GCV[2]**	**Heritability**	**Genetic advance**	**GA (%)**
Days to heading (75 %)	161.27	1.59	1.21	57.82	4.02	2.49
Days to maturity (75 %)	201.61	1.39	1.13	66.15	4.69	2.33
Plant height (cm)	83.46	15.79	13.94	77.94	23.96	28.71
Tiller number per meter	158.29	34.39	21.72	39.9	70.82	44.74
Spike length (cm)	8.39	11.53	9.66	70.13	1.67	19.89
Grain spike^{-1}	38.5	35.98	34.95	94.39	27.72	72
1000 GW (g)	48.13	8.43	5.71	45.97	5.67	11.77
Yield per plot (g)	308.8	29.98	19.18	40.92	122.01	39.51

[1]Phenotypic coefficient of variation; [2]genotypic coefficient of variation; heritability is expressed in the broad sense (%); GA = genetic advance; GA % = genetic advance as a percentage of the trait mean.

yield per plot, indicating diverse performance across genotypes. Phenotypic coefficient of variation was higher than GCV in all traits, suggesting a considerable influence of the environment. The observed differences between PCV and GCV further indicate that environmental factors contributed to the expression of several traits, particularly yield and tiller number per meter. Such environmental sensitivity is commonly reported for complex quantitative traits in cereals and reflects the interaction between genotype performance and local agro-climatic conditions under which the evaluation is conducted.

High heritability estimates were observed for grains per spike (94.39 %), plant height (77.94 %) and spike length (70.13 %), suggesting additive gene action. These traits also showed high genetic advance as a percentage of the mean (72.00 %, 28.71 % and 19.89 %, respectively). Genetic advance as a percentage of the mean (GA %) was highest for tiller number per meter (44.74 %) and yield per plot (39.51 %), even though both traits exhibited only moderate heritability (39.9 % and 40.92 %, respectively). This combination indicates that additive genetic effects play a major role and these traits can be effectively improved through direct or indirect selection. In contrast, days to heading and days to maturity showed comparatively lower heritability and genetic advance, suggesting limited scope for improvement through conventional selection methods. Notably, although tiller number per meter and yield per plot were non-significant in the ANOVA, both traits exhibited high phenotypic coefficients of variation and substantial genetic advance as a percentage of the mean. This combination indicates the presence of exploitable genetic variability that may not be fully captured by significance testing under unreplicated conditions, but is nonetheless relevant for selection when supported by multivariate analyses and trait associations.

Correlation analysis

The correlation analysis among eight key agronomic traits in 51 barley genotypes revealed significant relationships, highlighting opportunities for indirect selection and trait improvement (Fig. 1).

