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Cluster analysis of phenotypic diversity in barley genotypes for yield improvement under rainfed environment

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ABSTRACT

Barley (*Hordeum vulgare* L.) is an important cereal crop cultivated in diverse agroecological regions, while yield stability under rainfed conditions remains a major challenge due to environmental variability and limited water availability. This research evaluated twenty barley genotypes to assess phenotypic diversity, trait correlations, and genotype classification based on agronomic and morphometric traits via a field trial under the Moghan (39°38′ N, 47°54′ E) conditions under rainfed conditions characterized by an average annual rainfall of 335 mm. Ten traits were recorded, including plant height (PH), spike height (SH), number of tillers per plant (NTP), number of fertile tillers (NFT), straw weight (SW), biomass weight (BW), number of grains per plant (NGP), grain yield per plant (GYP), grain yield (GY), and thousand-grain weight (TGW). Correlation analysis demonstrated that GY was positively associated with BW and TGW, indicating that both vegetative growth and grain size are key contributors to yield, while a significant negative correlation was observed between spike height (SH) and thousand-grain weight (TGW), indicating a trade-off between spike architectural traits and grain size development. Genotypic clustering divided the barley genotypes into three distinct groups. Cluster-I comprised genotypes with high grain yield and favorable per-plant productivity, Cluster-II included low-performing genotypes with limited biomass and yield potential, and Cluster-III consisted of tall, biomass-rich genotypes with competitive grain yield. Thus, Cluster-III genotypes offer potential for enhancing biomass production, plant vigor, and morphological traits associated with environmental adaptation such as plant height and spike architecture. Genotypes from Cluster-I may be prioritized for direct yield improvement, whereas Cluster-III genotypes offer potential for enhancing biomass production, plant vigor, and adaptive traits. This research provides a framework for genotype selection and supports breeding strategies aimed at improving yield in rainfed barley production systems.

Highlights

- Efficient biomass partitioning is critical for maintaining grain yield under arid conditions.
- High thousand grain weight enhances yield stability in water-limited environments.
- Short- to medium-stature genotypes outperform tall, biomass-rich types for grain production in semi-arid zones.
- Stable tiller fertility is more important than high tiller number under moisture stress.
- Cluster-based selection enables identification of drought-adapted barley genotypes for semi-arid regions.

1. Introduction

As the fourth most important cereal crop globally after wheat, rice, and maize, barley is primarily utilized for animal feeding and the malt industry. Owing to its wide adaptability, high nutritional value, and relative environmental tolerance, barley is cultivated across diverse

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agroecological regions (Meng et al., 2023). Despite this adaptability, drought stress remains one of the most severe abiotic constraints limiting barley productivity, particularly in semi-arid and rainfed regions, where water scarcity significantly reduces agricultural production efficiency. Genetic improvement has played a crucial role in enhancing crop yield under stress conditions by simultaneously improving multiple quantitative traits associated with stress tolerance across different growth stages (Panahi et al., 2025). Grain yield, the primary target trait in most breeding programs, is a complex and quantitatively inherited character influenced by numerous morphological and physiological processes. Direct selection for grain yield is often inefficient due to its low heritability and strong environmental influence. Therefore, indirect selection through easily measurable, heritable traits that show positive and significant correlations with grain yield can substantially improve selection efficiency (Li et al., 2023).

Although barley is generally more tolerant to drought stress compared with other cereals, it is particularly sensitive to water deficits during critical developmental stages, especially stem elongation and grain formation (Bello et al., 2022). Drought stress during these stages leads to pronounced yield reductions, whereas water stress before pollination has been reported to reduce the number of grains per spike. Water shortage stress during and shortly after pollination primarily decreases grain weight, while drought occurring at early grain-filling stages exerts more severe effects, resulting in grain shriveling and reduced grain size (Bartosiewicz and Jadczyszyn, 2021). Several studies have demonstrated that yield reduction under moisture stress conditions is mainly attributable to decreases in thousand-grain weight, spikelets, and grains per plant (Berki et al., 2025). Genetic variation exists among barley genotypes for drought tolerance, providing opportunities for selecting and breeding stress-resilient cultivars. Although drought stress generally does not affect plant density per unit area, it can significantly reduce the harvest index, particularly when stress occurs during pollination, depending on cultivar sensitivity (Zia et al., 2021; Oguz et al., 2022).