Phenology traits

Days to heading showed a strong positive correlation with days to

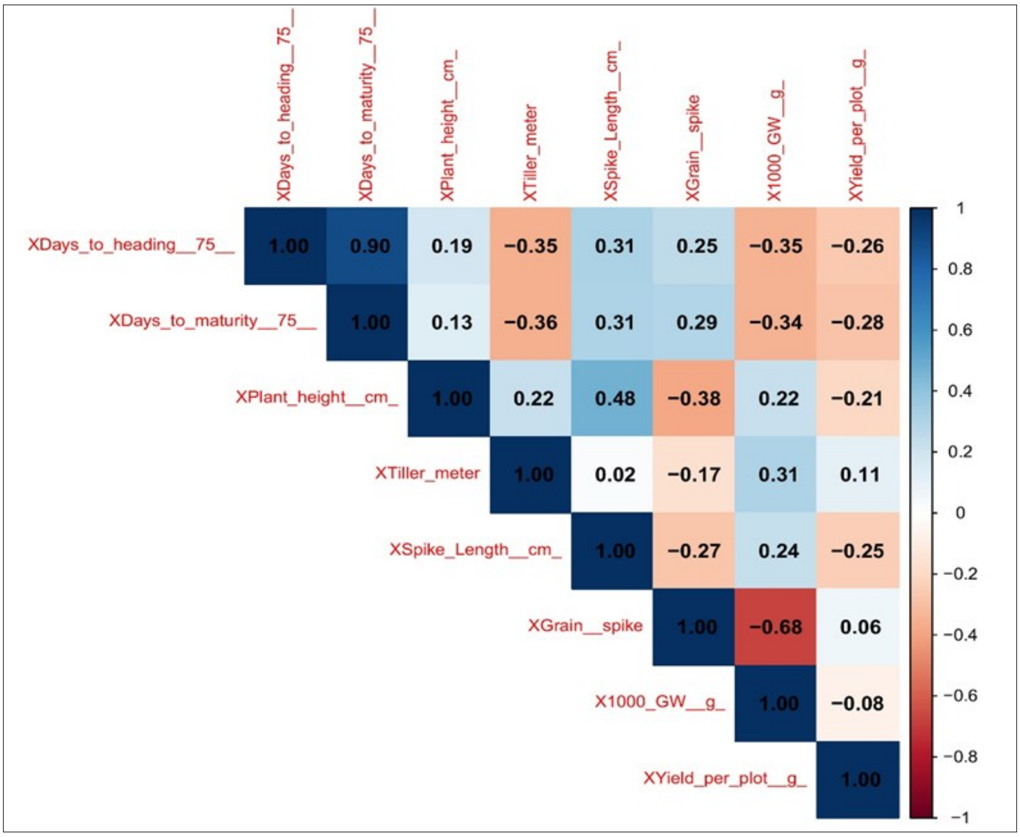

Fig. 1. Heatmap showing phenotypic correlation coefficients among eight agronomic and yield-related traits in 51 barley (*Hordeum vulgare*) genotypes evaluated under temperate conditions.

Red and blue colors indicate positive and negative correlations, respectively, with color intensity reflecting the strength of association.

maturity, indicating that early heading genotypes also tend to mature earlier. This relationship is particularly important in environments with terminal drought or short growing seasons, where early maturity can be advantageous.

Morphological traits

Plant height exhibited moderate positive correlations with spike length and grain per spike, suggesting that taller plants may contribute to improved reproductive traits.

Yield-related traits

Tiller number per meter was positively correlated with yield per plot and 1000-grain weight, underscoring its importance in determining overall productivity. In contrast, spike length showed weak to moderate negative correlations with 1000-grain weight and grain per spike, implying a possible trade-off between grain number and grain size.

Direct yield associations

Yield per plot displayed significant positive correlations with tiller number per meter, spike length and grain per spike. These findings confirm that these traits can serve as reliable selection criteria for improving yield in barley breeding programs.

Principal component analysis

Principal component analysis (PCA) was conducted on 8 agronomic traits from 51 barley genotypes to explore trait variation and interrelationships (Fig. 2). The first two principal components (PC1 and PC2) accounted for 56.7 % of the total phenotypic variation, signifying that a considerable amount of the overall heterogeneity across the 51 barley genotypes was represented by these components. Principal component 1 accounted for 34.7 % of the total variation and PC2 contributed an additional 22.0 %, culminating in an overall explanation of about 60 % of the total variance. The indicated level of explained variance implies that the PCA successfully condensed the multidimensional trait dataset into a limited number of meaningful axes appropriate for genotype differentiation and analysis.

Principal component 1 was primarily driven by traits such as days to heading, days to maturity and grains per spike, indicating that genotypes scoring high along this component were typically late-maturing and had more grains per spike. Principal component 2 was mainly influenced by yield-contributing traits, specifically tiller number per meter and yield per plot, demonstrating their distinct role in genotype differentiation. Traits such as spike length, grain per spike and plant height contributed moderately to both components, whereas 1000-grain weight had minimal loading on either axis, indicating limited influence on major trait variability.

The PCA biplot grouped genotypes with similar trait expressions and revealed key associations among traits. Genotypes such as IBYT-HI-2021-14, IBON-HI-2021-10 and IBYT-HI-2021-9 were positioned near the yield and tiller number vectors, indicating their strong association with productivity-related traits. In contrast, HUB113 and DWRB637 appeared as outliers, showing unique trait