Genetic diversity is a fundamental prerequisite for effective plant breeding, as it enables the identification of suitable parents and the development of superior progeny. Various approaches have been employed to assess genetic diversity in plant species; among them, field evaluation based on agro-morphological traits remains a widely used and practical method for characterizing and classifying genetic resources (Kumar et al., 2025). Because multivariate statistical methods can simultaneously analyze multiple traits, they are particularly effective for evaluating genetic diversity using morphological, biochemical, and molecular data. Among these methods, cluster analysis is the most applied and informative technique. Khan et al. (2021) evaluated genetic diversity among 40 barley landraces using morphological traits and analysis of seed storage proteins, and showed variation in plant height, seed weight, flowering time, and biomass, while clustering categorized the accessions into seven distinct clusters, demonstrating considerable variation. Verma et al. (2021) assessed morphological diversity under heat stress and found substantial genome-wide genetic diversity, with two subpopulations in barley, while days to heading and maturity, spike traits, and grain yield were important traits in stress tolerance.

Gadissa et al. (2021) studied 120 barley landraces to assess agronomical diversity and reported high genotypic and phenotypic variation with low broad-sense heritability, indicating a strong environmental influence on trait expression. Also, the geographical origin of landraces showed little association with genetic grouping, suggesting extensive gene flow, while high morphological diversity was found, but the need for multi-environment evaluations was emphasized to effectively utilize genetic variation for barley breeding and conservation (Gadissa et al., 2021). Previous studies have reported substantial genetic variability in barley germplasm for yield and related agronomic traits, and several have applied multivariate approaches to assess trait relationships and genetic diversity. However, most of these studies have treated trait evaluation and genotype classification separately, and there is still a lack of integrated analyses combining trait interrelationships, trait-based clustering, and genotype grouping under specific rainfed field conditions. In particular, few studies have simultaneously linked phenotypic diversity with functional trait clustering and provided explicit breeding-oriented interpretation for semi-arid rainfed environments such as those of northwestern Iran. Addressing this gap is essential for improving selection efficiency and identifying superior genotypes for breeding programs. This study addresses the following research question: Which agronomic and morphometric traits most strongly contribute to yield variation among barley genotypes under rainfed conditions? We hypothesize that significant genetic variability exists among barley genotypes for yield-related traits, and that specific morphological and yield components are significantly associated with grain yield, allowing effective indirect selection under moisture-limited environments. Therefore, the objectives of this study were: (i) to evaluate phenotypic variability among barley genotypes under rainfed conditions; (ii) to determine key agronomic traits associated with grain yield through correlation analysis; and (iii) to classify both traits and genotypes using multivariate clustering methods to support the identification of superior breeding material.

2. Materials and methods

2.1. Experiment

A collection of 20 barley genotype samples was obtained from the Seed and Plant Improvement Institute, Iran (Table 1). A field trial was carried out at the research location, Moghan (39°38′ N, 47°54′ E), at an elevation of 32 m, due to its proven suitability for barley production. The soil of the experimental site was predominantly clay loam. However, soil moisture content and field water-holding capacity were not directly measured during the study, which is acknowledged as a limitation of the present

experiment. The experiment was conducted under rainfed conditions during the main cropping season (November-May) (Table 2). Seasonal precipitation data for the experimental period were obtained from the local meteorological station and have been added to better characterize the environmental conditions of the study site. Experiments were conducted during the primary cropping season, spanning November to May. The site experiences an average annual rainfall of 335 mm and mean temperatures ranging annually from 1.4 °C to 31.4 °C, with predominantly clay loam soils. A randomized complete block design with three replicates was utilized. Each plot consisted of six rows, each 3.0 m in length, with 0.30 m spacing between rows, resulting in a total plot size of 5.4 m^2 (6 × 3.0 m × 0.30 m). Each barley genotype was sown with 0.10 m spacing between plants on the row and 0.30 m spacing between rows. Sowing was conducted at the onset of the rainy season (November) on well-prepared soil. Agronomic practices were uniformly applied, including weeding and fertilization, while pest control measures were applied as needed. A total of ten agronomic/morphometric traits were recorded using the barley descriptor, whereas most traits were measured from ten tagged middle plants per plot per replication, and plot-based traits such as yield performance were recorded collectively. Thus, plant height (PH), spike height (SH), number of tillers per plant (NTP), number of fertile tillers (NFT), straw weight (SW), number of grains per plant (NGP), and grain yield per plant (GYP) were measured. For harvesting, all aboveground materials from the central area were harvested and weighed to determine biomass weight (BW) and grain yield (GY). Finally, Thousand-grain weight (TGW) was calculated as the mean weight of three randomly selected samples of 1000 seeds per plot. Biomass weight (BW) and straw weight (SW) were recorded on a per-plot basis and expressed as dry weight after oven-drying plant samples at 65-70°C until constant weight was achieved. For statistical presentation, values were standardized and reported in appropriate units (kg ha^{-1}).

Table 1. The pedigree of 20 barley genotypes evaluated under rainfed condition

#	Pedigree
1	Mahour
2	CBSS97M00850T-G-2M-1Y-2M-0Y
3	CB06M00193T-C-9M-0AP-0AP-0AP
4	CBSS07Y00696S-0AP-0AP
5	CBSS05M00256S-2M-0Y-0M-0AP-0TR
6	CBSS97M00850T-G-2M-1Y-2M-0Y
7	CBSS06Y00079S-29Y-0M-0AP-0TR
8	ICB86-0512-1AP-0TR-4AP-0TR-0AP
9	ICB05-0498-5AP-0AP
10	ICB05-0421-0AP-6AP-0AP
11	ICB95-0204-0AP-16AP-0AP-4AP-0AP-9AP-0AP
12	LBIRAN/UNA80//LIGNEE640/6/Vmorales
13	CBSS05Y00158S- 25Y-0M-0Y-0M-4AP
14	CBSW01WM00107T-0TOPY-6Y-1M-2Y-1M-0Y
15	CBSS97M00850T-G-2M-1Y-2M-0Y
16	ICB08-0124- OAP - OG-OG-OG- 5G
17	CBSS05M00680D-K-2M-0M-0AP-0TR
18	ICB09-1436-0AP-0TR-0AP-0TR-0AP-0TR
19	TR145/I95039
20	M91178002/MAHIGAN

Table 2. Monthly climatic conditions during the barley growing season at Moghan

Month	Rainfall (mm)	Mean Temp (°C)	Min Temp (°C)	Max Temp (°C)
November	53	9.5	4.2	15.3
December	33	6.8	1.5	11.8
January	41	5.2	-0.8	9.7
February	44	4.8	-0.3	10.2
March	30	7.5	2.6	13.8
April	22	10.6	6.1	17.9
May	12	16.1	10.2	23.8

2.2. Statistical analysis

Frequency distributions of traits were checked, and descriptive statistics as well as correlations were computed using Minitab 17.0. Prior to clustering analysis, all traits were standardized (mean = 0, standard deviation = 1) to eliminate scale effects. Clustering analyses were performed using genotype mean values across replications to ensure that grouping reflected genotypic performance rather than plot-level environmental variation. Agglomerative hierarchical clustering was conducted to classify both genotypes and traits based on standardized squared Euclidean distances. The clusters were formed using Ward's minimum variance method, and dendrograms were constructed to illustrate the relationships among genotypes and traits using Minitab 17.0. Ward's minimum variance method was selected because it minimizes within-cluster variance and generates more homogeneous and interpretable clusters for continuous agronomic traits. This approach is widely recommended for morpho-agronomic datasets compared with alternative linkage methods such as UPGMA, which may produce less compact clusters. To validate the number of clusters and ensure meaningful separation, multivariate analysis of variance was performed on the original dataset for all recorded traits. Statistical

significance of the cluster boundaries was evaluated using the largest root of Roy and lambda of Wilks, as well as traces of Hotelling and Pillai, in the Multivariate procedure of SPSS version 27.0. Cluster-based selection enables identification of barley genotypes with superior performance under rainfed (moisture-limited) conditions.