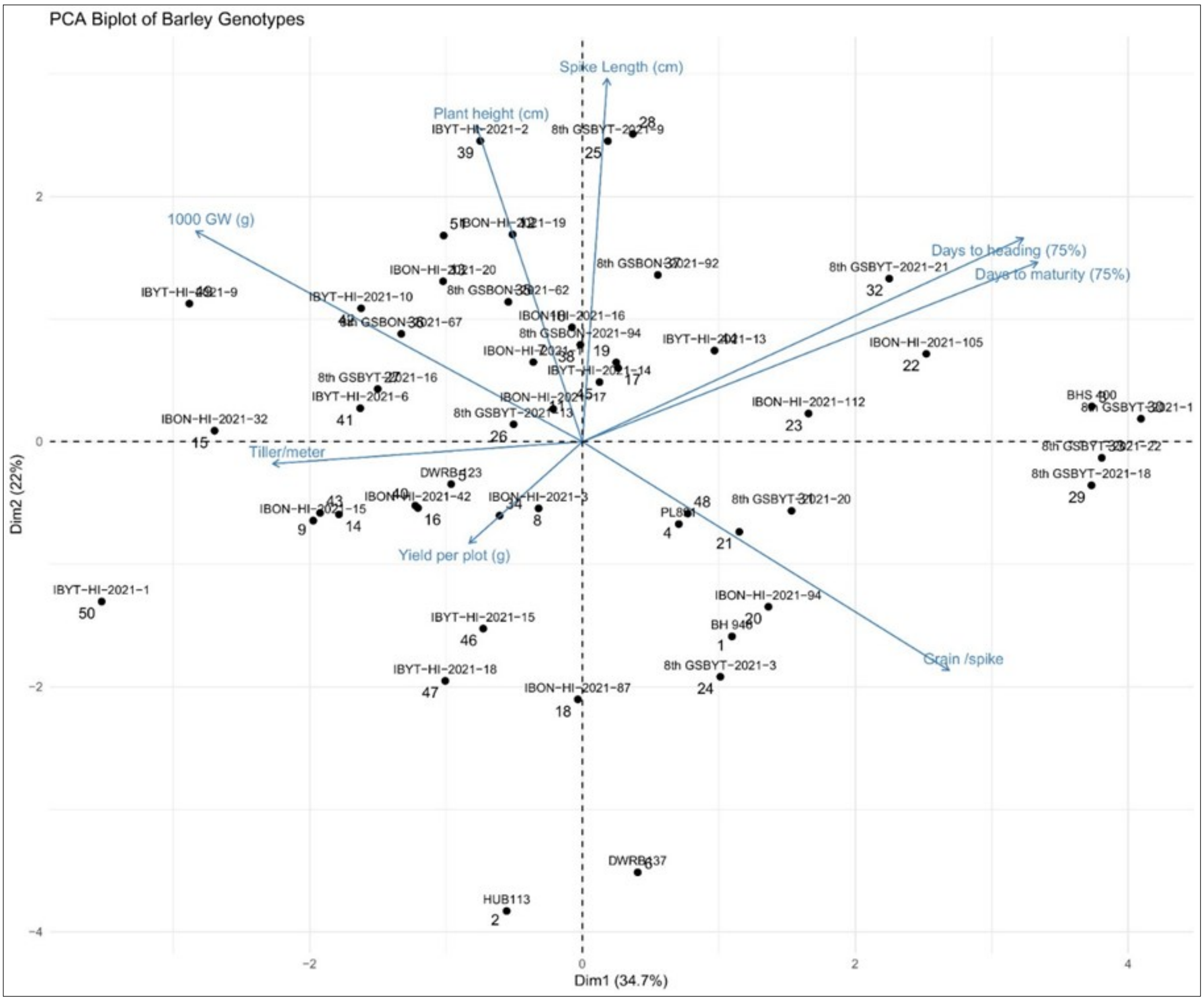

Fig. 2. PCA biplot based on eight agronomic and yield-related traits of 51 barley (*Hordeum vulgare*) genotypes evaluated under temperate agro-climatic conditions. The first two principal components (PC1 and PC2) explain the maximum proportion of total phenotypic variation, with vectors indicating trait contributions and points representing individual genotypes.

combinations and potential as sources of novel variation. A close alignment was observed between tiller number per meter and yield per plot vectors, suggesting a strong positive association. Yield was nearly orthogonal to phenological traits such as days to heading and maturity, indicating a weak or negative correlation.

Cluster analysis

The hierarchical cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA) method grouped the 51 barley genotypes into 5 distinct clusters, indicating significant genetic divergence among them (Fig. 3). The unequal distribution of genotypes across clusters underscores underlying phenotypic diversity. The dendrogram structure clearly visualises the relative genetic proximity among genotypes, with shorter branches indicating higher similarity and longer branches signifying greater divergence.

Cluster I included 12 genotypes, cluster II had 19, cluster III had 12, cluster IV and cluster V each consisted of 4 genotypes, showing an unequal distribution across groups. Inter-cluster distances were generally larger than intra-cluster distances, suggesting higher genetic variation between groups than within groups. The highest inter-cluster distance was observed between cluster I and cluster V (10.8). In contrast, the smallest inter-cluster distance was found between cluster IV and cluster V (3.2), showing minimal divergence among genotypes and potentially limited recombinant value if crossed. Traits such as grains per spike, spike length, tiller number per meter and yield per plot contributed significantly to the grouping pattern, as revealed by earlier PCA loadings. Cluster V, including IBYT-HI-2021-14, showed high yield potential and were grouped with others exhibiting superior agronomic performance, making this cluster a priority for selection. While clusters I and IV included early-maturing and low-yielding lines, cluster V stood out for its superior yield potential and tillering capacity. Cluster II was notable for productivity and taller stature and cluster III offered broad variability across traits.

The average linkage (UPGMA) method effectively captured the hierarchical relationships among genotypes, supporting informed decisions for genotype selection and crossbreeding strategies. The clustering results, validated by corresponding trait heatmaps confirmed clear differences in trait expression across clusters, reinforcing the reliability of clustering results (Fig. 4). Genotypes within the same cluster exhibited similar trait expressions, while those in distant clusters were more divergent.

Selection of superior genotypes

The multivariate analysis based on average standardised trait values enabled the identification of superior barley genotypes with high yield potential and favorable trait combinations (Fig. 5). Among these, IBYT-HI-2021-12, IBON-HI-2021-19 and 8th GSBYT-2021-13 consistently exhibited above-average standardised scores for yield per plot, tiller number per meter, grain per spike and plant height. The superior genotypes IBYT-HI-2021-14, IBYT-HI-2021-12, IBON-HI-2021-19 and 8th GSBYT-2021-13 recorded yields of 485 g plot^{-1}, 390 g plot^{-1}, 320 g plot^{-1} and 460 g plot^{-1}, respectively. They also showed tiller numbers of 268, 252, 115 and 120 m^{-1}, respectively. These values are higher than the overall mean yield of 308.8 g plot^{-1} and the mean tiller number of 158 per meter, confirming that these genotypes are numerically superior in both seed yield and tiller production.

The distribution of these genotypes across distant clusters highlights the presence of substantial genetic diversity, which is a critical prerequisite for exploiting heterosis in hybridisation programs. Such diversity ensures that crossing these genotypes could generate transgressive segregants with enhanced yield stability and adaptability. Principal component analysis further confirmed their high scores on PC1 and PC2, which captured the majority of trait variability. Selection criteria combined genotypic performance, clustering results and trait loadings to ensure both productivity and genetic divergence. This approach ensures that selected lines can serve as ideal parents for future breeding