3. Results

3.1. Descriptive statistics

The barley genotypes exhibited a relatively wide range of phenotypic variation across traits, reflecting remarkable diversity (Table 3). The coefficient of variation (CV) of grain yield (GY) was 18.46%, indicating moderate yield variability among genotypes. Biomass weight (BW) and straw weight (SW) recorded CVs of 24.54 and 31.61%, respectively, highlighting pronounced differences in vegetative growth patterns. Grain yield per plant (GYP) ranged from 1.30 to 2.22 g, with a moderate CV (14.13%), while the number of grains per plant (NGP) also displayed noticeable variation, suggesting differential sink capacity among genotypes (Table 3). Thousand grain weight (TGW) exhibited low CV (8.46%), while the number of tillers per plant (NTP) and number of fertile tillers (NFT) showed moderate variation, with CV values of 15.78% and 12.24%, respectively (Table 3). Morphological traits were less variable than yield and biomass traits, whereas spike height (SH) and plant height (PH) showed CV values of 10.11% and 7.64%, respectively, reflecting relatively uniform plant stature across the tested genotypes. The magnitude of variation observed for the measured traits confirms the presence of adequate phenotypic diversity for effective multivariate analysis and supports the application of cluster analysis to classify the barley genotypes.

3.2. Correlations of traits

Considering significant associations, GY was positively correlated with BW and showed a strong positive association with TGW (Table 4), indicating that both biomass accumulation and grain size were major contributors to yield variation among the barley genotypes. Biomass weight exhibited a very strong positive correlation with SW, reflecting their close physiological relationship and shared contribution to total above-ground biomass. Grain yield per plant was positively associated with NGP (Table 4), demonstrating that grain number was the principal determinant of per-plant yield, while NTP was correlated with NFT, indicating high efficiency in tiller survival and fertility. Spike height showed a significant positive correlation with NGP, but was negatively associated with TGW (Table 4). A significant negative correlation was observed between spike height (SH) and thousand-grain weight (TGW), indicating a trade-off between spike architectural traits and grain size development. Plant height was positively correlated with BW and SW, indicating that taller plants tended to accumulate greater vegetative biomass. These correlations highlighted trait interdependencies that contributed to genotype differentiation in the next cluster analysis. Recent morphological diversity research on barley (Aka et al., 2025) also found significant positive correlations between GY and TGW, PH, NTP, and NGP, pointing to similar yield component relationships in barley germplasm studies. The negative association found between SH and TGW highlighted potential trade-offs between reproductive structures and grain weight.

Table 3. Descriptive statistics of measured traits in 20 barley genotypes

Statistics	GY	BW	SW	GYP	NGP	TGW	NTP	NFT	SH	PH
Mean	2118.3	7567.2	5448.9	1.79	55.20	32.46	2.89	2.23	5.88	79.79
SE†	87.5	415.3	385.1	0.06	1.76	0.61	0.10	0.06	0.13	1.36
SD‡	391.1	1857.3	1722.3	0.25	7.88	2.75	0.46	0.27	0.59	6.09
Range	1313.3	7331.8	6021.8	0.92	32.00	8.91	1.74	1.10	2.53	19.74
Minimum	1386.7	3668.2	2281.6	1.30	41.00	27.94	2.33	1.77	4.87	68.93
Maximum	2700.0	11000.0	8303.3	2.22	73.00	36.85	4.07	2.87	7.40	88.67
CV	18.46	24.54	31.61	14.13	14.27	8.46	15.78	12.24	10.11	7.64

†Standard Error of Mean, ‡ Standard Deviation

Traits were GY, grain yield; BW, biomass weight; SW, straw weight; GYP, grain yield per plant; NGP, number of grains per plant; TGW, thousand grain weight; NTP, number of tillers per plant; NFT, number of fertile tillers; SH, spike height; and PH, plant height.

Biomass weight (BW) and straw weight (SW) were recorded on a per-plot basis and expressed as dry weight after oven-drying plant samples at 65-70°C until constant weight was achieved. For statistical presentation, values were standardized and reported in appropriate units (kg ha^{-1}).

Table 4. Pearson's correlations for measured traits across 20 barley genotypes

	GY	BW	SW	GYP	NGP	TGW	NTP	NFT	SH
BW	0.44								
SW	0.25	0.98							
GYP	0.43	0.28	0.21						
NGP	0.08	0.11	0.10	0.80					
TGW	0.59	0.23	0.12	0.33	-0.29				
NTP	0.27	0.25	0.21	0.12	0.10	0.07			
NFT	0.37	0.36	0.30	0.44	0.32	0.21	0.73		
SH	-0.35	0.02	0.10	0.25	0.60	-0.58	0.07	-0.09	
PH	-0.30	0.44	0.54	0.05	0.16	-0.23	0.23	0.18	0.42

Critical amounts $P<0.05$ and $P<0.01$ (with 18 degrees of freedom) are 0.44 and 0.56, respectively.