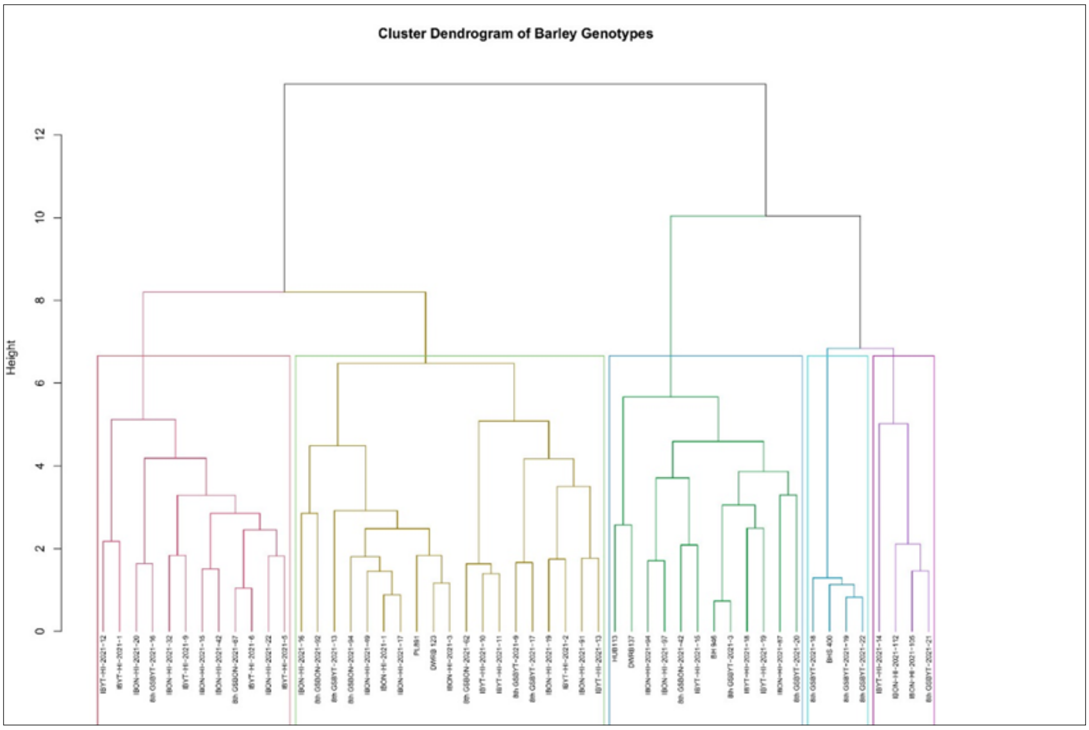

Fig. 3. Hierarchical cluster dendrogram illustrating genetic relationships among 51 barley (*Hordeum vulgare*) genotypes based on 8 agronomic and yield-related traits. Clustering was performed using the UPGMA method and genotypes were grouped into five distinct clusters indicating varying degrees of genetic divergence.

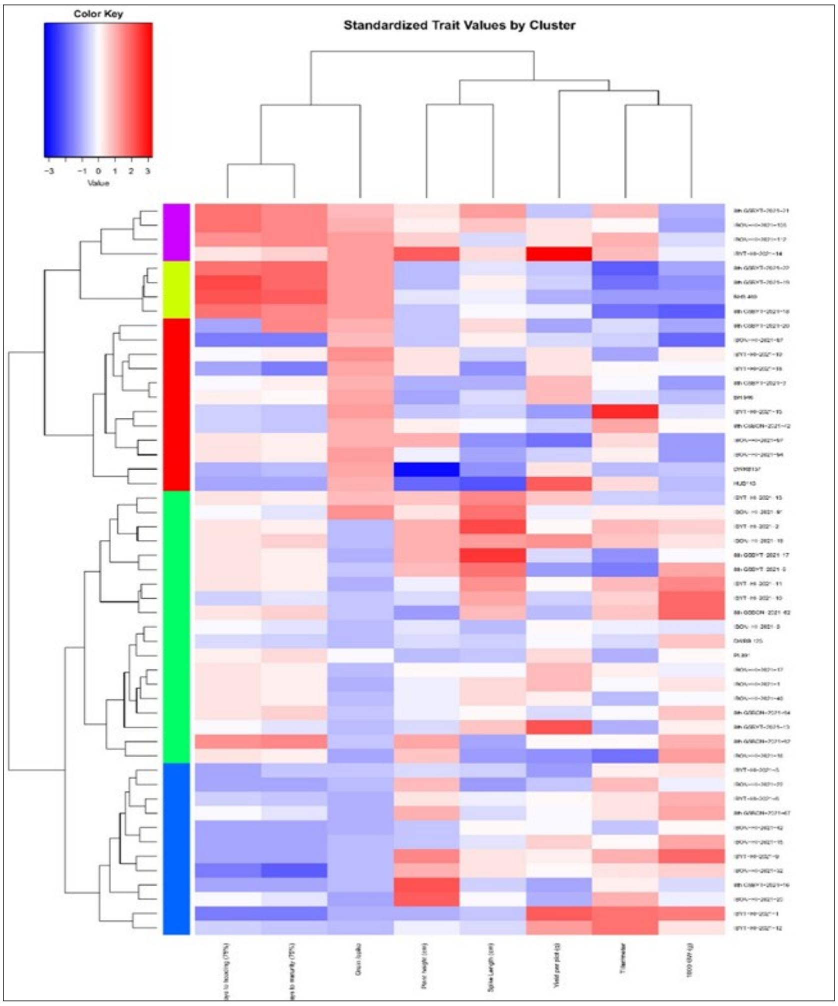

Fig. 4. Heatmap depicting cluster-wise mean performance of eight agronomic and yield-related traits across five clusters of 51 barley (*Hordeum vulgare*) genotypes. Color gradients represent relative trait expression levels, facilitating comparison of trait patterns among clusters.