Traits were GY, grain yield; BW, biomass weight; SW, straw weight; GYP, grain yield per plant; NGP, number of grains per plant; TGW, thousand grain weight; NTP, number of tillers per plant; NFT, number of fertile tillers; SH, spike height; and PH, plant height.

3.3. Clustering of traits

The dendrogram was constructed to explore the structural relationships among barley traits (Figure 1), and the selection of the cutoff line was validated using four multivariate ANOVA indices (Table 5). The largest root of Roy and lambda of Wilks, as well as traces of Hotelling and Pillai, were all statistically significant, confirming the appropriateness of the chosen cutoff level in the dendrogram. Based on this analysis, the barley traits were classified into three distinct clusters; Cluster-A consisted of GY, TGW, NTP, and NFT, Cluster-B included BW and SW, whereas Cluster-C contained GYP, NGP, SH, and PH (Figure 1). Cluster-A grouped traits related to yield, its components, and tiller traits, which is aligned with the correlations, as GY had a positive association with TGW and reflected the role of grain size in yield, while NTP and NFT correlations indicated efficient tiller fertility; thus, Cluster-A reflected traits that directly drive productivity. Cluster-B grouped biomass-related traits, which makes sense given their very strong positive correlation, and represented the vegetative growth and above-ground biomass accumulation of the plant. The clustering pattern of Cluster-B captured the physiological similarity between straw and total biomass. Cluster-C collected traits related to per-plant productivity (GYP, NGP) and plant architecture (SH, PH). Although PH was correlated with BW and SW (Cluster-B), it grouped here because it was more closely related to grain number and spike structure, reflecting a combination of yield components and plant morphology. This cluster captured the structural and reproductive aspects of the plant rather than absolute yield or biomass. The clusters reflected the correlation structure, grouping traits by their physiological and agronomic roles: yield, biomass, and reproductive/structural traits, while also resolving traits that interact across multiple physiological processes.

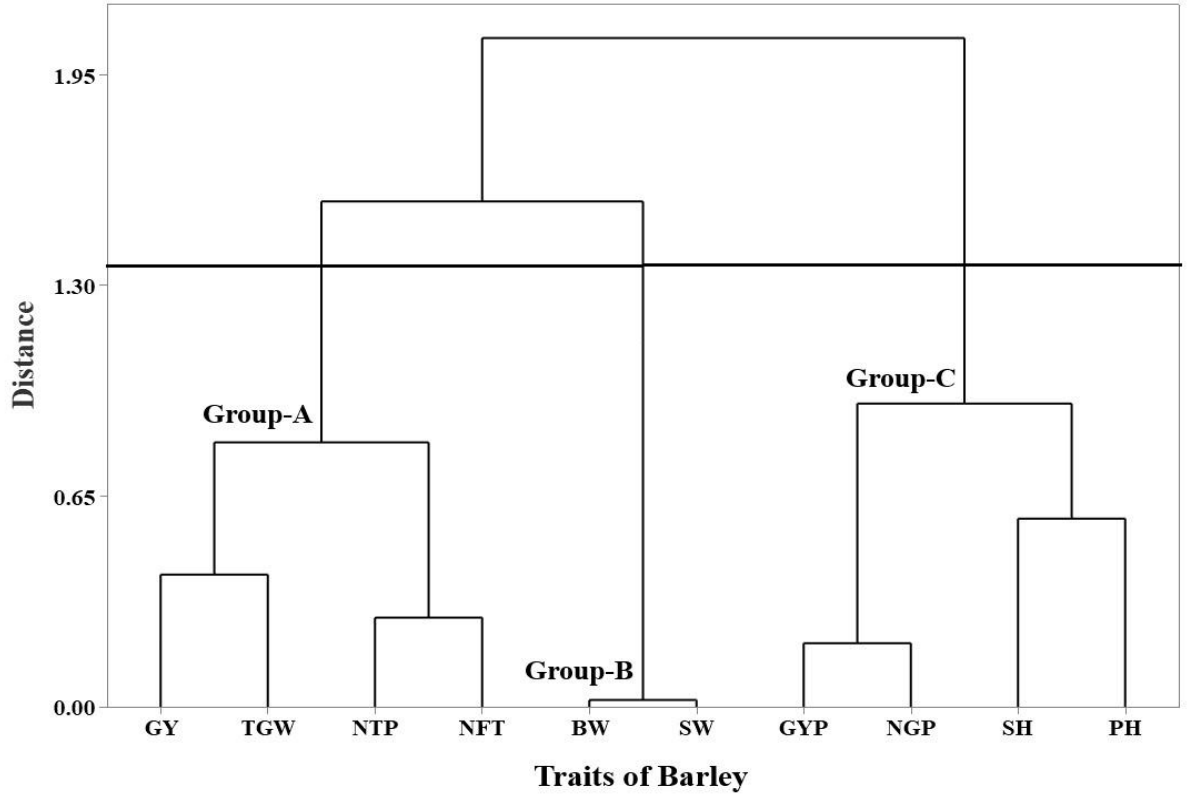


Figure 1. The dendrogram of cluster analysis in traits barley genotypes

Traits were GY, grain yield; BW, biomass weight; SW, straw weight; GYP, grain yield per plant; NGP, number of grains per plant; TGW, thousand grain weight; NTP, number of tillers per plant; NFT, number of fertile tillers; SH, spike height; and PH, plant height.

3.4. Clustering of genotypes

The dendrogram was constructed to examine the grouping pattern among barley genotypes (Figure 2). The selection of the cutoff line was validated using the largest root of Roy, lambda of Wilks, and traces of Hotelling and Pillai, all of which were statistically significant (Table 5). Based on this cutoff, the barley genotypes were classified into three clusters. Cluster I comprised seven genotypes (G1, G2, G4, G6, G7, G8, and G12) and included the commercial variety Mahour. This cluster showed the highest mean grain yield (2280.00 kg ha^{-1}) and grain yield per plant (1.92 g). It also had a higher mean number of grains per plant (58.48) and thousand-grain weight (33.12) compared with Cluster II. Biomass weight, straw weight, spike height, and plant height showed lower mean values in this cluster (Table 6). Cluster II included six genotypes (G3, G5, G9, G10, G15, and G20). This cluster recorded the lowest mean grain yield (1778.33 kg ha^{-1}) and the lowest grain yield per plant (1.48 g), along with a lower number of grains per plant (47.61). Biomass weight and straw weight were also lower compared with Clusters I and III (Table 6). Cluster III consisted of seven genotypes (G11,

G13, G14, G16, G17, G18, and G19). This cluster showed the highest biomass weight (9542.74) and straw weight (7294.65). It also recorded the highest plant height (84.80 cm) and spike height (6.09 cm). Grain yield (2248.09 kg ha^{-1}) and grain yield per plant (1.92 g) were comparable to Cluster I (Table 6).