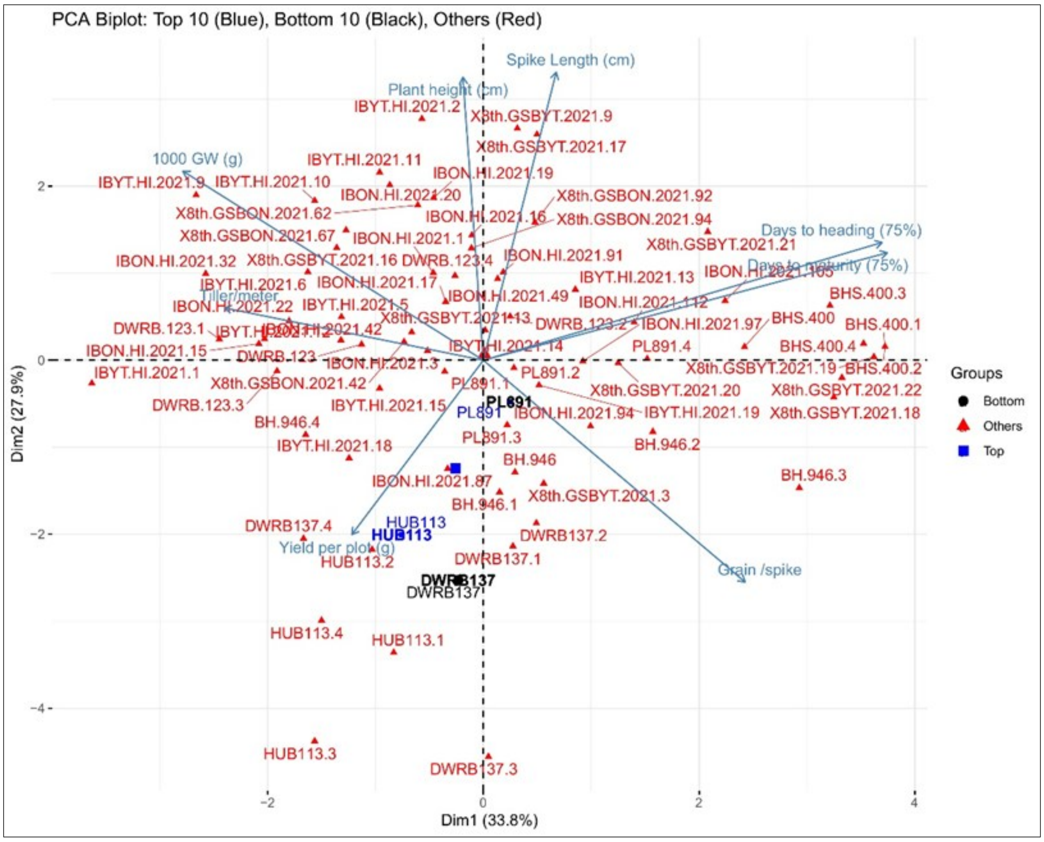

Fig. 5. Identification of superior barley (*Hordeum vulgare*) genotypes based on combined multivariate analysis, including yield per plot, tiller number per meter, grains per spike and principal component scores. Genotypes highlighted exhibit superior agronomic performance and genetic divergence, indicating their potential utility as parents in future breeding programs.

programs targeting improved yield and agronomic traits. These findings provide a solid foundation for advanced selection and crossbreeding strategies aimed at developing high-yielding, stress-resilient barley varieties.

Discussion

The present study reveals significant genetic variability among barley genotypes, particularly for traits related to yield and plant architecture. Despite the evaluation being performed at a singular site and during a certain season, the impact of environmental factors on trait expression must be acknowledged when interpreting the findings. Barley yield and associated attributes are significantly influenced by environmental variability and genotype × environment interactions can considerably modify genotype rankings across different locales. Consequently, although the discovered superior genotypes exhibited commendable performance under the temperate circumstances of Khudwani, their stability of performance across varied environments cannot be deduced from the existing dataset alone.

The narrow variation in phenological traits such as days to heading and maturity indicates stability but may limit selection scope for earliness. In contrast, broader variability in traits, such as plant height, tiller number and yield components highlights the potential for selecting superior genotypes. The extent of genetic diversity observed among the evaluated barley genotypes underscores the effectiveness of the germplasm set in capturing meaningful variation for key agronomic traits. Rather than reiterating the general importance of diversity, the present findings demonstrate how this variability was structured into distinct clusters and principal components, providing a practical framework for parent selection and targeted hybridisation in barley breeding programs.

Although the genotype effect was not statistically significant for traits, such as tiller number per meter, 1000-grain weight and yield per plot, these traits still exhibited notable variation, warranting further investigation through multivariate analyses. In augmented trials with unreplicated test entries, significance testing for certain traits may be conservative due to the limited replication of test lines and the consequent precision structure, while multivariate analyses can still uncover biologically significant patterns of variation across traits and genotypes. Statistical analyses of augmented block designs demonstrate the influence of control allocation and block arrangement on the variance of pairwise contrasts, advocating for careful interpretation of non-significant ANOVA results in conjunction with further multivariate evidence (16).