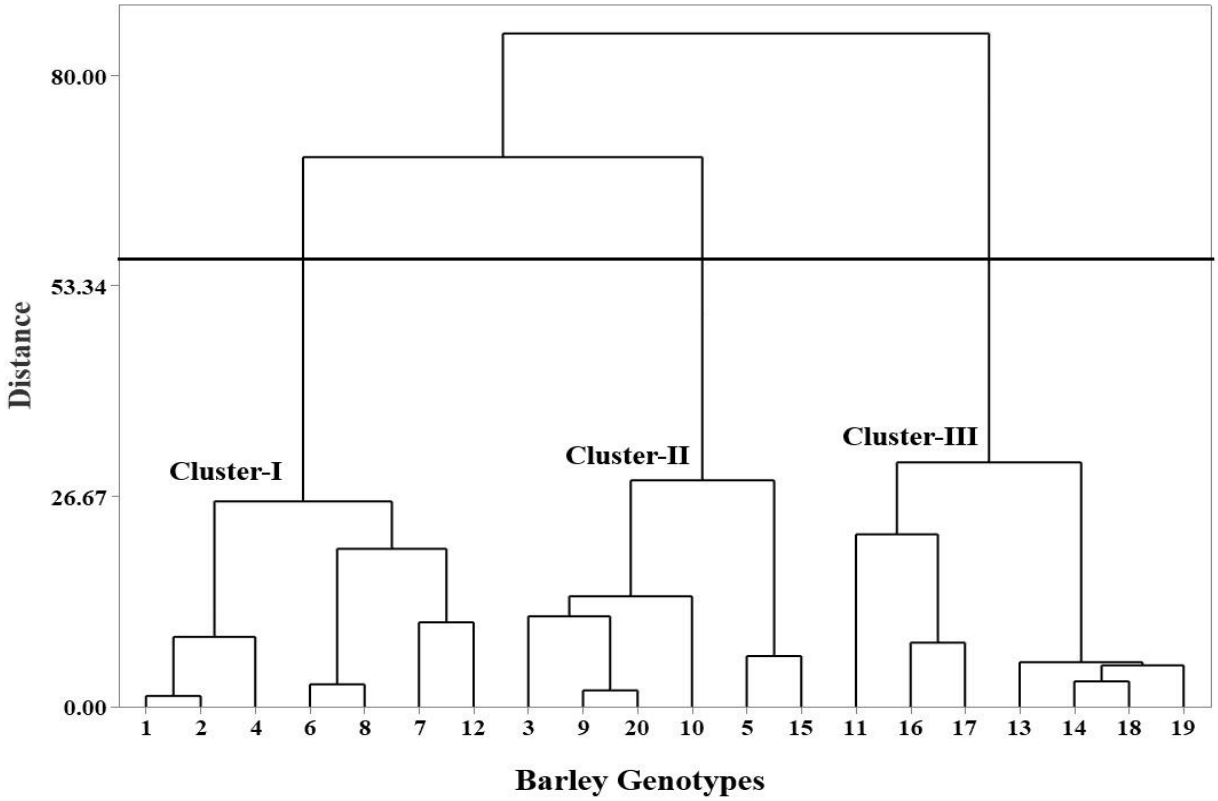


Figure 2. The dendrogram of cluster analysis in 20 barley genotypes

Table 5. Multivariate indices for determining the cutoff line of the dendrogram for categorizing genotypes and traits

Statistics	Hypothesis df†	Error df	Value	F-test
Genotypes				
Pillai's Trace	18	20	1.61	4.57**
Wilks' Lambda	18	18	0.04	4.15**
Hotelling's Trace	18	16	8.38	3.72**
Roy's Largest Root	9	10	4.83	5.37**
Traits				
Pillai's Trace	14	4	1.84	3.30**
Wilks' Lambda	14	2	0.02	30.94**
Hotelling's Trace	14	1	72.25	15.01**
Roy's Largest Root	7	2	71.98	20.14**

†df, degrees of freedom. ** significant at 0.01 probability level.

Table 6. The averages of measured traits across three identified clusters in barley genotypes

Traits	Cluster-I	Cluster-II	Cluster-III
GY	2280.00 ± 395.77	1778.33 ± 339.25	2248.09 ± 257.24
BW	6328.49 ± 692.09	6707.54 ± 1744.14	9542.74 ± 909.55
SW	4048.49 ± 557.29	4929.21 ± 1528.23	7294.65 ± 776.85
GYP	1.92 ± 0.16	1.48 ± 0.16	1.92 ± 0.15
NGP	58.48 ± 8.46	47.61 ± 5.02	58.43 ± 4.65
TGW	33.12 ± 2.56	31.27 ± 2.27	32.81 ± 3.31
NTP	2.96 ± 0.43	2.72 ± 0.18	2.96 ± 0.64
NFT	2.32 ± 0.23	2.06 ± 0.27	2.30 ± 0.27
SH	5.79 ± 0.57	5.73 ± 0.38	6.09 ± 0.78
PH	75.48 ± 5.50	78.99 ± 5.38	84.80 ± 3.42

Traits were GY, grain yield; BW, biomass weight; SW, straw weight; GYP, grain yield per plant; NGP, number of grains per plant; TGW, thousand grain weight; NTP, number of tillers per plant; NFT, number of fertile tillers; SH, spike height; and PH, plant height.

4. Discussion

The present study revealed high phenotypic variation among barley genotypes, particularly for yield- and biomass-related traits, indicating a wide genetic base suitable for selection under rainfed conditions. In contrast, morphological traits such as plant height and spike height showed relatively low variability, suggesting greater phenotypic stability and limited environmental sensitivity.

Thousand-grain weight also exhibited low variability, indicating that grain size is a relatively conserved trait within the evaluated germplasm. These observations are consistent with previous studies. According to Naresh and Kumar (2023), biomass-related traits (BW and SW) showed high variability, while grain yield per plant (GYP), number of tillers per plant (NTP), and number of fertile tillers (NFT) exhibited moderate variation. In contrast, traits related to grain size and plant morphology, including thousand-grain weight (TGW), spike height (SH), and plant height (PH), showed low coefficients of variation. These authors concluded that yield and biomass traits are more variable than morphological traits, indicating greater potential for selection, whereas morphological traits tend to be more stable due to lower environmental influence. Similarly, Tilahun et al. (2025) reported high variability for grains per spike, spike grain weight, grain yield performance, biological yield, and harvest index, while spike length and fertile tillers showed moderate variability, and plant height and thousand-grain weight exhibited low variability, confirming that yield-related traits are generally more responsive to selection than structural traits.