Block effects were not significant for most traits, indicating uniformity across experimental blocks. The residual error was relatively low, supporting the reliability of the results. Overall, the results confirm that the observed variations are primarily due to genetic factors rather than environmental influences. Therefore, it supports further multivariate analysis and genotype selection aiming at enhancing barley yield and agronomic performance.

Traits with high heritability and genetic advance such as grains per spike, spike length and plant height, suggest additive gene action and are ideal for direct selection, consistent with earlier findings (18, 19). The moderate heritability coupled with high genetic advance observed for tiller number per meter and yield per plot suggests that these traits are under the combined influence of genetic and environmental factors. Such a pattern indicates that while environmental variation contributes to their expression, the additive genetic component is sufficiently strong to ensure reliable progress through selection. This makes them particularly valuable in advanced generations, where the fixation of favorable alleles can lead to sustained yield improvement. Previous studies have similarly reported that traits with moderate heritability but high genetic advance are ideal candidates for selection, as they reflect the predominance of additive gene action over non-additive effects.

In wheat breeding programs, tiller number and plot yield are directly linked to productivity and resource-use efficiency, reinforcing their importance as selection criteria. The consistency of these findings across different environments underscores their robustness and highlights the potential of integrating them into breeding pipelines aimed at enhancing yield stability and adaptability. Future research could further dissect the genetic architecture of these traits using molecular markers, thereby strengthening their utility in marker-assisted selection and genomic prediction models (20–22). Tiller number per meter and yield per plot exhibited high genetic advance despite moderate heritability, suggesting they can be improved through direct or indirect selection. Traits such as days to heading and maturity showed lower heritability and genetic advance, reflecting greater environmental influence.

The lack of notable genotype effects on tiller number, 1000-grain weight and yield per plot, despite their crucial importance in productivity, can wheat ascribed to both statistical and biological causes. These characteristics are intrinsically intricate and are recognised to be extremely responsive to environmental factors, including soil fertility, moisture availability and microclimatic fluctuations. The implementation of an augmented block design with unreplicated test entries diminishes the precision of variance estimation for these features, elevating residual variance and constraining the identification of significant differences. Thus, multivariate methodologies and variability metrics offer supplementary data that elucidates the inherent genetic potential of these productivity-associated variables.

The significant and consistent relationships among key agronomic traits observed in this study support the use of indirect selection in barley breeding programs. The strong correlation between days to heading and days to maturity indicates a shared genetic or physiological basis for phenological traits, which is particu1larly valuable in environments with terminal drought or short cropping seasons. The positive association of plant height with spike length and grain number per spike suggests that taller genotypes may exhibit enhanced reproductive development—a trend also noted previously (23, 24). Moreover, the strong correlation between tiller number and both yield and 1000-grain weight highlights tillering as a vital yield component, echoing earlier findings, where the study demonstrated similar patterns in grain yield correlations with spike and tillering traits (25).

Negative correlations between spike length and 1000-grain weight or grain number may point to resource allocation trade-offs, as suggested in prior studies, where spike elongation sometimes came at the expense of grain fill. The overall significant positive correlations of yield with tiller number, spike traits and grains per spike support earlier, reported similar correlations in barley across various environments and populations (18, 26–31). The negative association observed between spike length and 1000-grain weight can be interpreted as a consequence of resource allocation trade-offs during spike development and grain filling. Longer spikes often accommodate a greater number of spikelets or grains, which can intensify intra-spike competition for assimilates, ultimately reducing the average grain weight. Similar trade-offs between grain number–related traits and grain weight have been reported in barley and other cereals, reflecting physiological constraints on assimilate partitioning under finite source availability. This relationship highlights the importance of balancing spike size and grain filling efficiency when selecting for yield improvement.

The PCA successfully reduced the dimensionality of the dataset while preserving key sources of variation among genotypes, which is particularly valuable in identifying patterns not readily visible through univariate analyses. The first two principal components explaining approximately 60 % of the total variance indicates a robust summary of trait variation, which is comparable to or higher than values commonly reported in multivariate studies of barley germplasm and other self-pollinated cereal crops. The dominant role of phenological traits in PC1 and yield-related traits in PC2 suggests that these two sets of traits are relatively independent, providing opportunities for concurrent selection without confounding effects.