The correlation structure in the present study further demonstrated that grain yield is jointly determined by biomass accumulation and grain size, as evidenced by its positive associations with biomass weight and thousand-grain weight. This indicates that yield formation in barley is governed by both source capacity (biomass production) and sink strength (grain size and grain number). The strong relationship between tiller number and fertile tillers further suggests efficient reproductive allocation and stability of tiller fertility among genotypes. However, yield formation is also constrained by physiological trade-offs among traits. The negative association between spike height and thousand-grain weight highlights a trade-off between spike architecture and grain size development. Similar antagonistic relationships among yield components have been reported in barley by Czembor et al. (2022). More broadly, research on barley genotype diversity has shown that traits such as plant height and grain filling duration may exhibit opposite loadings to yield and grain weight traits, reinforcing the presence of complex physiological trade-offs in phenotypic expression (Serrago et al., 2025).

Trait clustering further clarified the functional organization of agronomic traits. The grouping of traits into yield/tillering, biomass, and plant architecture/per-plant productivity clusters reflects their physiological integration. Such clustering patterns are consistent with previous studies, where yield-related traits tend to form distinct functional groups. For example, Güngör et al. (2024), evaluating 75 barley genotypes, reported that yield performance, thousand-grain weight, and grains per spike consistently clustered together, reflecting strong interdependence among yield components. Similarly, Abdelghany et al. (2025) observed clear separation between phenological traits and yield components in Egyptian barley landraces, highlighting the physiological distinction between developmental timing and yield formation processes. Genotypic clustering revealed three distinct performance groups with contrasting agronomic profiles. High-yielding genotypes were characterized by efficient partitioning of assimilates toward reproductive structures, balanced tillering, and favorable yield components. Low-performing genotypes showed consistently poor expression of both biomass and yield traits, reflecting limited genetic potential. In contrast, biomass-rich genotypes exhibited vigorous vegetative growth and tall stature, with moderate to high yield performance, indicating their value as parental material for improving plant vigor and biomass-related traits. Similar patterns have also been observed in other cereals such as wheat and rye, where vigorous biomass producers are used to enhance structural traits in breeding programs (Aharon et al., 2021; Hackauf et al., 2022). However, such genotypes may require further refinement, particularly reduction in plant height, to improve harvest index and grain production efficiency. Finally, the integration of phenotypic variability, trait correlations, and clustering analysis provides a comprehensive framework for understanding yield formation in barley under rainfed conditions. The results emphasize that yield improvement requires simultaneous consideration of biomass production, grain weight, and plant architecture. Moreover, the presence of trade-offs among traits highlights the importance of balanced multi-trait selection strategies that account for physiological constraints and trait interdependencies. Collectively, these findings provide a robust basis for targeted barley breeding aimed at improving yield stability, biomass productivity, and agronomic performance under water-limited (rainfed) environments.

5. Conclusions

The present study demonstrated substantial phenotypic diversity among barley genotypes under rainfed conditions, particularly for yield- and biomass-related traits, while morphological traits were relatively stable. Trait correlations indicated that grain yield is primarily governed by the combined effects of biomass accumulation and grain size, whereas notable trade-offs exist between spike architecture and grain weight. Cluster analysis effectively separated traits into functional groups and identified distinct genotype classes with contrasting agronomic performance. The study does not represent a controlled drought-stress experiment; rather, it reflects genotype performance under rainfed conditions typical of semi-arid environments, where intermittent moisture limitation may occur. Therefore, the results should be interpreted as indicators of performance under water-limited field conditions rather than explicit drought tolerance. From a breeding perspective, high-yielding genotypes with balanced yield components (Cluster I) are recommended for direct selection, while biomass-rich genotypes (Cluster III) represent valuable genetic resources for improving plant vigor and structural traits. The integration of phenotypic variability, trait interrelationships, and clustering analysis provides a robust framework for selecting superior barley genotypes adapted to rainfed agricultural systems.

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