The identification of high-yielding genotypes such as IBYT-HI-2021-14 and IBON-HI-2021-10 near the yield and tillering vectors supports their value for direct selection in breeding programs. PCA is effective in identifying trait-driven genotype groupings and selecting superior individuals based on multivariate profiles (32–34). The orthogonality between yield and phenological traits such as days to heading and maturity highlights a potential trade-off between earliness and productivity, which has practical implications for breeding under varying environmental conditions. For instance, in environments with terminal drought stress, early maturity is critical, whereas in favourable environments, longer crop durations may enhance yield potential.

Furthermore, the minimal contribution of 1000-grain weight to the major components suggests that this trait may not significantly differentiate genotypes within this specific germplasm set, or that it varies independently of the primary yield determinants captured in PC1 and PC2. Overall, the PCA provided a comprehensive understanding of trait interactions and enabled the identification of superior genotypes with desirable trait combinations. This supports the use of PCA as a powerful selection tool in multivariate breeding strategies for barley improvement. Cluster analysis revealed substantial genetic divergence, especially between Clusters I and V, which can be exploited in hybridisation programs to generate heterosis (35). The identification of elite genotypes from divergent clusters also aligns with genetic gains (29, 36). These findings align with recent studies that emphasise the utility of multivariate approaches in dissecting complex trait interactions. It was reported that integrating univariate and multivariate stability indices provides a more reliable framework for identifying barley genotypes with both high yield and stability across environments (37). Similarly, it was also reported that moderate heritability combined with high genetic advance in yield-related traits reflects the predominance of additive gene action, making them ideal candidates for selection (22). The above-average standardised values observed in the present study reinforce this interpretation, as they suggest that these genotypes not only perform well under specific conditions but also possess genetic potential for consistent improvement.

From a breeding perspective, the identification of genotypes distributed across distant clusters is particularly significant. Genetic divergence among high-performing lines increases the likelihood of heterotic responses when used as parents in hybridisation, thereby accelerating genetic gain. This is consistent with earlier reports that emphasize the importance of exploiting genetic diversity to broaden the breeding base and enhance resilience to environmental fluctuations. Moreover, the favorable trait combinations observed-such as high tiller number coupled with increased grain per spike-are directly linked to yield improvement strategies in barley, underscoring their relevance for both conventional and molecular breeding pipelines.

Locally and regionally adapted barley genetic resources are essential for expanding the genetic foundation of breeding programs, especially in varied and stress-prone conditions, such as temperate agro-climatic zones. Indigenous genotypes and regionally adapted lines frequently possess distinctive genes linked to adaptation to particular climatic conditions, soil types and biotic stresses, which may be absent in elite or extensively farmed germplasm. The evident genetic divergence among clusters in this study indicates that both local and exotic entrants significantly contributed to total diversity. Integrating locally adapted genotypes from diverse clusters into breeding programs can boost allelic diversity and resilience to environmental variability, hence facilitating the creation of robust, high-yielding barley cultivars.

Nonetheless, multivariate techniques such as PCA and cluster analysis offer significant first insights into genotype adaptation by pinpointing genotypes that exhibit balanced performance across various characteristics. Genotypes that demonstrate advantageous placements along principal components and are part of high-performing clusters may have greater adaptation capacity; yet, validation of yield stability necessitates assessment across several locations and years. The combined application of variability parameters, correlation studies, PCA and cluster analysis enabled the identification of superior genotypes with both high yield potential and genetic diversity. These findings provide a solid foundation for future barley improvement programs under temperate conditions and support the utility of multivariate tools in modern breeding pipelines.

Conclusion

This study demonstrated substantial genetic variability among 51 barley genotypes, with key yield-related traits such as tiller number, grains per spike and yield per plot showing high genetic advance despite moderate heritability, underscoring their potential for improvement through selection. Multivariate analyses identified superior genotypes (e.g., IBYT-HI-2021-14, IBON-HI-2021-19) and revealed broad genetic divergence, highlighting their value as parental lines for hybridisation and heterosis exploitation. However, as the evaluation was conducted in a single year and location, the stability and adaptability of these genotypes remain provisional. The findings provide a solid foundation for advancing barley breeding strategies aimed at high yield, stability and adaptability in temperate environments.

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Authors' contributions

SHW, NRS and ZAD conceptualised, planned the experiment and wrote the manuscript. GHK, SG, GA and SMU, conducted the experiment and carried out the statistical analyses. SHW, RRM and MAK were involved in data interpretation and selection of promising genotypes. MR was involved in data analysis and interpretation. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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